1 Amino Acids, Peptides, Proteins

1.1 Foreword

Amino acids, peptides and proteins are important constituents of food. They supply the required building blocks for protein biosynthesis. In addition, they directly contribute to the flavor of food and are precursors for aroma compounds and colors formed during thermal or enzymatic reactions in production, processing and storage of food. Other food constituents, e. g., carbohydrates, also take part in such reactions. Proteins also contribute significantly to the physical properties of food through their ability to build or stabilize gels, foams, emulsions and fibrillar structures. The nutritional energy value of proteins (17 kJ/g or 4 kcal/g) is as high as that of carbohydrates.

The most important sources of protein are grain, oilseeds and legumes, followed by meat and milk. In addition to plants and animals, protein producers include algae (*Chlorella, Scenedesmus, Spirulina* spp.), yeasts and bacteria (single-cell proteins [SCP]). Among the C sources we use are glucose, molasses, starch, sulfite liquor, waste water, the higher n-alkanes, and methanol. Yeast of the genus *Candida* grow on paraffins, for example, and supply about 0.75 t of protein per t of carbohydrate. Bacteria of the species *Pseudomonas* in aqueous methanol produce about 0.30 t of protein per t of alcohol. Because of the high nucleic acid content of yeasts and bacteria (6–17% of dry weight), it is necessary to isolate protein from the cell mass. The future importance of single-cell proteins depends on price and on the technological properties.

In other raw materials, too, protein enrichment occurs for various reasons: protein concentration in the raw material may be too low for certain purposes, the sensory characteristics of the material (color, taste) may not be acceptable, or undesirable constituents may be present. Some products rich in protein also result from other processes, e. g., in oil and starch production. Enrichment results from the extraction of the constituents (protein concentrate) or from extraction and subsequent separation of protein from the solution, usually through thermal coagulation or isoelectric precipitation (protein isolate). Protein concentrates and protein isolates serve to enhance the nutritional value and to achieve the enhancement of the above mentioned physical properties of foods. They are added, sometimes after modification (cf. 1.4.6.1), to traditional foods, such as meat and cereal products, but they are also used in the production of novel food items such as meat, fish and milk substitutes. Raw materials in which protein enrichment takes place include:

- Legumes such as soybeans (cf. 16.3.1.2.1) and broad beans;
- Wheat and corn, which provide gluten as a by-product of starch production;
- Potatoes; from the natural sap left over after starch production, proteins can be isolated by thermal coagulation;
- Eggs, which are processed into different whole egg, egg white and egg yolk products (cf. 11.4);
- Milk, which supplies casein (cf. 10.2.9 and whey protein (cf. 10.2.10);
- Fish, which supplies protein concentrates after fat extraction (cf. 13.1.6.13 and 1.4.6.3.2);
- Blood from slaughter animals, which is processed into blood meal, blood plasma concentrate (cf. 12.6.1.10) and globin isolate.
- Green plants grown for animal fodder, such as alfalfa, which are processed into leaf protein concentrates through the thermal coagulation of cell sap proteins.

1.2 Amino Acids

1.2.1 General Remarks

There are about 20 amino acids in a protein hydrolysate. With a few exceptions, their general

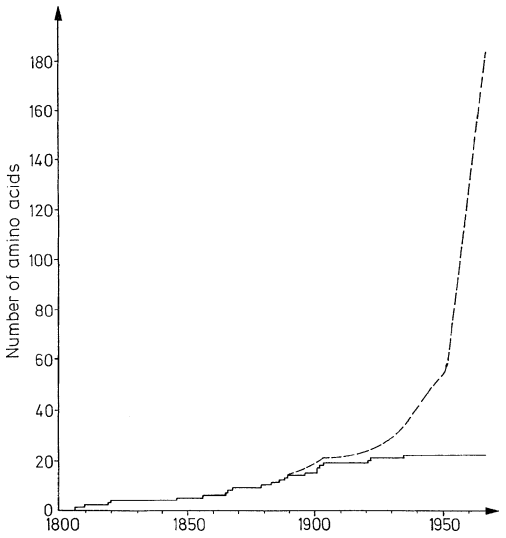
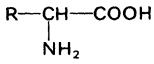


Fig. 1.1. Discovery of naturally occurring amino acids (according to *Meister*, 1965).--- Amino acids, total; — protein constituents

structure is:



(1.0)

In the simplest case, R=H (aminoacetic acid or glycine). In other amino acids, R is an aliphatic, aromatic or heterocyclic residue and may incorporate other functional groups. Table 1.1 shows the most important "building blocks" of proteins. There are about 200 amino acids found in nature (Fig. 1.1). Some of the more uncommon ones, which occur mostly in plants in free form, are covered in Chap. 17 on vegetables.

1.2.2 Classification, Discovery and Occurrence

1.2.2.1 Classification

There are a number of ways of classifiying amino acids. Since their side chains are the deciding factors for intra- and intermolecular interactions in proteins, and hence, for protein properties, amino acids can be classified as:

- Amino acids with nonpolar, uncharged side chains: e. g., glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan and methionine.
- Amino acids with uncharged, polar side chains: e. g., serine, threonine, cysteine, tyrosine, asparagine and glutamine.
- Amino acids with charged side chains: e. g., aspartic acid, glutamic acid, histidine, lysine and arginine.

Based on their nutritional/physiological roles, amino acids can be differentiated as:

- Essential amino acids:
  Valine, leucine, isoleucine, phenylalanine, tryptophan, methionine, threonine, histidine (essential for infants), lysine and arginine ("semi-essential").
- Nonessential amino acids:
  Glycine, alanine, proline, serine, cysteine, tyrosine, asparagine, glutamine, aspartic acid and glutamic acid.

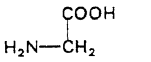
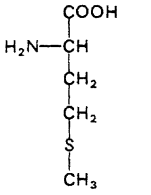
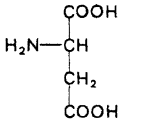
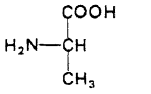
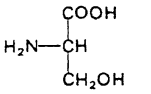
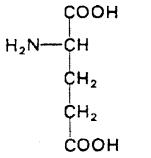
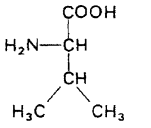
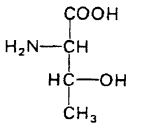
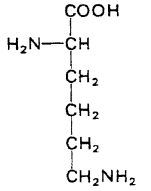
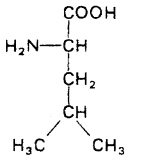
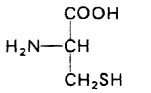
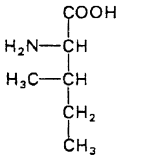
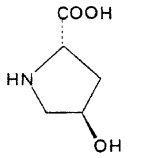
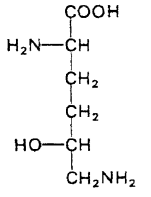
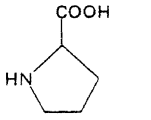
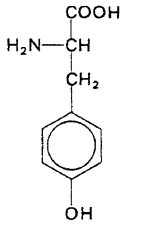
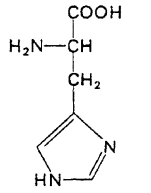
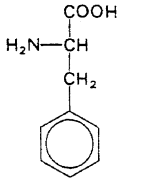
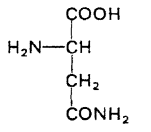
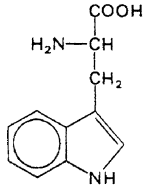
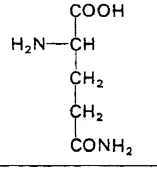
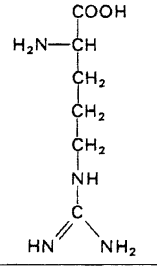
1.2.2.2 Discovery and Occurrence

Alanine was isolated from silk fibroin by *Weyl* in 1888. It is present in most proteins and is particularly enriched in silk fibroin (35%). Gelatin and zein contain about 9% alanine, while its content in other proteins is 2–7%. Alanine is considered nonessential for humans.

Arginine was first isolated from lupin seedlings by *Schulze* and *Steiger* in 1886. It is present in all proteins at an average level of 3–6%, but is particularly enriched in protamines. The arginine content of peanut protein is relatively high (11%). Biochemically, arginine is of great importance as an intermediary product in urea synthesis. Arginine is a semi-essential amino acid for humans. It appears to be required under certain metabolic conditions.

Asparagine from asparagus was the first amino acid isolated by *Vauguelin* and *Robiquet* in 1806. Its occurrence in proteins (edestin) was confirmed by *Damodaran* in 1932. In glycoproteins the carbohydrate component may be bound N-glycosidically to the protein moiety through the amide group of asparagine (cf. 11.2.3.1.1 and 11.2.3.1.3).

Table 1.1. Amino acids (protein building blocks) with their corresponding three ond one letter symbols

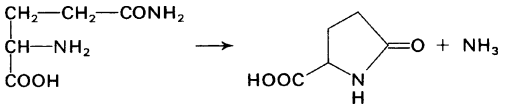
 Glycine (Gly. G)	 L-Methionine (Met. M)	 L-Aspartic acid (Asp. D)
 L-Alanine (Ala. A)	 L-Serine (Ser. S)	 L-Glutamic acid (Glu. E)
 L-Valine (Val. V)	 L-Threonine (Thr. T)	 L-Lysine (Lys. K)
 L-Leucine (Leu. L)	 L-Cysteine (Cys. C)	
 L-Isoleucine (Ile. I)	 L-4-Hydroxy-proline	 L-5-Hydroxy-lysine
 L-Proline (Pro. P)	 L-Tyrosine (Tyr. Y)	 L-Histidine (His. H)
 L-Phenylalanine (Phe. F)	 L-Asparagine[a] (Asn. N)	
 L-Tryptophan (Trp. W)	 L-Glutamine[a] (Gln. Q)	 L-Arginine (Arg. R)

[a] When no distinction exists between the acid and its amide then the symbols (Asx, B) and (Glx, Z) are valid.

Aspartic Acid was isolated from legumes by *Ritthausen* in 1868. It occurs in all animal proteins, primarily in albumins at a concentration of 6–10%. Alfalfa and corn proteins are rich in aspartic acid (14.9% and 12.3%, respectively) while its content in wheat is low (3.8%). Aspartic acid is nonessential.

Cystine was isolated from bladder calculi by *Wolaston* in 1810 and from horns by *Moerner* in 1899. Its content is high in keratins (9%). Cystine is very important since the peptide chains of many proteins are connected by two cysteine residues, i. e. by disulfide bonds. A certain conformation may be fixed within a single peptide chain by disulfide bonds. Most proteins contain 1–2% cystine. Although it is itself nonessential, cystine can partly replace methionine which is an essential amino acid.

Glutamine was first isolated from sugar beet juice by *Schulze* and *Bosshard* in 1883. Its occurrence in protein (edestin) was confirmed by *Damodaran* in 1932. Glutamine is readily converted into pyrrolidone carboxylic acid, which is stable between pH 2.2 and 4.0, but is readily cleaved to glutamic acid at other pH's:



(1.1)

Glutamic Acid was first isolated from wheat gluten by *Ritthausen* in 1866. It is abundant in most proteins, but is particularly high in milk proteins (21.7%), wheat (31.4%), corn (18.4%) and soya (18.5%). Molasses also contains relatively high amounts of glutamic acid. Monosodium glutamate is used in numerous food products as a flavor enhancer.

Glycine is found in high amounts in structural protein. Collagen contains 25–30% glycine. It was first isolated from gelatin by *Braconnot* in 1820. Glycine is a nonessential amino acid although it does act as a precursor of many compounds formed by various biosynthetic mechanisms.

Histidine was first isolated in 1896 independently by *Kossel* and by *Hedin* from protamines occurring in fish. Most proteins contain 2–3% histidine. Blood proteins contain about 6%. Histidine is essential in infant nutrition.

5-Hydroxylysine was isolated by *van Slyke et al.* (1921) and *Schryver et al.* (1925). It occurs in collagen. The carbohydrate component of glycoproteins may be bound O-glycosidically to the hydroxyl group of the amino acid (cf. 12.3.2.3.1).

4-Hydroxyproline was first obtained from gelatin by *Fischer* in 1902. Since it is abundant in collagen (12.4%), the determination of hydroxyproline is used to detect the presence of connective tissue in comminuted meat products. Hydroxyproline is a nonessential amino acid.

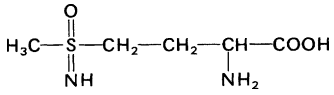
Isoleucine was first isolated from fibrin by *Ehrlich* in 1904. It is an essential amino acid. Meat and ceral proteins contain 4–5% isoleucine; egg and milk proteins, 6–7%.

Leucine was isolated from wool and from muscle tissue by *Braconnot* in 1820. It is an essential amino acid and its content in most proteins is 7–10%. Cereal proteins contain variable amounts (corn 12.7%, wheat 6.9%). During alcoholic fermentation, fusel oil is formed from leucine and isoleucine.

Lysine was isolated from casein by *Drechsel* in 1889. It makes up 7–9% of meat, egg and milk proteins. The content of this essential amino acid is 2–4% lower in cereal proteins in which prolamin is predominant. Crab and fish proteins are the richest sources (10–11%). Along with threonine and methionine, lysine is a limiting factor in the biological value of many proteins, mostly those of plant origin. The processing of foods results in losses of lysine since its ε-amino group is very reactive (cf. *Maillard* reaction).

Methionine was first isolated from casein by *Mueller* in 1922. Animal proteins contain 2–4% and plant proteins contain 1–2% methionine. Methionine is an essential amino acid and in many biochemical processes its main role is as a methyl-donor. It is very sensitive to oxygen and heat treatment. Thus, losses occur in many food processing operations such as drying, kiln-drying, puffing, roasting or treatment with oxidizing agents. In the bleaching of flour

with NCl_3 (nitrogen trichloride), methionine is converted to the toxic methionine sulfoximide:



(1.2)

Phenylalanine was isolated from lupins by *Schulze* in 1881. It occurs in almost all proteins (averaging 4–5%) and is essential for humans. It is converted *in vivo* into tyrosine, so phenylalanine can replace tyrosine nutritionally.

Proline was discovered in casein and egg albumen by *Fischer* in 1901. It is present in numerous proteins at 4–7% and is abundant in wheat proteins (10.3%), gelatin (12.8%) and casein (12.3%). Proline is nonessential.

Serine was first isolated from sericin by *Cramer* in 1865. Most proteins contain about 4–8% serine. In phosphoproteins (casein, phosvitin) serine, like threonine, is a carrier of phosphoric acid in the form of O-phosphoserine. The carbohydrate component of glycoproteins may be bound O-glycosidically through the hydroxyl group of serine and/or threonine [cf. 10.1.2.1.1 (κ-casein) and 13.1.4.2.4].

Threonine was discovered by *Rose* in 1935. It is an essential amino acid, present at 4.5–5% in meat, milk and eggs and 2.7–4.7% in cereals. Threonine is often the limiting amino acid in proteins of lower biological quality. The "bouillon" flavor of protein hydrolysates originates partly from a lactone derived from threonine (cf. 5.3.1.3).

Tryptophan was first isolated from casein hydrolysates, prepared by hydrolysis using pancreatic enzymes, by *Hopkins* in 1902. It occurs in animal proteins in relatively low amounts (1–2%) and in even lower amounts in cereal proteins (about 1%). Tryptophan is exceptionally abundant in lysozyme (7.8%). It is completely destroyed during acidic hydrolysis of protein. Biologically, tryptophan is an important essential amino acid, primarily as a precursor in the biosynthesis of nicotinic acid.

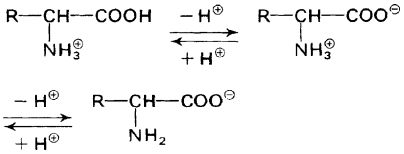
Tyrosine was first obtained from casein by *Liebig* in 1846. Like phenylalanine, it is found in almost all proteins at levels of 2–6%. Silk fibroin can have as much as 10% tyrosine. It is converted through dihydroxyphenylalanine by enzymatic oxidation into brown-black colored melanins.

Valine was first isolated by Schutzenberger in 1879. It is an essential amino acid and is present in meat and cereal proteins (5–7%) and in egg and milk proteins (7–8%). Elastin contains notably high concentrations of valine (15.6%).

1.2.3 Physical Properties

1.2.3.1 Dissociation

In aqueous solution amino acids are present, depending on pH, as cations, zwitterions or anions:



(1.3)

With the cation denoted as ^{+}A, the dipolar zwitterion as $^{+}A^{-}$ and the anion as A^{-}, the dissociation constant can be expressed as:

$$\frac{[^{\oplus}A^{\ominus}][H^{\oplus}]}{[^{\oplus}A]} = K_1 \qquad \frac{[A^{\ominus}][H^{\oplus}]}{[^{\oplus}A^{\ominus}]} = K_2 \tag{1.4}$$

At a pH where only dipolar ions exist, i. e. the isoelectric point, pI, $[^{+}A] = [A^{-}]$:

$$[^{\oplus}A] = \frac{[^{\oplus}A^{\ominus}][H^{\oplus}]}{K_1} = [A^{\ominus}] = \frac{[^{\oplus}A^{\ominus}]K_2}{[H^{\oplus}]}$$

$$[H^{\oplus}] = (K_1 \cdot K_2)^{0,5}$$

$$pI = 0{,}5\,(pK_1 + pK_2) \tag{1.5}$$

The dissociation constants of amino acids can be determined, for example, by titration of the acid. Figure 1.2 shows titration curves for glycine, histidine and aspartic acid. Table 1.2 lists the dissociation constants for some amino acids. In amino acids the acidity of the carboxyl group is higher and the basicity of the amino group lower than in the corresponding carboxylic acids and amines (cf. pK values for propionic acid, 2-propylamine and alanine). As illustrated by the comparison of pK values of 2-aminopropionic acid (alanine) and 3-aminopropionic acid (β-alanine), the pK is influenced by the distance between the two functional groups.

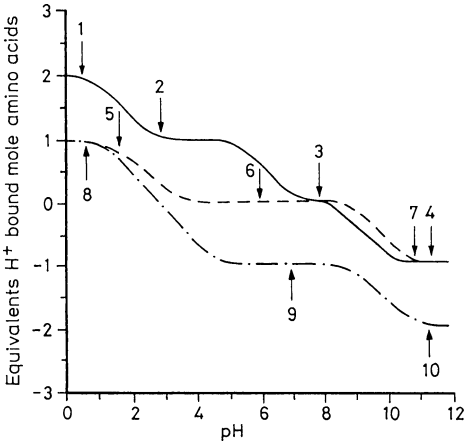
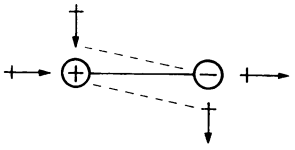


Fig. 1.2. Calculated titration curves for glycine (---), histidine (—) and aspartic acid (-·-·-). Numerals on curves are related to charge of amino acids in respective pH range: 1 $^{++}$His, 2 $^{++}$His^{-}, 3 $^{+}$His^{-}, 4 His^{-}, 5 $^{+}$Gly, 6 $^{+}$Gly^{-}, 7 Gly^{-}, 8 $^{+}$Asp. 9 $^{+}$Asp^{--}, 10 Asp^{--}

Table 1.2. Amino acids: dissociation constants and isoelectric points at 25 °C

Amino acid	pK_1	pK_2	pK_3	pK_4	pI
Alanine	2.34	9.69			6.0
Arginine	2.18	9.09	12.60		10.8
Asparagine	2.02	8.80			5.4
Aspartic acid	1.88	3.65	9.60		2.8
Cysteine	1.71	8.35	10.66		5.0
Cystine	1.04	2.10	8.02	8.71	5.1
Glutamine	2.17	9.13			5.7
Glutamic acid	2.19	4.25	9.67		3.2
Glycine	2.34	9.60			6.0
Histidine	1.80	5.99	9.07		7.5
4-Hydroxyproline	1.82	9.65			5.7
Isoleucine	2.36	9.68			6.0
Leucine	2.36	9.60			6.0
Lysine	2.20	8.90	10.28		9.6
Methionine	2.28	9.21			5.7
Phenylalanine	1.83	9.13			5.5
Proline	1.99	10.60			6.3
Serine	2.21	9.15			5.7
Threonine	2.15	9.12			5.6
Tryptophan	2.38	9.39			5.9
Tyrosine	2.20	9.11	10.07		5.7
Valine	2.32	9.62			6.0
Propionic acid	4.87				
2-Propylamine	10.63				
β-Alanine	3.55	10.24			6.9
γ-Aminobutyric acid	4.03	10.56			7.3

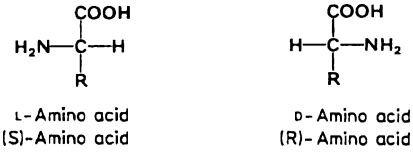
The reasons for this are probably as follows: in the case of the cation → zwitterion transition, the inductive effect of the ammonium group; in the case of the zwitterion → anion transition, the stabilization of the zwitterion through hydration caused by dipole repulsion (lower than in relation to the anion).



(⊕—⊖, zwitterion; ⟷ water dipole) (1.6)

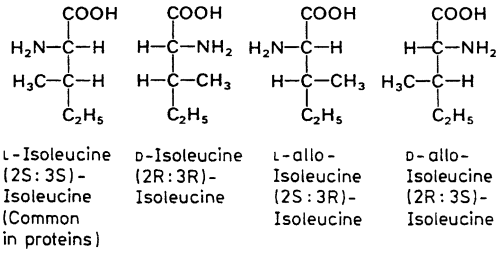
1.2.3.2 Configuration and Optical Activity

Amino acids, except for glycine, have at least one chiral center and, hence, are optically active. All amino acids found in proteins have the same configuration on the α-C-atom: they are considered L-amino acids or (S)-amino acids* in the *Cahn-Ingold-Prelog* system (with L-cysteine an exception; it is in the (R)-series). D-amino acids (or (R)-amino acids) also occur in nature, for example, in a number of peptides of microbial origin:



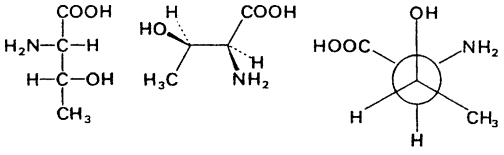
(1.7)

Isoleucine, threonine and 4-hydroxyproline have two asymmetric C-atoms, thus each has four isomers:



(1.8)

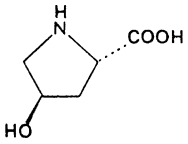
* As with carbohydrates, D,L-nomenclature is preferred with amino acids.



(Fischer-projection) (dotted line-wedge) (Newman-projection)

L-Threonine, (2S : 3R)-Threonine (Common in proteins)

(1.9)



L-4-Hydroxyproline, (2S: 4R)-Hydroxyproline (Common in proteins) (1.10)

The specific rotation of amino acids in aqueous solution is strongly influenced by pH. It passes through a minimum in the neutral pH range and rises after addition of acids or bases (Table 1.3). There are various possible methods of separating the racemates which generally occur in amino acid synthesis (cf. 1.2.5). Selective crystallization of an over-saturated solution of racemate after seeding with an enantiomer is used, as is the fractioned crystallization of diastereomeric salts or other derivatives, such as (S)-phenylethylammonium salts of N-acetylamino acids. With enzymatic methods, asymmetric synthesis is used, e. g., of acylamino acid anilides from acylamino acids and aniline through papain:

$$\text{D,L—R—CO—NH—CHR}^1\text{—COOH} \xrightarrow[\text{Papain}]{\text{Aniline}}$$
$$\text{L—R—CO—NH—CHR}^1\text{—CO—NH—C}_6\text{H}_5$$
$$+\ \text{D—R—CO—NH—CHR}^1\text{—COOH} \qquad (1.11)$$

or asymmetric hydrolysis, e. g., of amino acid esters through esterases, amino acid amides through amidases or N-acylamino acids through amino-acylases:

$$\text{D,L—H}_2\text{N—CHR—COOR}^1 \xrightarrow{\text{Esterase}}$$
$$\text{L—H}_2\text{NCHR—COOH} + \text{D—H}_2\text{N—CHR—COOR}^1$$
$$\text{D,L—H}_2\text{N—CHR—CONHR}^1 \xrightarrow{\text{Amidase}}$$
$$\text{L—H}_2\text{N—CHR—COOH} + \text{D—H}_2\text{N—CHR—CONHR}^1$$
$$\text{D,L—R—CO—NH—CHR}^1\text{—COOH} \xrightarrow{\text{Acylase}}$$
$$\text{L—H}_2\text{N—CHR}^1\text{—COOH}$$
$$+\ \text{D—R—CO—NH—CHR}^1\text{—COOH} \qquad (1.12)$$

The detection of D-amino acids is carried out by enantioselective HPLC or GC of chiral amino acid derivatives. In a frequently applied method, the derivatives are produced in a precolumn by reaction with o-phthalaldehyde and a chiral thiol (cf. 1.2.4.2.4). Alternatively, the amino acids can be transformed into trifluoroacetylamino acid-2-(R,S)-butylesters. Their GC separation is shown in Fig. 1.3.

Table 1.3. Amino acids: specific rotation ($[\alpha]_D^t$)

Amino acid	Solvent system	Temperature (°C)	$[\alpha]_D$
L-Alanine	0.97 M HCl	15	+14.7°
	water	22	+ 2.7°
	3 M NaOH	20	+ 3.0°
L-Cystine	1.02 M HCl	24	−214.4°
L-Glutamic acid	6.0 M HCl	22.4	+31.2°
	water	18	+11.5°
	1M NaOH	18	+10.96°
L-Histidine	6.0 M HCl	22.7	+13.0°
	water	25.0	−39.01°
	0.5 M NaOH	20	−10.9°
L-Leucine	6.0 M HCl	25.9	+15.1°
	water	24.7	−10.8°
	3.0 M NaOH	20	+7.6°

1.2.3.3 Solubility

The solubilities of amino acids in water are highly variable. Besides the extremely soluble proline, hydroxyproline, glycine and alanine are also quite soluble. Other amino acids (cf. Table 1.4) are significantly less soluble, with cystine and tyrosine having particularly low solubilities. Addition of acids or bases improves the solubility through salt formation. The presence of other amino acids, in general, also brings about

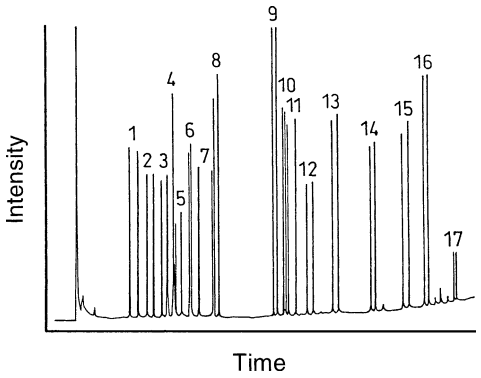


Fig. 1.3. Gas chromatogram of N-pentafluoropropanoylDL-amino acid isopropylesters on Chirasil-Val (N-propionyl-L-valine-tert-butylamide-polysiloxane) (1: D-, L-Ala, 2: D-, L-Val, 3. D-, L-Thr, 4: Gly, 5: D-, L-Ile, 6: D-, L-Pro, 7: D-, L-Leu, 8: D-, L-Ser, 9: D-, L-Cys, 10: D-, L-Asp, 11: D-, L-Met, 12: D-, L-Phe, 13: D-, L-Glu, 14: D-, L-Tyr, 15: D-, L-Orn, 16: D-, L-Lys, 17: D-, L-Trp; according to *Frank* et al., 1977)

Table 1.4. Solubility of amino acids in water ($g/100$ g H_2O)

	Temperature (°C)				
Amino acid	0	25	50	75	100
L-Alanine	12.73	16.51	21.79	28.51	37.30
L-Asparatic acid	0.209	0.500	1.199	2.875	6.893
L-Cystine	0.005	0.011	0.024	0.052	0.114
L-Glutamic acid	0.341	0.843	2.186	5.532	14.00
Glycine	14.18	24.99	39.10	54.39	67.17
L-Histidine	–	4.29	–	–	–
L-Hydroxy-proline	28.86	36.11	45.18	51.67	–
L-Isoleucine	3.791	4.117	4.818	6.076	8.255
L-Leucine	2.270	2.19	2.66	3.823	5.638
D,L-Methi-onine	1.818	3.381	6.070	10.52	17.60
L-Phenyl-alanine	1.983	2.965	4.431	6.624	9.900
L-Proline	127.4	162.3	206.7	239.0	–
D,L-Serine	2.204	5.023	10.34	19.21	32.24
L-Tryptophan	0.823	1.136	1.706	2.795	4.987
L-Tyrosine	0.020	0.045	0.105	0.244	0.565
L-Valine	8.34	8.85	9.62	10.24	–

an increase in solubility. Thus, the extent of solubility of amino acids in a protein hydrolysate is different than that observed for the individual components.

The solubility in organic solvents is not very good because of the polar characteristics of the amino acids. All amino acids are insoluble in ether. Only cysteine and proline are relatively soluble in ethanol (1.5 g/100 g at 19 °C). Methionine, arginine, leucine (0.0217 g/100 g; 25 °C), glutamic acid (0.00035 g/100 g; 25 °C), phenylalanine, hydroxy-proline, histidine and tryptophan are sparingly soluble in ethanol. The solubility of isoleucine in hot ethanol is relatively high (0.09 g/100 g at 20 °C; 0.13 g/100 g at 78–80 °C).

1.2.3.4 UV-Absorption

Aromatic amino acids such as phenylalanine, tyrosine and tryptophan absorb in the UV-range of the spectrum with absorption maxima at 200–230 nm and 250–290 nm (Fig. 1.4). Dissociation of the phenolic HO-group of tyrosine shifts the absorption curve by about 20 nm towards longer wavelengths (Fig. 1.5).

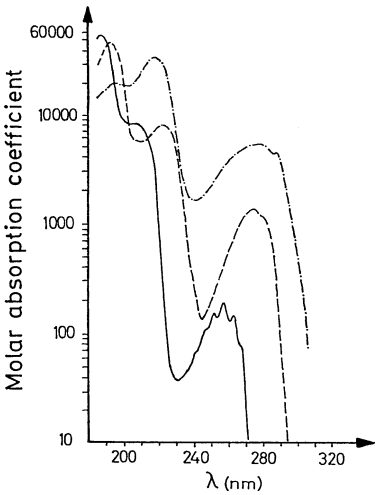


Fig. 1.4. Ultraviolet absorption spectra of some amino acids. (according to *Luebke, Schroeder* and *Kloss*, 1975). -----Trp. ---Tyr. —Phe

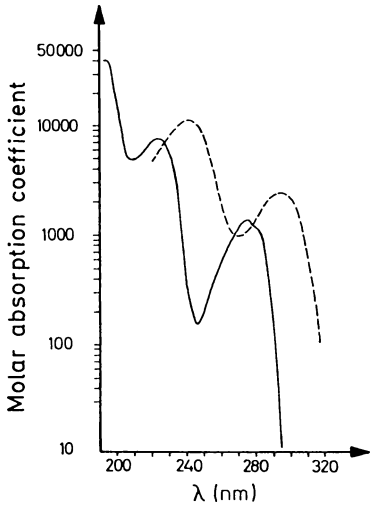


Fig. 1.5. Ultraviolet absorption spectrum of tyrosine as affected by pH. (according to *Luebke, Schroeder* and *Kloss*, 1975) — 0.1 mol/l HCl, --- 0.1 mol/l NaOH

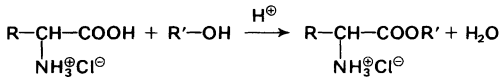
Absorption readings at 280 nm are used for the determination of proteins and peptides. Histidine, cysteine and methionine absorb between 200 and 210 nm.

1.2.4 Chemical Reactions

Amino acids show the usual reactions of both carboxylic acids and amines. Reaction specificity is due to the presence of both carboxyl and amino groups and, occasionally, of other functional groups. Reactions occurring at 100–220 °C, such as in cooking, frying and baking, are particularly relevant to food chemistry.

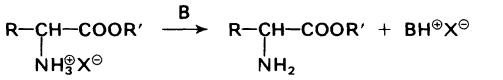
1.2.4.1 Esterification of Carboxyl Groups

Amino acids are readily esterified by acid-catalyzed reactions. An ethyl ester hydrochloride is obtained in ethanol in the presence of HCl:



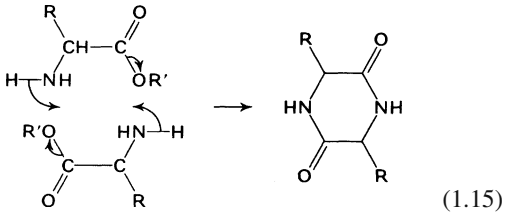
(1.13)

The free ester is released from its salt by the action of alkali. A mixture of free esters can then be separated by distillation without decomposition. Fractional distillation of esters is the basis of a method introduced by *Emil Fischer* for the separation of amino acids:

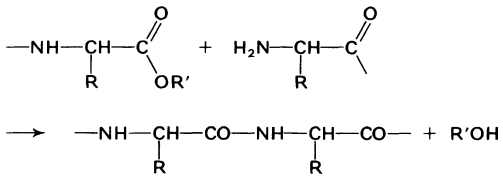


(1.14)

Free amino acid esters have a tendency to form cyclic dipeptides or open-chain polypeptides:



(1.15)



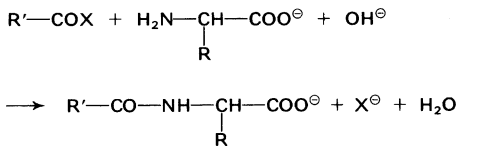
(1.16)

tert-butyl esters, which are readily split by acids, or benzyl esters, which are readily cleaved by HBr/glacial acetic acid or catalytic hydrogenation, are used as protective groups in peptide synthesis.

1.2.4.2 Reactions of Amino Groups

1.2.4.2.1 Acylation

Activated acid derivatives, e. g., acid halogenides or anhydrides, are used as acylating agents:

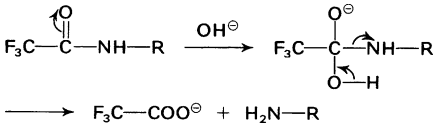


(1.17)

N-acetyl amino acids are being considered as ingredients in chemically-restricted diets and for fortifying plant proteins to increase their biological value. Addition of free amino acids to food which must be heat treated is not problem free. For example, methionine in the presence of a reducing sugar can form methional by a *Strecker* degradation mechanism, imparting an off-flavor to food. Other essential amino acids, e. g., lysine or threonine, can lose their biological value through similar reactions. Feeding tests with rats have shown that N-acetyl-L-methionine and N-acetyl-L-threonine have nutritional values equal to those of the free amino acids (this is true also for humans with acetylated methionine). The growth rate of rats is also increased significantly by the α- or ε-acetyl or α,ε-diacetyl derivatives of lysine.

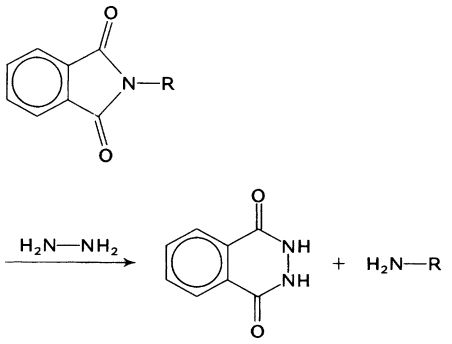
Some readily cleavable acyl residues are of importance as temporary protective groups in peptide synthesis.

The trifluoroacetyl residue is readily removed by mild base-catalyzed hydrolysis:



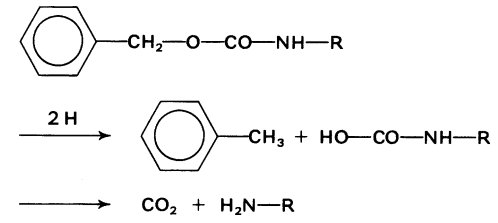
(1.18)

The phthalyl residue can be readily cleaved by hydrazinolysis:

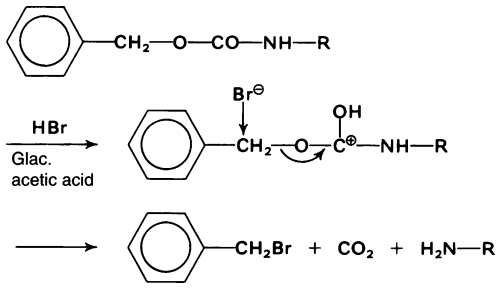


(1.19)

The benzyloxycarbonyl group can be readily removed by catalytic hydrogenation or by hydrolysis with HBr/glacial acetic acid:

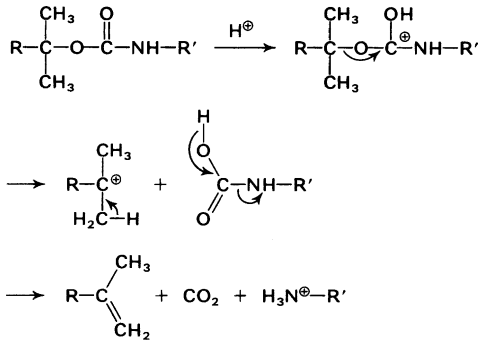


(1.20)



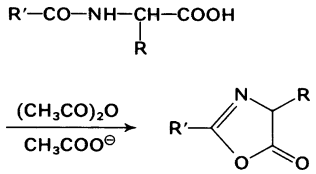
(1.21)

The *tert*-alkoxycarbonyl residues, e. g., the *tert*-butyloxycarbonyl groups, are cleaved under acid-catalyzed conditions:



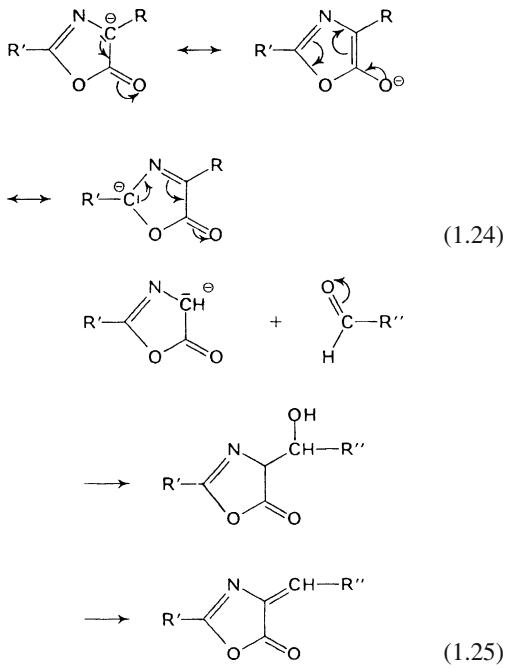
(1.22)

N-acyl derivatives of amino acids are transformed into oxazolinones (azlactones) by elimination of water:



(1.23)

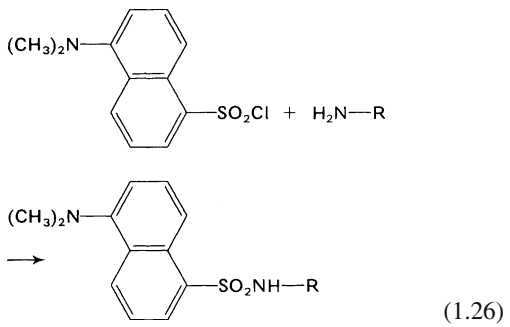
These are highly reactive intermediary products which form a mesomerically stabilized anion. The anion can then react, for example, with aldehydes. This reaction is utilized in amino acid synthesis with glycine azlactone as a starting compound:



(1.24)

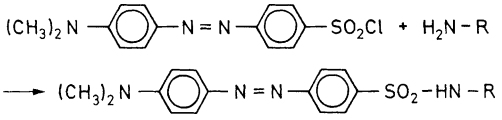
(1.25)

Acylation of amino acids with 5-dimethylaminonaphthalene-1-sulfonyl chloride (dansyl chloride, DANS-Cl) is of great analytical importance:

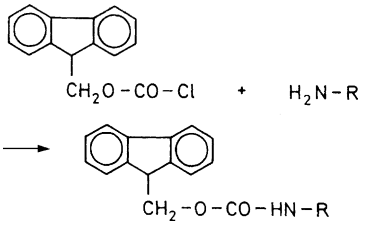


(1.26)

The aryl sulfonyl derivatives are very stable against acidic hydrolysis. Therefore, they are suitable for the determination of free N-terminal amino groups or free ε-amino groups of peptides or proteins. Dansyl derivatives which fluoresce in UV-light have a detection limit in the nanomole range, which is lower than that of 2,4-dinitrophenyl derivatives by a factor of 100. Dimethylaminoazobenzenesulfonylchloride (DABS-Cl) and 9-fluoroenylmethylchloroformate (FMOC) detect amino acids (cf. Formula 1.27 and 1.28) including proline and hydroxyproline. The fluorescent derivatives can be quantitatively determined after HPLC separation.



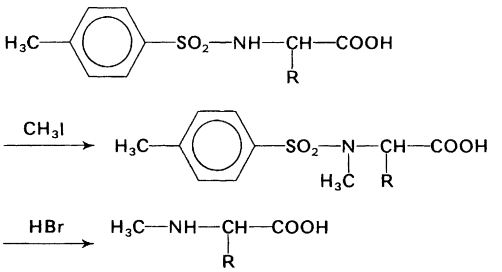
(1.27)



(1.28)

1.2.4.2.2 Alkylation and Arylation

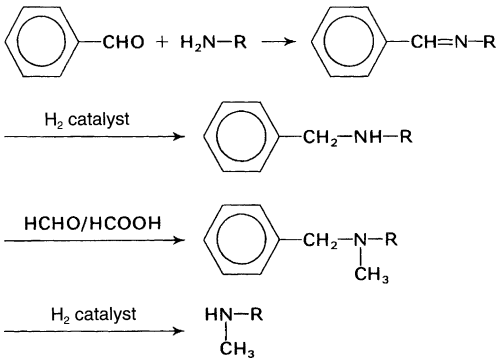
N-methyl amino acids are obtained by reaction of the N-tosyl derivative of the amino acid with methyl iodide, followed by removal of the tosyl substituent with HBr:



(1.29)

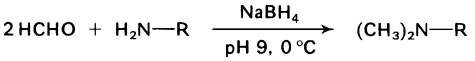
The N-methyl compound can also be formed by methylating with HCHO/HCOOH the benzylidene derivative of the amino acid, formed initially by reaction of the amino acid with benzaldehyde. The benzyl group is then eliminated

by hydrogenolysis:



(1.30)

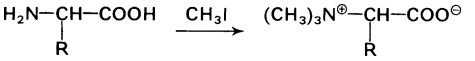
Dimethyl amino acids are obtained by reaction with formaldehyde, followed by reduction with sodium borohydride:



(1.31)

The corresponding reactions with proteins are being considered as a means of protecting the ε-amino groups and, thus, of avoiding their destruction in food through the *Maillard* reaction (cf. 1.4.6.2.2).

Direct reaction of amino acids with methylating agents, e. g. methyl iodide or dimethyl sulfate, proceeds through monomethyl and dimethyl compounds to trimethyl derivatives (or generally to N-trialkyl derivatives) denoted as betaines:



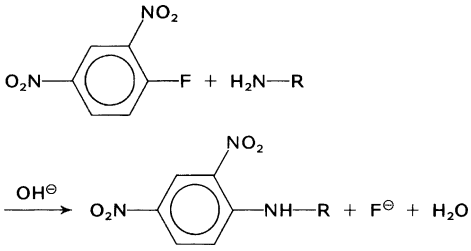
(1.32)

As shown in Table 1.5, betaines are widespread in both the animal and plant kingdoms.

Derivatization of amino acids by reaction with 1-fluoro-2,4-dinitrobenzene (FDNB) yields N-2,4-dinitrophenyl amino acids (DNP-amino acids), which are yellow compounds and crystallize readily. The reaction is important for labeling N-terminal amino acid residues and free ε-amino groups present in peptides and proteins; the DNP-amino acids are stable under conditions of acidic hydrolysis (cf. Reaction 1.33).

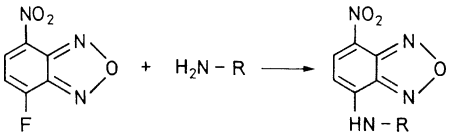
Table 1.5. Occurrence of trimethyl amino acids $(CH_3)_3N^+$-CHR-COO^- (betaines)

Amino acid	Betaine	Occurrence
β-Alanine	Homobetaine	Meat extract
γ-Amino-butyric acid	Actinine	Mollusk (shell-fish)
Glycine	Betaine	Sugar beet, other samples of animal and plant origin
Histidine	Hercynine	Mushrooms
β-Hydroxy-γ-amino-butyric acid	Carnitine	Mammals muscle tissue, yeast, wheat germ, fish, liver, whey, mollusk (shell-fish)
4-Hydroxy-proline	Betonicine	Jack beans
Proline	Stachydrine	Stachys, orange leaves, lemon peel, alfalfa, Aspergillus oryzae



(1.33)

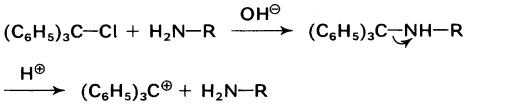
Another arylation reagent is 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazol (NBD-F), which is also used as a chlorine compound (NBD-Cl) and which leads to derivatives that are suited for an amino acid analysis through HPLC separation:



(1.34)

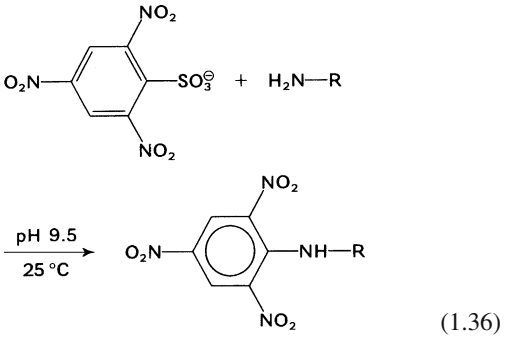
Reaction of amino acids with triphenylmethyl chloride (tritylchloride) yields N-trityl derivatives, which are alkali stable. However, the derivative is cleaved in the presence of acid,

giving a stable triphenylmethyl cation and free amino acid:



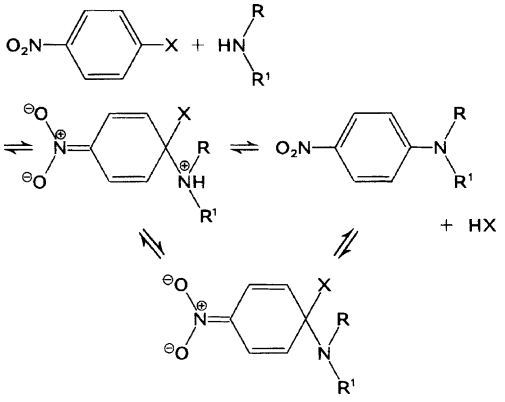
(1.35)

The reaction with trinitrobenzene sulfonic acid is also of analytical importance. It yields a yellow-colored derivative that can be used for the spectrophotometric determination of protein:



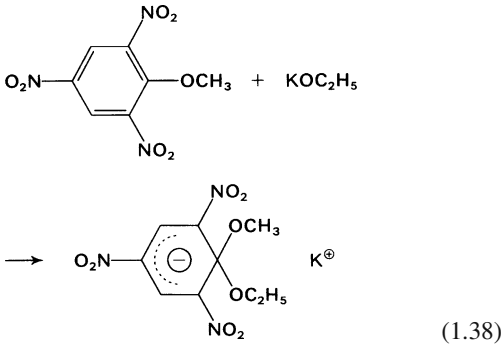
(1.36)

The reaction is a nucleophilic aromatic substitution proceeding through an intermediary addition product (*Meisenheimer* complex). It occurs under mild conditions only when the benzene ring structure is stabilized by electron-withdrawing substituents on the ring (cf. Reaction 1.37).



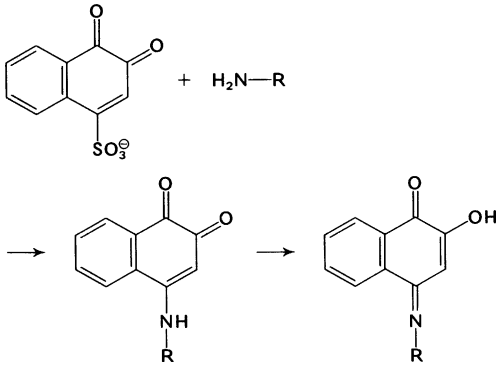
(1.37)

The formation of the *Meisenheimer* complex has been verified by isolating the addition product from the reaction of 2,4,6-trinitroanisole with potassium ethoxide (cf. Reaction 1.38).



(1.38)

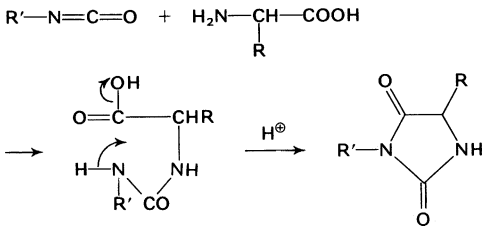
An analogous reaction occurs with 1,2-naphthoquinone-4-sulfonic acid (*Folin* reagent) but, instead of a yellow color (cf. Formula 1.36), a red color develops:



(1.39)

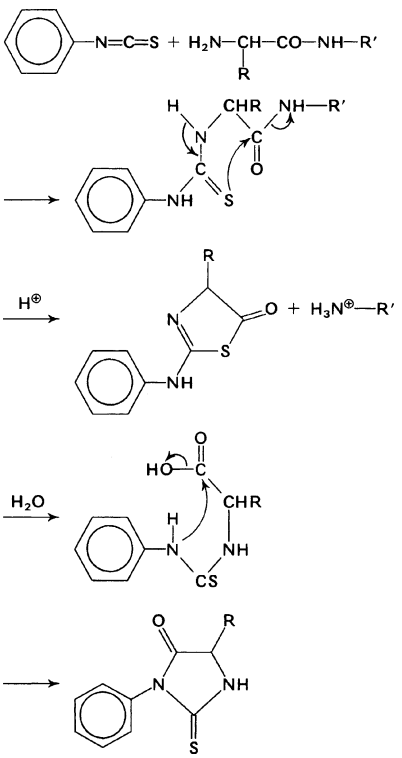
1.2.4.2.3 Carbamoyl and Thiocarbamoyl Derivatives

Amino acids react with isocyanates to yield carbamoyl derivatives which are cyclized into 2,4-dioxoimidazolidines (hydantoins) by boiling in an acidic medium:

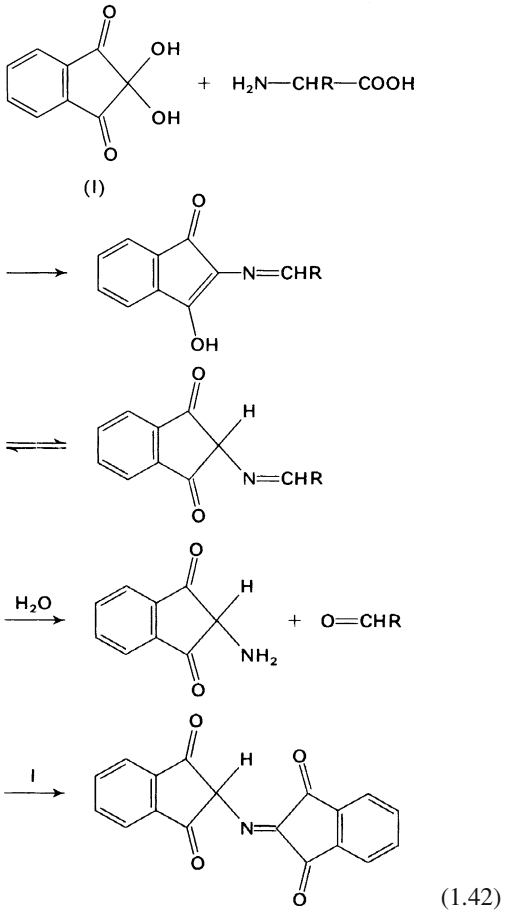


(1.40)

A corresponding reaction with phenylisothiocyanate can degrade a peptide in a stepwise fashion (*Edman* degradation). The reaction is of great importance for revealing the amino acid sequence in a peptide chain. The phenylthiocarbamoyl derivative (PTC-peptide) formed in the first step (coupling) is cleaved non-hydrolytically in the second step (cleavage) with anhydrous trifluoroacetic acid into anilinothiazolinone as derivative of the N-terminal amino acid and the remaining peptide which is shortened by the latter. Because of its instability, the thiazolinone is not suited for an identification of the N-terminal amino acid and is therefore – after separation from the remaining peptide, in the third step (conversion) – converted in aqueous HCl via the phenylthiocarbamoylamino acid into phenyl-thiohydantoin, while the remaining peptide is fed into a new cycle.



(1.41)

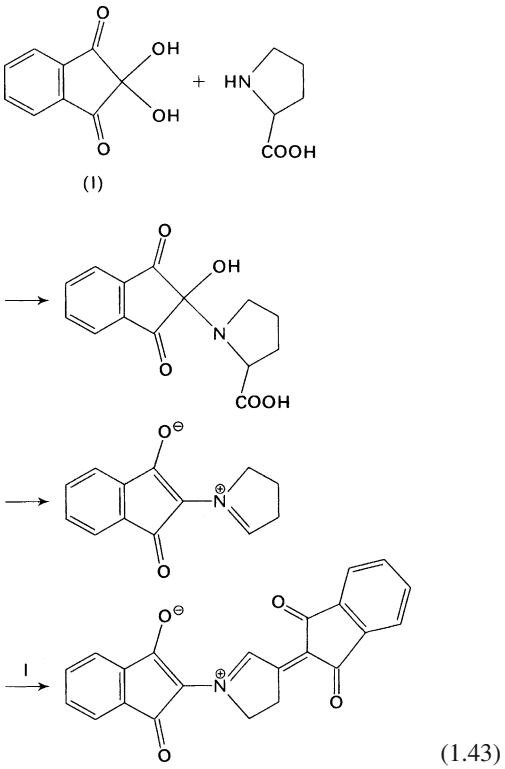


(1.42)

1.2.4.2.4 Reactions with Carbonyl Compounds

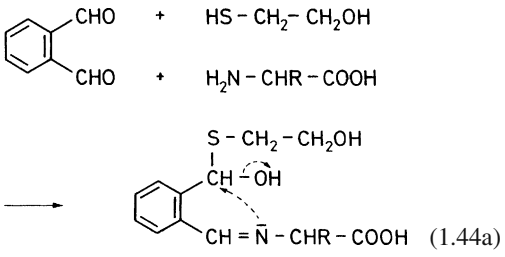
Amino acids react with carbonyl compounds, forming azomethines. If the carbonyl compound has an electron-withdrawing group, e. g., a second carbonyl group, transamination and decarboxylation occur. The reaction is known as the *Strecker* degradation and plays a role in food since food can be an abundant source of dicarbonyl compounds generated by the *Maillard* reaction (cf. 4.2.4.4.7). The aldehydes formed from amino acids (*Strecker* aldehydes) are aroma compounds (cf. 5.3.1.1). The ninhydrin reaction is a special case of the *Strecker* degradation. It is an important reaction for the quantitative determination of

amino acids using spectrophotometry (cf. Reaction 1.42). The detection limit lies at 1–0.5 nmol. The resultant blue-violet color has an absorption maximum at 570 nm. Proline yields a yellow-colored compound with $\lambda_{max} = 440$ nm (Reaction 1.43):

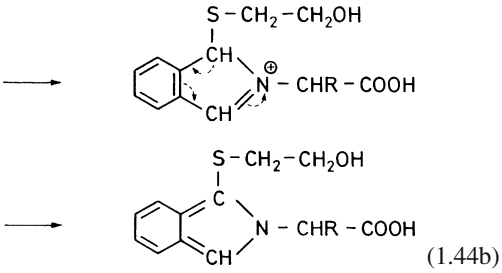


(1.43)

The reaction of amino acids with o-phthaldialdehyde (OPA) and mercaptoethanol leads to fluorescent isoindole derivatives ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 455$ nm) (Reaction 1.44a).



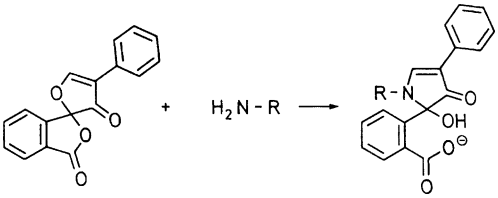
(1.44a)



(1.44b)

The derivatives are used for amino acid analysis via HPLC separation. Instead of mercaptoethanol, a chiral thiol, e. g., N-isobutyryl-L-cysteine, is used for the detection of D-amino acids. The detection limit lies at 1 pmol. The very fast racemizing aspartic acid is an especially suitable marker. One disadvantage of the method is that proline and hydroxyproline are not detected. This method is applied, e. g., in the analysis of fruit juices, in which high concentrations of D-amino acids indicate bacterial contamination or the use of highly concentrated juices. Conversely, too low concentrations of D-amino acids in fermented foods (cheese, soy and fish sauces, wine vinegar) indicate unfermented imitations.

Fluorescamine reacts with primary amines and amino acids – at room temperature under alkaline conditions – to form fluorescent pyrrolidones ($\lambda_{ex} = 390$ nm, $\lambda_{em} = 474$ nm). The detection limit lies at 50–100 pmol:



(1.45)

The excess reagent is very quickly hydrolyzed into water-soluble and non-fluorescent compounds.

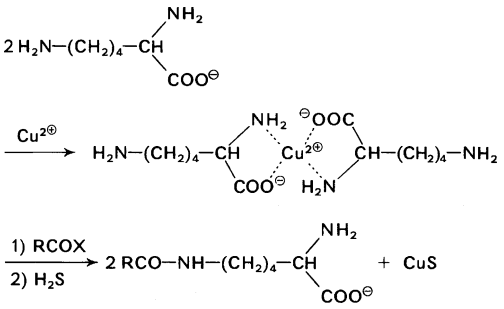
1.2.4.3 Reactions Involving Other Functional Groups

The most interesting of these reactions are those in which α-amino and α-carboxyl groups are

blocked, that is, reactions occurring with peptides and proteins. These reactions will be covered in detail in sections dealing with modification of proteins (cf. 1.4.4 and 1.4.6.2). A number of reactions of importance to free amino acids will be covered in the following sections.

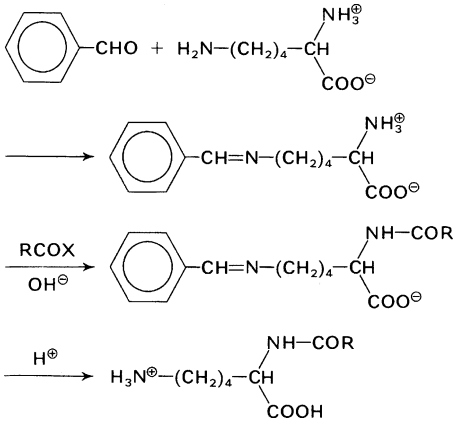
1.2.4.3.1 Lysine

A selective reaction may be performed with either of the amino groups in lysine. Selective acylation of the ε-amino group is possible using the lysine-Cu^{2+} complex as a reactant:



(1.46)

Selective reaction with the α-amino group is possible using a benzylidene derivative:

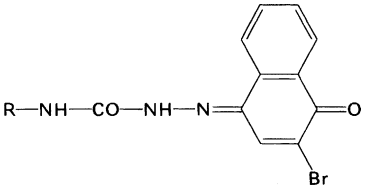


(1.47)

ε-N-benzylidene-L-lysine and ε-N-salicylidene-L-lysine are as effective as free lysine in growth feeding tests with rats. Browning reactions of these derivatives are strongly retarded, hence they are of interest for lysine fortification of food.

1.2.4.3.2 Arginine

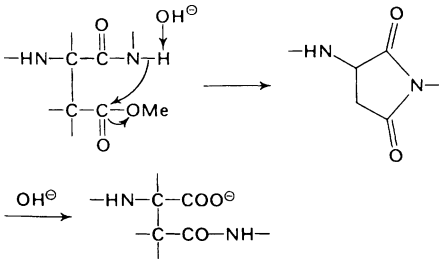
In the presence of α-naphthol and hypobromite, the guanidyl group of arginine gives a red compound with the following structure:



(1.48)

1.2.4.3.3 Aspartic and Glutamic Acids

The higher esterification rate of β- and γ-carboxyl groups can be used for selective reactions. On the other hand the β- and γ-carboxyl groups are more rapidly hydrolyzed in acid-catalyzed hydrolysis since protonation is facilitated by having the ammonium group further away from the carboxyl group. Alkali-catalyzed hydrolysis of methyl or ethyl esters of aspartic or glutamic acids bound to peptides can result in the formation of isopeptides.

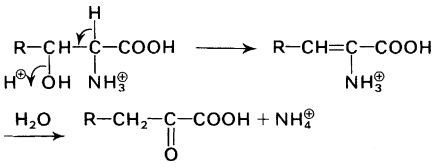


(1.49)

Decarboxylation of glutamic acid yields γ-aminobutyric acid. This compound, which also occurs in wine (cf. 20.2.6.9), tastes sour and produces a dry feeling in the mouth at concentrations above its recognition threshold (0.02 mmol/l).

1.2.4.3.4 Serine and Threonine

Acidic or alkaline hydrolysis of protein can yield α-keto acids through β-elimination of a water molecule:

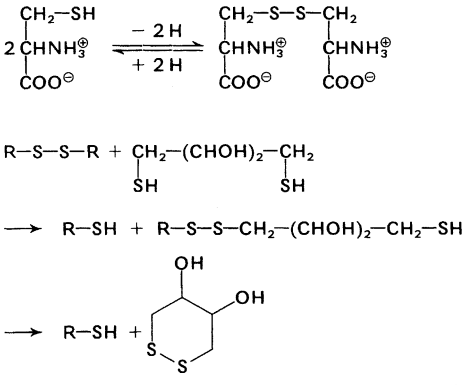


(1.50)

In this way, α-ketobutyric acid formed from threonine can yield another amino acid, α-aminobutyric acid, via a transamination reaction. Reaction 1.51 is responsible for losses of hydroxy amino acids during protein hydrolysis.
Reliable estimates of the occurrence of these amino acids are obtained by hydrolyzing protein for varying lengths of time and extrapolating the results to zero time.

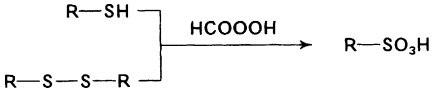
1.2.4.3.5 Cysteine and Cystine

Cysteine is readily converted to the corresponding disulfide, cystine, even under mild oxidative conditions, such as treatment with I_2 or potassium hexacyanoferrate (III). Reduction of cystine to cysteine is possible using sodium borohydride or thiol reagents (mercaptoethanol, dithiothreitol):



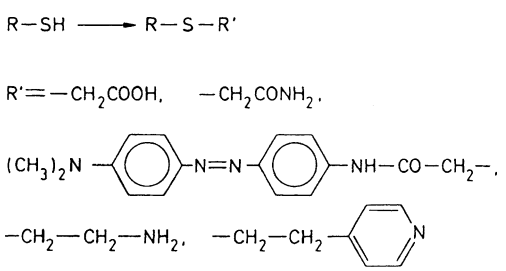
(1.51)

The equilibrium constants for the reduction of cystine at pH 7 and 25 °C with mercaptoethanol or dithiothreitol are 1 and 10^4, respectively.
Stronger oxidation of cysteine, e. g., with performic acid, yields the corresponding sulfonic acid, cysteic acid:



(1.52)

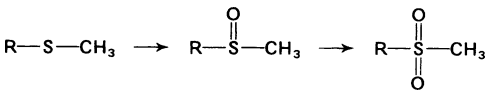
Reaction of cysteine with alkylating agents yields thioethers. Iodoacetic acid, iodoacetamide, dimethylaminoazobenzene iodoacetamide, ethylenimine and 4-vinylpyridine are the most commonly used alkylating agents:



(1.53)

1.2.4.3.6 Methionine

Methionine is readily oxidized to the sulfoxide and then to the sulfone. This reaction can result in losses of this essential amino acid during food processing:

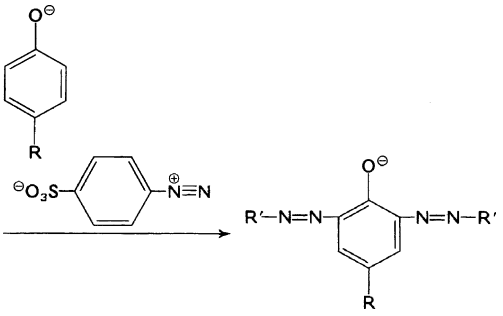


(1.54)

1.2.4.3.7 Tyrosine

Tyrosine reacts, like histidine, with diazotized sulfanilic acid (*Pauly* reagent). The coupled-

reaction product is a red azo compound:



(1.55)

1.2.4.4 Reactions of Amino Acids at Higher Temperatures

Reactions at elevated temperatures are important during the preparation of food. Frying, roasting, boiling and baking develop the typical aromas of many foods in which amino acids participate as precursors. Studies with food and model systems have shown that the characteristic odorants are formed via the *Maillard* reaction and that they are subsequent products, in particular of cysteine, methionine, ornithine and proline (cf. 12.9.3).

1.2.4.4.1 Acrylamide

The toxic compound acrylamide is one of the volatile compounds formed during the heating of food (cf. 9.7.3). Model experiments have shown that it is produced in reactions of asparagine with reductive carbohydrates or from the resulting cleavage products (e. g., 2-butanedione, 2-oxopropanal).

The formation is promoted by temperatures >100 °C and/or longer reaction times. Indeed, model experiments have shown that the highest yields based on asparagine are ca. 0.1–1 mol%. Cysteine and methionine also form acrylamide in the presence of glucose, but the yields are considerably lower than those from asparagine. The thermal reaction of acrolein with ammonia also produces acrylamide, but again only in small amounts.

Although from a purely stoichiometric standpoint, it would be possible that the degradation of asparagine by the cleavage of CO_2 und NH_3 directly produces acrylamide, the course of formation is quite complex. Indeed, various proposals exist for the mechanism of this formation. It was shown that considerable amounts of 3-aminopropionamide are produced in the reaction of asparagine with α-dicarbonyl compounds with the formation of the *Schiff* base and subsequent decarboxylation and hydrolysis in the sense of a *Strecker* reaction (Fig. 1.6). It could be shown in model studies and in additional experiments with foods (cocoa, cheese) that the splitting-off of ammonia from 3-aminopropionamide occurs relatively easily at higher temperatures and even in the absence of carbohydrates results in very high yields of acrylamide (>60 mol%). Therefore, 3-aminopropionamide, which is to be taken as the biogenic amine of asparagine, represents a transient intermediate in the formation of acrylamide in foods. In the meantime, this compound has also been identified in different foods.

Another mechanism (Fig. 1.7, right) starts out from the direct decomposition of the *Schiff* base obtained from a reductive carbohydrate and asparagine via instable analytically undetectable intermediates. It is assumed that the ylide formed by the decarboxylation of the *Schiff* base directly decomposes on cleavage of the

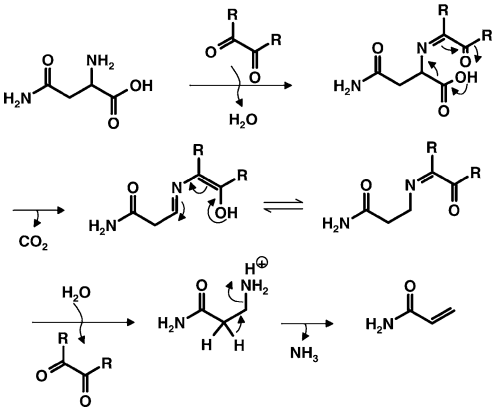


Fig. 1.6. Formation of 3-aminopropionamide (3-APA) from the Strecker reaction of asparagine and subsequent deamination to acrylamide (according to *Granvogl* et al., 2006)

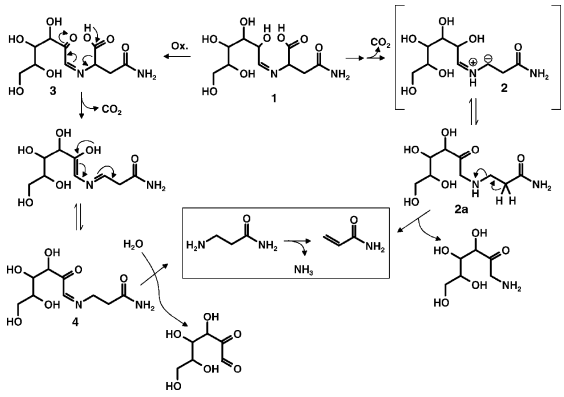
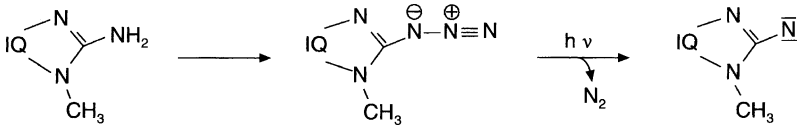


Fig. 1.7. Reaction paths from the *Schiff* base of asparagine and glucose which result in acrylamide (according to *Stadler* et al., 2004 and *Granvogl* et al., 2006)

C-N bond to give acrylamide and a 1-amino-2-hexulose. Another proposed mechanism (Fig. 1.7, left) is the oxidation of the *Schiff* base and subsequent decarboxylation. Here, an intermediate is formed which can decompose to 3-aminopropionamide after enolization and hydrolysis. 3-Aminopropionamide can then be converted to acrylamide after the splitting-off of ammonia.



(1.56)

1.2.4.4.2 Mutagenic Heterocyclic Compounds

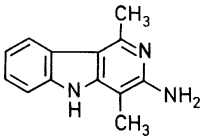
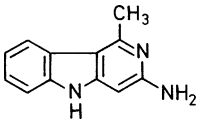
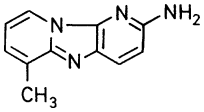
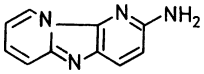
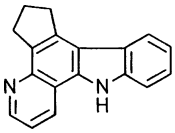
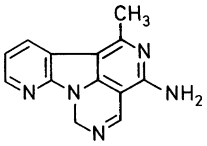
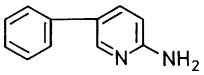
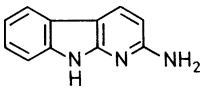
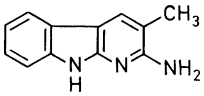
In the late 1970s it was shown that charred surface portions of barbecued fish and meat as well as the smoke condensates captured in barbecuing have a highly mutagenic effect in microbial tests (*Salmonella typhimurium* tester strain TA 98). In model tests it could be demonstrated that pyrolyzates of amino acids and proteins are responsible for that effect. Table 1.6 lists the mutagenic compounds isolated from amino acid pyrolyzates. They are pyridoindoles, pyridoimidazoles and tetra-azafluoroanthenes.

At the same time, it was found that mutagenic compounds of amino acids and proteins can also be formed at lower temperatures. The compounds listed in Table 1.7 were obtained from meat extract, deep-fried meat, grilled fish and heated model mixtures on the basis of creatine, an amino acid (glycine, alanine, threonine) and glucose. For the most part they were imidazoquinolines and imidazoquinoxalines. The highest concentrations (μ/kg) were found in meat extract: IQ (0−15), MeIQ (0−6), MeIQx (0−80). A model experiment directed at processes in meat shows that heterocyclic amines are detectable at temperatures around 175 °C after only 5 minutes. It is assumed that they are formed from creatinine, subsequent products of the *Maillard* reaction (pyridines, pyrazines, cf. 4.2.4.4.3) and amino acids as shown in Fig. 1.8.

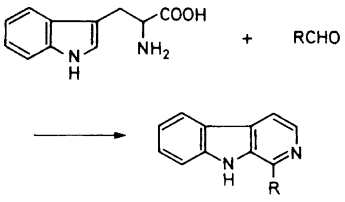
The toxicity is based on the heteroaromatic amino function. The amines are genotoxic after oxidative metabolic conversion to a strong electrophile, e. g., a nitrene. Nitrenes of this type are synthesized for model experiments as shown in Formula 1.56. According to these experiments, MeIQ, IQ and MeIQx have an especially high genotoxic potential. The compounds listed in Table 1.6 can be deaminated by nitrite in weakly acid solution and thus inactivated.

The β-carbolines norharmane (I, R=H) and harmane (I, R=CH_3) are well known as components

Table 1.6. Mutagenic compounds from pyrolysates of amino acids and proteins

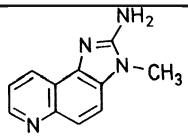
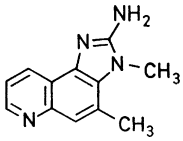
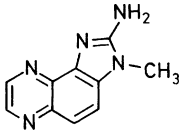
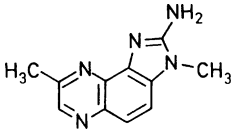
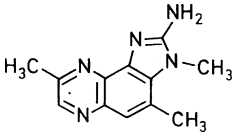
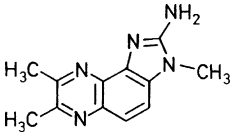
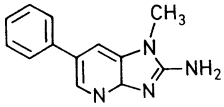
Mutagenic compound	Short form	Pyrolized compound	Structure
3-Amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole	Trp-P-1	Tryptophan	
3-Amino-1-methyl-5H-pyrido[4,3-*b*]indole	Trp-P-2	Tryptophan	
2-Amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole	Glu-P-1	Glutamic acid	
2-Aminodipyrido[1,2-*a*:3',2'-*d*]imidazole	Glu-P-2	Glutamic acid	
3,4-Cyclopentenopyrido[3,2-*a*]carbazole	Lys-P-1	Lysine	
4-Amino-6-methyl-1H-2,5,10,10*b*-tetraazafluoranthene	Orn-P-1	Ornithine	
2-Amino-5-phenylpyridine	Phe-P-1	Phenylalanine	
2-Amino-9H-pyrido[2,3-*b*]indole	AαC	Soya globulin	
2-Amino-3-methyl-9H-pyrido[2,3-*b*] indole	MeAαC	Soya globulin	

of tobacco smoke. They are formed by a reaction of tryptophan and formaldehyde or acetaldehyde:



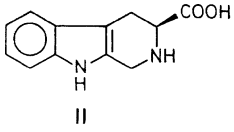
I (1.57)

Table 1.7. Mutagenic compounds from various heated foods and from model systems

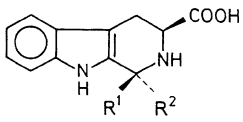
Mutagenic compound	Short form	Food Model system[a]	Structure
2-Amino-3-methylimidazo-[4,5-*f*]quinoline	IQ	1,2,3	
2-Amino-3,4-dimethylimidazo-[4,5-*f*]quinoline	MeIQ	3	
2-Amino-3-methylimidazo-[4,5-*f*]quinoxaline	IQx	2	
2-Amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline	MeIQ2x	2,3	
2-Amino-3,4,8-trimethyl-imidazo-[4,5-*f*]quinoxaline	4,8-Di MeIQx	2,3,5,6	
2-Amino-3,7,8-trimethyl-imidazo-[4,5-*f*]quinoxaline	7,8-Di MeIQx	4	
2-Amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine	PhIP	2	

[a] 1: Meat extract; 2: Grilled meat; 3: Grilled fish; 4: Model mixture of creatinine, glycine, glucose; 5: as 4, but alanine; 6: as 4, but threonine

Tetrahydro-β-carboline-3-carboxylic acid (II) and (1S, 3S)-(III) and (1R, 3S)-methyltetrahydro-β-carboline-3-carboxylic acid (IV) were detected in beer (II: 2–11 mg/L, III + IV: 0.3–4 mg/L) and wine (II: 0.8–1.7 mg/L, III + IV: 1.3–9.1 mg/L). The ratio of diastereomers III and IV (Formula 1.58) was always near 2:1:



II



III $R^1 = CH_3$, $R^2 = H$
IV $R^1 = H$, $R^2 = CH_3$

(1.58)

The compounds are pharmacologically active.

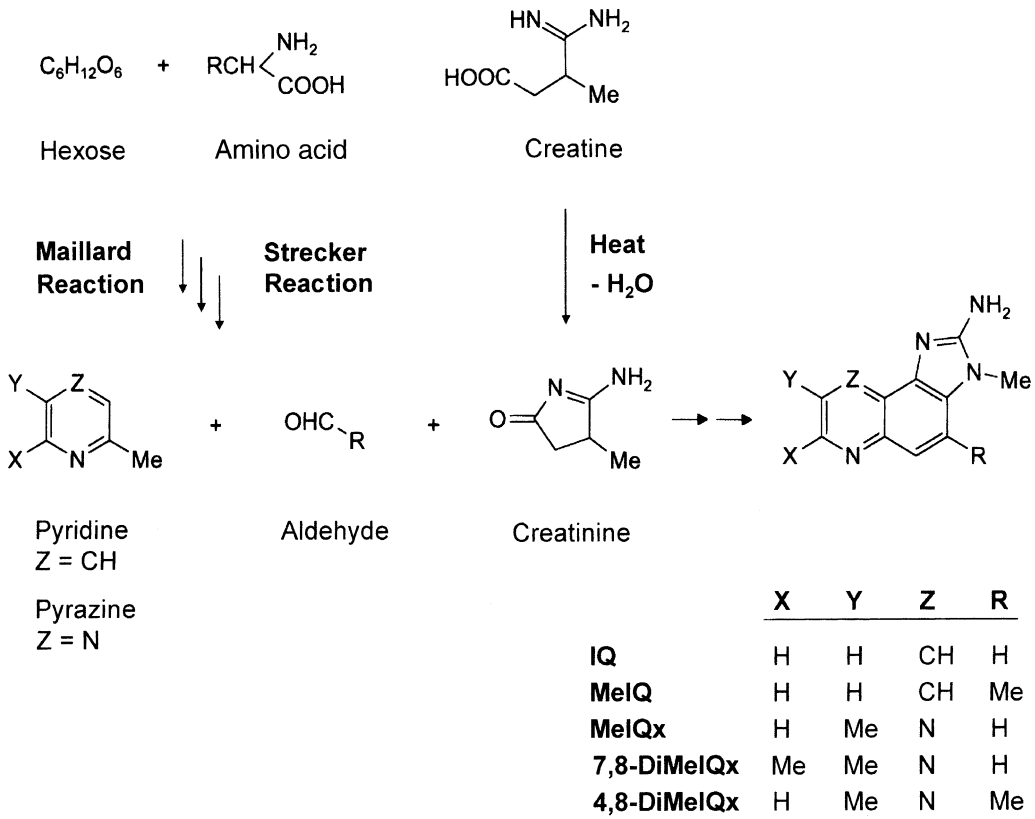


Fig. 1.8. Formation of heterocyclic amines by heating a model system of creatine, glucose and an amino acid mixture corresponding to the concentrations in beef (according to *Arvidsson* et al., 1997). For abbreviations, see Table 1.7

1.2.5 Synthetic Amino Acids Utilized for Increasing the Biological Value of Food (Food Fortification)

The daily requirements of humans for essential amino acids and their occurrence in some important food proteins are presented in Table 1.8. The biological value of a protein (g protein formed in the body/100 g food protein) is determined by the absolute content of essential amino acids, by the relative proportions of essential amino acids, by their ratios to nonessential amino acids and by factors such as digestibility and availability. The most important (more or less expensive) *in vivo* and *in vitro* methods for determining the biological valence are based on the following principles:

- Replacement of endogenous protein after protein depletion.
  The test determines the amount of endogenous protein that can be replaced by 100 g of food protein. The test person is given a non-protein diet and thus reduced to the absolute N minimum. Subsequently, the protein to be examined is administered, and the N balance is measured. The biological valence (BV) follows from

$$\mathrm{BV} = \frac{\text{Urea-N(non-protein diet)} + \text{N balance}}{\text{N intake}} \times 100, \qquad (1.59)$$

  "Net protein utilization" (NPU) is based on the same principle and is determined in animal experiments. A group of rats

Table 1.8. Adult requirement for essential amino acids and their occurrence in various food

Amino acid	1	2	3	4	5	6	7	8	9
Isoleucine	10–11	3.5	4.0	4.6	3.9	3.6	3.4	5.0	3.5
Leucine	11–14	4.2	5.3	7.1	4.3	5.1	6.5	8.2	5.4
Lysine	9–12	3.5	3.7	4.9	3.6	4.4	2.0	3.6	5.4
Methionine + Cystine	11–14	4.2	3.2	2.6	1.9	2.1	3.8	3.4	1.9
Methionine		2.0	1.9	1.9	1.2	0.9	1.4	2.2	0.8
Phenylalanine + Tyrosine	13–14	4.5	6.1	7.2	5.8	5.5	6.7	8.9	6.0
Phenylalanine		2.4	3.5	3.5	3.1	3.3	4.6	4.7	2.5
Threonine	6–7	2.2	2.9	3.3	2.9	2.7	2.5	3.7	3.8
Tryptophan	3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Valine	11–14	4.2	4.3	5.6	3.6	3.3	3.8	6.4	4.1
Tryptophan[a]			1.7	1.4	1.4	1.5	1.1	1.0	1.3

1: Daily requirement in mg/kg body weight.
2–8: Relative value related to Trp = 1 (pattern).
2: Daily requirements, 3: eggs, 4: bovine milk, 5: potato, 6: soya, 7: wheat flour, 8: rice, and 9: *Torula*-yeast.
[a] Tryptophan (%) in raw protein.

is fed a non-protein diet (Gr 1), while the second group is fed the protein to be examined (Gr 2). After some time, the animals are killed, and their protein content is analyzed. The biological valence follows from

$$\text{NPU} = \frac{\text{Protein content Gr 2} - \text{protein content Gr 1}}{\text{Protein intake}} \times 100$$

- Utilization of protein for growth.
  The growth value (protein efficiency ratio = PER) of laboratory animals is calculated according to the following formula:

$$\text{PER} = \frac{\text{Weight gain (g)}}{\text{Available protein (g)}}$$

- Maintenance of the N balance.
- Plasma concentration of amino acids.
- Calculation from the amino acid composition.
- Determination by enzymatic cleavage *in vitro*.

Table 1.9 lists data about the biological valence of some food proteins, determined according to different methods.

The highest biological value observed is for a blend of 35% egg and 65% potato proteins. The biological value of a protein is generally limited by:

- Lysine: deficient in proteins of cereals and other plants
- Methionine: deficient in proteins of bovine milk and meat

Table 1.9. Biological valence of some food proteins determined according to different methods[a]

Protein from	Biological valence			Limiting amino acid
	BV	NPU	PER	
Chicken egg	94	93	3.9	
Cow's milk	84	81	3.1	Met
Fish	76	80	3.5	Thr
Beef	74	67	2.3	Met
Potatoes	73	60	2.6	Met
Soybeans	73	61	2.3	Met
Rice	64	57	2.2	Lys, Tyr
Beans	58	38	1.5	Met
Wheat flour (white)	52	57	0.6	Lys, Thr

[a] The methods are explained in the text.

Table 1.10. Increasing the biological valence (PER[a]) of some food proteins through the addition of amino acids

Protein from	Addition(%)					
	without	0.2 Lys	0.4 Lys	0.4 Lys 0.2 Thr	0.4 Lys 0.07 Thr	0.4 Lys 0.07 Thr 0.2 Thr
Casein (Reference)	2.50					
Wheat flour	0.65	1.56	1.63	2.67		
Corn	0.85		1.08		2.50	2.59

[a] The method is explained in the text.

- Threonine: deficient in wheat and rye
- Tryptophan: deficient in casein, corn and rice.

Since food is not available in sufficient quantity or quality in many parts of the world, increasing its biological value by addition of essential amino acids is gaining in importance. Illuminating examples are rice fortification with L-lysine and L-threonine, supplementation of bread with L-lysine and fortification of soya and peanut protein with methionine. Table 1.10 lists data about the increase in biological valence of some food proteins through the addition of amino acids. Synthetic amino acids are used also for chemically defined diets which can be completely absorbed and utilized for nutritional purposes in space travel, in pre-and post-operative states, and during therapy for maldigestion and malabsorption syndromes.

The fortification of animal feed with amino acids (0.05–0.2%) is of great significance. These demands have resulted in increased production of amino acids. Table 1.11 gives data for world production in 1982. The production of L-glutamic acid, used to a great extent as a flavor enhancer, is exceptional. Production of methionine and lysine is also significant.

Four main processes are distinguished in the production of amino acids: chemical synthesis, isolation from protein hydrolysates, enzymatic and microbiological methods of production, which is currently the most important. The following sections will further elucidate the important

Table 1.11. World production of amino acids, 1982

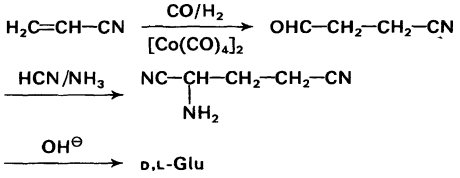
Amino acid	t/year	Process[a]				Mostly used as
		1	2	3	4	
L-Ala	130		+		+	Flavoring compound
D,L-Ala	700	+				Flavoring compound
L-Arg	500			+	+	Infusion Therapeutics
L-Asp	250		+		+	Therapeutics Flavoring compound
L-Asn	50				+	Therpeutics
L-CySH	700				+	Baking additive Antioxidant
L-Glu	270,000			+		Flavoring compound flavor enhancer
L-Gln	500			+		Therapeutics
Gly	6.000	+				Sweetener
L-His	200			+	+	Therapeutics
L-Ile	150			+	+	Infusion
L-Leu	150			+	+	Infusion
L-Lys	32,000		+	+		Feed ingredient
L-Met	150		+			Therapeutics
D,L-Met	110,000	+				Feed ingredient
L-Phe	150		+	+		Infusion
L-Pro	100			+	+	Infusion
L-Ser	50			+	+	Cosmetics
L-Thr	160			+	+	Food additive
L-Trp	200		+	+		Infusion
L-Tyr	100			+		Infusion
L-Val	150		+		+	Infusion

[a] 1: Chemical synthesis, 2: protein hydrolysis, 3: microbiological procedure, 4: isolation from raw materials.

industrial processes for a number of amino acids.

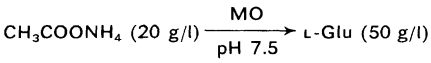
1.2.5.1 Glutamic Acid

Acrylnitrile is catalytically formylated with CO/H_2 and the resultant aldehyde is transformed through a *Strecker* reaction into glutamic acid dinitrile which yields D,L-glutamic acid after alkaline hydrolysis. Separation of the racemate is achieved by preferential crystallization of the L-form from an oversaturated solution after seeding with L-glutamic acid:



(1.60)

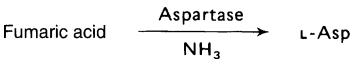
A fermentation procedure with various selected strains of microorganisms (*Brevibacterium flavum, Brev. roseum, Brev. saccharolyticum*) provides L-glutamic acid in yields of 50 g/l of fermentation liquid:



(1.61)

1.2.5.2 Aspartic Acid

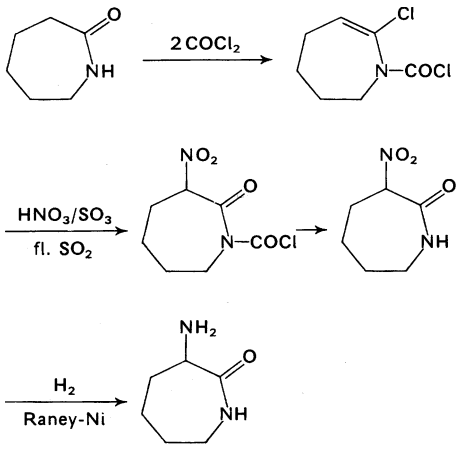
Aspartic acid is obtained in 90% yield from fumaric acid by using the aspartase enzyme:



(1.62)

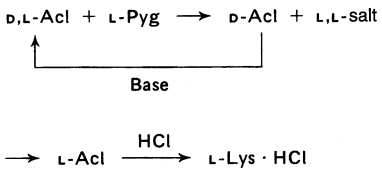
1.2.5.3 Lysine

A synthetic procedure starts with caprolactam, which possesses all the required structural features, except for the α-amino group which is introduced in several steps:



(1.63)

Separation of isomers is done at the α-amino caprolactam (Acl) step through the sparingly soluble salt of the L-component with L-pyrrolidone carboxylic acid (Pyg):

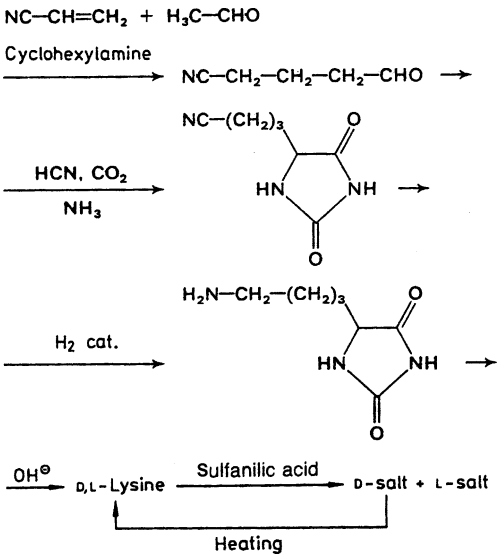


(1.64)

More elegant is selective hydrolysis of the L-enantiomer by an L-α-amino-ε-caprolactamase which occurs in several yeasts, for example in *Cryptococcus laurentii*. The racemization of the remaining D-isomers is possible with a racemase of *Achromobacter obae*. The process can be performed as a one-step reaction: the racemic aminocaprolactam is incubated with intact cells of *C. laurentii* and *A. obae*, producing almost 100% L-lysine.

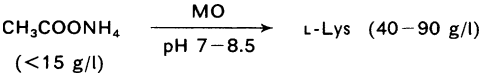
In another procedure, acrylnitrile and ethanal react to yield cyanobutyraldehyde which is then transformed by a *Bucherer* reaction into cyanopropylhydantoin. Catalytic hydrogenation of the nitrile group, followed by alkaline hydrolysis yields D,L-lysine.

The isomers can be separated through the sparingly soluble L-lysine sulfanilic acid salt:



(1.65)

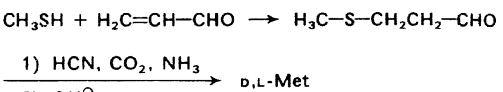
Fermentation with a pure culture of *Brevibacterium lactofermentum or Micrococcus glutamicus* produces L-lysine directly:



(1.66)

1.2.5.4 Methionine

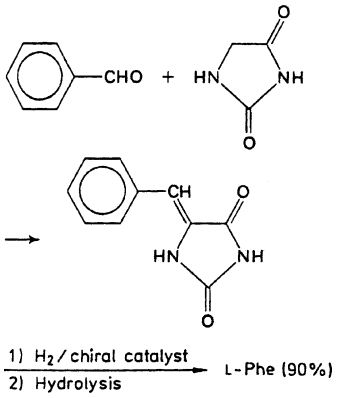
Interaction of methanethiol with acrolein produces an aldehyde which is then converted to the corresponding hydantoin through a *Bucherer* reaction. The product is hydrolyzed by alkaline catalysis. Separation of the resultant racemate is usually not carried out since the D-form of methionine is utilized by humans via transamination:



(1.67)

1.2.5.5 Phenylalanine

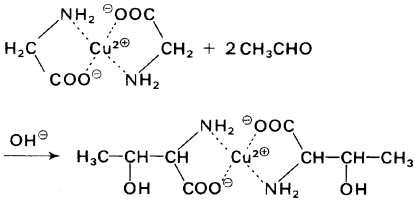
Benzaldehyde is condensed with hydantoin, then hydrogenation using a chiral catalyst gives a product which is about 90% L-phenylalanine:



(1.68)

1.2.5.6 Threonine

Interaction of a copper complex of glycine with ethanal yields the *threo* and *erythro* isomers in the ratio of 2:1. They are separated on the basis of their differences in solubility:



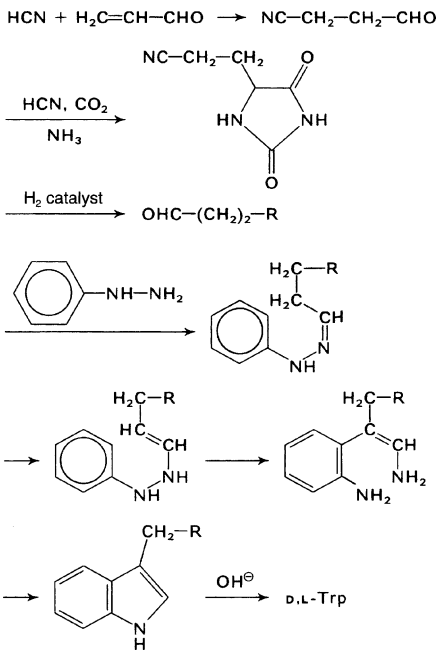
(1.69)

D,L-threonine is separated into its isomers through its N-acetylated form with the help of an acylase enzyme.
Threonine is also accessible via microbiological methods.

1.2.5.7 Tryptophan

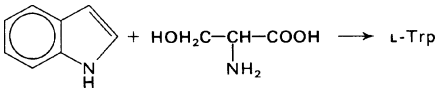
Tryptophan is obtained industrially by a variation of the *Fischer* indole synthesis. Addition of hydrogen cyanide to acrolein gives 3-cyanopropanal which is converted to hydantoin through a *Bucherer* reaction. The nitrile group is then

reduced to an aldehyde group. Reaction with phenylhydrazine produces an indole derivative. Lastly, hydantoin is saponified with alkali:



(1.70)

L-Tryptophan is also produced through enzymatic synthesis from indole and serine with the help of tryptophan synthase:



(1.71)

1.2.6 Sensory Properties

Free amino acids can contribute to the flavor of protein-rich foods in which hydrolytic processes occur (e. g. meat, fish or cheese).

Table 1.12 provides data on taste quality and taste intensity of amino acids. Taste quality is influenced by the molecular configuration: sweet amino acids are primarily found among members of the D-series, whereas bitter amino acids are generally within the L-series. Consequently amino acids with a cyclic side chain (1-aminocycloalkane-1-carboxylic acids) are sweet and bitter.

The taste intensity of a compound is reflected in its recognition threshold value. The recognition threshold value is the lowest concentration needed to recognize the compound reliably, as assessed by a taste panel. Table 1.12 shows that the taste intensity of amino acids is dependent on the hydrophobicity of the side chain.

L-Tryptophan and L-tyrosine are the most bitter amino acids with a threshold value of $c_{t\,bitter} = 4-6\,mmol/l$. D-Tryptophan, with $c_{t\,sweet} = 0.2-0.4\,mmol/l$, is the sweetest amino acid. A comparison of these threshold values with those of caffeine ($c_{t\,bi} = 1-1.2\,mmole/l$) and sucrose ($c_{t\,sw} = 10-12\,mmol/l$) shows that caffeine is about 5 times as bitter as L-tryptophan and that D-tryptophan is about 37 times as sweet as sucrose.

L-Glutamic acid has an exceptional position. In higher concentrations it has a meat broth flavor, while in lower concentrations it enhances the characteristic flavor of a given food (flavor enhancer, cf. 8.6.1). L-Methionine has a sulfur-like flavor.

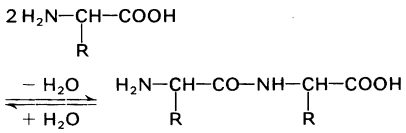
The bitter taste of the L-amino acids can interfere with the utilization of these acids, e. g., in chemically defined diets.

1.3 Peptides

1.3.1 General Remarks, Nomenclature

Peptides are formed by binding amino acids together through an amide linkage.

On the other hand peptide hydrolysis results in free amino acids:



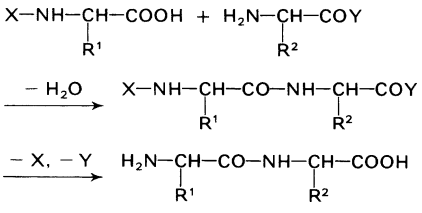
(1.72)

Functional groups not involved in the peptide synthesis reaction should be blocked. The protecting or blocking groups must be removed after synthesis under conditions which retain the stability of the newly formed peptide bonds:

Table 1.12. Taste of amino acids in aqueous solution at pH 6–7 sw – sweet, bi – bitter, neu – neutral

Amino acid	Taste			
	L-Compound		D-Compound	
	Quality	Intensity[a]	Quality	Intensity[a]
Alanine	sw	12−18	sw	12−18
Arginine	bi		neu	
Asparagine	neu		sw	3−6
Aspartic acid	neu		neu	
Cystine	neu		neu	
Glutamine	neu		sw	
Glutamic acid	meat broth like (3.0)		neu	
Glycine[b]	sw	25−35		
Histidine	bi	45−50	sw	2−4
Isoleucine	bi	10−12	sw	8−12
Leucine	bi	11−13	sw	2−5
Lysine	sw		sw	
	bi	80−90		
Methionine	sulphurous		sulphurous	
			sw	4−7
Phenylalanine	bi	5−7	sw	1−3
Proline	sw	25−40	neu	
	bi	25−27		
Serine	sw	25−35	sw	30−40
Threonine	sw	35−45	sw	40−50
Tryptophan	bi	4−6	sw	0.2−0.4
Tyrosine	bi	4−6	sw	1−3
1-Aminocycloalkane-1-carboxylic acid[b]				
Cyclobutane derivative	sw	20−30		
Cyclopentane derivative	sw	3−6		
	bi	95−100		
Cyclohexane derivative	sw	1−3		
	bi	45−50		
Cyclooctane derivative	sw	2−4		
	bi	2−5		
Caffeine	bi	1−1.2		
Saccharose	sw	10−12		

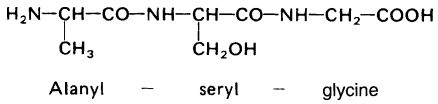
[a] Recognition threshold value (mmol/l).
[b] Compounds not optically active.



(1.73)

Peptides are denoted by the number of amino acid residues as di-, tri-, tetrapeptides, etc., and the term "oligopeptides" is used for those with 10 or less amino acid residues. Higher molecular weight peptides are called polypeptides. The transition of "polypeptide" to "protein" is rather undefined, but the limit is commonly assumed to be at a molecular weight of about 10 kdal, i. e., about 100 amino acid residues are needed in the chain for it to be called a protein. Peptides are interpreted as acylated amino

acids:



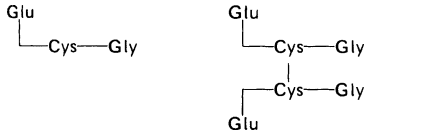
(1.74)

The first three letters of the amino acids are used as symbols to simplify designation of peptides (cf. Table 1.1). Thus, the peptide shown above can also be given as:

Ala—Ser—Gly or ASG (1.75)

One-letter symbols (cf. Table 1.1) are used for amino acid sequences of long peptide chains.

D-Amino acids are denoted by the prefix D-. In compounds in which a functional group of the side chain is involved, the bond is indicated by a perpendicular line. The tripeptide glutathione (γ-glutamyl-cysteinyl-glycine) is given as an illustration along with its corresponding disulfide, oxidized glutathione:



(1.76)

By convention, the amino acid residue with the free amino group is always placed on the left. The amino acids of the chain ends are denoted as N-terminal and C-terminal amino acid residues. The peptide linkage direction in cyclic peptides is indicated by an arrow, i. e., —CO → NH-.

1.3.2 Physical Properties

1.3.2.1 Dissociation

The pK values and isoelectric points for some peptides are listed in Table 1.13. The acidity of the free carboxyl groups and the basicity of the free amino groups are lower in peptides than in the corresponding free amino acids. The amino acid sequence also has an influence (e. g., Gly-Asp/Asp-Gly).

Table 1.13. Dissociation constants and isoelectric points of various peptides (25 °C)

Peptide	pK_1	pK_2	pK_3	pK_4	pK_5	pI
Gly-Gly	3.12	8.17				5.65
Gly-Gly-Gly	3.26	7.91				5.59
Ala-Ala	3.30	8.14				5.72
Gly-Asp	2.81	4.45	8.60			3.63
Asp-Gly	2.10	4.53	9.07			3.31
Asp-Asp	2.70	3.40	4.70	8.26		3.04
Lys-Ala	3.22	7.62	10.70			9.16
Ala-Lys-Ala	3.15	7.65	10.30			8.98
Lys-Lys	3.01	7.53	10.05	11.01		10.53
Lys-Lys-Lys	3.08	7.34	9.80	10.54	11.32	10.93
Lys-Glu	2.93	4.47	7.75	10.50		6.10
His-His	2.25	5.60	6.80	7.80		7.30

1.3.3 Sensory Properties

While the taste quality of amino acids does depend on configuration, peptides, except for the sweet dipeptide esters of aspartic acid (see below), are neutral or bitter in taste with no relationship to configuration (Table 1.14). As

Table 1.14. Taste threshold values of various peptides: effect of configuration and amino acid sequence (tested in aqueous solution at pH 6–7); bi – bitter

Peptide[a]	Taste	
	Quality	Intensity[b]
Gly-Leu	bi	19–23
Gly-D-Leu	bi	20–23
Gly-Phe	bi	15–17
Gly-D-Phe	bi	15–17
Leu-Leu	bi	4–5
Leu-D-Leu	bi	5–6
D-Leu-D-Leu	bi	5–6
Ala-Leu	bi	18–22
Leu-Ala	bi	18–21
Gly-Leu	bi	19–23
Leu-Gly	bi	18–21
Ala-Val	bi	60–80
Val-Ala	bi	65–75
Phe-Gly	bi	16–18
Gly-Phe	bi	15–17
Phe-Gly-Phe-Gly	bi	1.0–1.5
Phe-Gly-Gly-Phe	bi	1.0–1.5

[a] L-Configuration if not otherwise designated.
[b] Recognition threshold value in mmol/l.

Table 1.15. Bitter taste of dipeptide A–B: dependence of recognition threshold value (mmol/l) on side chain hydrophobicity (0: sweet or neutral taste)

A/B	Asp	Glu	Asn	Gln	Ser	Thr	Gly	Ala	Lys	Pro	Val	Leu	Ile	Phe	Tyr	Trp	
	0	0	0	0	0	0	0	0	85	26	21	12	11	6	5	5	
Gly	0[a]	–	–	–	–	–	–	0	0	–	45	75	21	20	16	17	13
Ala	0	–	–	–	–	–	–	0	0	–	–	70	20	–	–	–	–
Pro	26	–	–	–	–	–	–	–	–	–	–	–	6	–	–	–	–
Val	21	–	–	–	–	–	–	65	70	–	–	20	10	–	–	–	–
Leu	12	–	–	–	–	–	–	20	20	–	–	–	4.5	–	–	3.5	0.4
Ile	11	43	43	33	33	33	33	21	21	23	4	9	5.5	5.5	–	–	0.9
Phe	6	–	–	–	–	–	–	17	–	–	2	–	1.4	–	0.8	0.8	–
Tyr	5	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–
Trp	5	–	28	–	–	–	–	–	–	–	–	–	–	–	–	–	–

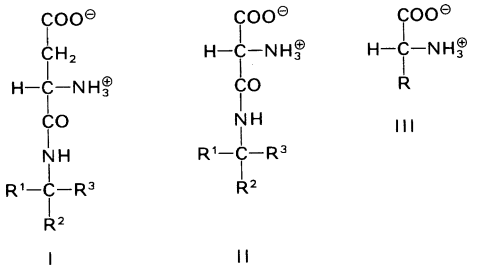
[a] Threshold of the amino acid (cf. Table 1.12).

with amino acids, the taste intensity is influenced by the hydrophobicity of the side chains (Table 1.15). The taste intensity does not appear to be dependent on amino acid sequence (Table 1.14).

Bitter tasting peptides can occur in food after proteolytic reactions. For example, the bitter taste of cheese is a consequence of faulty ripening. Therefore, the wide use of proteolytic enzymes to achieve well-defined modifications of food proteins, without producing a bitter taste, causes some problems. Removal of the bitter taste of a partially hydrolyzed protein is outlined in the section dealing with proteins modified with enzymes (cf. 1.4.6.3.2).

The sweet taste of aspartic acid dipeptide esters (I) was discovered by chance in 1969 for α-L-aspartyl-L-phenylalanine methyl ester ("Aspartame", "NutraSweet"). The corresponding peptide ester of L-aminomalonic acid (II) is also sweet.

A comparison of structures I, II and III reveals a relationship between sweet dipeptides and



(1.77)

sweet D-amino acids. The required configuration of the carboxyl and amino groups and the side chain substituent, R, is found only in peptide types I and II.

Since the discovery of the sweetness of compounds of type I, there has been a systematic study of the structural prerequisites for a sweet taste.

The presence of L-aspartic acid was shown to be essential, as was the peptide linkage through the α-carboxyl group.

R^1 may be an H or CH_3 group[2], while the R^2 and R^3 groups are variable within a certain range. Several examples are presented in Table 1.16. The sweet taste intensity passes through a maximum with increasing length and volume of the R^2 residue (e. g., COO-fenchyl ester is $22-23 \times 10^3$ times sweeter than sucrose). The size of the R^3 substituent is limited to a narrow range. Obviously, the R^2 substituent has the greatest influence on taste intensity.

The following examples show that R^2 should be relatively large and R^3 relatively small: L-Asp-L-Phe-OMe (aspartame, R^2—$CH_2C_6H_5$, R_3 = COOMe) is almost as sweet ($f_{sac,g}(1) = 180$) as L-Asp-D-Ala-OPr ($f_{sac,g}(0.6) = 170$), while L-Asp-D-Phe-OMe has a bitter taste.

In the case of acylation of the free amino group of aspartic acid, the taste characteristics depend on the introduced group. Thus, D-Ala-L-Asp-L-Phe-OMe is sweet ($f_{sac,g}(0.6) = 170$), while L-Ala-L-Asp-L-Phe-OMe is not. It should be noted that superaspartame is extremely sweet (cf. 8.8.15.2).

[2] Data are not yet available for compounds with $R^1 > CH_3$.

Table 1.16. Taste of dipeptide esters of aspartic acid[a] and of amino malonic acid[b]

R^2	R^3	Taste[c]
Asparagin acid derivate		
$COOCH_3$	H	8
n-C_3H_7	$COOCH_3$	4
n-C_4H_9	$COOCH_3$	45
n-C_4H_9	$COOC_2H_5$	5
n-C_6H_{13}	CH_3	10
n-C_7H_{15}	CH_3	neutral
$COOCH(CH_3)_2$	n-C_3H_7	17
$COOCH(CH_3)_2$	n-C_4H_9	neutral
$COOCH_3$	$CH_2C_6H_5$	bitter
$CH(CH_3)C_2H_5$	$COOCH_3$	bitter
$CH_2CH(CH_3)_2$	$COOCH_3$	bitter
$CH_2C_6H_5$	$COOCH_3$	140
COO-2-methyl-cyclohexyl	$COOCH_3$	5–7000
COO-fenchyl	$COOCH_3$	22–33,000
D,L-Aminomalon acid derivate		
$COOiC_3H_7$	CH_3	58
CH_3	$COOiC_3H_7$	neutral

[a] Formula 1.77 I, $R^1 = H$.
[b] Formula 1.77 II, $R^1 = H$.
[c] For sweet compounds the factor $f_{sac,g}$ is given, related to the threshold value of a 10% saccharose solution (cf. 8.8.1.1).

The intensity of the salty taste of Orn-β-Ala depends on the pH (Table 1.18). Some peptides exhibit a salty taste, e. g. ornithyl-β-alanine hydrochloride (Table 1.17) and may be used as substitutes for sodium chloride.

Table 1.17. Peptides with a salty taste

Peptide[a]	Taste	
	Threshold (mmol/l)	Quality[b]
Orn-βAla.HCl	1.25	3
Orn-γAbu.HCl	1.40	3
Orn-Tau.HCl	3.68	4
Lys-Tau.HCl	5.18	4
NaCl	3.12	3

[a] Abbreviations: Orn, ornithine; β-Ala, β-alanine, γ-Abu, γ-aminobutyric acid; Tau, taurine.
[b] The quality of the salty taste was evaluated by rating it from 0 to 5 on a scale in comparison with a 6.4 mmol/L NaCl solution (rated 3); 4 is slightly better, 5 clearly better than the control solution.

Table 1.18. Effect of HCl on the salty taste of Orn-β-Ala[a]

Equivalents HCl	pH	Taste	
		salty[b]	sour[c]
0	8.9	0	
0.79	7.0	0	
0.97	6.0	1	
1.00	5.5	2	
1.10	4.7	3	+/−
1.20	4.3	3.5	+
1.30	4.2	3	++

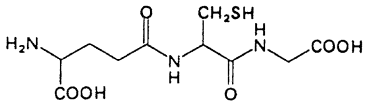
[a] Peptide solution: 30 mmol/L.
[b] The values 1, 3 and 5 correspond in intensity to 0.5%, 0.25% and 0.1% NaCl solutions respectively.
[c] Very weak (+) and slightly sour (++).

1.3.4 Individual Peptides

Peptides are widespread in nature. They are often involved in specific biological activities (peptide hormones, peptide toxins, peptide antibiotics). A number of peptides of interest to food chemists are outlined in the following sections.

1.3.4.1 Glutathione

Glutathione (γ-L-glutamyl-L-cysteinyl-glyci-ne) is widespread in animals, plants and microorganisms. Beef (200), broccoli (140), spinach (120), parsley (120), chicken (95), cauliflower (74), potatoes (71), paprika (49), tomatoes (49) and oranges (40) are especially rich in glutathione (mg/kg). A noteworthy feature is the binding of glutamic acid through its γ-carboxyl group. The peptide is the coenzyme of glyoxalase.



(1.78)

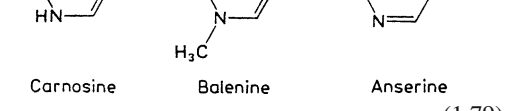
It is involved in active transport of amino acids and, due to its ready oxidation, is also involved in many redox-type reactions. It influences the rheological properties of wheat flour dough through thiol-disulfide interchange with wheat gluten. High concentrations of reduced glutathione in flour bring about reduction

of protein disulfide bonds and a corresponding decrease in molecular weight of some of the protein constituents of dough gluten (cf. 15.4.1.4.1).

1.3.4.2 Carnosine, Anserine and Balenine

These peptides are noteworthy since they contain a β-amino acid, β-alanine, bound to L-histidine or 1-methyl- or 3-methyl-L-histidine, and are present in meat extract and in muscle of vertebrates (cf. Formula 1.79).

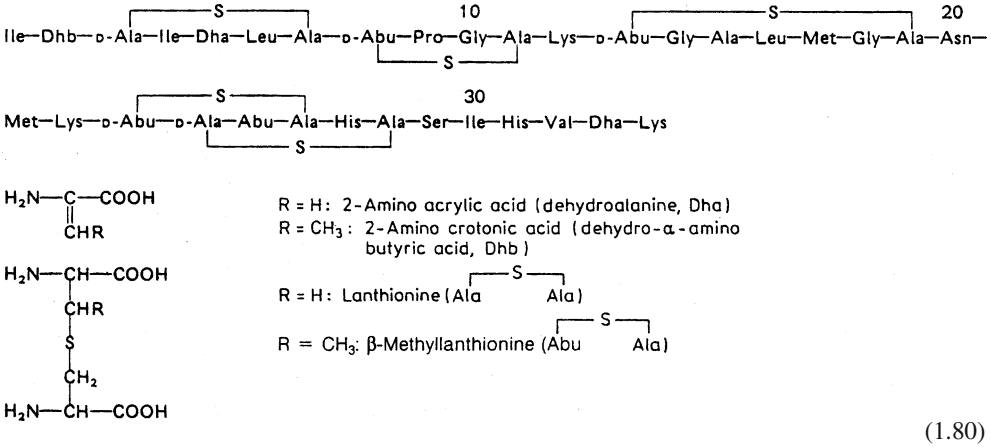
Data on the amounts of these peptides present in meat are given in Table 1.19. Carnosine is predominant in beef muscle tissue, while anserine is predominant in chicken meat. Balenine is a characteristic constituent of whale muscle, although it appears that sperm whales do not have this dipeptide. The amounts found in commercial sperm whale meat extract are probably due to the presence of meat from other whale species. These peptides are used analytically to identify the meat extract. Their physiological roles are not clear. Their buffering capacity in the pH range of 6–8 may be of some importance. They may also be involved in revitalizing exhausted muscle, i. e. in the muscle regaining its excitability and ability to contract. Carnosine may act as a neurotransmitter for nerves involved in odor perception.



(1.79)

1.3.4.3 Nisin

This peptide is formed by several strains of *Streptococcus lactis* (Langfield-N-group). It contains a number of unusual amino acids, namely dehydroalanine, dehydro-β-methyl-alanine, lanthionine, β-methyl-lanthionine, and therefore also five thioether bridges (cf. Formula 1.80).



(1.80)

The peptide subtilin is related to nisin. Nisin is active against Gram-positive microorganisms (lactic acid bacteria, *Streptococci, Bacilli, Clostridia* and other anaerobic spore-forming microorganisms). Nisin begins to act against the cytoplasmic membrane as soon as the spore has germinated. Hence, its action is more pronounced against spores than against vegetative cells. Nisin is permitted as a preservative in several countries. It is used to suppress anaerobes in cheese and cheese products, especially in hard cheese and processed cheese to inhibit butyric acid fermentation. The use of nisin in the canning of vegetables allows mild sterilization conditions.

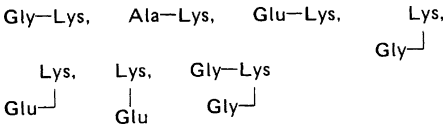
Table 1.19. Occurrence of carnosine, anserine and balenine (%) in meat[a]

Meat	Carnosine	Anserine	Balenine	Σ[b]
Beef muscle tissue	0.15–0.35	0.01–0.05		0.2–0.4
Beef meat extract	3.1–5.7	0.4–1.0		4.4–6.2
Chicken meat[c]	0.01–0.1	0.05–0.25		
Chicken meat extract	0.7–1.2	2.5–3.5		
Whale meat				ca. 0.3
Whale meat extract a[d]	3.1–5.9	0.2–0.6	13.5–23.0	16–30
Whale meat extract b[e]	2.5–4.5	1.2–3.0	0–5.2	3.5–12

[a] The results are expressed as % of the moist tissue weight, or of commercially available extracts containing 20% moisture.
[b] β-Alanine peptide sum.
[c] Lean and deboned chiken meat.
[d] Commercial extract mixture of various whales.
[e] Commercial extract mixture, with sperm whale prevailing.

1.3.4.4 Lysine Peptides

A number of peptides, such as:



(1.81)

have been shown to be as good as lysine in rat growth feeding tests. These peptides substantially retard the browning reaction with glucose (Fig. 1.9), hence they are suitable for lysine fortification of sugar-containing foods which must be heat treated.

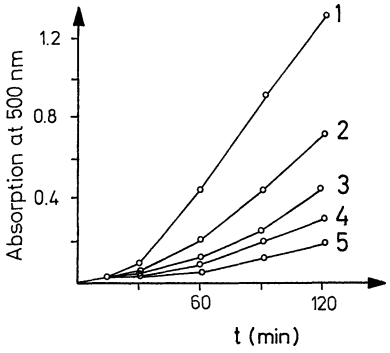


Fig. 1.9. Browning of some lysine derivatives (0.1 M lysine or lysine derivative, 0.1 M glucose in 0.1 M phosphate buffer pH 6.5 at 100 °C in sealed tubes, (according to *Finot* et al., 1978.) 1 Lys, 2 Ala-Lys, 3 Gly–Lys, 4 Glu–Lys, 5 Lys)

1.3.4.5 Other Peptides

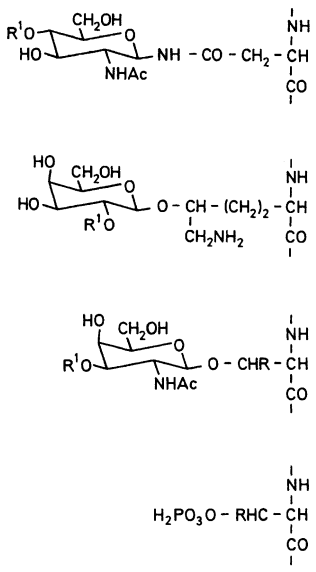
Other peptides occur commonly and in variable levels in protein rich food as degradation products of proteolytic processes.

1.4 Proteins

Like peptides, proteins are formed from amino acids through amide linkages. Covalently bound hetero constituents can also be incorporated into proteins. For example, phosphoproteins such as milk casein (cf. 10.1.2.1.1) or phosvitin of egg yolk (cf. 11.2.4.1.2) contain phosphoric acid esters of serine and threonine residues.

The structure of a protein is dependent on the amino acid sequence (the primary structure)

which determines the molecular conformation (secondary and tertiary structures). Proteins sometimes occur as molecular aggregates which are arranged in an orderly geometric fashion (quaternary structure). The sequences and conformations of a large number of proteins have been elucidated and recorded in several data bases.



R : H, CH_3 ; R^1 : H, Sugar residue; Ac: Acetyl (1.82)

Glycoproteins, such as $\varkappa$-casein (cf. 10.1.2.1.1), various components of egg white (cf. 11.2.3.1) and egg yolk (cf. 11.2.4.1.2), collagen from connective tissue (cf. 12.3.2.3.1) and serum proteins of some species of fish (cf. 13.1.4.2.4), contain one or more monosaccharide or oligosaccharide units bound O-glycosidically to serine, threonine or δ-hydroxylysine or N-glycosidically to asparagine (Formula 1.82). In glycoproteins, the primary structure of the protein is defined genetically. The carbohydrate components, however, are enzymatically coupled to the protein in a co- or post-transcriptional step. Therefore, the carbohydrate composition of glycoproteins is inhomogeneous (microheterogeneity).

1.4.1 Amino Acid Sequence

1.4.1.1 Amino Acid Composition, Subunits

Sequence analysis can only be conducted on a pure protein. First, the amino acid composition is determined after acidic hydrolysis. The procedure (separation on a single cation-exchange resin column and color development with ninhydrin reagent or fluorescamine) has been standardized and automated (amino acid analyzers). Figure 1.10 shows a typical amino acid chromatogram.

As an alternative to these established methods, the derivatization of amino acids with the subsequent separation and detection of derivatives is possible (pre-column derivatization). Various derivatization reagents can be selected, such as:

- 9-Fluorenylmethylchloroformate (FMOC, cf. 1.2.4.2.1)
- Phenylisothiocyanate (PITC, cf. 1.2.4.2.3)
- Dimethylaminoazobenzenesulfonylchloride (DABS-Cl, cf. 1.2.4.2.1)
- Dimethylaminonaphthalenesulfonylchloride (DANS-Cl, cf. 1.2.4.2.1)
- 7-Fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBDF, cf. 1.2.4.2.1)
- 7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBDCl, cf. 1.2.4.2.1)
- o-Phthaldialdehyde (OPA, cf. 1.2.4.2.4)

It is also necessary to know the molecular weight of the protein. This is determined by gel column chromatography, ultracentrifugation or SDS-PAG electrophoresis. Furthermore, it is necessary to determine whether the protein is a single molecule or consists of a number of identical or different polypeptide chains (subunits) associated through disulfide bonds or noncovalent forces. Dissociation into subunits can be accomplished by a change in pH, by chemical modification of the protein, such as by succinylation, or with denaturing agents (urea, guanidine hydrochloride, sodium dodecyl sulfate). Disulfide bonds, which are also found in proteins which consist of only one peptide chain, can be cleaved by oxidation of cystine to cysteic acid or by reduction to cysteine with subsequent alkylation of the thiol group

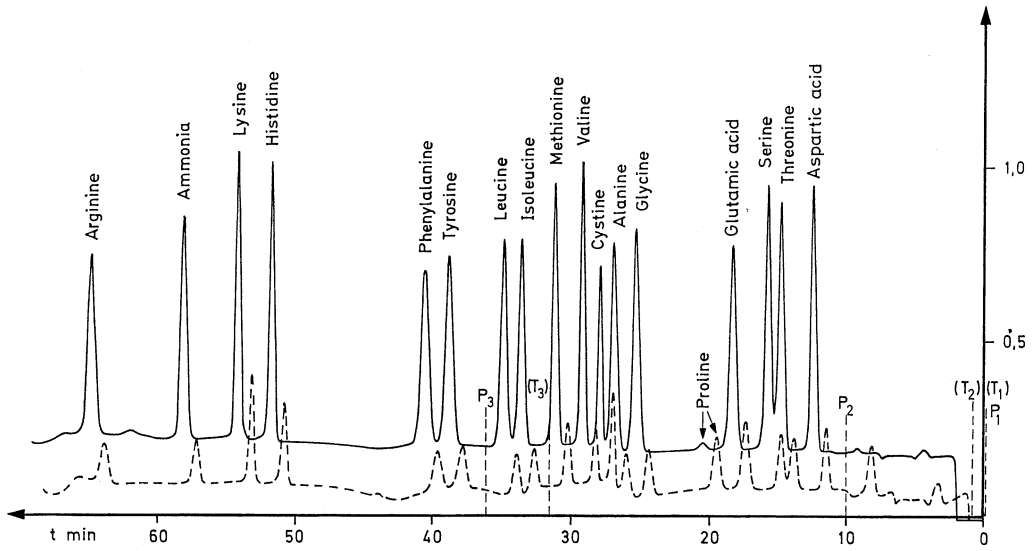


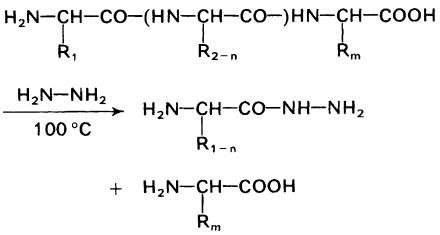
Fig. 1.10. Amino acid chromatogram. Separation of a mixture of amino acids (10 nmol/amino acid) by an amino acid analyzer. Applied is a single ion exchange column: Durrum DC-4A, 295 × 4 mm buffers $P_1/P_2/P_3$: 0.2 N Na-citrate pH 3.20/0.2 N Na-citrate pH 4.25/1.2 N Na-citrate and NaCl of pH 6.45. Temperatures $T_1/T_2/T_3$: 48/56/80 °C. Flow rate: 25 ml/h; absorbance reading after color development with ninhydrin at 570/440 nm: —/– – – –

(cf. 1.2.4.3.5) to prevent reoxidation. Separation of subunits is achieved by chromatographic or electrophoretic methods.

1.4.1.2 Terminal Groups

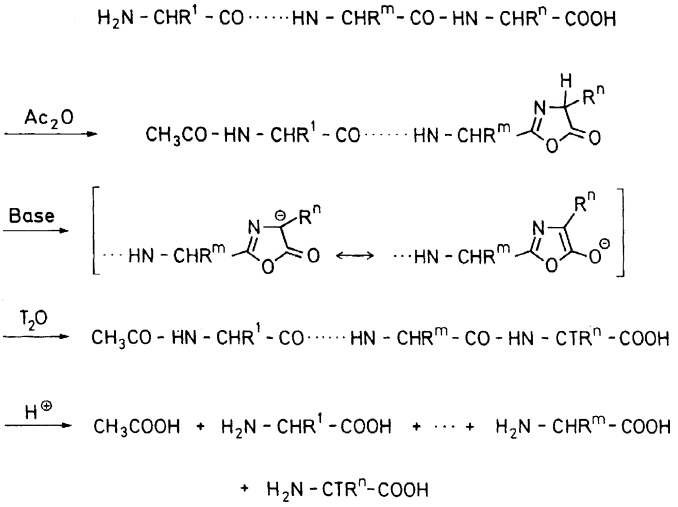
N-terminal amino acids can be determined by treating a protein with l-fluoro-2,4-dinitrobenzene (*Sanger's* reagent; cf. 1.2.4.2.2) or 5-dimethylaminonaphthalene-1-sulfonyl chloride (dansyl chloride; cf. 1.2.4.2.1). Another possibility is the reaction with cyanate, followed by elimination of the N-terminal amino acid in the form of hydantoin, and separation and recovery of the amino acid by cleavage of the hydantoin (cf. 1.2.4.2.3). The N-terminal amino acid (and the amino acid sequence close to the N-terminal) is accessible by hydrolysis with aminopeptidase, in which case it should be remembered that the hydrolysis rate is dependent on amino acid side chains and that proline residues are not cleaved. A special procedure is required when the N-terminal residue is acylated (N-formyl- or N-acetyl amino acids, or pyroglutamic acid).

Determination of C-terminal amino acids is possible via the hydrazinolysis procedure recommended by *Akabori:*



(1.83)

The C-terminal amino acid is then separated from the amino acid hydrazides, e. g., by a cation exchange resin, and identified. It is possible to mark the C-terminal amino acid through selective titration via oxazolinone:

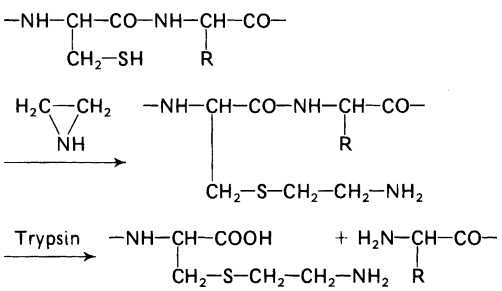


(1.84)

The C-terminal amino acids can be removed enzymatically by carboxypeptidase A which preferentially cleaves amino acids with aromatic and large aliphatic side chains, carboxypeptidase B which preferentially cleaves lysine, arginine and amino acids with neutral side chains or carboxypeptidase C which cleaves with less specificity but cleaves proline.

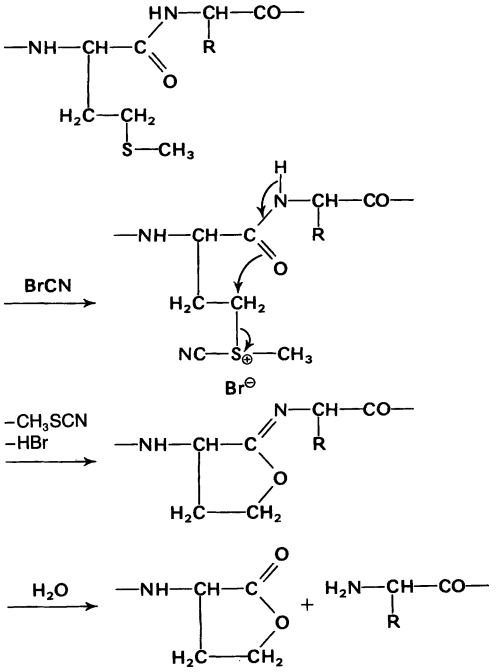
1.4.1.3 Partial Hydrolysis

Longer peptide chains are usually fragmented. The fragments are then separated and analyzed individually for amino acid sequences. Selective enzymatic cleavage of peptide bonds is accomplished primarily with trypsin, which cleaves exclusively Lys-X- and Arg-X-bonds, and chymotrypsin, which cleaves peptide bonds with less specificity (Tyr-X, Phe-X, Trp-X and Leu-X). The enzymatic attack can be influenced by modification of the protein. For example, acylation of the ε-amino group of lysine limits its tryptic hydrolysis to Arg-X (cf. 1.4.4.1.3 and 1.4.4.1.4), whereas substitution of the SH-group of a cysteine residue with an aminoethyl group introduces a new cleavage position for trypsin into the molecule "pseudolysine residue"):



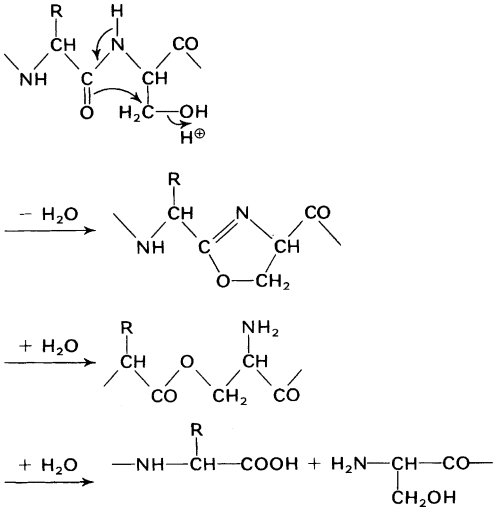
(1.85)

Also suited for the specific enzymatic hydrolysis of peptide chains is the endoproteinase Glu-C from *Staphylococcus aureus* V8. It cleaves Glu-X bonds (ammonium carbonate buffer pH 7.8 or ammonium acetate buffer pH 4.0) as well as Glu-X plus Asp-X bonds (phosphate buffer pH 7.8).
The most important chemical method for selective cleavage uses cyanogen bromide (BrCN) to attack Met-X-linkages (Reaction 1.86).
Hydrolysis of proteins with strong acids reveals a difference in the rates of hydrolysis of peptide bonds depending on the adjacent amino acid side chain. Bonds involving amino groups of serine and threonine are particularly susceptible to hydrolysis. This effect is due to



(1.86)

N → O-acyl migration via the oxazoline and subsequent hydrolysis of the ester bond:



(1.87)

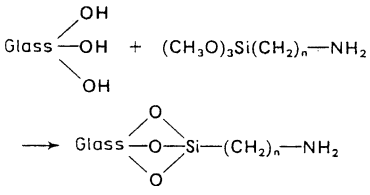
Hydrolysis of proteins with dilute acids preferentially cleaves aspartyl-X-bonds.

Separation of peptide fragments is achieved by gel and ion-exchange column chromatography using a volatile buffer as eluent (pyridine, morpholine acetate) which can be removed by freeze-drying of the fractions collected. The separation of peptides and proteins by reversed-phase HPLC has gained great importance, using volatile buffers mixed with organic, water-soluble solvents as the mobile phase.

The fragmentation of the protein is performed by different enzymic and/or chemical techniques, at least by two enzymes of different specifity. The arrangement of the obtained peptides in the same order as they occur in the intact protein is accomplished with the aid of overlapping sequences. The principle of this method is illustrated for subtilisin BPN′ as an example in Fig. 1.11.

1.4.1.4 Sequence Analysis

The classical method is the *Edman* degradation reaction. It involves stepwise degradation of peptides with phenylisothiocyanate (cf. 1.2.4.2.3) or suitable derivatives, e. g. dimethylaminoazobenzene isothiocyanate (DABITC). The resultant phenylthiohydantoin is either identified directly or the amino acid is recovered. The stepwise reactions are performed in solution or on peptide bound to a carrier, i. e. to a solid phase. Both approaches have been automated ("sequencer"). Carriers used include resins containing amino groups (e. g. amino polystyrene) or glass beads treated with amino alkylsiloxane:



(1.88)

The peptides are then attached to the carrier by carboxyl groups (activation with carbodiimide or carbonyl diimidazole, as in peptide synthesis) or by amino groups. For example, a peptide

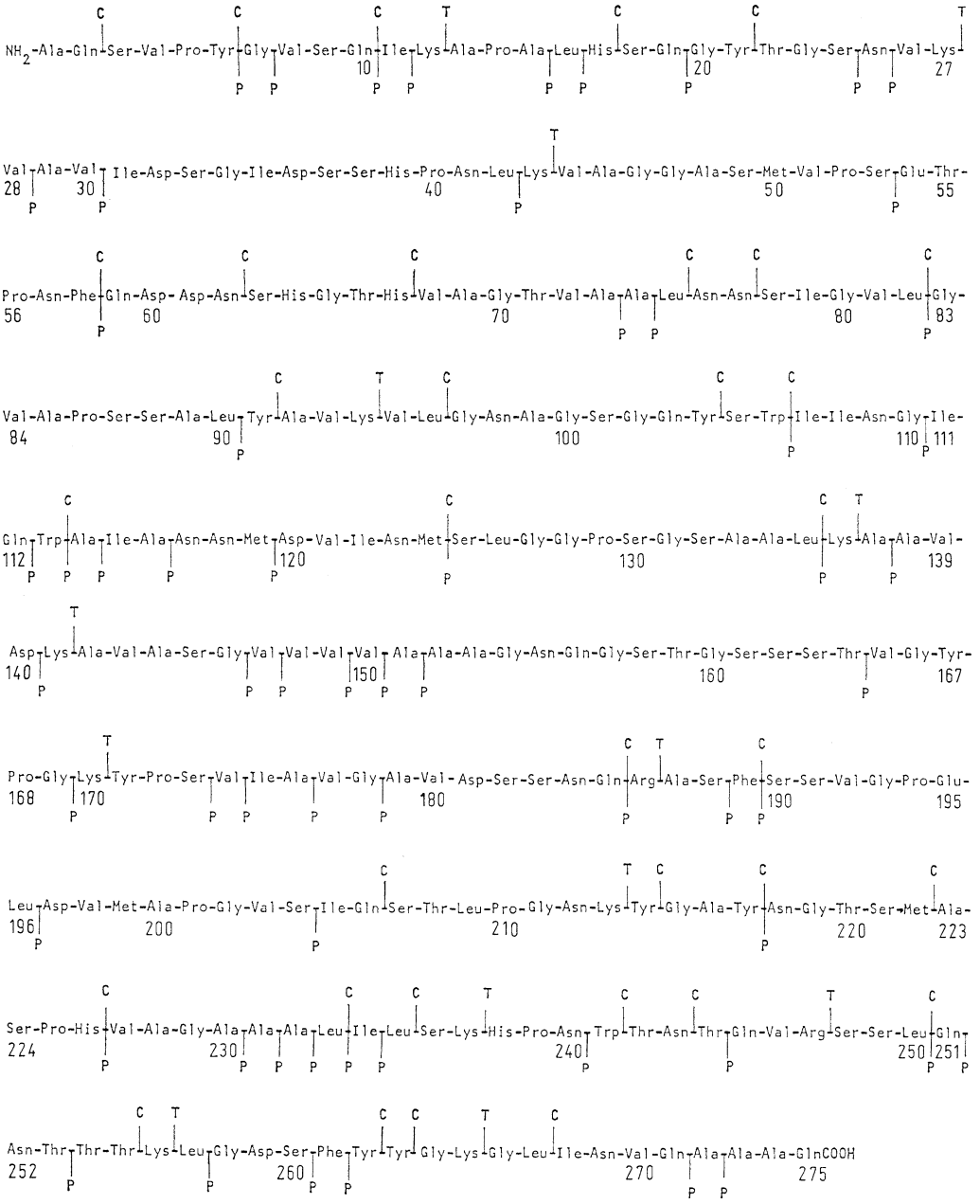
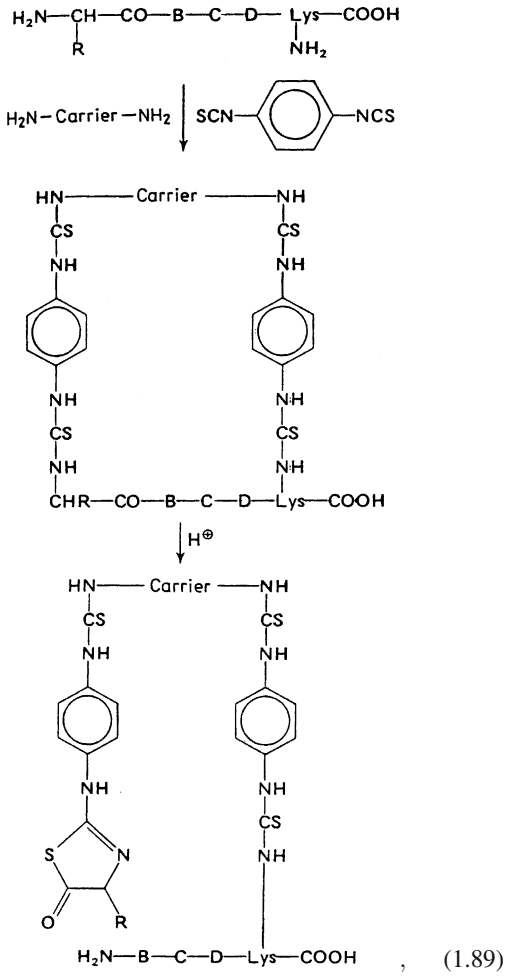


Fig. 1.11. Subtilisin BPN; peptide bonds hydrolyzed by trypsin (T), chymotrypsin (C), and pepsin (P)

segment from the hydrolysis of protein by trypsin has lysine as its C-terminal amino acid. It is attached to the carrier with p-phenylene-diisothiocyanate through the α- and ε-amino groups. Mild acidic treatment of the carrier under conditions of the *Edman* degradation splits the first peptide bond. The *Edman* procedure is then performed on the shortened peptide

through second, third and subsequent repetitive reactions:



(1.89)

Microvariants allow working in the picomole range. In the reaction chamber, the protein is fixed on a glass-fiber disc, and the coupling and cleaving reagents are added and removed in a carrier gas stream (vapour-phase sequentiation).

Apart from the *Edman* degradation, other methods can give valuable additional information on sequence analysis. These methods include the hydrolysis with amino- and carboxypeptidases as mentioned in the case of end group analysis and the fragmentation of suitable volatile peptide derivatives in a mass spectrometer.

1.4.1.5 Derivation of Amino Acid Sequence from the Nucleotide Sequence of the Coding Gene

The number of proteins for which the coding gene in the genome has been characterized is increasing steadily. However, a considerable part of the amino acid sequences known today has already been derived from the nucleotide sequences in question.

The background of this process will be briefly described here. The nucleotides consist of four different bases as well as 2-deoxyribose and phosphoric acid. They are the building blocks of the high-molecular deoxyribonucleic acid (DNA). The nucleotides are linked via 2-deoxyribose and phosphoric acid as $3' \rightarrow 5'$-diesters. In DNA, two polynucleotide strands are linked together in each case via hydrogen bridge bonds to give a double helix. The bases thymine and adenine as well as cytosine and guanine are complementary (cf. Formula 1.90). DNA is the carrier of the genetic information which controls protein biosynthesis via transcription to messenger ribonucleic acid (RNA). In translation into proteins, the sequence of bases codes the primary sequence of amino acids. Here, three of the four bases adenine, guanine, cytosine and thymine (abbreviated AGCT) in each case determines one amino acid, e. g., UGG codes for tryptophan (cf. Fig. 1.12).

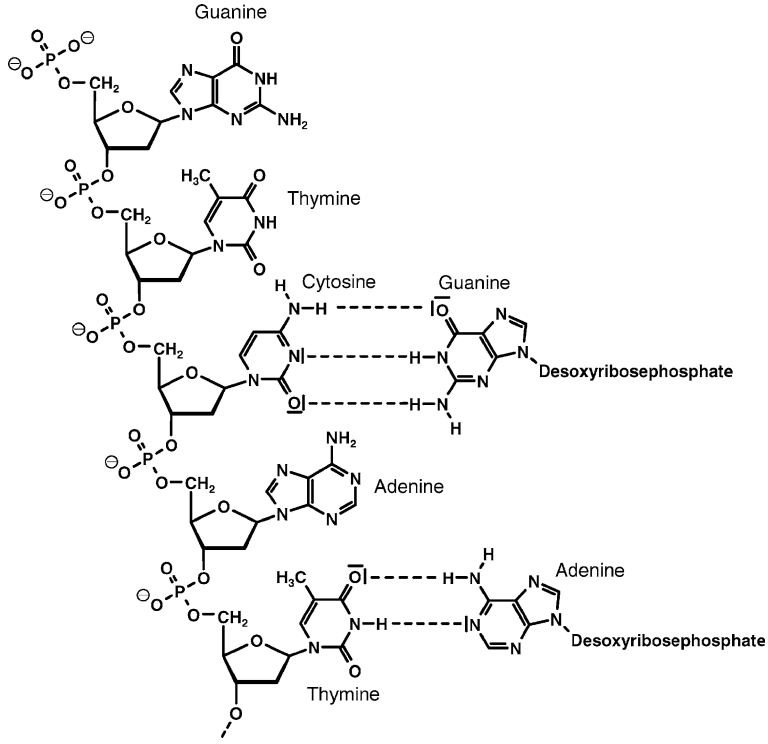
UUU Phe	UCU Ser	UAU Tyr	UGU Cys
UUC Phe	UCC Ser	UAC Tyr	UGC Cys
UUA Phe	UCA Ser	UAA Stop	UGA Stop
UUG Phe	UCG Ser	UAG Stop	UGG Trp
CUU Leu	CCU Pro	CAU His	CGU Arg
CUC Leu	CCC Pro	CAC His	CGC Arg
CUA Leu	CCA Pro	CAA Gln	CGA Arg
CUG Leu	CCG Pro	CAG Gln	CGG Arg
AUU Ile	ACU Thr	AAU Asn	AGU Ser
AUC Ile	ACC Thr	AAC Asn	AGC Ser
AUA Ile	ACA Thr	AAA Lys	AGA Arg
AUG Met	ACG Thr	AAG Lys	AGG Arg
GUU Val	GCU Ala	GAU Asp	GGU Gly
GUC Val	GCC Ala	GAC Asp	GGC Gly
GUA Val	GCA Ala	GAA Glu	GGA Gly
GUG Val	GCG Ala	GAG Glu	GGG Gly

Fig. 1.12. The genetic code

Therefore, the primary sequence of a protein can be derived from the nucleotide (base) sequence.

For the sequencing of DNA, the method of choice is the dideoxy process (chain termination process) introduced by *Fred Sanger* in 1975. The principle is based on the specific termination of the enzymatic synthesis of a DNA strand by means of DNA polymerase by using a 2′,3′-dideoxynucleotide, i. e., to prevent polymerization with the formation of the 3′ → 5′-phosphodiester at the position of the base in question. For instance, if the 2′,3′-dideoxynucleotide of guanine is used, the biosynthesis is stopped at guanine in each case. To detect all the guanine residues, only about 0.5 mol% of the dideoxynucleotide in question (based on the 2-deoxynucleotide) is used. In this way, DNA fragments of varying length are obtained which all have the same 5′-terminal and thus mark the position of the base.

The starting material is a hybrid of the single-stranded DNA to be sequenced and a primer consisting of about 20 nucleotides. This is lengthened with the help of DNA polymerase and a mixture of the 4 nucleotides and one 2′,3′-dideoxynucleotide in each case. The primer serves as a defined starting position and also as an initiator for the start of the synthesis of the complementary DNA strand. The DNA fragments of different length obtained in four experiments are then separated electrophoretically according to molecular size. For detection, either the primer can be labelled with four different fluorescent dyes (TAG) or the four dideoxynucleotides are labelled with different fluorescent dyes. In the former case, 4 series of experiments are carried out with differently labelled primers and one of the 4 dideoxynucleotides in each case. The charges are combined and electrophoretically separated together. The primary sequence is determined from the signals measured at different wave lengths (Fig. 1.13). When 4 differently labelled dideoxynucleotides are used, the primer is not labelled. Alternatively, the dideoxynucleotides can also be radioactively labelled (e. g., with ^{32}P). In this case, four separate DNA syntheses are also required.



(1.90)

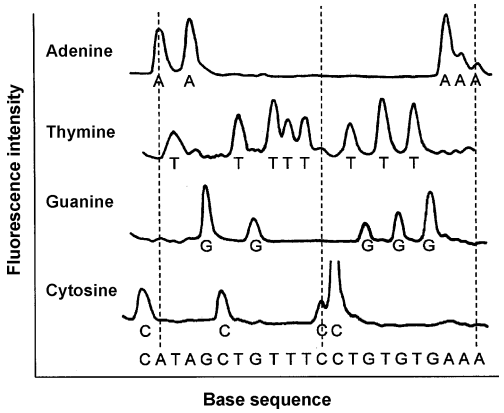


Fig. 1.13. Fluorescence detection of electrophoretically separated DNA fragments obtained by using the dideoxy method (according to *Smith* et al., 1986)

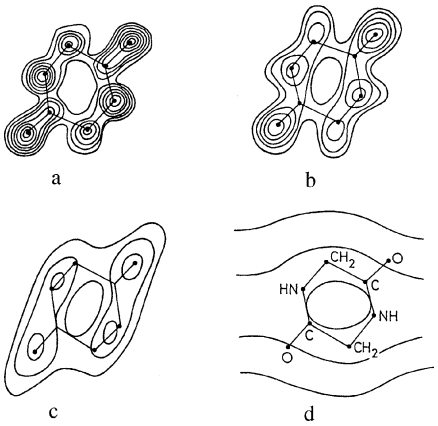


Fig. 1.14. Electron density distribution patterns for 2,5-dioxopiperazine with varying resolution extent. **a** 0.11 nm, **b** 0.15 nm, **c** 0.20 nm, **d** 0.60 nm (after *Perutz*, 1962)

1.4.2 Conformation

Information about conformation is available through X-ray crystallographic analysis of protein crystals and by measuring the distance ($\leq$30 nm) between selected protons of the peptide chain (NH_i–NH_{i+1}, NH_{i+1}–$C_\alpha H_i$, NH_{i+1}–$C_\beta H_i$, $C_\alpha H_i$–$C_\alpha H_{i+1}$, $C_\alpha H_i$–$C_\beta H$) by means of H-NMR spectroscopy in solution. This assumes that, in many cases, the conformation of the protein in crystalline form is similar to that of the protein in solution. As an example the calculated electron

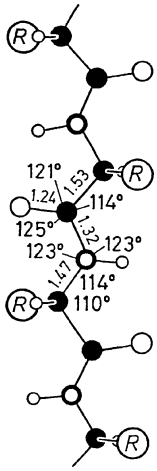
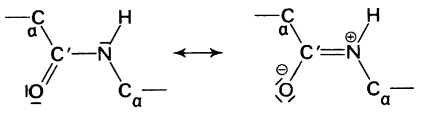


Fig. 1.15. Structure of an elongated peptide chain. ● Carbon, ◯ oxygen, ○ nitrogen, ○ hydrogen and ® side chain

density distributions of 2,5-dioxopiperazine based on various degrees of resolution are presented in Fig. 1.14. Individual atoms are well revealed at 0.11 nm. Such a resolution has not been achieved with proteins. Reliable localization of the C_α-atom of the peptide chain requires a resolution of less than 0.3 nm.

1.4.2.1 Extended Peptide Chains

X-ray structural analysis and other physical measurements of a fully extended peptide chain reveal the lengths and angles of bonds (see the "ball and stick" representation in Fig. 1.15). The peptide bond has partial (40%) double bond character with π electrons shared between the C′—O and C′—N bonds. The resonance energy is about 83.6 kJ/mole:



(1.91)

Normally the bond has a trans-configuration, i. e. the oxygen of the carbonyl group and the hydrogen of the NH group are in the trans-position; a cis-configuration which has 8 kJ mol^{-1} more energy occurs only in exceptional cases (e. g. in

small cyclic peptides or in proteins before proline residues).

Thus in ribonuclease A, two X-Pro bonds have trans-conformation (Pro-42 and Pro-117), and two have cis-conformation (Pro-93 and Pro-114). The equilibrium between the two isomers is catalyzed by specific enzymes (peptidyl-prolyl-cis/trans-isomerases). This accelerates the folding of a peptide chain (cf. 1.4.2.3.2), which in terms of the biosynthesis occurs initially in all-trans-conformation.

Six atoms of the peptide bonds, C_i^{α}, C_i', O_i, N_{i+1}, C_{i+1}^{α} and H_{i+1}, lie in one plane (cf. Fig. 1.16). For a trans-peptide bond, ω_i is 180°. The position of two neighboring planes is determined by the numerical value of the angles ψ_i (rotational bond between a carbonyl carbon and an α-carbon) and ϕ_i (rotational bond between an amide-N and an α-carbon). For an extended peptide chain, $\psi_i = 180°$ and $\phi_i = 180°$. The position of side chains can also be described by a series of angles χ_i^{1-n}.

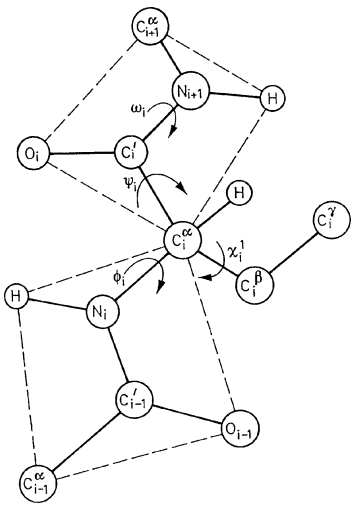


Fig. 1.16. Definitions for torsion angles in a peptide chain
$\omega_i = 0°$ for $C_i^{\alpha}-C_i'/N_{i+1}-C_{i+1}^{\alpha} \rightarrow$ cis,
$\psi_i = 0°$ for $C_i^{\alpha}-N_i/C_i'-O_i \rightarrow$ trans,
$\phi_i = 0°$ for $C_i'-C_i'/N_i-H \rightarrow$ trans,
$\chi_i = 0°$ for $C_i^{\alpha}-N_iC_i^{\beta}-C_i \rightarrow$ cis.
The angles are positive when the rotation is clockwise and viewed from the N-terminal side of a bond or (for X) from the atom closer to the main chain respectively. (according to *Schulz* and *Schirmer*, 1979)

1.4.2.2 Secondary Structure (Regular Structural Elements)

The primary structure gives the sequence of amino acids in a protein chain while the secondary structure reveals the arrangement of the chain in space. The peptide chains are not in an extended or unfolded form ($\psi_i, \phi_i = 180°$). It can be shown with models that ψ_i and ϕ_i, at a permissible minimum distance between non-bonding atoms (Table 1.20), can assume only particular angles. Figure 1.17 presents the

Table 1.20. Minimal distances for nonbonded atoms (Å)

	C	N	O	H
C	3.20[a]	2.90	2.80	2.40
	(3.00)[b]	(2.80)	(2.70)	(2.20)
N		2.70	2.70	2.40
		(2.60)	(2.60)	(2.20)
O			2.70	2.40
			(2.60)	(2.20)
H				2.00
				(1.90)

[a] Normal values.
[b] Extreme values.

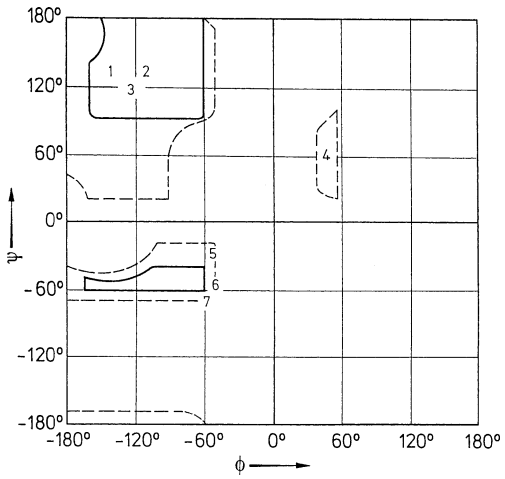


Fig. 1.17. ϕ, ψ-Diagram (*Ramachandran* plot). Allowed conformations for amino acids with a C^{β}-atom obtained by using normal (−) and lower limit (- - -) contact distances for non-bonded atoms, from Table 1.20. β-Sheet structures: antiparallel (1); parallel (2), twisted (3). Helices: α-, left-handed (4), 3_{10} (5), α, right-handed (6), π (7)

Table 1.21. Regular structural elements (secondary structures) in polypeptides

Structure	Φ (°)	ψ (°)	n[a]	d[b] (Å)	r[c] (Å)	Comments
β-Pleated sheet, parallel	−119	+113	2.0	3.2	1.1	Occurs occasionally in neighbouring chain sectors of globular proteins
β-Pleated sheet, antiparallel	−139	+135	2.0	3.4	0.9	Common in proteins and synthetic polypeptides
3_{10}-Helix	−49	−26	2.3	2.0	1.9	Observed at the ends of α-helixes
α-Helix, left-handed coiling	+57	+47	3.6	1.5	2.3	Common in globular proteins, as α "coiled coil" in fibrous proteins
α-Helix, right-handed coiling	−57	−47	3.6	1.5	2.3	Poly-D-amino acids poly-(β-benzyl)-L-aspartate
π-Helix	−57	−70	4.4	1.15	2.8	Hypothetical
Polyglycine II	−80	+150	3.0	3.1		Similar to antiparallel β-pleated-sheet formation
Polyglycine II, left-handed coiling	+80	−150	3.0	3.1		Synthetic polyglycine is a mixture of right- and left-handed helices; in some silk fibroins, the left-handed helix occurs
Poly-L-proline I	−83	+158	3.3	1.9		Synthetic poly-L-proline, only cis-peptide bonds
Poly-L-proline II	−78	+149	3.0	3.1		As left-handed polyglycine II, as triple helix in collagen

[a] Amino acid residues per turn.
[b] The rise along the axis direction, per residue.
[c] The radius of the helix.

permissible ranges for amino acids other than glycine (R ≠ H). The range is broader for glycine (R = H). Figure 1.18 demonstrates that most of 13 different proteins with a total of about 2500 amino acid residues have been shown empirically to have values of ψ, ϕ-pairs within the permissible range. When a multitude of equal ψ, ϕ-pairs occurs consecutively in a peptide chain, the chain acquires regular repeating structural elements. The types of structural elements are compiled in Table 1.21.

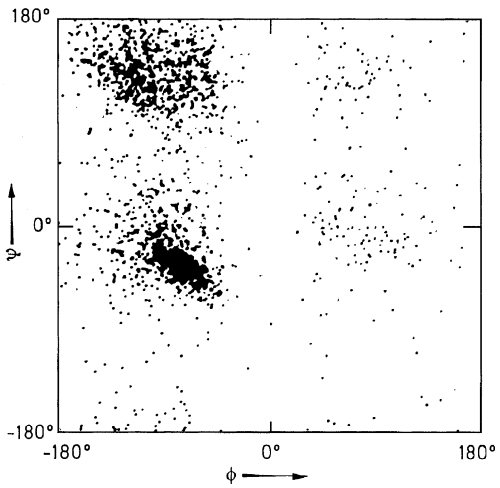


Fig. 1.18. ϕ, ψ-Diagram for observed values of 13 different proteins containing a total of 2500 amino acids. (according to *Schulz* and *Schirmer*, 1979)

1.4.2.2.1 β-Sheet

Three regular structural elements (pleated-sheet structures) have values in the range of $\phi = -120\,°C$ and $\psi = +120\,°C$. The peptide chain is always lightly folded on the C_α atom (cf. Fig. 1.19), thus the R side chains extend perpendicularly to the extension axis of the chain, i. e. the side chains change their projections alternately from +z to −z. Such a pleated structure is stabilized when more chains are present.
Subsequently, adjacent chains interact along the x-axis by hydrogen bonding, thus providing the cross-linking required for stability. When adjacent chains run in the same direction, the peptide chains are parallel. This provides a stabilized,

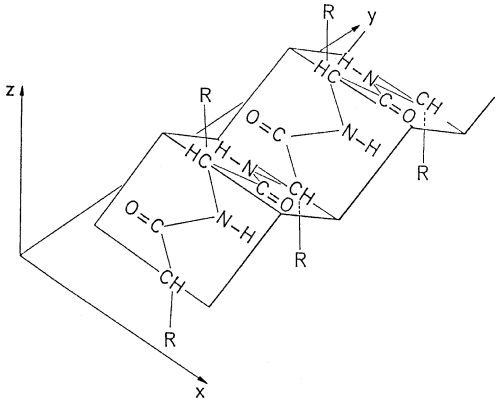


Fig. 1.19. A pleated sheet structure of a peptide chain

planar, parallel sheet structure. When the chains run in opposite directions, a planar, antiparallel sheet structure is stabilized (Fig. 1.20). The lower free energy, twisted sheet structures, in which the main axes of the neighboring chains are arranged at an angle of 25 °C (Fig. 1.21), are more common than planar sheet structures.
The β structures can also be regarded as special helix with a continuation of 2 residues per turn. With proline, the formation of a β structure is not possible.

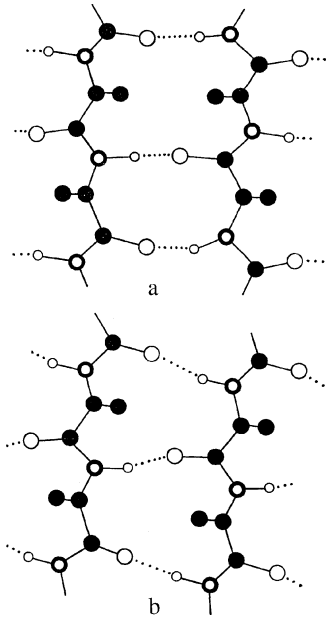


Fig. 1.20. Diagrammatic presentation of antiparallel (**a**) and parallel (**b**) peptide chain arrangements

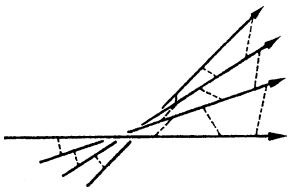


Fig. 1.21. Diagrammatic presentation of a twisted sheet structure of parallel peptide chains (according to *Schulz* and *Schirmer*, 1979)

1.4.2.2.2 Helical Structures

There are three regular structural elements in the range of $\phi = -60°$ and $\psi = -60°$ (cf. Fig. 1.17) in which the peptide chains are coiled like a threaded screw. These structures are stabilized by intrachain hydrogen bridges which extend almost parallel to the helix axis, crosslinking the CO and NH groups, i. e., the CO group of amino acid residue i with the NH group of residue $i + 3$ (3_{10}-helix), $1 + 4$ (α-helix) or $i + 5$ (π-helix).
The most common structure is the α-helix and for polypeptides from L-amino acids, exclusively the right-handed α-helix (Fig. 1.22). The left-handed α-helix is energetically unfavourable for L-amino acids, since the side chains here are in close contact with the backbone. No α-helix is possible with proline. The 3_{10}-helix was observed only at the ends of α-helices but not as an independent regular structure. The π-helix is hypothetical. Two helical conformations are known of polyproline (I and II). Polyproline I contains only cispeptide bonds and is right-handed, while polyproline II contains trans-peptide bonds and is left-handed. The stability of the two conformations depends on the solvent and other factors. In water, polyproline II predominates. Polyglycine can also occur in two conformations. Polyglycine I is a β-structure, while polyglcine II corresponds largely to the polyproline II-helix. A helix is characterized by the angles ϕ and ψ, or by the parameters derived from these angles: n, the number of amino acid residues per turn; d, the rise along the main axis per amino acid residue; and r, the radius of the helix. Thus, the equation for the pitch, p, is $p = n \cdot d$. The parameters n and d are presented within a ϕ, ψ plot in Fig. 1.23.

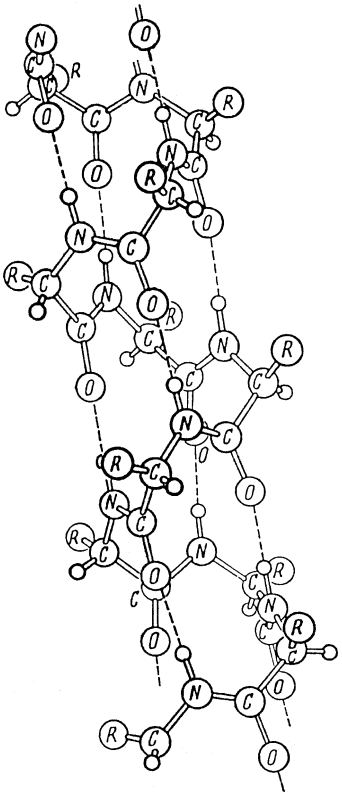


Fig. 1.22. Right-handed α-helix

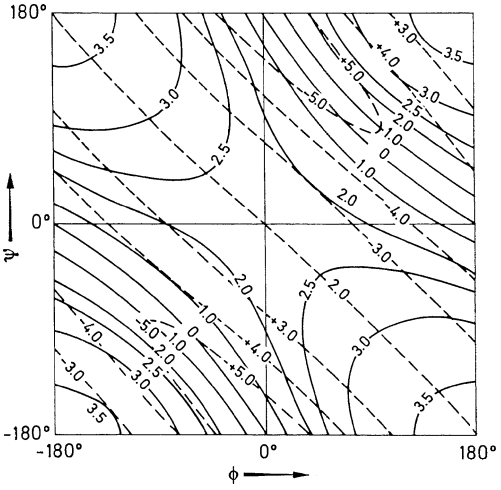


Fig. 1.23. φ, ψ-Diagram with marked helix parameters n (---) and d (—). (according to *Schulz* and *Schirmer*, 1979)

Table 1.22. β-Turns in the peptide chain of egg white lysozyme

Residue Number	Sequence			
20–23	Y	R	G	Y
36–39	S	N	F	N
39–42	N	T	Q	A
47–50	T	D	G	S
54–57	G	I	L	E
60–63	S	R	W	W
66–69	D	G	R	T
69–72	T	P	G	S
74–77	N	L	C	N
85–88	S	S	D	I
100–103	S	D	G	D
103–106	D	G	M	N

1.4.2.2.3 Reverse Turns

An important conformational feature of globular proteins are the reverseturns β-turns and β-bends. They occur at "hairpin" corners, where the peptide chain changes direction abruptly. Such corners involve four amino acid residues often including proline and glycine. Several types of turns are known; of greatest importance are type I (42% of 421 examined turns), type II (15%) and type III (18%); see Fig. 1.24.

In type I, all amino acid residues are allowed, with the exception of proline in position 3. In type II, glycine is required in position 3. In type III, which corresponds to a 3_{10}-helix, all amino acids are allowed. The sequences of the β-bends of lysozyme are listed in Table 1.22 as an example.

1.4.2.2.4 Super-Secondary Structures

Analysis of known protein structures has demonstrated that regular elements can exist in combined forms. Examples are the coiled-coil α-helix (Fig. 1.25, a), chain segments with antiparallel β-structures (β-meander structure; Fig. 1.25, b) and combinations of α-helix and β-structure (e. g., βαβαβ; Fig. 1.25 c).

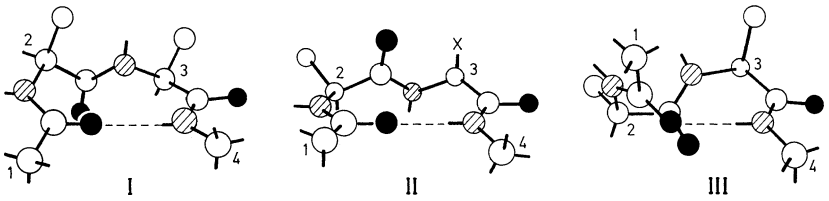


Fig. 1.24. Turns of the peptide chains (β-turns), types I–III. ○ = carbon, ⊘ = nitrogen, ● = oxygen. The α-C atoms of the amino acid residues are marked 1–4. X = no side chain allowed

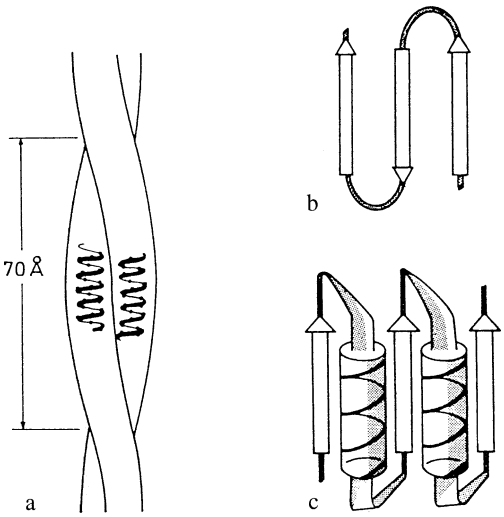


Fig. 1.25. Superhelix secondary structure (according to *Schulz* and *Schirmer*, 1979). **a** coiled-coil α-helix, **b** β-meander, **c** βαβαβ-structure

1.4.2.3 Tertiary and Quaternary Structures

Proteins can be divided into two large groups on the basis of conformation: (a) fibrillar (fibrous) or scleroproteins, and (b) folded or globular proteins.

1.4.2.3.1 Fibrous Proteins

The entire peptide chain is packed or arranged within a single regular structure for a variety of fibrous proteins. Examples are wool keratin (α-helix), silk fibroin (β-sheet structure) and collagen (a triple helix). Stabilization of these structures is achieved by intermolecular bonding (electrostatic interaction and disulfide linkages, but primarily hydrogen bonds and hydrophobic interactions).

1.4.2.3.2 Globular Proteins

Regular structural elements are mixed with randomly extended chain segments (randomly coiled structures) in globular proteins. The proportion of regular structural elements is highly variable: 20–30% in casein, 45% in lysozyme and 75% in myoglobin (Table 1.23). Five structural subgroups are known in this group of proteins: (1) α-helices

Table 1.23. Proportion of "regular structural elements" present in various globular proteins

Protein	α-Helix	β-Structure	n_G	n	%
Myoglobin	3–16[a]			14	
	20–34			15	
	35–41			7	
	50–56			7	
	58–77			20	
	85–93			9	
	99–116			18	
	123–145			23	
			151	173	75
Lysozyme	5–15			11	
	24–34			11	
		41–54		14	
	80–85			6	
	88–96			9	
	97–101			5	
	109–125			7	
			129	63	49
α_{s1}-Casein			199		ca. 30
β-Casein			209		ca. 20

[a] Position number of the amino acid residue in the sequence.
n_G: Total number of amino acid residues.
n: Amino acid residues within the regular structure.
%: Percentage of the amino acid residues present in regular structure.

occur only; (2) β-structures occur only; (3) α-helical and β-structural portions occur in separate segments on the peptide chain; (4) α-helix and β-structures alternate along the peptide chain; and (5) α-helix and β-structures do not exist.

The process of peptide chain folding is not yet fully understood. It begins spontaneously, probably arising from one center or from several centers of high stability in larger proteins. The tendency to form regular structural elements shows a very different development in the various amino acid residues. Table 1.24 lists data which were derived from the analysis of globular proteins of known conformation. The data indicate, for example, that Met, Glu, Leu and Ala are strongly helix-forming. Gly and Pro on the other hand show a strong helix-breaking tendency. Val, Ile and Leu promote the formation of pleated-sheet structures, while Asp, Glu and Pro prevent them. Pro and Gly are important building blocks of turns. Arginine does not prefer any of the three structures. By means of such data it is possible to forecast the expected conformations for a given amino acid sequence.

Table 1.24. Normalized frequencies[a] of amino acid residues in the regular structural elements of globular proteins

Amino acid	α-Helix (P_α)	Pleated sheet (P_β)	β-Turn (P_t)
Ala	1.29	0.90	0.78
Cys	1.11	0.74	0.80
Leu	1.30	1.02	0.59
Met	1.47	0.97	0.39
Glu	1.44	0.75	1.00
Gln	1.27	0.80	0.97
His	1.22	1.08	0.69
Lys	1.23	0.77	0.96
Val	0.91	1.49	0.47
Ile	0.97	1.45	0.51
Phe	1.07	1.32	0.58
Tyr	0.72	1.25	1.05
Trp	0.99	1.14	0.75
Thr	0.82	1.21	1.03
Gly	0.56	0.92	1.64
Ser	0.82	0.95	1.33
Asp	1.04	0.72	1.41
Asn	0.90	0.76	1.28
Pro	0.52	0.64	1.91
Arg	0.96	0.99	0.88

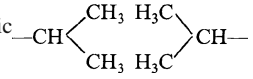
[a] Shown is the fraction of an amino acid in a regular structural element, related to the fraction of all amino acids of the same structural element. $P = 1$ means random distribution; $P > 1$ means enrichment, $P < 1$ means depletion. The data are based on an analysis of 66 protein structures.

The hydrophobic interaction of the nonpolar regions of the peptide chains also plays an important role in protein folding. These interactions are responsible for the fact that nonpolar groups are folded to a great extent towards the interior of the protein globule. The surface areas accessible to water molecules have been calculated for both unfolded and native folded forms for a number of monomeric proteins with known conformations. The proportion of the accessible surface in the stretched state, which tends to be burried in the interior of the globule as a result of folding, is a simple linear function of the molecular weight (M). The gain in free energy for the folded surface is

Folding of the peptide chain packs it densely by formation of a large number of intermolecular noncovalent bonds. Data on the nature of the bonds involved are provided in Table 1.25.

The H-bonds formed between main chains, main and side chains and side-side chains are of particular importance for folding. The portion of polar groups involved in H-bond buildup in proteins of Mr $>$ 8.9 kdal appears to be fairly constant at about 50%.

Table 1.25. Bond-types in proteins

Type	Examples	Bond strength (kJ/mole)
Covalent bonds	–S–S–	ca. −230
Electrostatic bonds	$-COO-H_3N^+-$	−21
Hydrogen bonds	>C=O O=C<	+1.3
	–O–H··· O<	−16.7
	>N–H··· O=C<	−12.5
Hydrophobic bonds		0.01[b]
	–Ala ··· Ala–	−3
	–Val ··· Val–	−8
	–Leu ··· Leu–	−9
	–Phe ··· Phe–	−13
	–Trp ··· Trp–	−19

[a] For $\varepsilon = 4$.

[b] Per $Å^2$-surface area.

$10\,kJnm^{-2}$. Therefore, the total hydrophobic contribution to free energy due to folding is:

$$\Delta G_{HP} = 88\ M + 79 \cdot 10^{-5}\ M^2\ [J \cdot mol^{-1}] \qquad (1.92)$$

This relation is valid for a range of $6108 \leq M \leq 34{,}409$, but appears to be also valid for larger molecules since they often consist of several loose associations of independent globular portions called structural domains (Fig. 1.26).

Proteins with disulfide bonds fold at a significantly slower rate than those without disulfide bonds. Folding is not limited by the reaction rate of disulfide formation. Therefore the folding process of disulfide-containing proteins seems to proceed in a different way. The reverse process, protein unfolding, is very much slowed down by the presence of disulfide bridges which generally impart great stability to globular proteins. This stability is particularly effective against denaturation. An example is the *Bowman-Birk* inhibitor from soybean (Fig. 1.27) which inhibits the activity of trypsin and chymotrypsin. Its tertiary structure is stabilized by seven disulfide bridges. The reactive sites of inhibition are Lys^{16}-Ser^{17} and Leu^{43}-Ser^{44}, i.e. both sites are located in relatively small rings, each of which consists of nine amino acid residues held in ring form by a disulfide bridge. The thermal stability of this inhibitor is high.

As examples of the folding of globular proteins, Fig. 1.28 shows schematically the course of the peptide chains in the β-chain of hemoglobin, in triosephosphate isomerase and carboxypeptidase. Other protein conformations are shown in the following figures:

- Fig. 8.7 (cf. 8.8.4): Thaumatin and monellin (two-dimensional)
- Fig. 8.8 (cf. 8.8.5): Thaumatin and monellin (three-dimensional)
- Fig. 11.3 (cf. 11.2.3.1.4): Lysozyme

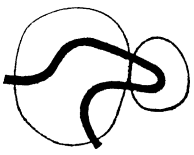


Fig. 1.26. Globular protein with two-domain structure (according to *Schulz* and *Schirmer*, 1979)

1.4.2.3.3 BSE

The origination of transmissible spongiform encephalopathies (TESs) is explained by a change in the protein conformation. (The name refers to the spongy deformations which occur in the brain in this disease. The resulting defects interrupt the transmission of signals). One of the TESs is bovine spongiform encephalopathy (BSE). According to the current hypothesis, TESs are caused by pathogenic prion proteins (PrPp), which can be present in the animal meal used as feed. PrPp are formed from normal prion proteins (PrPn) found in all mammalian cells.

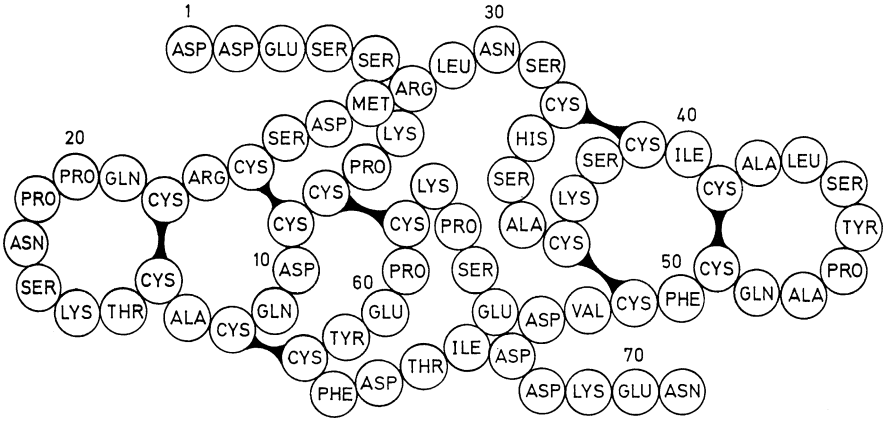


Fig. 1.27. *Bowman-Birk* inhibitor from soybean (according to *Ikenaka* et al., 1974)

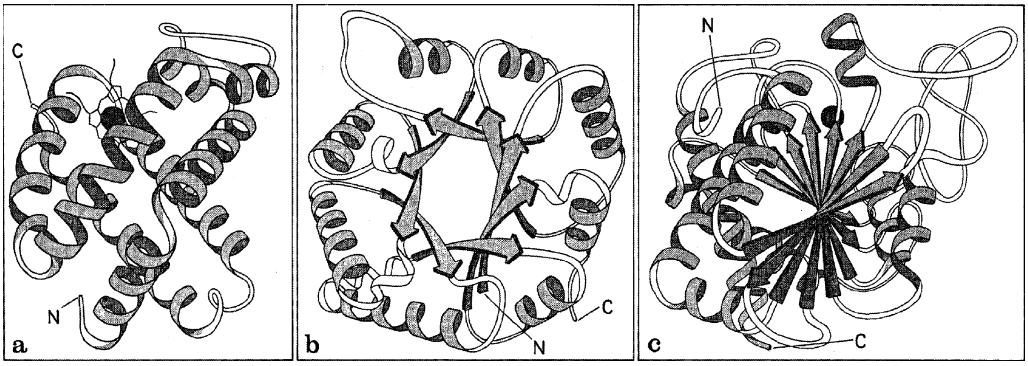


Fig. 1.28. Tertiary structures (schematic: spiral: α-helix, arrow: pleated sheet) of the β-chain of hemoglobin (**a**), of triosephosphate isomerase (**b**) and carboxypeptidase (**c**). (according to *Walton*, 1981)

However, a PrPp forces the pathogenic conformation on a PrPn. The stability towards serine proteinase K from the fungus *Tritirachium album* is used to differentiate between PrPp and PrPn. Serine proteinase K, which attacks the carboxyl side of hydrophobic amino acids, largely hydrolyzes PrPn while a characteristic peptide (M_r 27-30 kDa) is released from PrPp. This marker can be identified using the sandwich ELISA (cf. 2.6.3).

1.4.2.3.4 Quaternary Structures

In addition to the free energy gain by folding of a single peptide chain, association of more than one peptide chain (subunit) can provide further gains in free energy. For example, hemoglobin (4 associated peptide chains) $\Delta G^0 = -46\,\text{kJ mole}^{-1}$ and the trypsin-trypsin inhibitor complex (association of 2 peptide chains) $\Delta\ G^0 = -75.2\,\text{kJ mole}^{-1}$. In principle such associations correspond to the folding of a larger peptide chain with several structural domains without covalently binding the subunits. Table 1.26 lists some proteins which partially exhibit quaternary structures.

1.4.2.4 Denaturation

The term denaturation denotes a reversible or irreversible change of native conformation (tertiary structure) without cleavage of covalent bonds (except for disulfide bridges). Denaturation is possible with any treatment that cleaves hydrogen

Table 1.26. Examples of globular proteins

Name	Origin	Molecular weight (Kdal)	Number of subunits
Lysozyme	Chicken egg	14.6	1
Papain	*Papaya latex*	20.7	1
α-Chymotrypsin	Pancreas (beef)	23	1
Trypsin	Pancreas (beef)	23.8	1
Pectinesterase	Tomato	27.5	
Chymosin	Stomach (calf)	31	
β-Lactoglobulin	Milk	35	2
Pepsin A	Stomach (swine)	35	1
Peroxidase	Horseradish	40	1
Hemoglobin	Blood	64.5	4
Avidin	Chicken egg	68.3	4
Alcohol-dehydrogenase	Liver (horse)	80	2
	Yeast	150	4
Hexokinase	Yeast	104	2
Lactate dehydrogenase	Heart (swine)	135	4
Glucose oxidase	*P. notatum*	152	
Pyruvate kinase	Yeast	161	8
	A. niger	186	
β-Amylase	Sweet potato	215	4
Catalase	Liver (beef)	232	4
	M. lysodeikticus	232	
Adenosine triphosphatase	Heart (beef)	284	6
Urease	Jack beans	483	6
Glutamine synthetase	*E. coli*	592	12
Arginine decarboxylase	*E. coli*	820	10

bridges, ionic or hydrophobic bonds. This can be accomplished by: changing the temperature, adjusting the pH, increasing the interface area, or adding organic solvents, salts, urea, guanidine hyrochloride or detergents such as sodium dodecyl sulfate. Denaturation is generally reversible when the peptide chain is stabilized in its unfolded state by the denaturing agent and the native conformation can be reestablished after removal of the agent. Irreversible denaturation occurs when the unfolded peptide chain is stabilized by interaction with other chains (as occurs for instance with egg proteins during boiling). During unfolding reactive groups, such as thiol groups, that were buried or blocked, may be exposed. Their participation in the formation of disulfide bonds may also cause an irreversible denaturation.

An aggregation of the peptide chains caused by the folding of globular proteins is connected with reduced solubility or swellability. Thus the part of wheat gluten that is soluble in acetic acid diminishes as heat stress increases (Fig. 1.29). As a result of the reduced rising capacity of gluten caused by the pre-treatment, the volume of bread made of recombined flours is smaller (Fig. 1.30). In the case of fibrous proteins, denaturation, through destruction of the highly ordered structure, generally leads to increased solubility or rising capacity. One example is the thermally caused collagen-to-gelatin conversion, which occurs when meat is cooked (cf. 12.3.2.3.1).

The thermal denaturation of the whey proteins β-lactoglobulin and α-lactalbumin has been well-studied. The data in Table 1.27 based on reaction kinetics and the *Arrhenius* diagram (Fig. 1.31) indicate that the activation energy of the overall reaction in the range of 80–90 °C changes. The higher E_a values at lower temperatures must be attributed to folding, which is the partial reaction that determines the reaction rate at temperatures <90 °C. At higher temperatures (>95 °C), the aggregation to which the lower activation energy corresponds predominates.

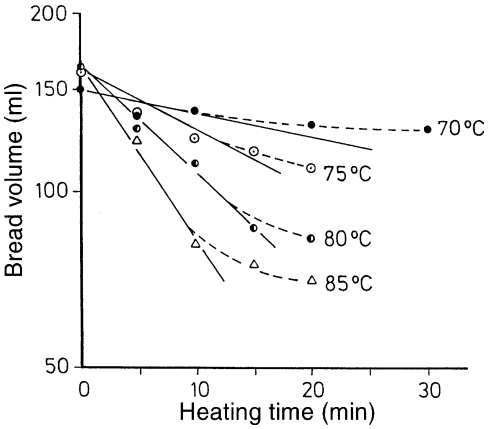


Fig. 1.30. Volume of white bread of recombined flours using thermally treated liquid gluten (wheat) (according to *Pence* et al., 1953)

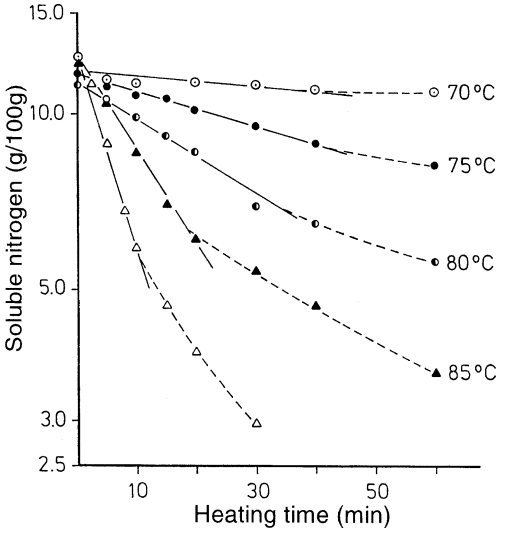


Fig. 1.29. Solubility of moist gluten (wheat) in diluted acetic acid after various forms of thermal stress (according to *Pence* et al., 1953)

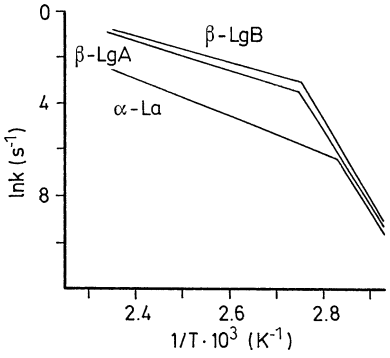


Fig. 1.31. *Arrhenius* diagram for the denaturation of the whey proteins β-lactoglobulin A, β-lactoglobulin B and α-lactalbumin B (according to *Kessler*, 1988)

Table 1.27. Denaturation of β-lactoglobulins A and B (β-LG-A, β-LG-B) and of α-lactalbumin (a-LA)

Protein	n	ϑ (°C)	E_a (kJ mol^{-1})	ln(k_o) (s^{-1})	$\Delta S^{\neq}$ (kJ mol^{-1}) K^{-1}
β-LG-A	1.5	70–90	265.21	84.16	0.445
		95–150	54.07	14.41	−0.136
β-LG-B	1.5	70–90	279.96	89.43	0.487
		95–150	47.75	12.66	−0.150
α-LA	1.0	70–80	268.56	84.92	0.452
		85–150	69.01	16.95	−0.115

n: reaction order, δ: temperature, E_a: activation energy, k_o: reaction rate constant, $\Delta S^{\neq}$: activation entropy.

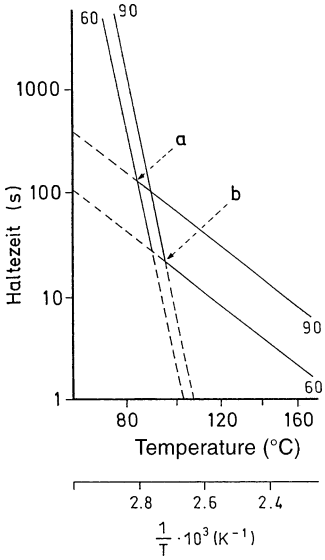


Fig. 1.32. Lines of equal denaturation degrees of β-lactoglobulin B. [The *steeper lines* correspond to the folding (60%, 90%), the *flatter lines* to aggregation (60%, 90%); at point a, 60% are folded and 90% can be aggregated, corresponding to an overall reaction of 60%; at point b, 90% are folded and 60% can be aggregated, corresponding to an overall reaction of 60%; according to *Kessler*, 1988]

The values in Table 1.27 determined for activation entropy also support the above mentioned attribution. In the temperature range of 70–90 °C, $\Delta S^{\#}$ is always positive, which indicates a state of greater disorder than should be expected with the predominance of the folding reaction. On the other hand, the negative $\Delta S^{\#}$ values at 95–105 °C indicate a state of greater order than should be expected considering that aggregation predominates in this temperature range. Detailed studies of the kind described above allow optimal control of thermal processes. In the case of milk processing, the data have made it possible, for example, to avoid the separation of whey proteins in heating equipment and to optimize the properties of yogurt gels (cf. 10.1.3.3 and 10.2.1.2).

Figure 1.32 shows the denaturation of β-LG in a diagram that combines the heating period with the temperature (cf. 2.5.4.3) in the form of straight lines of equal denaturation degrees. This allows us to read directly the time/temperature combinations required for a certain desired effect. At 85 °C/136 s for example, only 60% of the β-LG-B are folded, so that only 60% can aggregate, although 90% would be potentially able to aggregate: at this temperature, the folding determines the overall reaction, as shown above. Conversely, 90% of the protein is potentially folded at 95 °C/21 s while only 60% can be aggregated. At this temperature, aggregation determines the overall reaction.

Denaturation of biologically active proteins is usually associated with loss of activity. The fact that denatured proteins are more readily digested by proteolytic enzymes is also of interest.

1.4.3 Physical Properties

1.4.3.1 Dissociation

Proteins, like amino acids, are amphoteric. Depending on pH, they can exist as polyvalent cations, anions or zwitter ions. Proteins differ in their α-carboxyl and α-amino groups – since these groups are linked together by peptide bonds, the uptake or release of protons is limited to free terminal groups. Therefore, most of the dissociable functional groups are derived from side chains. Table 1.28 lists pK values of some protein groups. In contrast to free amino acids, these values fluctuate greatly for proteins since the dissociation is influenced by neighboring groups in the macromolecule. For example, in lysozyme the γ-carboxyl group of Glu^{35} has a pK

Table 1.28. pK values of protein side chains

Group	pK (25 °C)	Group	pK (25 °C)
α-Carboxyl-	3–4	Imidazolium-	4–8
β, γ-Carboxyl-	3–5	Hydroxy-	
α-Ammonium-	7–8	(aromatic)	9–12
ε-Ammonium-	9–11	Thiol	8–11
Guanidinium-	12–13		

of 6–6.5, while the pK of the β-carboxyl group of Asp^{66} is 1.5–2, of Asp^{52} is 3–4.6 and of Asp^{101} is 4.2–4.7.

The total charge of a protein, which is the absolute sum of all positive and negative charges, is differentiated from the so-called net charge which, depending on the pH, may be positive, zero or negative. By definition the net charge is zero and the total charge is maximal at the isoelectric point. Lowering or raising the pH tends to increase the net charge towards its maximum, while the total charge always becomes less than at the isolectric point.

Since proteins interact not only with protons but also with other ions, there is a further differentiation between an isoionic and an isoelectric point. The isoionic point is defined as the pH of a protein solution at infinite dilution, with no other ions present except for H^+ and HO^-. Such a protein solution can be acquired by extensive dialysis (or, better, electrodialysis) against water. The isoionic point is constant for a given substance while the isoelectric point is variable depending on the ions present and their concentration. In the presence of salts, i. e. when binding of anions is stronger than that of cations, the isoelectric point is lower than the isoionic point. The reverse is true when cationic binding is dominant. Figure 1.33 shows the shift in pH of an isoionic serum albumin solution after addition of various salts. The shift in pH is consistently positive, i. e. the protein binds more anions than cations.

The titration curve of β-lactoglobulin at various ionic strengths (Fig. 1.34) shows that the isoelectric point of this protein, at pH 5.18, is independent of the salts present. The titration curves are, however, steeper with increasing ionic strength, which indicates greater suppression of the electrostatic interaction between protein molecules.

At its isoelectric point a protein is the least soluble and the most likely to precipitate ("isoelectric precipitation") and is at its maximal crystallization capacity. The viscosity of solubilized proteins and the swelling power of insoluble proteins are at a minimum at the isoelectric point.

When the amino acid composition of a protein is known, the isoelectric point can be estimated according to the following formula:

$$pI = -10 \log Q_{pI} + 7.0 \qquad (1.93)$$

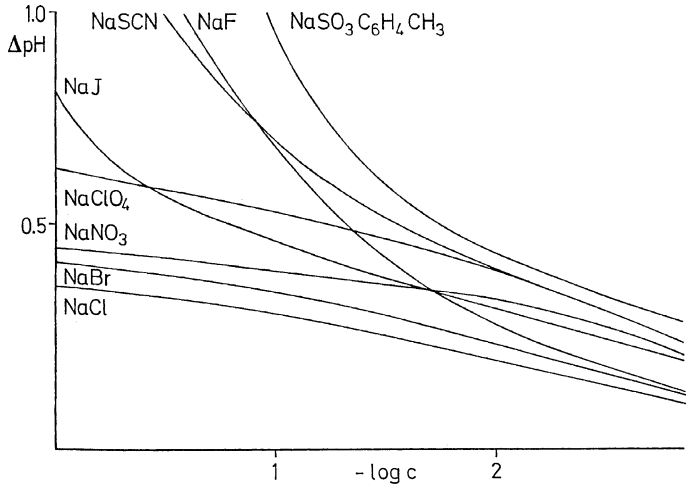


Fig. 1.33. pH-shift of isoionic serum albumin solutions by added salts. (according to *Edsall* and *Wymann*, 1958)

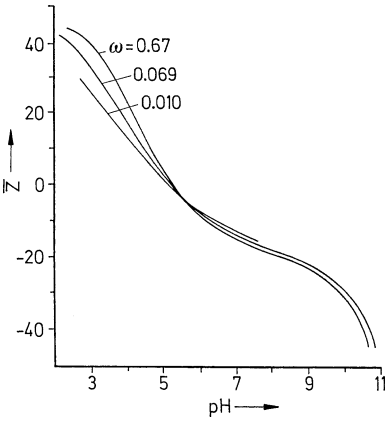


Fig. 1.34. Titration curves for β-lactoglobulin at various ionic strengths ω. (according to *Edsall* and *Wyman*, 1958)

where QpI is the sum of deviations of the isoelectric points of all participating amino acids from the neutral point:

$$Q_{pI} = \frac{4.2 \cdot n\ \text{Asp} + 3.8 m\ \text{Glu}}{3.8 q\ \text{Arg} + 2.6 r\ \text{Lys} + 0.5 s\ \text{His}} \qquad (1.94)$$

The formula fails when acid or alkaline groups occur in masked form.

1.4.3.2 Optical Activity

The optical activity of proteins is due not only to asymmetry of amino acids but also to the chirality resulting from the arrangement of the peptide chain. Information on the conformation of proteins can be obtained from a recording of the optical rotatory dispersion (ORD) or the circular dichroism (CD), especially in the range of peptide bond absorption wavelengths (190–200 nm). The *Cotton* effect occurs in this range and reveals quantitative information on secondary structure. An α-helix or a β-structure gives a negative *Cotton* effect, with absorption maxima at 199 and 205 nm respectively, while a randomly coiled conformation shifts the maximum to shorter wavelengths, i. e. results in a positive *Cotton* effect (Fig. 1.35).

1.4.3.3 Solubility, Hydration and Swelling Power

Protein solubility is variable and is influenced by the number of polar and apolar groups and their arrangement along the molecule. Generally, proteins are soluble only in strongly

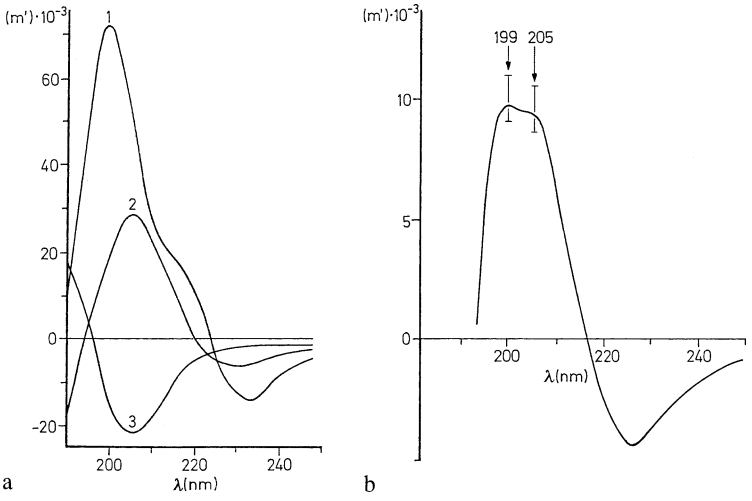


Fig. 1.35. *Cotton* effect. **a** Polylysine α-helix (1, pH 11–11.5) β-sheet structure (2, pH 11–11.3 and heated above 50 °C) and random coiled (3, pH 5–7). **b** Ribonuclease with 20% α-helix, 40% β-sheet structure and 40% random coiled region. (according to *Luebke, Schroeder*, and *Kloss*, 1975)

polar solvents such as water, glycerol, formamide, dimethylformamide or formic acid. In a less polar solvent such as ethanol, proteins are rarely noticeably soluble (e. g. prolamines). The solubility in water is dependent on pH and on salt concentration. Figure 1.36 shows these relationships for β-lactoglobulin.

At low ionic strengths, the solubility rises with increase in ionic strength and the solubility minimum (isoelectric point) is shifted from pH 5.4 to pH 5.2. This shift is due to preferential binding of anions to the protein.

If a protein has enough exposed hydrophobic groups at the isoelectric point, it aggregates due to the lack of electrostatic repulsion via intermolecular hydrophobic bonds, and (isoelectric) precipitation will occur. If on the other hand, intermolecular hydrophobic interactions are only poorly developed, a protein will remain in solution even at the isoelectric point, due to hydration and steric repulsion.

As a rule, neutral salts have a two-fold effect on protein solubility. At low concentrations they increase the solubility ("salting in" effect) by suppressing the electrostatic protein-protein interaction (binding forces).

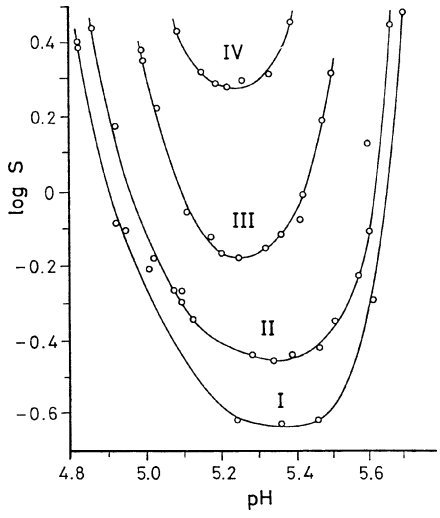


Fig. 1.36. β-Lactoglobulin solubility as affected by pH and ionic strength I. 0.001, II. 0.005, III. 0.01, IV. 0.02

The log of the solubility (S) is proportional to the ionic strength (μ) at low concentrations (cf. Fig. 1.36.):

$$\log S = k \cdot \mu \,. \tag{1.95}$$

Protein solubility is decreased ("salting out" effect) at higher salt concentrations due to the ion hydration tendency of the salts. The following relationship applies (S_0: solubility at $\mu = 0$; K: salting out constant):

$$\log S = \log S_0 - K \cdot \mu \,. \tag{1.96}$$

Cations and anions in the presence of the same counter ion can be arranged in the following orders (*Hofmeister* series) based on their salting out effects:

$$K^+ > Rb^+ > Na^+ > Cs^+ > Li^+ > NH_4^+ \,;$$
$$SO_4^{2-} > \text{citrate}^{2-} > \text{tratrate}^{2-} > \text{acetate}^- > Cl^- > NO_3^- > Br^- > J^- > CNS^- \,. \tag{1.97}$$

Multivalent anions are more effective than monovalent anions, while divalent cations are less effective than monovalent cations.

Since proteins are polar substances, they are hydrated in water. The degree of hydration (g water of hydration/g protein) is variable. It is 0.22 for ovalbumin (in ammonium sulfate), 0.06 for edestin (in ammonium sulfate), 0.8 for β-lactoglobulin and 0.3 for hemoglobin. Approximately 300 water molecules are sufficient to cover the surface of lysozyme (about 6000 $Å^2$), that is one water molecule per 20 $Å^2$.

The swelling of insoluble proteins corresponds to the hydration of soluble proteins in that insertion of water between the peptide chains results in an increase in volume and other changes in the physical properties of the protein. For example, the diameter of myofibrils (cf. 12.2.1) increases to 2.5 times the original value during rinsing with 1.0 mol/L NaCl, which corresponds to a six-fold volume increase (cf. 12.5). The amount of water taken up by swelling can amount to a multiple of the protein dry weight. For example, muscle tissue contains 3.5–3.6 g water per g protein dry matter.

The water retention capacity of protein can be estimated with the following formula:

$$a = f_c + 0.4 f_p + 0.2 f_n \quad (1.98)$$

(a: g water/g protein; f_c, f_p, f_n: fraction of charged, polar, neutral amino acid residues).

1.4.3.4 Foam Formation and Foam Stabilization

In several foods, proteins function as foam-forming and foam-stabilizing components, for example in baked goods, sweets, desserts and beer. This varies from one protein to another. Serum albumin foams very well, while egg albumin does not. Protein mixtures such as egg white can be particularly well suited (cf. 11.4.2.2). In that case, the globulins facilitate foam formation. Ovomucin stabilizes the foam, egg albumin and conalbumin allow its fixation through thermal coagulation.

Foams are dispersions of gases in liquids. Proteins stabilize by forming flexible, cohesive films around the gas bubbles. During impact, the protein is adsorbed at the interface via hydrophobic areas; this is followed by partial unfolding (surface denaturation). The reduction of surface tension caused by protein adsorption facilitates the formation of new interfaces and further gas bubbles. The partially unfolded proteins associate while forming stabilizing films.

The more quickly a protein molecule diffuses into interfaces and the more easily it is denatured there, the more it is able to foam. These values in turn depend on the molecular mass, the surface hydrophobicity, and the stability of the conformation.

Foams collapse because large gas bubbles grow at the expense of smaller bubbles (disproportionation). The protein films counteract this disproportionation. That is why the stability of a foam depends on the strength of the protein film and its permeability for gases. Film strength depends on the adsorbed amount of protein and the ability of the adsorbed molecules to associate. Surface denaturation generally releases additional amino acid side chains which can enter into intermolecular interactions. The stronger the cross-linkage, the more stable the film. Since the smallest possible net charge promotes association, the pH of the system should lie in the range of the isoelectric points of the proteins that participate in film formation.

In summary, the ideal foam-forming and foam-stabilizing protein is characterized by a low molecular weight, high surface hydrophobicity, good solubility, a small net charge in terms of the pH of the food, and easy denaturability.

Foams are destroyed by lipids and organic solvents such as higher alcohols, which due to their hydrophobicity displace proteins from the gas bubble surface without being able to form stable films themselves. Even a low concentration of egg yolk, for example, prevents the bursting of egg white. This is attributed to a disturbance of protein association by the lecithins.

The foam-forming and foam-stabilizing characteristics of proteins can be improved by chemical and physical modification. Thus a partial enzymatic hydrolysis leads to smaller, more quickly diffusing molecules, better solubility, and the release of hydrophobic groups. Disadvantages are the generally lower film stability and the loss of thermal coagulability. The characteristics can also be improved by introducing charged or neutral groups (cf. 1.4.6.2) and by partial thermal denaturation (e. g. of whey proteins). Recently, the addition of strongly alkaline proteins (e. g. clupeines) is being tested, which apparently increases the association of protein in the films and allows the foaming of fatty systems.

1.4.3.5 Gel Formation

Gels are disperse systems of at least two components in which the disperse phase in the dispersant forms a cohesive network. They are characterized by the lack of fluidity and elastic deformability. Gels are placed between solutions, in which repulsive forces between molecules and the disperse phase predominate, and precipitates, where strong intermolecular interactions predominate. We differentiate between two types of gel, the *polymeric networks* and the *aggregated dispersions*, although intermediate forms are found as well.

Examples of *polymeric networks* are the gels formed by gelatin (cf. 12.3.2.3.1) and polysaccharides such as agarose (cf. 4.4.4.1.2) and

carrageenan (4.4.4.3.2). Formation of a three-dimensional network takes place through the aggregation of unordered fibrous molecules via partly ordered structures, e. g. while double helices are formed (cf. 4.4.4.3.2, Fig. 4.14, Fig. 12.21). Characteristic for gels of this type is the low polymer concentration (∼1%) as well as transparency and fine texture. Gel formation is caused by setting a certain pH, by adding certain ions, or by heating/cooling. Since aggregation takes place mostly via intermolecular hydrogen bonds which easily break when heated, polymeric networks are thermo-reversible, i. e. the gels are formed when a solution cools, and they melt again when it is heated.

Examples of *aggregated dispersions* are the gels formed by globular proteins after heating and denaturation. The thermal unfolding of the protein leads to the release of amino acid side chains which may enter into intermolecular interactions. The subsequent association occurs while small spherical aggregates form which combine into linear strands whose interaction establishes the gel network. Before gel can be formed in the unordered type of aggregation, a relatively high protein concentration (5–10%) is necessary. The aggregation rate should also be slower than the unfolding rate, since otherwise coarse and fairly unstructured gels are formed, such as in the area of the iso-electric point. The degree of denaturation necessary to start aggregation seems to depend on the protein. Since partial denaturation releases primarily hydrophobic groups, intermolecular hydrophobic bonds generally predominate, which results in the thermoplastic (thermo-irreversible) character of this gel type, in contrast to the thermoreversible gel type stabilized by hydrogen bonds. Thermoplastic gels do not liquefy when heated, but they can soften or shrink. In addition to hydrophobic bonds, disulfide bonds formed from released thiol groups can also contribute to cross-linkage, as can intermolecular ionic bonds between proteins with different isoelectric points in heterogeneous systems (e. g. egg white).

Gel formation can be improved by adding salt. The moderate increase in ionic strength increases interaction between charged macro-molecules or molecule aggregates through charge shielding without precipitation occurring. An example is the heat coagulation of soybean curd (tofu, cf. 16.3.1.2.3) which is promoted by calcium ions.

1.4.3.6 Emulsifying Effect

Emulsions are disperse systems of one or more immiscible liquids. They are stabilized by emulsifiers – compounds which form interface films and thus prevent the disperse phases from flowing together (cf. 8.15). Due to their amphipathic nature, proteins can stabilize o/w emulsions such as milk (cf. 10.1.2.3). This property is made use of on a large scale in the production of food preparations.

The adsorption of a protein at the interface of an oil droplet is thermodynamically favored because the hydrophobic amino acid residues can then escape the hydrogen bridge network of the surrounding water molecules. In addition, contact of the protein with the oil droplet results in the displacement of water molecules from the hydrophobic regions of the oil-water boundary layer. Therefore, the suitability of a protein as an emulsifier depends on the rate at which it diffuses into the interface and on the deformability of its conformation under the influence of interfacial tension (surface denaturation). The diffusion rate depends on the temperature and the molecular weight, which in turn can be influenced by the pH and the ionic strength. The adsorbability depends on the exposure of hydrophilic and hydrophobic groups and thus on the amino acid profile, as well as on the pH, the ion strength and the temperature. The conformative stability depends in the amino acid composition, the molecular weight and the intramolecular disulfide bonds. Therefore, a protein with ideal qualities as an emulsifier for an oil-in-water emulsion would have a relatively low molecular weight, a balanced amino acid composition in terms of charged, polar and nonpolar residues, good water solubility, well-developed surface hydrophobicity, and a relatively stable conformation. The β-casein molecule meets these requirements because of less pronounced secondary structures and no crosslinks due to the lack of SH groups (cf. 10.1.2.1.1). The apolar “tail” of this flexible molecule is adsorbed by the oil phase of the

boundary layer and the polar "head", which projects into the aqueous medium, prevents coalescence.
The solubility and emulsifying capacity of some proteins can be improved by limited enzymatic hydrolysis.

1.4.4 Chemical Reactions

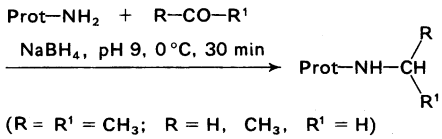
The chemical modification of protein is of importance for a number of reasons. It provides derivatives suitable for sequence analysis, identifies the reactive groups in catalytically active sites of an enzyme, enables the binding of protein to a carrier (protein immobilization) and provides changes in protein properties which are important in food processing. In contrast to free amino acids and except for the relatively small number of functional groups on the terminal amino acids, only the functional groups on protein side chains are available for chemical reactions.

1.4.4.1 Lysine Residue

Reactions involving the lysine residue can be divided into several groups, (a) reactions leading to a positively charged derivative, (b) reactions eliminating the positive charge, (c) derivatizations introducing a negative charge, and (d) reversible reactions. The latter are of particular importance.

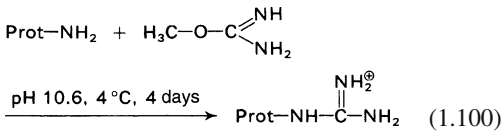
1.4.4.1.1 Reactions Which Retain the Positive Charge

Alkylation of the free amino group of lysine with aldehydes and ketones is possible, with a simultaneous reduction step:



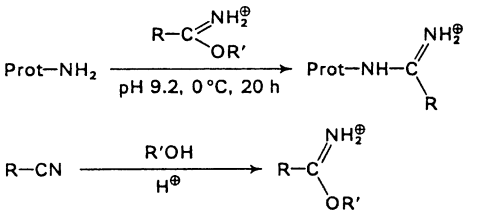
(1.99)

A dimethyl derivative [Prot−$N(CH_3)_2$] can be obtained with formaldehyde (R=R_1=H) (cf. 1.2.4.2.2).
Guanidination can be accomplished by using O-methylisourea as a reactant. α-Amino groups react at a much slower rate than ε-amino groups:



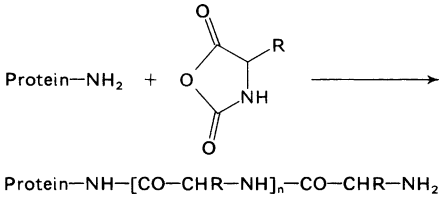
(1.100)

This reaction is used analytically to assess the amount of biologically available ε-amino groups and for measuring protein digestibility.
Derivatization with imido esters is also possible. The reactant is readily accessible from the corresponding nitriles:



(1.101)

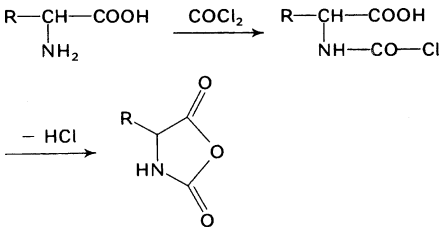
Proteins can be cross-linked with the use of a bifunctional imido ester (cf. 1.4.4.10).
Treatment of the amino acid residue with amino acid carboxyanhydrides yields a polycondensation reaction product:



(1.102)

The value n depends on reaction conditions. The carboxyanhydrides are readily accessible through interaction of the amino acid with

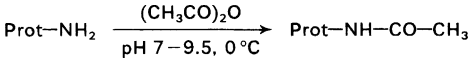
phosgene:



(1.103)

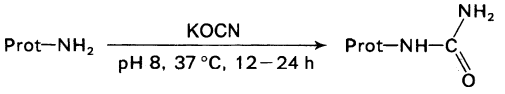
1.4.4.1.2 Reactions Resulting in a Loss of Positive Charge

Acetic anhydride reacts with lysine, cysteine, histidine, serine, threonine and tyrosine residues. Subsequent treatment of the protein with hydroxylamine (1 M, 2 h, pH 9, 0 °C) leaves only the acetylated amino groups intact:



(1.104)

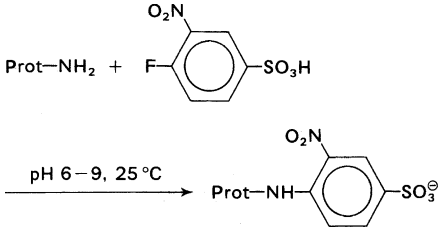
Carbamoylation with cyanate attacks α- and ε-amino groups as well as cysteine and tyrosine residues. However, their derivatization is reversible under alkaline conditions:



(1.105)

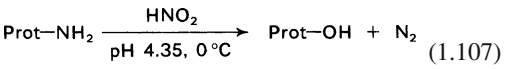
Arylation with 1-fluoro-2,4-dinitrobenzene (*Sanger's* reagent; FDNB) and trinitrobenzene sulfonic acid was outlined in Section 1.2.4.2.2. FDNB also reacts with cysteine, histidine and tyrosine.

4-Fluoro-3-nitrobenzene sulfonic acid, a reactant which has good solubility in water, is also of interest for derivatization of proteins:



(1.106)

Deamination can be accomplished with nitrous acid:

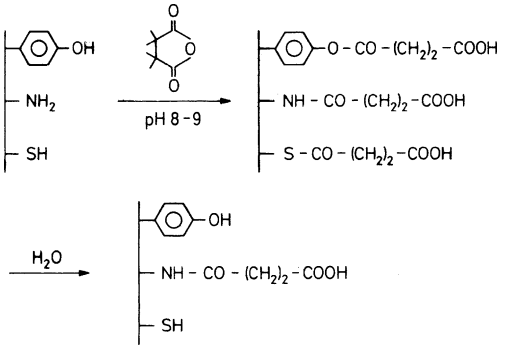


(1.107)

This reaction involves α- and ε-amino groups as well as tryptophan, tyrosine, cysteine and methionine residues.

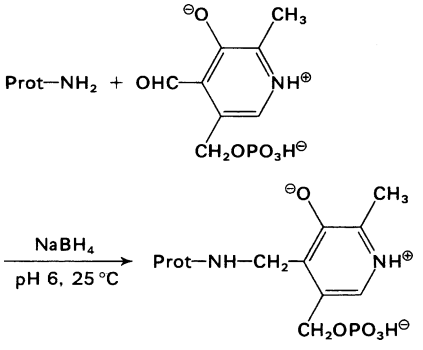
1.4.4.1.3 Reactions Resulting in a Negative Charge

Acylation with dicarboxylic acid anhydrides, e. g. succinic acid anhydride, introduces a carboxyl group into the protein:



(1.108)

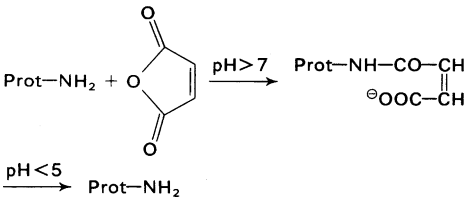
Introduction of a fluorescent acid group is possible by interaction of the protein with pyridoxal phosphate followed by reduction of the intermediary *Schiff* base:



(1.109)

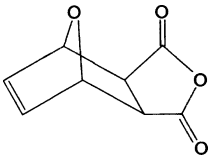
1.4.4.1.4 Reversible Reactions

N-Maleyl derivatives of proteins are obtained at alkaline pH by reaction with maleic acid anhydride. The acylated product is cleaved at pH < 5, regenerating the protein:



(1.110)

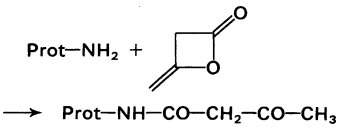
The half-life (τ) of ε-N-maleyl lysine is 11 h at pH 3.5 and 37 °C. More rapid cleavage is observed with the 2-methyl-maleyl derivative (τ < 3 min at pH 3.5 and 20 °C) and the 2,2,3,3-tetrafluoro-succinyl derivative (τ very low at pH 9.5 and 0 °C). Cysteine binds maleic anhydride through an addition reaction. The S-succinyl derivative is quite stable. This side reaction is, however, avoided when protein derivatization is done with exo-cis-3,6-end-oxo-1,2,3,6- tetrahydrophthalic acid anhydride:



(1.111)

For ε-N-acylated lysine, τ = 4–5 h at pH 3 and 25 °C.

Acetoacetyl derivatives are obtained with diketene:

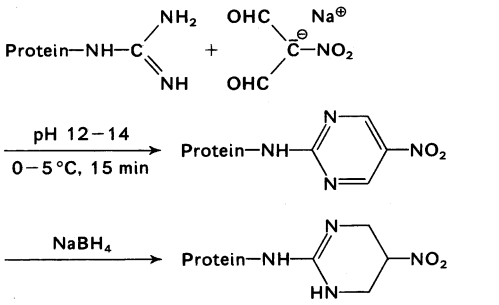


(1.112)

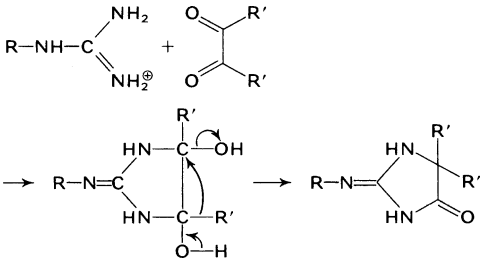
This is type of reaction also occurs with cysteine and tyrosine residues. The acyl group is readily split from tyrosine at pH 9.5. Complete release of protein from its derivatized form is possible by treatment with phenylhydrazine or hydroxylamine at pH 7.

1.4.4.2 Arginine Residue

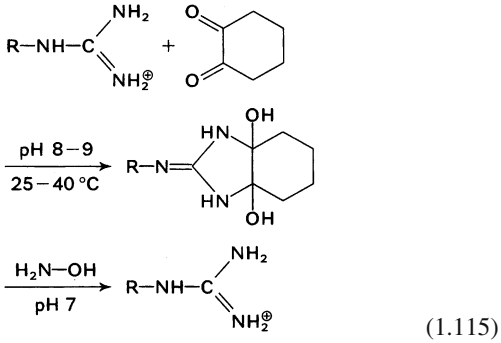
The arginine residue of proteins reacts with α- or β-dicarbonyl compounds to form cyclic derivatives:



(1.113)



(1.114)



(1.115)

The nitropyrimidine derivative absorbs at 335 nm. The arginyl bond of this derivative is not cleaved by trypsin but it is cleaved in its tetrahydro form, obtained by reduction with $NaBH_4$ (cf. Reaction 1.113). In the reaction with benzil, an iminoimidazolidone derivative is obtained after a benzilic acid rearrangement (cf. Reaction 1.114).

Reaction of the arginine residue with 1,2-cyclohexanedione is highly selective and proceeds under mild conditions. Regeneration of the arginine residue is again possible with hydroxylamine (cf. Reaction 1.115).

1.4.4.3 Glutamic and Aspartic Acid Residues

These amino acid residues are usually esterified with methanolic HCl. There can be side reactions, such as methanolysis of amide derivatives or N,O-acyl migration in serine or threonine residues:

$$\text{Protein—COOH} \xrightarrow[0\,^\circ\text{C}]{\text{CH}_3\text{OH/HCl}} \text{Protein—COOCH}_3 \qquad (1.116)$$

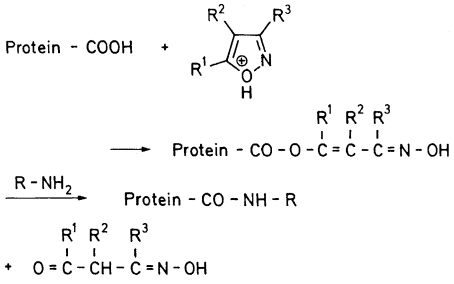
Diazoacetamide reacts with a carboxyl group and also with the cysteine residue:

$$\text{Protein—COOH} + \text{N}_2\text{CH—CONH}_2 \longrightarrow \text{R—COOCH}_2\text{CONH}_2 \qquad (1.117)$$

Amino acid esters or other similar nucleophilic compounds can be attached to a carboxyl group of a protein with the help of a carbodiimide:

$$\text{Protein—COOH} + \text{H}_2\text{N—CH}_2\text{—COOCH}_3 \xrightarrow{\text{R—N=C=N—R}} \text{Protein—CO—NH—CH}_2\text{—COOCH}_3 \qquad (1.118)$$

Amidation is also possible by activating the carboxyl group with an isooxazolium salt (*Woodward* reagent) to an enolester and its conversion with an amine.



(1.119)

1.4.4.4 Cystine Residue (cf. also Section 1.2.4.3.5)

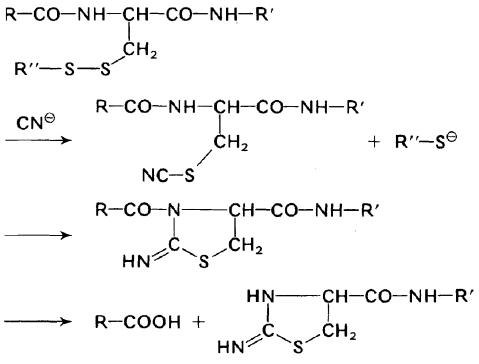
Cleavage of cystine is possible by a nucleophilic attack:

$$\text{Protein—S—S—Protein} + \text{Y}^{\ominus} \longrightarrow \text{Protein—S—Y} + \text{Protein—S}^{\ominus} \qquad (1.120)$$

The nucleophilic reactivity of the reagents decreases in the series: hydride > arsenite and phosphite > alkanethiol > aminoalkanethiol > thiophenol and cyanide > sulfite > OH^- > p-nitrophenol > thiosulfate > thiocyanate. Cleavage with sodium borohydride and with thiols was covered in Section 1.2.4.3.5. Complete cleavage with sulfite requires that oxidative agents (e. g. Cu^{2+}) be present and that the pH be higher than 7:

$$\text{RSSR} + \text{SO}_3^{2\ominus} \longrightarrow \text{RSSO}_3^{\ominus} + \text{RS}^{\ominus}$$
$$2\,\text{RS}^{\ominus} \xrightarrow{\text{Cu}^{2\oplus}} \text{RSSR} \qquad (1.121)$$

The resultant S-sulfo derivative is quite stable in neutral and acidic media and is fairly soluble in water. The S-sulfo group can be eliminated with an excess of thiol reagent.
Cleavage of cystine residues with cyanides (nitriles) is of interest since the thiocyanate formed in the reaction is cyclized to a 2-iminothiazolidine derivative with cleavage of the N-acyl bond:



(1.122)

This reaction can be utilized for the selective cleavage of peptide chains. Initially, all the disulfide bridges are reduced with dithiothreitol, and then are converted to mixed disulfides through

reaction with 5,5′-dithio-bis-(2-nitro-benzoic acid). These mixed disulfides are then cleaved by cyanide at pH 7.

Electrophilic cleavage occurs with Ag^+ and Hg^+ or Hg^{2+} as follows:

$$2\,Ag^{\oplus} + 2\,RSSR \longrightarrow 2\,RSAg + 2\,RS^{\oplus}$$
$$2\,RS^{\oplus} + 2\,OH^{\ominus} \longrightarrow 2\,RSOH \longrightarrow RSO_2H + RSH$$
$$RSH + Ag^{\oplus} \longrightarrow RSAg + H^{\oplus}$$
$$3\,Ag^{\oplus} + 2\,RSSR + 2\,OH^{\ominus} \longrightarrow 3\,RSAg + RSO_2H + H^{\oplus} \quad (1.123)$$

Electrophilic cleavage with H^+ is possible only in strong acids (e. g. 10 mol/L HCl). The sulfenium cation which is formed can catalyze a disulfide exchange reaction:

$$RSSR + H^{\oplus} \longrightarrow RSH + RS^{\oplus}$$
$$RS^{\oplus} + R^1SSR^1 \longrightarrow RSSR^1 + R^1S^{\oplus} \quad (1.124)$$

In neutral and alkaline solutions a disulfide exchange reaction is catalyzed by the thiolate anion:

$$RSSR + OH^{\ominus} \rightleftharpoons R{-}SOH + RS^{\ominus}$$
$$R^1SSR^1 + RS^{\ominus} \rightleftharpoons R^1SSR + R^1S^{\ominus} \quad (1.125)$$

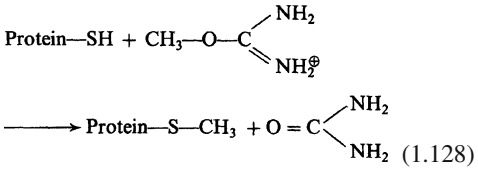
1.4.4.5 Cysteine Residue (cf. also Section 1.2.4.3.5)

A number of alkylating agents yield derivatives which are stable under the conditions for acidic hydrolysis of proteins. The reaction with ethylene imine giving an S-aminoethyl derivative and, hence, an additional linkage position in the protein for hydrolysis by trypsin, was mentioned in Section 1.4.1.3. Iodoacetic acid, depending on the pH, can react with cysteine, methionine, lysine and histidine residues:

$$Protein{-}SH \xrightarrow{ICH_2COOH} Protein{-}S{-}CH_2{-}COOH \quad (1.126)$$

The introduction of methyl groups is possible with methyl iodide or methyl isourea, and the introduction of methylthio groups with methylthiosulfonylmethane:

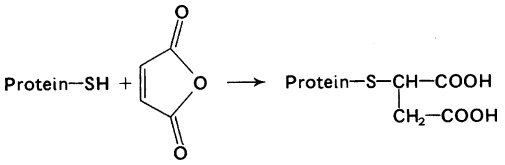
$$Protein\text{-}SH \xrightarrow{CH_3I} Protein{-}S{-}CH_3 \quad (1.127)$$



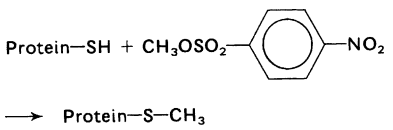
(1.128)

$$Protein{-}SH + CH_3{-}S{-}SO_2{-}CH_3 \longrightarrow Protein{-}S{-}S{-}CH_3 + CH_3SO_3H \quad (1.129)$$

Maleic acid anhydride and methyl-p-nitrobenzene sulfonate are also alkylating agents:

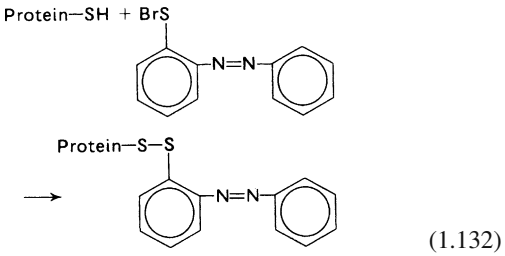


(1.130)



(1.131)

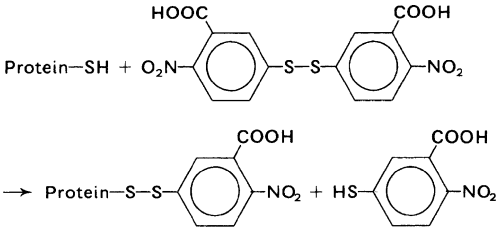
A number of reagents make it possible to measure the thiol group content spectrophotometrically. The molar absorption coefficient, ε, for the derivative of azobenzene-2-sulfenylbromide, ε_{353}, is 16,700 $M^{-1}cm^{-1}$ at pH 1:



(1.132)

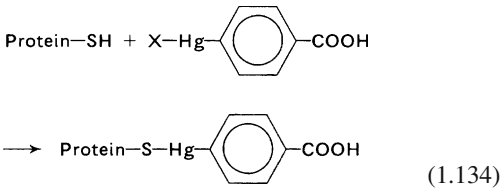
5,5′-Dithiobis-(2-nitrobenzoic acid) has a somewhat lower ε_{412} of 13,600 at pH 8 for its product,

a thionitrobenzoate anion:

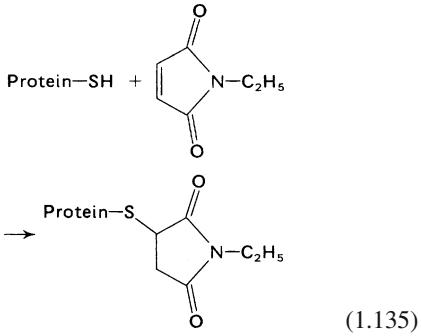


(1.133)

The derivative of p-hydroxymercuribenzoate has an ε_{250} of 7500 at pH 7, while the derivative of N-ethylmaleic imide has an ε_{300} of 620 at pH 7:

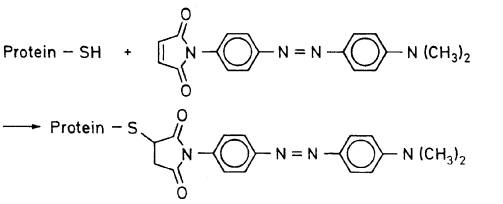


(1.134)



(1.135)

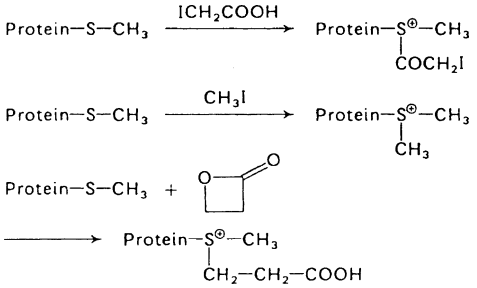
Especially suitable for the specific isolation of cysteine-containing peptides of great sensitivity is N-dimethylaminoazobenzenemaleic acid imide (DABMA).



(1.136)

1.4.4.6 Methionine Residue

Methionine residues are oxidized to sulfoxides with hydrogen peroxide. The sulfoxide can be reduced, regenerating methionine, using an excess of thiol reagent (cf. 1.2.4.3.6). α-Halogen carboxylic acids, β-propiolactone and alkyl halogenides convert methionine into sulfonium derivatives, from which methionine can be regenerated in an alkaline medium with an excess of thiol reagent:

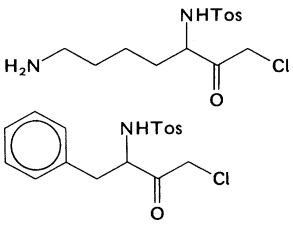


(1.137)

Reaction with cyanogen bromide (BrCN), which splits the peptide bond on the carboxyl side of the methionine molecule, was outlined in Section 1.4.1.3.

1.4.4.7 Histidine Residue

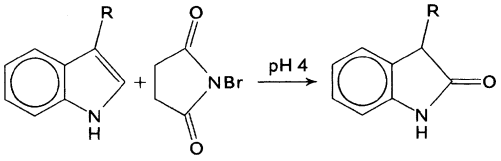
Selective modification of histidine residues present on active sites of serine proteinases is possible. Substrate analogues such as halogenated methyl ketones inactivate such enzymes (for example, 1-chloro-3-tosylamido-7-aminoheptan-2-one inactivates trypsin and 1-chloro-3-tosylamido-4-phenylbutan-2-one inactivates chymotrypsin) by N-alkylation of the histidine residue:



(1.138)

1.4.4.8 Tryptophan Residue

N-Bromosuccinimide oxidizes the tryptophan side chain and also tyrosine, histidine and cysteine:

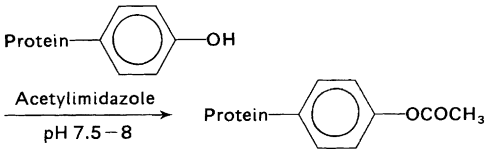


(1.139)

The reaction is used for the selective cleavage of peptide chains and the spectrophotometric determination of tryptophan.

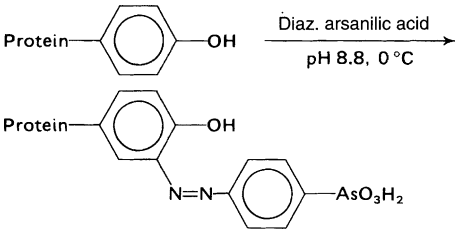
1.4.4.9 Tyrosine Residue

Selective acylation of tyrosine can occur with acetylimidazole as a reagent:



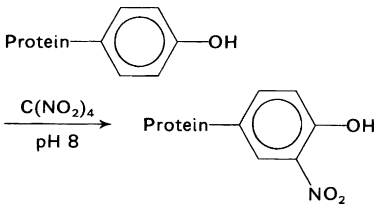
(1.140)

Diazotized arsanilic acid reacts with tyrosine and with histidine, lysine, tryptophan and arginine:



(1.141)

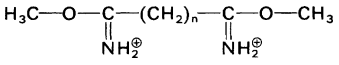
Tetranitromethane introduces a nitro group into the ortho position:



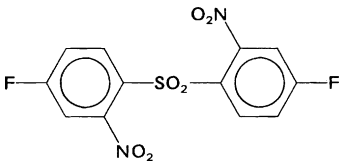
(1.142)

1.4.4.10 Bifunctional Reagents

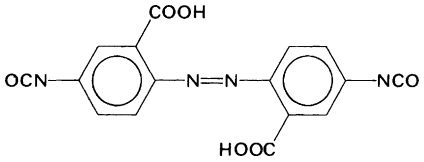
Bifunctional reagents enable intra- and intermolecular cross-linking of proteins. Examples are bifunctional imidoester, fluoronitrobenzene, isocyanate derivatives and maleic acid imides:



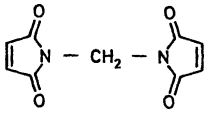
(1.143)



(1.144)



(1.145)



(1.146)

1.4.4.11 Reactions Involved in Food Processing

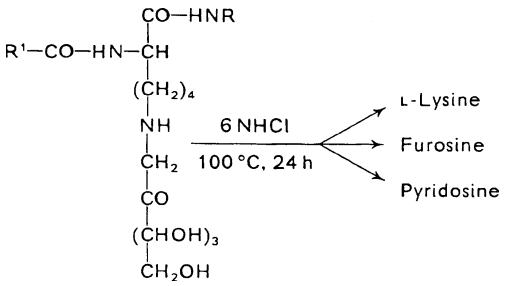
The nature and extent of the chemical changes induced in proteins by food processing depend on a number of parameters, for example, composition of the food and processing conditions, such as temperature, pH or the presence of oxygen. As a consequence of these reactions, the biological value of proteins may be decreased:

- Destruction of essential amino acids
- Conversion of essential amino acids into derivatives which are not metabolizable
- Decrease in the digestibility of protein as a result of intra- or interchain cross-linking.

Formation of toxic degradation products is also possible. The nutritional/physiological and toxicological assessment of changes induced by processing of food is a subject of some controversy and opposing opinions.

The *Maillard* reaction of the ε-amino group of lysine prevails in the presence of reducing sugars,

for example, lactose or glucose, which yield protein-bound ε-N-deoxylactulosyl-1-lysine or ε-N-deoxyfructosyl-1-lysine, respectively. Lysine is not biologically available in these forms. Acidic hydrolysis of such primary reaction products yields lysine as well as the degradation products furosine and pyridosine in a constant ratio (cf. 4.2.4.4):

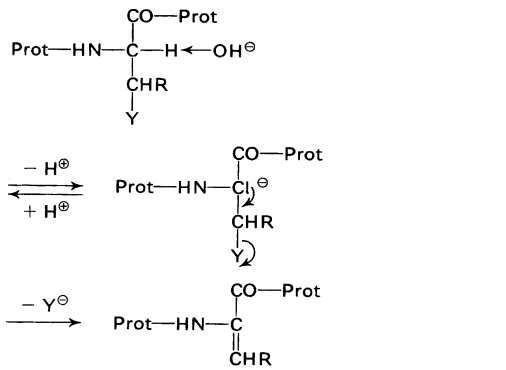


(1.147)

A nonreducing sugar (e. g. sucrose) can also cause a loss of lysine when conditions for sugar hydrolysis are favorable.

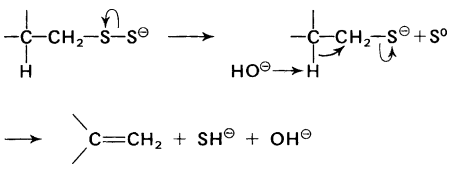
Losses of available lysine, cystine, serine, threonine, arginine and some other amino acids occur at higher pH values. Hydrolysates of alkali-treated proteins often contain some unusual compounds, such as ornithine, β-aminoalanine, lysinoalanine, ornithinoalanine, lanthionine, methyllanthionine and D-alloiso-leucine, as well as other D-amino acids.

The formation of these compounds is based on the following reactions: 1,2-elimination in the case of hydroxy amino acids and thio amino acids results in 2-amino-acrylic acid (dehydroalanine) or 2-aminocrotonic acid (dehydro-aminobutyric acid):



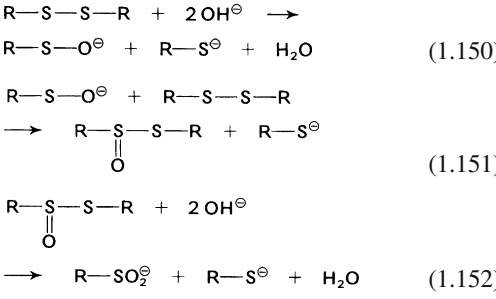
R = H, CH_3; Y = OH, OPO_3H_2, SH, SR^1, SSR^1 (1.148)

In the case of cystine, the eliminated thiolcysteine can form a second dehydroalanine residue:

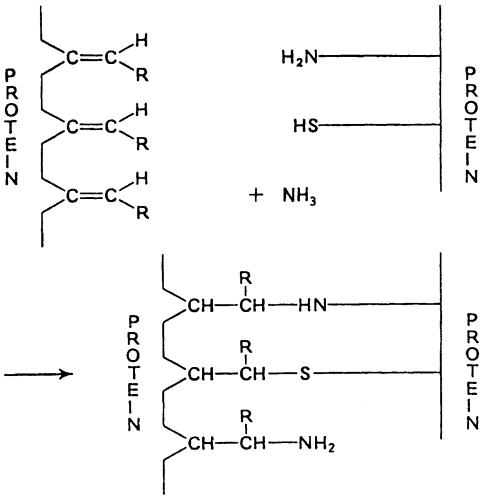


(1.149)

Alternatively, cleavage of the cystine disulfide bond can occur by nucleophilic attack on sulfur, yielding a dehydroalanine residue via thiol and sulfinate intermediates:



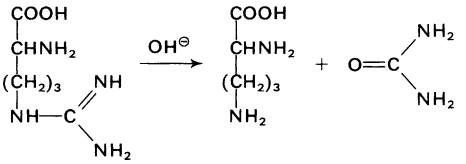
Intra- and interchain cross-linking of proteins can occur in dehydroalanine reactions involving additions of amines and thiols. Ammonia may also react via an addition reaction:



(1.153)

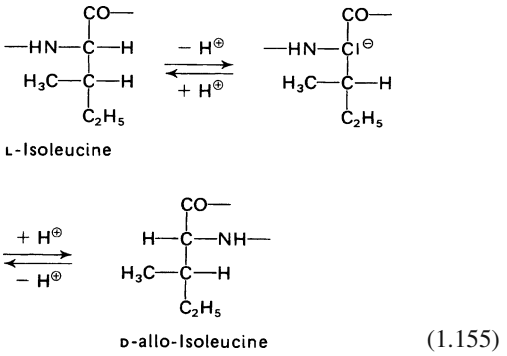
Acidic hydrolysis of such a cross-linked protein yields the unusual amino acids listed in

Table 1.29. Ornithine is formed during cleavage of arginine (Reaction 1.54).



(1.154)

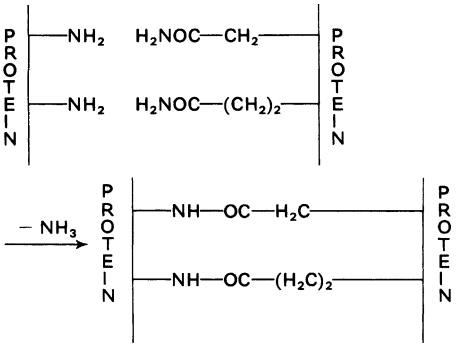
Formation of D-amino acids occurs through abstraction of a proton via a C2-carbanion. The reaction with L-isoleucine is particularly interesting. L-Isoleucine is isomerized to D-alloisoleucine which, unlike other D-amino acids, is a diastereoisomer and so has a retention time different from L-isoleucine, making its determination possible directly from an amino acid chromatogram:



L-Isoleucine

D-allo-Isoleucine (1.155)

Heating proteins in a dry state at neutral pH results in the formation of isopeptide bonds between the ε-amino groups of lysine residues and the β- or γ-carboxamide groups of asparagine and glutamine residues:



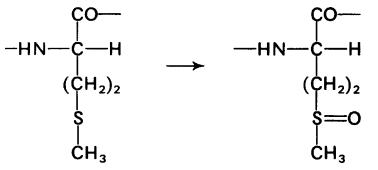
(1.156)

Table 1.29. Formation of unusual amino acids by alkali treatment of protein

Name	Formula
3-N^6-Lysinoalanine (R = H)	COOH | $CHNH_2$ | $CHR—NH—(CH_2)_4$
3-N^6-Lysino-3-methyl-alanine(R = CH_3)	COOH | $CHNH_2$ |
3-N^5-Ornithinoalanine (R = H)	COOH | $CHNH_2$ | $CHR—NH—(CH_2)_3$
3-N^5-Ornithino-3-methylalanine (R = CH_3)	COOH | $CHNH_2$ |
Lanthionine (R = H)	COOH | $CHNH_2$ | $CHR——S——CH_2$
3-Methyllanthionine (R = CH_3)	COOH |
3-Aminoalanine (R =H)	COOH |
2,3-Diamino butyric acid (R = CH_3)	$CHNH_2$ | $CHRNH_2$

These isopeptide bonds are cleaved during acidic hydrolysis of protein and, therefore, do not contribute to the occurrence of unusual amino acids. A more intensive heat treatment of proteins in the presence of water leads to a more extensive degradation.

Oxidative changes in proteins primarily involve methionine, which relatively readily forms methionine sulfoxide:



(1.157)

The formation of methionine sulfoxide was observed in connection with lipid peroxidation, phenol oxidation and light exposure in the presence of oxygen and sensitizers such as riboflavin.

After *in vivo* reduction to methionine, protein-bound methionine sulfoxide is apparently biologically available.

Figure 1.37 shows the effect of alkaline treatment of a protein isolate of sunflower seeds. Serine, threonine, arginine and isoleucine concentrations

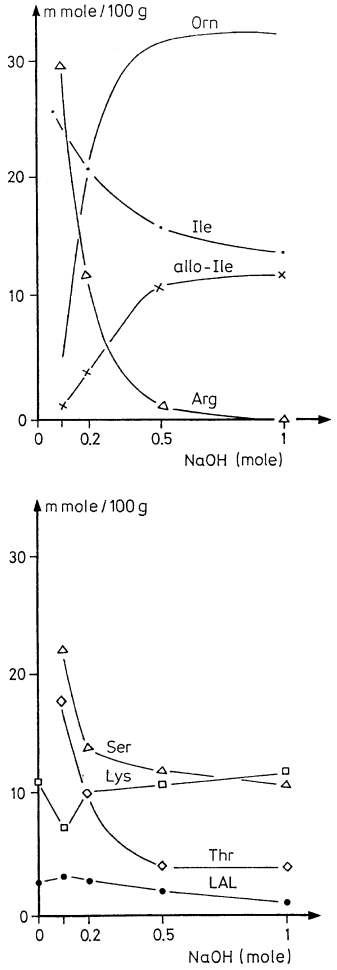


Fig. 1.37. Amino acid contents of a sunflower seed protein isolate heated in sodium hydroxide solutions at 80 °C for 16 h. (according to *Mauron*, 1975)

Table 1.30. Formation of D-amino acids by alkali treatment of proteins[a] (1% solution in 0.1 N NaOH, pH ~ 12.5, temperature 65 °C)

Protein	Heating time (h)	D-Asp (%)	D-Ala	D-Val	D-Leu	D-Pro	D-Glu	D-Phe
Casein	0	2.2	2.3	2.1	2.3	3.2	1.8	2.8
	1	21.8	4.2	2.7	5.0	3.0	10.0	16.0
	3	30.2	13.3	6.1	7.0	5.3	17.4	22.2
	8	32.8	19.4	7.3	13.6	3.9	25.9	30.5
Wheat gluten	0	3.3	2.0	2.1	1.8	3.2	2.1	2.3
	3	29.0	13.5	3.9	5.6	3.2	25.9	23.3
Promine D (soya protein)	0	2.3	2.3	2.6	3.3	3.2	1.8	2.3
	3	30.1	15.8	6.6	8.0	5.8	18.8	24.9
Lactal-bumin	0	3.1	2.2	2.9	2.7	3.1	2.9	2.3
	3	22.7	9.2	4.8	5.8	3.6	12.2	16.5

[a] Results in % correspond to D- + L-amino acids = 100%.

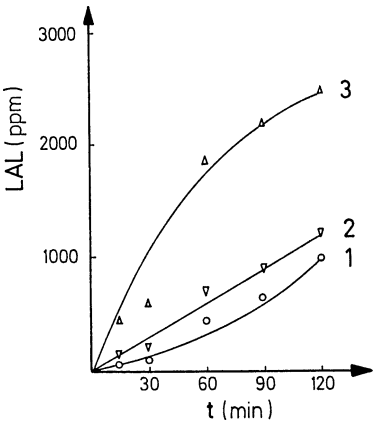


Fig. 1.38. Formation of lysinoalanine (LAL) by heating casein (5% solution at 100 °C) (according to *Sternberg* and *Kim*, 1977) 1 pH 5.0, 2 pH 7.0, 3 pH 8.0

are markedly decreased with increasing concentrations of NaOH. New amino acids (ornithine and alloisoleucine) are formed. Initially, lysine concentration decreases, but increases at higher concentrations of alkali. Lysinoalanine behaves in the opposite manner. The extent of formation of D-amino acids as a result of alkaline treatment of proteins is shown in Table 1.30.

Data presented in Figs. 1.38 and 1.39 clearly show that the formation of lysinoalanine is influenced not only by pH but also by the protein source. An extensive reaction occurs in casein even at pH 5.0 due to the presence of phosphorylated serine residues, while noticeable reactions occur in gluten from wheat or in zein from corn only in the pH range of 8–11. Figure 1.40 illustrates the dependence of the reaction on protein concentration.

Table 1.31 lists the contents of lysinoalanine in food products processed industrially or prepared under the "usual household conditions".

The contents are obviously affected by the food type and by the processing conditions.

In the radiation of food, o-hydroxyphenylalanine called o-tyrosine is formed through the re-

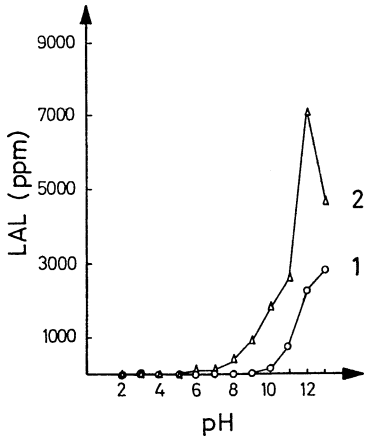


Fig. 1.39. Lysinoalanine (LAL) formation from wheat gluten (2) and corn gluten (1). Protein contents of the glutens: 70%; heated as 6.6% suspension at 100 °C for 4 h. (according to *Sternberg* and *Kim*, 1977)

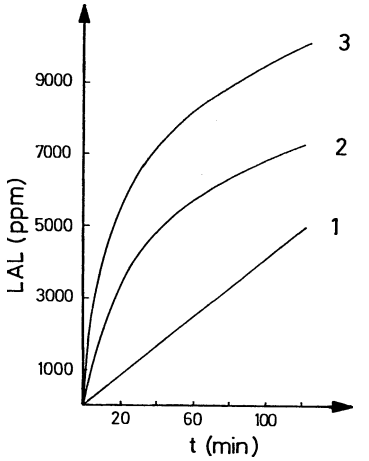


Fig. 1.40. Lysinoalanine (LAL) formation as influenced by casein concentration. (1): 5%, (2): 15%, and (3) 20% all at pH 12.8. (according to *Sternberg* and *Kim*, 1977)

action of phenylalanine with OH-radicals. In hydrolysates, the compound can be detected with the help of HPLC (fluorescence detection or electrochemical detection). It is under discussion as an indicator for food radiation. The amount formed depends on the irradiated dose and on the temperature. In samples of chicken and pork, fish and shrimps, <0.1 mg/kg (non-radiated controls), 0.5–0.8 mg/kg (5 kGy, −18 °C) and 0.8–1.2 mg/kg (5 kGy, 20 °C) were found.

1.4.5 Enzyme-Catalyzed Reactions

1.4.5.1 Foreword

A great number and variety of enzyme-catalyzed reactions are known with protein as a substrate. These include hydrolytic reactions (cleavage of peptide bonds or other linkages, e. g., the ester linkage in a phosphoprotein), transfer reactions (phosphorylation, incorporation of acyl residues, sugar residues and methyl groups) and redox reactions (thiol oxidation, disulfide reduction, amino group oxidation or incorporation of hydroxyl groups). Table 1.32 is a compilation of some examples. Some of these reactions are covered in Section 1.4.6.3 or in the sections related to individual foodstuffs. Only enzymes that are involved in hydrolysis of peptide bonds (proteolytic enzymes, peptidases) will be covered in the following sections.

1.4.5.2 Proteolytic Enzymes

Processes involving proteolysis play a role in the production of many foods. Proteolysis can occur as a result of proteinases in the food itself, e. g., autolytic reactions in meat, or due to microbial proteinases, e. g., the addition of pure cultures of selected microorganisms during the production of cheese.

This large group of enzymes is divided up as shown in Table 1.33. The two subgroups formed are: peptidases (exopeptidases) that cleave amino acids or dipeptides stepwise from the terminal ends of proteins, and proteinases (endopeptidases) that hydrolyze the linkages within the peptide chain, not attacking the terminal peptide bonds. Further division is possible, for example, by taking into account the presence of a given amino acid residue in the active site of the enzyme. The most important types of proteolytic enzymes are presented in the following sections.

1.4.5.2.1 Serine Endopeptidases

Enzymes of this group, in which activity is confined to the pH range of 7−11, are denoted as alkaline proteinases. Typical representatives

Table 1.31. Lysinoalanine content of various foods

Food	Origin/ Treatment		Lysinoalanine (mg/kg protein)
Frankfurter	CP[a]	Raw	0
		Cooked	50
		Roasted in oven	170
Chicken drums	CP	Raw	0
		Roasted in oven	110
		Roasted in micro wave oven	200
Egg white, fluid	CP		0
Egg white		Boiled	
		(3 min)	140
		(10 min)	270
		(30 min)	370
		Baked	
		(10 min/150 °C)	350
		(30 min/150 °C)	1100
Dried egg white	CP		160–1820[b]
Condensed milk, sweetened	CP		360–540
Condensed milk, unsweetened	CP		590–860
Milk product for infants	CP		150–640
Infant food	CP		<55 150
Soya protein isolate	CP		0–370
Hydrolyzed vegetable protein	CP		40–500
Cocoa powder	CP		130–190
Na-caseinate	CP		45–6900
Ca-caseinate	CP		250–4320

[a] Commercial product.
[b] Variation range for different brand name products.

from animal sources are trypsin, chymotrypsin, elastase, plasmin and thrombin. Serine proteinases are produced by a great number of bacteria and fungi, e. g. *Bacillus cereus, B. firmus, B. licheniformis, B. megaterium, B. subtilis, Serratia marcescens, Streptomyces fradiae, S. griseus, Trititrachium album, Aspergillus flavus, A. oryzae* and *A. sojae.*

These enzymes have in common the presence of a serine and a histidine residue in their active sites (for mechanism, see 2.4.2.5).

Inactivation of these enzymes is possible with reagents such as diisopropylfluorophosphate (DIFP) or phenylmethanesulfonylfluoride (PMSF). These reagents irreversibly acylate the serine residue in the active site of the enzymes:

$$\mathrm{E-CH_2OH + FY \rightarrow E-CH_2OY + HF}$$
$$(\mathrm{Y: -PO(iC_3H_7O)_2 - SO_2 - CH_2C_6H_6}) \quad (1.158)$$

Irreversible inhibition can also occur in the presence of halogenated methyl ketones which alkylate the active histidine residue (cf. 2.4.1.1), or as a result of the action of proteinase inhibitors, which are also proteins, by interaction with the enzyme to form inactive complexes. These natural inhibitors are found in the organs of animals and plants (pancreas, colostrum, egg white, potato tuber and seeds of many legumes; cf. 16.2.3). The specificity of serine

Table 1.32. Enzymatic reactions affecting proteins

Hydrolysis
– Endopeptidases
– Exopeptidases
Proteolytic induced aggregation
– Collagen biosynthesis
– Blood coagulation
– Plastein reaction
Cross-linking
– Disulfide bonds
Protein disulfide isomerase
Protein disulfide reductase (NAD(P)H)
Protein disulfide reductase (glutathione)
Sulfhydryloxidase
Lipoxygenase
Peroxidase
– ε(γ-Glutamyl)lysine
– Transglutaminase
– Aldol-, aldimine condensation and subsequent reactions (connective tissue)
Lysyloxidase
Phosphorylation, dephosphorylation
– Protein kinase
– Phosphoprotein phosphatase
Hydroxylation
– Proline hydroxylase
– Lysine hydroxylase
Glycosylation
– Glycoprotein-β-galactosyltransferase
Methylation and demethylation
– Protein(arginine)-methyl-transferase
– Protein(lysine)-methyl-transferase
– Protein-O-methyl-transferase
[2pt] Acetylation, deacetylation
– ε-N-Acetyl-lysine

endopeptidases varies greatly (cf. Table 1.34). Trypsin exclusively cleaves linkages of amino acid residues with a basic side chain (lysyl or arginyl bonds) and chymotrypsin preferentially cleaves bonds of amino acid residues which have aromatic side chains (phenylalanyl, tyrosyl or tryptophanyl bonds). Enzymes of microbial origin often are less specific.

1.4.5.2.2 Cysteine Endopeptidases

Typical representatives of this group of enzymes are: papain (from the sap of a tropical, melonlike fruit tree, *Carica papaya*), bromelain (from the sap and stem of pineapples, *Ananas comosus*), ficin (from *Ficus latex* and other *Ficus spp.*) and a Streptococcus proteinase. The range of activity of these enzymes is very wide and, depending on the substrate, is pH 4.5–10, with a maximum at pH 6–7.5.

The mechanism of enzyme activity appears to be similar to that of serine endopeptidases. A cysteine residue is present in the active site. A thioester is formed as a covalent intermediary product. The enzymes are highly sensitive to oxidizing agents. Therefore, as a rule they are used in the presence of a reducing agent (e. g., cysteine) and a chelating agent (e. g., EDTA).

Inactivation of the enzymes is possible with oxidative agents, metal ions or alkylating reagents (cf. 1.2.4.3.5 and 1.4.4.5). In general these enzymes are not very specific (cf. Table 1.34).

1.4.5.2.3 Metalo Peptidases

This group includes exopeptidases, carboxypeptidases A and B, aminopeptidases, dipeptidases, prolidase and prolinase, and endopeptidases from bacteria and fungi, such as *Bacillus cereus, B. megaterium, B. subtilits, B. thermoproteolyticus* (thermolysin), *Streptomyces griseus* (pronase; it also contains carboxy- and aminopeptidases) and *Aspergillus oryzae.*

Most of these enzymes contain one mole of Zn^{2+} per mole of protein, but prolidase and prolinase contain one mole of Mn^{2+}. The metal ion acts as a *Lewis* acid in carboxypeptidase A, establishing contact with the carbonyl group of the peptide bond which is to be cleaved. Figure 1.41 shows the arrangement of other participating residues in the active site, as revealed by X-ray structural analysis of the enzyme-substrate complex.

The enzymes are active in the pH 6–9 range; their specificity is generally low (cf. Table 1.34).

Inhibition of these enzymes is achieved with chelating agents (e. g. EDTA) or sodium dodecyl sulfate.

1.4.5.2.4 Aspartic Endopeptidases

Typical representatives of this group are enzymes of animal origin, such as pepsin and rennin

Table 1.33. Classification of proteolytic enzymes (peptidases)

EC-No.[a]	Enzyme group	Comments	Examples
	Exopeptidases	Cleave proteins/peptides stepwise from N- or C-terminals	
3.4.11.	Aminopeptidases	Cleave amino acids from N-terminal	Various aminopeptidases
3.4.13.	Dipeptidases	Cleave dipeptides	Various dipeptidases (carnosinase, anserinase)
3.4.14.	Dipeptidyl- and tripeptidylpeptidases	Cleave di- and tripeptides from N-terminal	Cathepsin C
3.4.15.	Peptidyl-dipeptidases	Cleave dipeptides from C-terminal	Carboxycathepsin,
3.4.16.	Serine carboxypeptidases	Cleave amino acids from C-terminal, serine in the active site	Carboxypeptidase C, cathepsin A
3.4.17.	Metalocarboxypeptidases	Cleave amino acids from C-terminal, Zn^{2+} or Co^{2+} in the active site	Carboxypeptidases A and B
3.4.18.	Cysteine carboxypeptidases	Cleave amino acids from C-terminal, cysteine in the active site	Lysosomal carboxypeptidase B
	Endopeptidases	Cleave protein/peptide bonds other than terminal ones	
3.4.21.	Serine endopeptidase	Serine in the active site	Chymotrypsins A, B and C, peptidase B alkaline proteinases α-and β-trypsin,
3.4.22.	Cysteine endopeptidase	Cysteine in the active site	Papain, ficin, bromelain, cathepsin B
3.4.23.	Aspartic endopeptidase	Aspartic acid (2 residues) in the active site	Pepsin, cathepsin D, rennin (chymosin)
3.4.24.	Metaloendopeptidase	Metal ions in the active site	Collagenase, microbial neutral proteinases

[a] cf. 2.2.7

Table 1.34. Specificity of proteolytic enzymes [based on cleavage of oxidized B chain of bovine insulin; strong cleavage: ↓, weak cleavage (↓)]

No.[a]	Phe	Val	Asn	Gln	His	Leu	Cys	Gly	Ser	His	Leu	Val	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Ala
Serine peptidases																														
1																						↓								↓
2																(↓)	↓									↓	↓			
3					↓		↓					↓				↓	(↓)	↓							↓	↓	(↓)			
4			↓	↓					↓	(↓)	↓								↓	(↓)										
5																							↓							↓
6			↓						↓						↓	↓						↓					↓			
7				(↓)						(↓)			↓	↓			↓				↓					↓	↓			↓
8				↓	↓			↓					(↓)	↓		↓	↓		↓					(↓)	↓					↓
Cysteine peptidases																														
9				↓			(↓)	(↓)	(↓)					↓	(↓)			↓		↓					↓	↓				
10									↓	↓					↓				↓					↓	↓					
11				↓			(↓)						↓	↓		↓			↓		↓	↓		↓	(↓)	(↓)				
Metalopeptidases																														
12		↓		↓					↓				↓	↓	↓	↓					↓	↓	↓			↓				
13					↓				↓					↓		↓	↓							↓	↓	↓				
14					↓				↓					↓		↓	↓						↓	↓	↓	↓				↓
Aspartic peptidases																														
15		(↓)		(↓)	(↓)				(↓)		↓		(↓)	(↓)	(↓)	↓						(↓)	↓	↓						
16	(↓)									(↓)		↓		↓	(↓)	↓							↓	↓						
17			↓									↓		↓				↓					↓							
18		↓														↓	↓							↓	↓	(↓)				
19		(↓)			(↓)					↓	↓	↓					(↓)				↓	↓	↓	↓						

[a] **Enzymes.**
1) Trypsin (bovine)
2) Chymotrypsin A (bovine)
3) Chymotrypsin B (porcine)
4) Aspergillopeptidase C *(Aspergillus oryzae)*
5) Endopeptidase from *Streptomyces griseus* (trypsin-like)
6) Subtilisin BPN′
7) Endopeptidase from *Aspergillus oryzae*
8) Endopeptidase from *Aspergillus flavus*
9) Papain *(Papaya carica)*
10) Ficin III *(Ficus glabrata)*
11) Chymopapain *(Charica papaya)*
12) Endopeptidase II from *(Aspergillus oryzae)*
13) Endopeptidase from *Bacillus subtilis*
14) Thermolysin *(Bacillus thermoproteolyticus Rokko)*
15) Pepsin A (porcine)
16) Renin (calf)
17) Endopeptidase from *Candida albicans*
18) Endopeptidase from *Mucor miehei*
19) Endopeptidase from *Rhizopus chinensis*

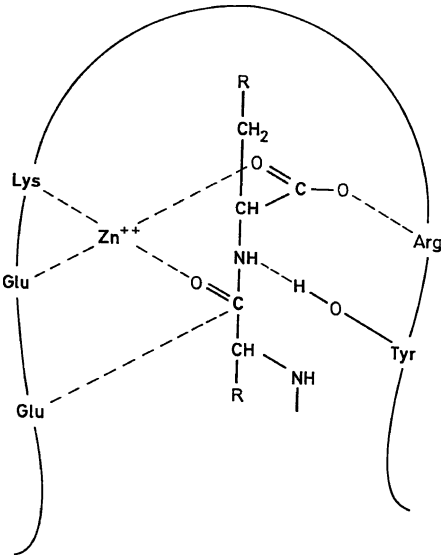
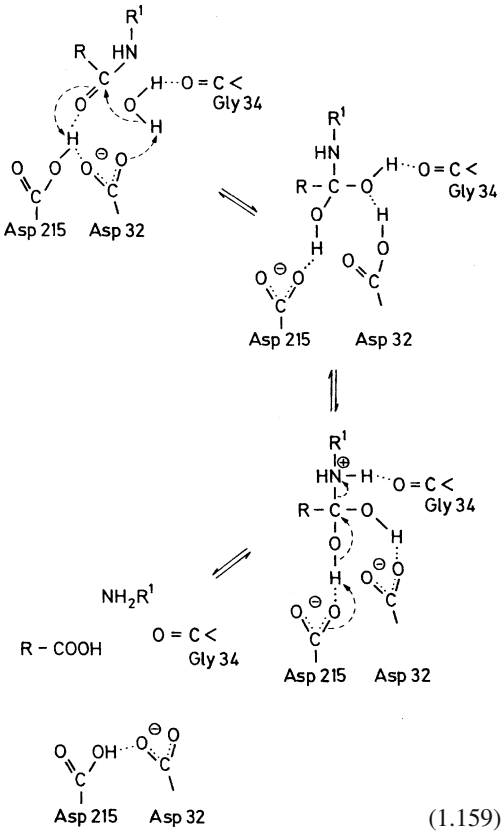


Fig. 1.41. Carboxypeptidase A active site. (according to *Lowe* and *Ingraham*, 1974)

(called Lab-enzyme in Europe), active in the pH range of 2–4, and cathepsin D, which has a pH optimum between 3 and 5 depending on the substrate and on the source of the enzyme. At pH 6–7 rennin cleaves a bond of κ-casein with great specificity, thus causing curdling of milk (cf. 10.1.2.1.1).

Aspartic proteinases of microbial origin can be classified as pepsin-like or rennin-like enzymes. The latter are able to coagulate milk. The pepsin-like enzymes are produced, for example, by *Aspergillus awamori, A. niger, A. oryzae, Penicillium spp.* and *Trametes sanguinea*. The rennin-like enzymes are produced, for example, by *Aspergillus usamii* and *Mucor spp.*, such as *M. pusillus.*

There are two carboxyl groups, one in undissociated form, in the active site of aspartic proteinases. The mechanism postulated for cleavage of peptide bonds is illustrated in Reaction 1.159. The nucleophilic attack of a water molecule on the carbonyl carbon atom of the peptide bond is catalyzed by the side chains of Asp-32 (basic catalyst) and Asp-215 (acid catalyst). The numbering of the amino acid residues in the active site applies to the aspartic proteinase from *Rhizopus chinensis.*



(1.159)

Inhibition of these enzymes is achieved with various diazoacetylamino acid esters, which apparently react with carboxyl groups on the active site, and with pepstatin. The latter is isolated from various *Streptomycetes* as a peptide mixture with the general formula (R: isovaleric or n-caproic acid; AHMHA: 4-amino-3-hydroxy-6-methyl heptanoic acid):

R – Val – Val – AHMHA – Ala – AHMAH (1.160)

The specifity of aspartic endopeptidases is given in Table 1.34.

1.4.6 Chemical and Enzymatic Reactions of Interest to Food Processing

1.4.6.1 Foreword

Standardization of food properties to meet nutritional/physiological and toxicological demands

and requirements of food processing operations is a perennial endeavor. Food production is similar to a standard industrial fabrication process: on the one hand is the food commodity with all its required properties, on the other hand are the components of the product, each of which supplies a distinct part of the required properties. Such considerations have prompted investigations into the relationships in food between macroscopic physical and chemical properties and the structure and reactions at the molecular level. Reliable understanding of such relationships is a fundamental prerequisite for the design and operation of a process, either to optimize the process or to modify the food components to meet the desired properties of the product.

Modification of proteins is still a long way from being a common method in food processing, but it is increasingly being recognized as essential, for two main reasons:

Firstly, proteins fulfill multipurpose functions in food. Some of these functions can be served better by modified than by native proteins.

Secondly, persistent nutritional problems the world over necessiate the utilization of new raw materials.

Modifying reactions can ensure that such new raw materials (e. g., proteins of plant or microbial origin) meet stringent standards of food safety, palatability and acceptable biological value. A review will be given here of several protein modifications that are being used or are being considered for use. They involve chemical or enzymatic methods or a combination of both. Examples have been selected to emphasize existing trends. Table 1.35 presents some protein properties which are of interest to food processing. These properties are related to the amino acid composition and sequence and the conformation of proteins. Modification of the properties of proteins is possible by changing the amino acid composition or the size of the molecule, or by removing or inserting hetero constituents. Such changes can be accomplished by chemical and/or enzymatic reactions. From a food processing point of view, the aims of modification of proteins are:

- Blocking the reactions involved in deterioration of food (e. g., the *Maillard* reaction)
- Improving some physical properties of proteins (e. g., texture, foam stability, whippability, solubility)
- Improving the nutritional value (increasing the extent of digestibility, inactivation of toxic or other undesirable constituents, introducing essential ingredients such as some amino acids).

Table 1.35. Properties of protein in food

Properties with	
nutritional/physiological relevance	processing relevance
Amino acid composition Availability of amino acids	Solubility, dispersibility Ability to coagulate Water binding/holding capacity Gel formation Dough formation, extensibility, elasticity Viscosity, adhesion, cohesion Whippability Foam stabilization Emulsifying ability Emulsion stabilization

1.4.6.2 Chemical Modification

Table 1.36 presents a selection of chemical reactions of proteins that are pertinent to and of current importance in food processing.

1.4.6.2.1 Acylation

Treatment with succinic anhydride (cf. 1.4.4.1.3) generally improves the solubility of protein.

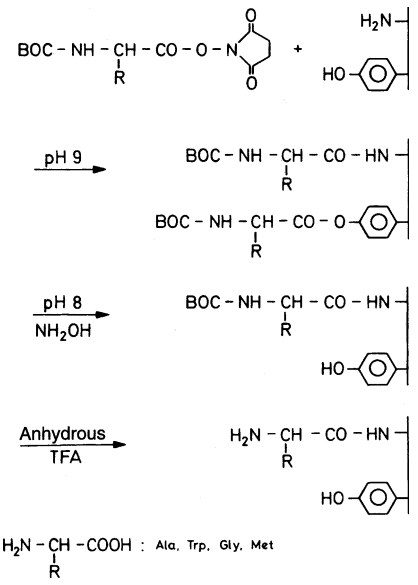
Table 1.36. Chemical reactions of proteins significant in food

Reactive group	Reaction	Product
—NH_2	Acylation	–NH–CO–R
—NH_2	Reductive alkylation with HCHO	—$N(CH_3)_2$
—$CONH_2$	Hydrolysis	—COOH
—COOH	Esterification	—COOR
—OH	Esterification	—O—CO—R
—SH	Oxidation	—S—S—
—S—S—	Reduction	—SH
—CO—NH—	Hydrolysis	—COOH + H_2N—

For example, succinylated wheat gluten is quite soluble at pH 5 (cf. Fig. 1.40). This effect is related to disaggregation of high molecular weight gluten fractions (cf. Fig. 1.41). In the case of succinylated casein it is obvious that the modification shifts the isoelectric point of the protein (and thereby the solubility minimum) to a lower pH (cf. Fig. 1.42). Succinylation of leaf proteins improves the solubility as well as the flavor and emulsifying properties.

Succinylated yeast protein has not only an increased solubility in the pH range of 4–6, but is also more heat stable above pH 5. It has better emulsifying properties, surpassing many other proteins (Table 1.37), and has increased whippability.

Introduction of aminoacyl groups into protein can be achieved by reactions involving amino acid carboxy anhydrides (Fig. 1.44), amino acids and carbodiimides (Fig. 1.46) or by BOC-amino acid hydroxysuccinimides with subsequent removal of the aminoprotecting group (BOC) (cf. 1.161):



(1.161)

Feeding tests with casein with attached methionine, as produced by the above method, have demonstrated a satisfactory availability of methionine (Table 1.38). Such covalent attachment of essential amino acids to a protein may avoid the problems associated with food supplementation with free amino acids: losses in processing, development of undesired aroma due to methional, etc.

Table 1.39, using β-casein as an example, shows to what extent the association of a protein is affected by its acylation with fatty acids of various chain lengths.

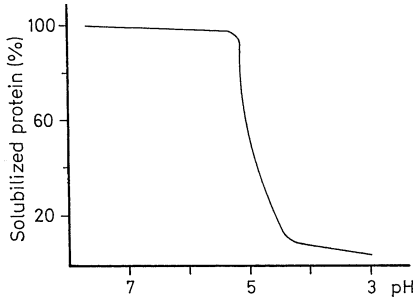


Fig. 1.42. Solubility of succinylated wheat protein as a function of pH (0.5% solution in water). (according to *Grant*, 1973)

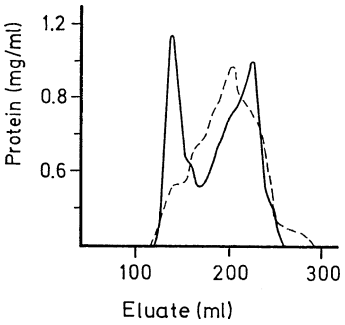


Fig. 1.43. Gel column chromatography of an acetic acid (0.2 mol/L) wheat protein extract. Column: Sephadex G-100 (— before and - - - after succinylation). (according to *Grant*, 1973)

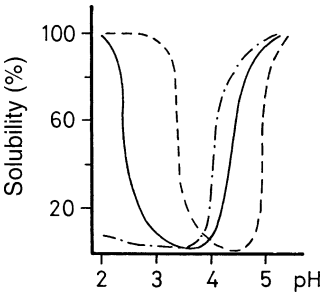


Fig. 1.44. Solubilities of native (—) and succinylated casein (— 50% and – · – · – 76%) as a function of pH. (according to *Schwenke* et al., 1977)

Table 1.37. Emulsifying property of various proteins[a]

Protein	Emulsifying Activity Index ($m^2 \times g^{-1}$)	
	pH 6.5	pH 8.0
Yeast protein (88%) succinylated	322	341
Yeast protein (62%) succinylated	262	332
Sodium dodecyl sulfate (0.1%)	251	212
Bovine serum albumin	–	197
Sodium caseinate	149	166
β-Lactoglobulin	–	153
Whey protein powder A	119	142
Yeast protein (24%) succinylated	110	204
Whey protein powder B	102	101
Soya protein isolate A	41	92
Hemoglobin	–	75
Soya protein isolate B	26	66
Yeast protein (unmodified)	8	59
Lysozyme	–	50
Egg albumin	–	49

[a] Protein concentration: 0.5% in phosphate buffer of pH 6.5.

Table 1.38. Feeding trial (rats) with modified casein: free amino acid concentration in plasma and PER value

Diet	μmole/100 ml plasma				
	Lys	Thr	Ser	Gly	Met
Casein	101	19	34	32	5
Met-casein[a]	96	17	33	27	39

	PER[b]
Casein (10%)	2.46
Casein (10%) + Met (0.2%)	3.15
Casein (5%) + Met-casein[a] (5%)	2.92

[a] Covalent binding of methionine to ε-NH_2 groups of casein.
[b] Protein Efficiency Ratio (cf. 1.2.5).

Table 1.39. Association of acylated β-casein A

Protein	SD[a]	Monomer	Polymer	$S^0_{20,w}$	$S^{1\%}_{20,w}$
	(%)	(%)	(%)	($S \cdot 10^{13}$)	($S \cdot 10^{13}$)
β-Casein A (I)	–	11	89	12.6	6.3
Acetyl-I	96	41	59	4.8	4.7
Propionyl-I	97	24	76	10.5	5.4
n-Butyryl-I	80	8	92	8.9	8.3
n-Hexanoyl-I	85	0	100	7.6	11.6
n-Octanoyl-I	89	0	100	6.6	7.0
n-Decanoyl-I	83	0	100	5.0	6.5

[a] Substitution degree.

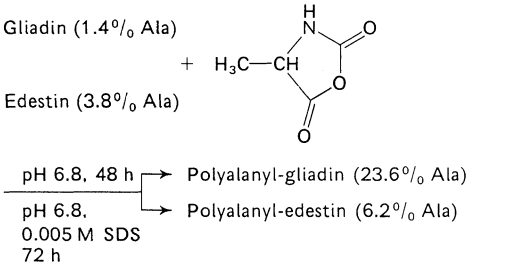


Fig. 1.45. Hydrolysis of a reductively methylated casein by bovine α-chymotrypsin. Modification extents: a 0%, b 33%, and c 52%. (according to *Galembeck* et al., 1977)

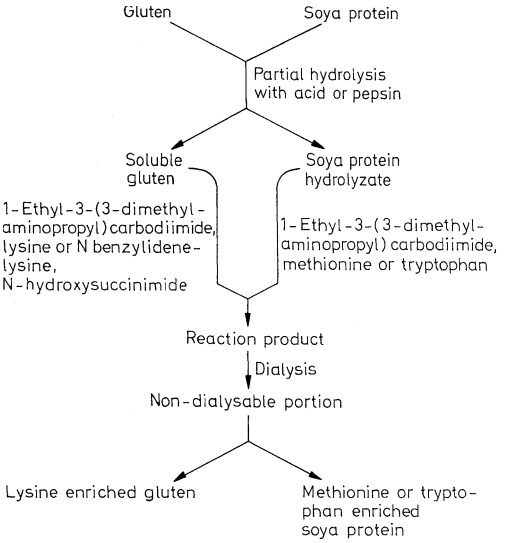


Fig. 1.46. Properties of modified wheat gluten. (according to *Lasztity*, 1975)

1.4.6.2.2 Alkylation

Modification of protein by reductive methylation of amino groups with formaldehyde/$NaBH_4$ retards *Maillard* reactions. The resultant methyl derivative, depending on the degree of substitution, is less accessible to proteolysis (Fig. 1.47). Hence, its value from a nutritional/physiological point of view is under investigation.

1.4.6.2.3 Redox Reactions Involving Cysteine and Cystine

Disulfide bonds have a strong influence on the properties of proteins. Wheat gluten can be

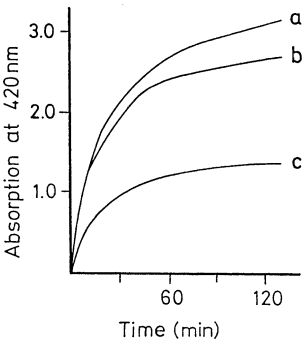


Fig. 1.47. Viscosity curves during reduction of different wheat glutens. For sample designation see Fig. 1.44. (according to *Lasztity*, 1975)

modified by reduction of its disulfide bonds to sulfhydryl groups and subsequent reoxidation of these groups under various conditions (Fig. 1.48). Reoxidation of a diluted suspension in the presence of urea results in a weak, soluble, adhesive product (gluten A), whereas reoxidation of a concentrated suspension in the presence of a higher concentration of urea yields an insoluble, stiff, cohesive product (gluten C). Additional viscosity data have shown that the disulfide bridges in gluten A are mostly intramolecular while those in gluten C are predominantly intermolecular (Fig. 1.49).

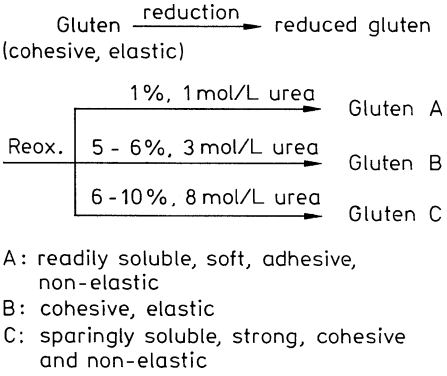


Fig. 1.48. Reaction of proteins with D,L-alanine carboxy anhydride. (according to *Sela* et al., 1962 and *St. Angelo* et al., 1966)

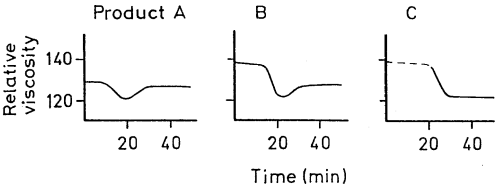


Fig. 1.49. Covalent binding of lysine to gluten (according to *Li-Chan* et al., 1979) and of methionine or tryptophan to soya protein (according to *Voutsinas* and *Nakai*, 1979), by applying a carbodiimide procedure

1.4.6.3 Enzymatic Modification

Of the great number of enzymatic reactions with protein as a substrate (cf. 1.4.5), only a small number have so far been found to be suitable for use in food processing.

1.4.6.3.1 Dephosphorylation

Figure 1.50 uses β-casein as an example to show that the solubility of a phosphoprotein in the presence of calcium ions is greatly improved by partial enzymatic dephosphorylation.

1.4.6.3.2 Plastein Reaction

The plastein reaction enables peptide fragments of a hydrolysate to join enzymatically through peptide bonds, forming a larger polypeptide of

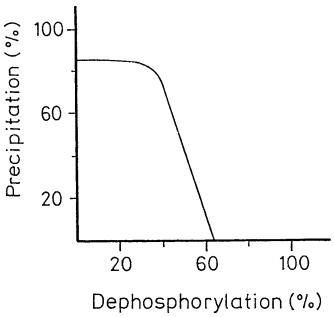
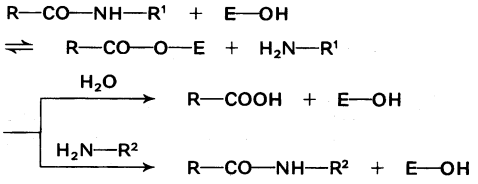


Fig. 1.50. Solubility of β-casein, partially dephosphorylated by phosphoprotein phosphatase: Precipitation: pH 7.1: 2.5 mg/ml protein: 10 mmol/L $CaCl_2$: 35 °C; 1 h. (according to *Yoshikawa* et al., 1974)

about 3 kdal:



(1.162)

The reaction rate is affected by, among other things, the nature of the amino acid residues. Hydrophobic amino acid residues are preferably linked together (Fig.1.51). Incorporation of amino acid esters into protein is affected by the alkyl chain length of the ester. Short-chain alkyl esters have a low rate of incorporation, while the long-chain alkyl esters have a higher rate of incorporation. This is especially important for the incorporation of amino acids with a short side chain, such as alanine (cf. Table 1.40).

The plastein reaction can help to improve the biological value of a protein. Figure 1.52 shows the plastein enrichment of zein with tryptophan, threonine and lysine. The amino acid composition of such a zein-plastein product is given in Table 1.41.

Enrichment of a protein with selected amino acids can be achieved with the corresponding amino acid esters or, equally well, by using suitable partial hydrolysates of another protein.

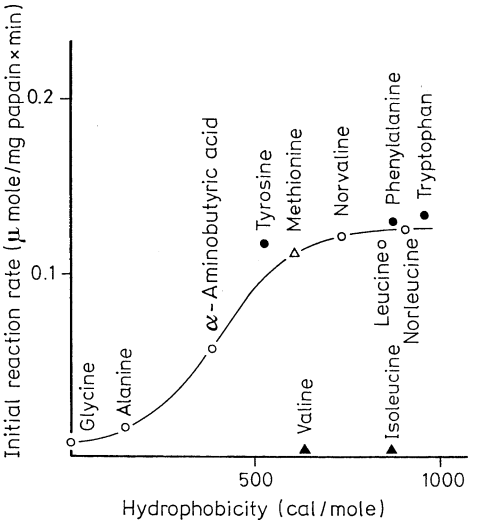


Fig. 1.51. Plastein reaction with papain: incorporation rates of amino acid esters as function of side chain hydrophobicity. (according to *Arai* et al., 1978)

Table 1.40. Plastein reaction catalyzed by papain: rate of incorporation of amino acid esters[a]

Aminoacyl residue	OEt	OnBu	OnHex	OnOct
L-Ala	0.016	0.054	0.133	0.135
D-Ala	0.0	–	0.0	–
α-Methylala	0.0	–	0.0	–
L-Val	0.005	–	0.077	–
L-Norval	0.122	–	0.155	–
L-Leu	0.119	–	0.140	–
L-Norleu	0.125	–	0.149	–
L-Ile	0.005	–	0.048	–

[a] μ mole × mg papain^{-1} × min^{-1}.

Figure 1.53 presents the example of soya protein enrichment with sulfur-containing amino acids through "adulteration" with the partial hydrolysate of wool keratin. The PER (protein efficiency ratio) values of such plastein products are significantly improved, as is seen in Table 1.42.

Figure 1.54 shows that the production of plastein with an amino acid profile very close to that recommended by FAO/WHO can be achieved from very diverse proteins.

The plastein reaction also makes it possible to improve the solubility of a protein, for example, by increasing the content of glutamic acid (Fig. 1.55). A soya protein with 25% glutamic acid yields a plastein with 42% glutamic acid.

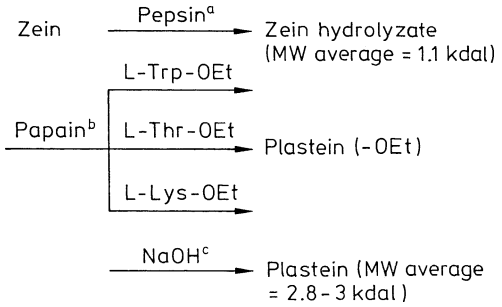


Fig. 1.52. Zein enrichment with Trp, Thr, and Lys by a plastein reaction. (according to *Aso* et al., 1974)
[a] 1% substrate, E/S = 1/50, pH 1.6 at 37 °C for 72 h
[b] 50% substrate, hydrolyzate/AS-OEt = 10/1, E/S = 3/100 at 37 °C for 48 h
[c] 0.1 mol/L in 50% ethanol at 25 °C for 5 h

Table 1.41. Amino acid composition of various plasteins (weight-%)

	1	2	3	4	5	6
Arg	1.56	1.33	1.07	1.06	1.35	1.74
His	1.07	0.95	0.81	0.75	0.81	1.06
Ile	4.39	6.39	6.58	5.49	6.23	5.67
Leu	20.18	23.70	23.05	23.75	25.28	23.49
Lys	0.20	0.20	0.24	2.14	3.24	0.19
Phe	6.63	7.26	6.82	7.34	7.22	6.98
Thr	2.40	2.18	9.23	2.36	2.46	2.13
Trp	0.38	9.71	0.25	0.40	0.42	0.33
Val	3.62	5.23	5.77	5.53	6.18	6.20
Met	1.58	1.87	1.67	1.89	2.06	2.04
Cys	1.00	0.58	0.88	0.81	0.78	0.92
Ala	7.56	7.51	8.05	7.97	7.93	8.77
Asp	4.61	3.38	3.42	3.71	3.60	3.91
Glu	21.70	12.48	14.03	14.77	12.95	13.02
Gly	1.48	1.15	1.23	1.29	1.27	1.52
Pro	10.93	8.42	9.10	9.73	9.14	9.37
Ser	4.42	3.40	3.89	3.93	3.74	4.28
Tyr	4.73	5.35	4.97	5.00	6.08	5.54

1) Zein hydrolyzate; 2) Trp-plastein; 3) Thr-plastein; 4) Lys-plastein; 5) Ac-Lys-plastein; 6) Control without addition of amino acid ethyl esters.

Table 1.42. PER-values for various proteins and plasteins

Protein	PER value (rats)
Casein	2.40
Soya protein (I)	1.20
Plastein SW[a] + I (1:2)	2.86
Plastein-Met[b] + I (1:3)	3.38

[a] From hydrolyzate I and wool keratin hydrolyzate.
[b] From hydrolyzate I and Met-OEt. PER (cf. 1.2.5).

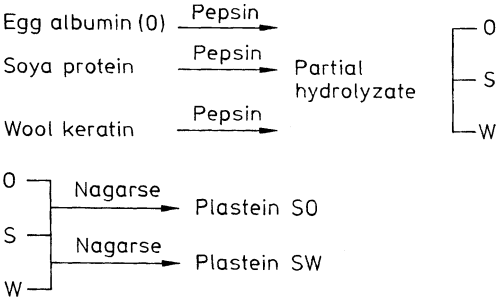


Fig. 1.53. Protein enrichment with sulfur amino acids applying plastein reaction. (according to *Yamashita* et al., 1971)

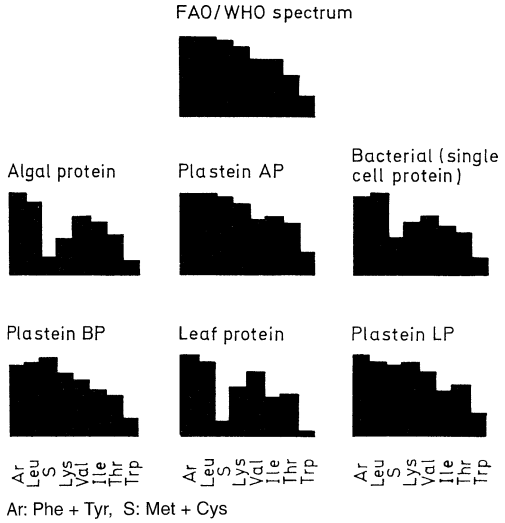


Fig. 1.54. Amino acid patterns of some proteins and their corresponding plasteins. (according to *Arai* et al., 1978)

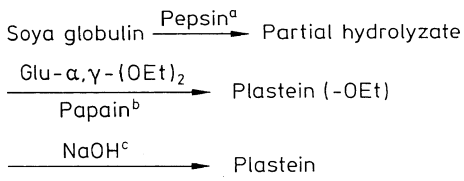


Fig. 1.55. Soy globulin enrichment with glutamic acid by a plastein reaction. (according to *Yamashita* et al., 1975)
[a] pH 1.6
[b] Partial hydrolyzate/Glu-α-γ-$(OEt)_2$ = 2:1, substrate concentration: 52.5%, E/S = 1/50, pH 5.5 at 37 °C for 24 h; sample contains 20% acetone
[c] 0.2 mol/L at 25 °C for 2 h

Soya protein has a pronounced solubility minimum in the pH range of 3–6. The minimum is much less pronounced in the case of the unmodified plastein, whereas the glutamic acidenriched soya plastein has a satisfactory solubility over the whole pH range (Fig. 1.56) and is also resistant to thermal coagulation (Fig. 1.57).

Proteins with an increased content of glutamic acid show an interesting sensory effect: partial hydrolysis of modified plastein does not result in a bitter taste, rather it generates a pronounced "meat broth" flavor (Table 1.43).

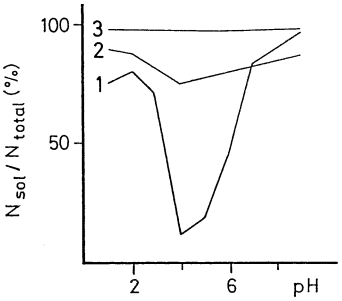


Fig. 1.56. Effect of pH on solubility of soy protein and modified products (1 g/100 ml water). 1 Soy protein, 24.1% Glu; 2 Plastein 24.8% Glu; 3 Glu-plastein with 41.9% Glu. (according to *Yamashita* et al., 1975)

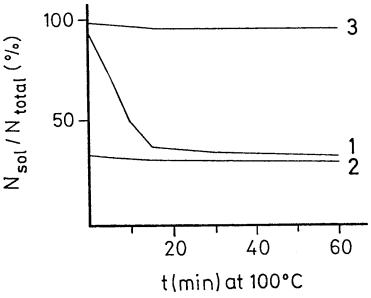


Fig. 1.57. Solubility of soy protein and modified products (800 mg/10 ml water) as a function of heating time at 100 °C. 1 Soy protein 24.1% Glu; 2 Plastein 24.8% Glu; 3 Glu-plastein, 41.9% Glu. (according to *Yamashita* et al., 1975)

Elimination of the bitter taste from a protein hydrolysate is also possible without incorporation of hydrophilic amino acids. Bitter-tasting peptides, such as Leu-Phe, which are released by partial hydrolysis of protein, react preferentially in the subsequent plastein reaction and are incorporated into higher molecular weight peptides with a neutral taste.

The versatility of the plastein reaction is also demonstrated by examples wherein undesired amino acids are removed from a protein. A phenylalanine-free diet, which can be prepared by mixing amino acids, is recommended for certain metabolic defects. However, the use of a phenylalanine-free higher molecular weight peptide is more advantageous with respect to sensory and osmotic properties. Such peptides can be prepared from protein by the plastein reaction. First, the protein is partially hydrolyzed with pepsin. Treatment with pronase under

Table 1.43. Taste of glutamic acid enriched plasteins

Enzyme	pH	Sub-strate[a]	Hydro-lysis[b]	Taste[c]	
				bitter	meat broth type
Pepsin	1.5	G	67	1	1.3
		P	73	4.5	1.0
α-Chymo-	8.0	G	48	1	1.0
trypsin		P	72	4.5	1.0
Molsin	3.0	G	66	1.0	5.0
		P	74	1.3	1.3
Pronase	8.0	G	66	1.0	4.3
		P	82	1.3	1.2

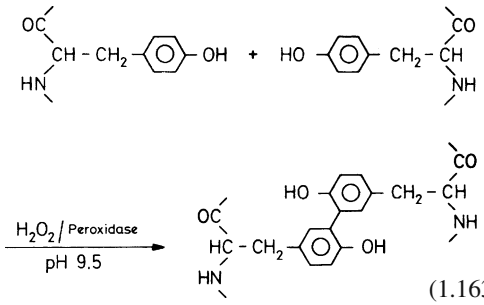
[a] G: Glu-plastein, P: plastein; 1 g/100 ml.
[b] N_{sol} (10% TCA)/N_{total} (%).
[c] 1: no taste, 5: very strong taste.

suitable conditions then preferentially releases amino acids with long hydrophobic side chains. The remaining peptides are separated by gel chromatography and then subjected to the plastein reaction in the presence of added tyrosine and tryptophan (Fig. 1.58). This yields a plastein that is practically phenylalanine-free and has a predetermined ratio of other amino acids, including tyrosine (Table 1.44).

The plastein reaction can also be carried out as a one-step process (Fig. 1.59), thus putting these reactions to economic, industrial-scale use.

1.4.6.3.3 Cross-Linking

Cross-linking between protein molecules is achieved with transglutaminase (cf. 2.7.2.4) and with peroxidase (cf. 2.3.2.2). The cross-linking occurs between tyrosine residues when a protein is incubated with peroxidase/H_2O_2 (cf. Reaction 1.163).



(1.163)

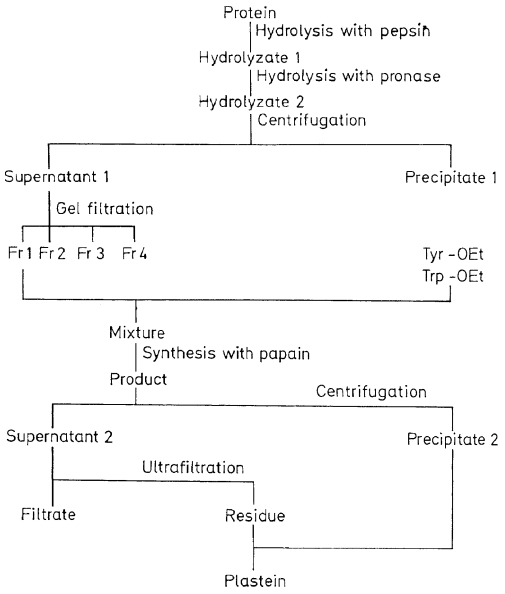


Fig. 1.58. Production of plasteins with high tyrosine and low phenylalanine contents. (according to *Yamashita* et al., 1976)

Table 1.44. Amino acid composition (weight-%) of plasteins with high tyrosine and low phenylalanine contents from fish protein concentrate (FPC) and soya protein isolate (SPI)

Amino acid	FPC	FPC-Plastein	SPI	SPI-Plastein
Arg	7.05	4.22	7.45	4.21
His	2.31	1.76	2.66	1.41
Ile	5.44	2.81	5.20	3.83
Leu	8.79	3.69	6.73	2.43
Lys	10.68	10.11	5.81	3.83
Thr	4.94	4.20	3.58	4.39
Trp	1.01	2.98	1.34	2.80
Val	5.88	3.81	4.97	3.24
Met	2.80	1.90	1.25	0.94
Cys	0.91	1.41	1.78	1.82
Phe	4.30	0.05	4.29	0.23
Tyr	3.94	7.82	3.34	7.96
Ala	6.27	4.82	4.08	2.56
Asp	11.13	13.67	11.51	18.00
Glu	17.14	27.17	16.94	33.56
Gly	4.42	3.94	4.88	3.89
Pro	3.80	4.25	6.27	2.11
Ser	4.59	3.58	5.45	4.67

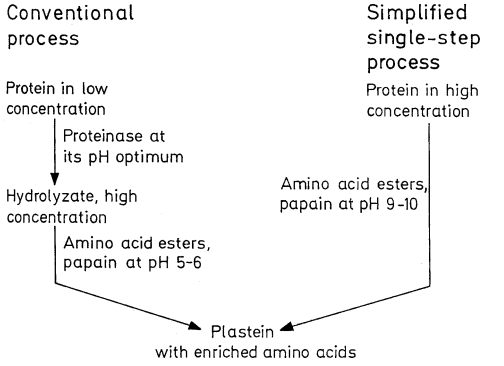
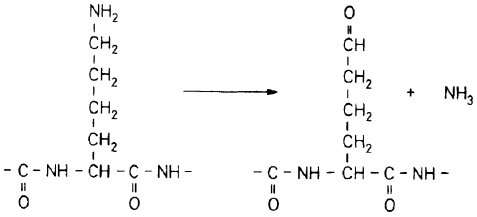


Fig. 1.59. An outline for two- and single-step plastein reactions. (according to *Yamashita* et al., 1979)

Incubation of protein with peroxidase/H_2O_2/catechol also results in cross-linking. The reactions in this case are the oxidative deamination of lysine residues, followed by aldol and aldimine condensations, i. e. reactions analogous to those catalyzed by lysyl oxidase in connective tissue:



(1.164)

Table 1.45 presents some of the proteins modified by peroxidase/H_2O_2 treatment and includes their ditryrosine contents.

1.4.7 Texturized Proteins

1.4.7.1 Foreword

The protein produced for nutrition in the world is currently about 20% from animal sources and 80% from plant sources. The plant proteins are primarily from cereals (57%) and oilseed meal (16%). Some nonconventional sources of protein (single cell proteins, leaves) have also acquired some importance.

Table 1.45. Content of dityrosine in some proteins after their oxidation with horseradish peroxidase/H_2O_2 (pH 9.5, 37 °C, 24 h. Substrate/enzyme = 20:1)

Protein	Tyrosine content prior to oxidation (g/100 g protein)	Tyrosine decrease (%)	Dityrosine content (g/100 g protein)
Casein	6.3	21.8	1.37
Soyamine[a]	3.8	11.5	0.44
Bovine serum albumin	4.56	30.7	1.40
Gliadin	3.2	5.4	0.17

[a] Protein preparation from soybean.

Proteins are responsible for the distinct physical structure of a number of foods, e. g. the fibrous structure of muscle tissue (meat, fish), the porous structure of bread and the gel structure of some dairy and soya products.
Many plant proteins have a globular structure and, although available in large amounts, are used to only a limited extent in food processing. In an attempt to broaden the use of such proteins, a number of processes were developed in the mid-1950's which confer a fiber-like structure to globular proteins. Suitable processes give products with cooking strength and a meat-like structure. They are marketed as meat extenders and meat analogues and can be used whenever a lumpy structure is desired.

1.4.7.2 Starting Material

The following protein sources are suitable for the production of texturized products: soya; casein; wheat gluten; oilseed meals such as from cottonseed, groundnut, sesame, sunflower, safflower or rapeseed; zein (corn protein); yeast; whey; blood plasma; or packing plant offal such as lungs or stomach tissue.
The required protein content of the starting material varies and depends on the process used for texturization. The starting material is often a mixture such as soya with lactalbumin, or protein plus acidic polysaccharide (alginate, carrageenan or pectin).
The suitability of proteins for texturization varies, but the molecular weight should be in the range of 10–50 kdal. Proteins of less than 10 kdal are weak fiber builders, while those higher than 50 kdal are disadvantageous due to their high viscosity and tendency to gel in the alkaline pH range. The proportion of amino acid residues with polar side chains should be high in order to enhance intermolecular binding of chains. Bulky side chains obstruct such interactions, so that the amounts of amino acids with these structures should be low.

1.4.7.3 Texturization

The globular protein is unfolded during texturization by breaking the intramolecular binding forces. The resultant extended protein chains are stabilized through interaction with neighboring chains. In practice, texturization is achieved in one of two ways:

- The starting protein is solubilized and the resultant viscous solution is extruded through a spinning nozzle into a coagulating bath (spin process).
- The starting protein is moistened slightly and then, at high temperature and pressure, is extruded with shear force through the orifices of a die (extrusion process).

1.4.7.3.1 Spin Process

The starting material (protein content >90%, e. g. a soya protein isolate) is suspended in water and solubilized by the addition of alkali. The 20% solution is then aged at pH 11 with constant stirring. The viscosity rises during this time as the protein unfolds. The solution is then pressed through the orifices of a die (5000–15,000 orifices, each with a diameter of 0.01–0.08 mm) into a coagulating bath at pH 2–3. This bath contains an acid (citric, acetic, phosphoric, lactic or hydrochloric) and, usually, 10% NaCl. Spinning solutions of protein and acidic polysaccharide mixtures also contain earth alkali salts. The protein fibers are extended further (to about 2- to 4-times the original length) in a "winding up" step and are bundled into thicker fibers with diameters

of 10–20 mm. The molecular interactions are enhanced during stretching of the fiber, thus increasing the mechanical strength of the fiber bundles.
The adherent solvent is then removed by pressing the fibers between rollers, then placing them in a neutralizing bath ($NaHCO_3 + NaCl$) of pH 5.5–6 and, occasionally, also in a hardening bath (conc. NaCl).
The fiber bundles may be combined into larger aggregates with diameters of 7–10 cm.
Additional treatment involves passage of the bundles through a bath containing a binder and other additives (a protein which coagulates when heated, such as egg protein; modified starch or other polysaccharides; aroma compounds; lipids). This treatment produces bundles with improved thermal stability and aroma. A typical bath for fibers which are to be processed into a meat analogue might consist of 51% water, 15% ovalbumin, 10% wheat gluten, 8% soya flour, 7% onion powder, 2% protein hydrolysate, 1% NaCl, 0.15% monosodium glutamate and 0.5% pigments.
Finally, the soaked fiber bundles are heated and chopped.

1.4.7.3.2 Extrusion Process

The moisture content of the starting material (protein content about 50%, e. g., soya flour) is adjusted to 30–40% and additives (NaCl, buffers, aroma compounds, pigments) are incorporated. Aroma compounds are added in fat as a carrier, when necessary, after the extrusion step to compensate for aroma losses. The protein mixture is fed into the extruder (a thermostatically controlled cylinder or conical body which contains a polished, rotating screw with a gradually decreasing pitch) which is heated to 120–180 °C and develops a pressure of 30–40 bar. Under these conditions the mixture is transformed into a plastic, viscous state in which solids are dispersed in the molten protein. Hydration of the protein takes place after partial unfolding of the globular molecules and stretching and rearrangement of the protein strands along the direction of mass transfer.
The process is affected by the rotation rate and shape of the screw and by the heat transfer and viscosity of the extruded material and its residence time in the extruder. As the molten material exits from the extruder, the water vaporizes, leaving behind vacuoles in the ramified protein strands.
The extrusion process is more economical than the spin process. However, it yields fiber-like particles rather than well-defined fibers. A great number and variety of extruders are now in operation. As with other food processes, there is a trend toward developing and utilizing high-temperature/short-time extrusion cooking.

1.5 References

Aeschbach, R., Amado, R., Neukom, H.: Formation of dityrosine cross-links in proteins by oxidation of tyrosine residues. Biochim. Biophys. Acta *439*, 292 (1976)

Arai, S., Yamashita, M., Fujimaki, M.: Nutritional improvement of food proteins by means of the plastein reaction and its novel modification. Adv. Exp. Med. Biol. *105*, 663 (1978)

Aso, K., Yamashita, M., Arai, S., Suzuki, J., Fujimaki, M.: Specificity for incorporation of α-amino acid esters during the plastein reaction by papain. J. Agric. Food Chem. *25*, 1138 (1977)

Belitz, H.-D., Wieser, H.: Zur Konfigurationsabhängigkeit des süßen oder bitteren Geschmacks von Aminosäuren und Peptiden. Z. Lebensm. Unters. Forsch. *160*, 251 (1976)

Biochemistry, 5th edition, Berg, J.M., Tymoczko, J.L., Stryer, L., W.H. Freeman and Company, New York, 2002

Bodanszky, M.: Peptide Chemistry. Springer-Verlag: Berlin, 1988

Boggs, R.W.: Bioavailability of acetylated derivatives of methionine, threonine and lysine. Adv. Exp. Med. Biol. *105*, 571 (1978)

Bosin, T.R., Krogh, S., Mais, D.: Identification and quantitation of 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in beer and wine. J. Agric. Food Chem. *34*, 843 (1986)

Bott, R.R., Davies, D.R.: Pepstatin binding to *Rhizopus chinensis* aspartyl proteinase. In: Peptides: Structure and function (Eds.: Hruby, V.J., Rich, D.H.), p. 531. Pierce Chemical Co.: Rockford, III. 1983

Brückner, H., Päzold, R.: Sind D-Aminosäuren gute molekulare Marker in Lebensmitteln? Pro und Kontra. Lebensmittelchemie *60*, 141 (2006)

Chen, C., Pearson, A.M., Gray, J.I.: Meat Mutagens. Adv. Food Nutr. Res. *34*, 387, (1990)

Cherry, J.P. (Ed.): Protein functionality in foods. ACS Symposium Series 147, American Chemical Society: Washington, D.C. 1981

Creighton, T.E.: Proteins: structures and molecular properties. W.H. Freeman and Co.: New York. 1983

Croft, L.R.: Introduction to protein sequence analysis, 2nd edn., John Wiley and Sons, Inc.: Chichester. 1980

Dagleish, D.G.: Adsorptions of protein and the stability of emulsions. Trends Food Sci. Technol. *8*, 1 (1997)

Dickinson, E.: Towards more natural emulsifiers. Trends Food Sci. Technol. *4*, 330 (1993)

Einsele, A.: Biomass from higher n-alkanes. In: Biotechnology (Eds.: Rehm, H.-J., Reed, G.), Vol. 3, p. 43, Verlag Chemie: Weinheim. 1983

Faust, U., Präve. P.: Biomass from methane and methanol. In: Biotechnology (Eds.: Rehm, H.-J., Reed, G.), Vol. 3, p. 83, Verlag Chemie: Weinheim. 1983

Felton, J.S., Pais, P., Salmon, C.P., Knize, M.G.: Chemical analysis and significance of heterocyclic aromatic amines. Z. Lebensm. Unters. Forsch. A *207*, 434 (1998)

Finot, P.-A., Mottu, F., Bujard, E., Mauron, J.: N-substituted lysines as sources of lysine in nutrition. Adv. Exp. Med. Biol. *105*, 549 (1978)

Friedman, M., Granvogl, M., Schieberle, P.: Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide. J. Agric. Food Chem. *54*, 5933-6938 (2006)

Galembeck, F., Ryan, D.S., Whitaker, J.R., Feeney, R.E.: Reactions of proteins with formaldehyde in the presence and absence of sodium borohydride. J. Agric. Food Chem. *25*, 238 (1977)

Glazer, A.N.: The chemical modification of proteins by group-specific and site-specific reagents. In: The proteins (Eds.: Neurath, H., Hill, R.L., Boeder, C.-L.), 3rd edn., Vol. II, p. 1, Academic Press: New York-London. 1976

Grant, D.R.: The modification of wheat flour proteins with succinic anhydride. Cereal Chem. *50* 417 (1973)

Gross, E., Morell, J.L.: Structure of nisin. J. Am. Chem. Soc. *93*, 4634 (1971)

Herderich, M., Gutsche, B.: Tryptophan-derived bioactive compounds in food. Food Rev. Int. *13*, 103 (1997)

Hudson, B.J.F. (Ed.): Developments in food proteins-1. Applied Science Publ.: London. 1982

Ikenaka, T., Odani, S., Koide, T: Chemical structure and inhibitory activities of soybean proteinase inhibitors. Bayer-Symposium V "Proteinase inhibitors", p. 325, Springer-Verlag: Berlin-Heidelberg. 1974

Jägerstad, M., Skog, K., Arvidson, P., Solyakov, A.: Chemistry, formation and occurrence of genotoxic heterocyclic amines identified in model systems and cooked foods. Z. Lebensm. Unters. Forsch A *207*, 419 (1998)

Kasai, H., Yamaizumi, Z., Shiomi, T., Yokoyama, S., Miyazawa, T., Wakabayashi, K., Nagao, M., Sugimura, T., Nishimura, S.: Structure of a potent mutagen isolated from fried beef. Chem. Lett. *1981*, 485

Kasai, H., Yamaizumi, Z., Wakabayashi, K., Nagao, M., Sugimura, T., Yokoyama, S., Miyazawa, T., Spingarn, N.E., Weisberger, J.H., Nishimura, S.: Potent novel mutagens produced by broiling fish under normal conditions. Proc. Jpn. Acad. Ser. B56, 278 (1980)

Kessler, H.G.: Lebensmittel- und Bioverfahrenstechnik; Molkereitechnologie. 3rd edn., Verlag A. Kessler: Freising, 1988

Kinsella, J.E.: Functional properties of proteins in foods: A survey. Crit. Rev. Food Sci. Nutr. *7*, 219 (1976)

Kinsella, J.E.: Texturized proteins: Fabrication, flavoring, and nutrition. Crit. Rev. Food Sci. Nutr. *10*, 147 (1978)

Kleemann. A., Leuchtenberger, W., Hoppe, B., Tanner. H.: Amino acids. In: Ullmann's encyclopedia of industrial chemistry, 5th Edition, Volume A2, p. 57, Verlag VCA. Weinheim, 1986

Klostermeier, H., et al.: Proteins. In: Ullmann's encyclopedia of industrial chemistry, 5th Edition, Volume A22, p. 289, Verlag VCH, Weinheim, 1993

Kostka, V. (Ed.): Aspartic proteinases and their inhibitors. Walter de Gruyter: Berlin. 1985

Lásztity, R.: Rheologische Eigenschaften von Weizenkleber und ihre Beziehungen zu molekularen Parametern. Nahrung *19*, 749 (1975)

Lottspeich, F., Henschen, A., Hupe, K.-P. (Eds.): High performance liquid chromatography in protein and peptide chemistry. Walter de Gruyter: Berlin. 1981

Lübke, K., Schröder, E., Kloss, G.: Chemie und Biochemie der Aminosäuren, Peptide und Proteine. Georg Thieme Verlag: Stuttgart. 1975

Masters, P.M., Friedman, M.: Racemization of amino acids in alkali-treated food proteins. J. Agric. Food Chem. *27*, 507 (1979)

Mauron, J.: Ernährungsphysiologische Beurteilung bearbeiteter Eiweißstoffe. Dtsch. Lebensm. Rundsch. *71*, 27 (1975)

Mazur, R.H., Goldkamp, A.H., James, P.A., Schlatter, J.M.: Structure-taste relationships of aspartic acid amides. J. Med. Chem. *13*, 1217 (1970)

Mazur, R.H., Reuter, J.A., Swiatek, K.A., Schlatter, J.M.: Synthetic sweetener. 3. Aspartyl dipeptide esters from L- and D-alkylglycines. J. Med. Chem. *16*, 1284 (1973)

Meister, A.: Biochemistry of the amino acid. 2nd edn., Vol. I, Academic Press: New York-London. 1965

Morrissay, P.A., Mulvihill, D.M., O'Neill, E.M.: Functional properties of muscle proteins. In: Development in Food Proteins – 5; (Ed.: Hudson, B.J.F.), p. 195, Elsevier Applied Science: London, 1987

Mottram, D.S., Wedzicha, B.L., Dodson, A.T.: Acrylamide is formed in the Maillard reaction. Nature 419, 448 (2002)

Nagao, M., Yahagi, T., Kawachi, T., Seino, Y., Honda, M., Matsukura, N., Sugimura, T., Wakabayashi, K., Tsuji, K., Kosuge, T.: Mutagens in foods, and especially pyrolysis products of protein. Dev. Toxicol. Environ. Sci. 2nd (Prog. Genet. Toxicol.), p. 259 (1977)

Nakai, S., Modler, H.W.: Food proteins. Properties and characterization. Verlag Chemie, Weinheim, 1996

Oura, E.: Biomass from carbohydrates. In: Biotechnology (Eds.: Rehm, H.-J., Reed, G.), Vol. 3, p. 3, Verlag Chemie: Weinheim, 1983

Pence, J.W., Mohammad, A., Mecham, D.K.: Heat denaturation of gluten. Cereal Chem. *30*, 115 (1953)

Pennington, S.R., Wilkins, M.R., Hochstrasser, D.F., Dunn, M.J.: Proteome analysis: from protein characterization to biological function. Trends in Cell Biology *7*, 168 (1997)

Perutz, M.F.: Proteins and nucleic acids. Elsevier Publ. Co.: Amsterdam. 1962

Phillips, E.G., Whitehead, D.M., Kinsella, J.: Structure-function properties of food proteins. Academic Press, London, 1994

Poindexter, E.H., Jr., Carpenter, R.D.: Isolation of harmane and norharmane from cigarette smoke. Chem. Ind. *1962*, 176

Puigserver, A.J., Sen, L.C., Clifford, A.J., Feeney, R.E., Whitaker, J.R.: A method for improving the nutritional value of food proteins: Covalent attachment of amino acids. Adv. Exp. Med. Biol. *105*, 587 (1978)

Repley, J.A., Careri, G.: Protein hydration and function. Adv. Protein Chem. *41*, 38 (1991)

Richmond, A.: Phototrophic microalgae. In: Biotechnology (Eds.: Rehm, H.-J., Reed G.). Vol. 3. p. 109, Verlag Chemie: Weinheim. 1983

Schmitz, M.: Möglichkeiten und Grenzen der Homoarginin-Markierungsmethode zur Messung der Proteinverdaulichkeit beim Schwein. Dissertation, Universität Kiel. 1988

Schulz, G.E., Schirmer, R.H.: Principle of protein structure. Springer-Verlag: Berlin-Heidelberg. 1979

Schwenke, K.D.: Beeinflussung funktioneller Eigenschaften von Proteinen durch chemische Modifizierung. Nahrung *22*, 101 (1978)

Seki, T., Kawasaki, Y., Tamura, M., Tada. M., Okai, H.: Further study on the salty peptide ornithyl-β-alanine. Some effects of pH and additive ions on saltiness. J. Agric. Food Chem. *38*, 25 (1990)

Severin, Th., Ledl, F.: Thermische Zersetzung von Cystein in Tributyrin. Chem. Mikrobiol. Technol. Lebensm. *1*, 135 (1972)

Shinoda, I., Tada, M., Okai, H.: A new salty peptide, ornithyl-β-alanine hydrochloride. Pept. Chem. 21st. Proceeding of the Symposium on Peptide Chemistry 1983, p. 43 (1984)

Soda, K., Tanaka, H., Esaki, N.: Amino acids. In: Biotechnology (Eds.: Rehm, H.-J. Reed. G.). Vol. 3, p. 479, Verlag Chemie: Weinheim. 1983

Stadler, R.H., Robert, F., Riediker, S., Varga, N., Davidek, T., Devaud, S., Goldmann, T., Hau, J., Blank I.: In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds in the Maillard reaction. J. Agric. Food Chem. *52*, 5550 (2004)

Sternberg, M., Kim, C.Y.: Lysinoalanine formation in protein food ingredients. Adv. Exp. Med. Biol. *86B*, 73 (1977)

Sugimura, T., Kawachi, T., Nagao, M., Yahagi, T., Seino, Y., Okamoto, T., Shudo, K., Kosuge, T., Tsuji, K. et al.: Mutagenic principle(s) in tryptophan and phenylalanine pyrolysis products. Proc. Jpn. Acad. *33*, 58 (1977)

Sulser, H.: Die Extraktstoffe des Fleisches. Wissenschaftliche Verlagsgesellschaft mbH: Stuttgart. 1978

Traub, W., Piez, K.A.: The chemistry and structure of collagen. Adv. Protein Chem. *25*, 267 (1971)

Treleano, R., Belitz, H.-D., Jugel, H., Wieser, H.: Beziehungen zwischen Struktur und Geschmack bei Aminosäuren mit cyclischen Seitenketten. Z. Lebensm. Unters. Forsch. *167*, 320 (1978)

Tschesche, H. (Ed.): Modern Methods in Protein Chemistry, Vol. 2, Walter de Gruyter: Berlin, 1985

Voutsinas, L.P., Nakai, S.: Covalent binding of methionine and tryptophan to soy protein. J. Food Sci. *44*, 1205 (1979)

Walton, A.G.: Polypeptides and protein structure, Elsevier North Holland, Inc., New York-Oxford. 1981

Watanabe, M., Arai, S.: The plastein reaction and its applications. In: Developments in Food Proteins – 6; (Ed.: Hudson, B.J.F.), p. 179, Elsevier Applied Science: London. 1988

Whitaker, J.R., Fujimaki, M. (Eds.): Chemical deterioration of proteins, ACS Symposium Series 123, American Chemical Society: Washington D.C. 1980

Wieser, H., Belitz, H.-D.: Zusammenhänge zwischen Struktur und Bittergeschmack bei Aminosäuren und Peptiden. I. Aminosäuren und verwandte Verbindungen. Z. Lebensm. Unters. Forsch. *159*, 65 (1975)

Wieser, H., Belitz, H.-D.: Zusammenhänge zwischen Struktur und Bittergeschmack bei Aminosäuren und Peptiden. II. Peptide und Peptidderivate. Z. Lebensm. Unters. Forsch. *160*, 383 (1976)

Wieser, H., Jugel, H., Belitz, H.-D.: Zusammenhänge zwischen Struktur und Süßgeschmack bei Aminosäuren. Z. Lebensm. Unters. Forsch. *164*, 277 (1977)

Wild, D., Kerdar, R.S.: The inherent genotoxic potency of food mutagens and other heterocyclic and carboxylic aromatic amines and corresponding azides. Z. Lebensm. Unters. Forsch. A *207*, 428 (1998)

Wittmann-Liebold, B., Salnikow, J., Erdmann, V.A. (Eds.): Advanced Methods in Protein Microsequence Analysis. Springer-Verlag: Berlin. 1986

Yamashita, M., Arai, S., Fujimaki, M.: A lowphenylalanine, high-tyrosine plastein as an acceptable dietetic food. J. Food Sci. *41*, 1029 (1976)

Yamashita, M., Arai, S., Amano, Y., Fujimaki, M.: A novel one-step process for enzymatic incorporation of amino acids into proteins: Application to soy protein and flour for enhancing their methionine levels. Agric. Biol. Chem. *43*, 1065 (1979)

Yamashita, M., Arai, S., Tsai, S.-J., Fujimaki, M.: Plastein reaction as a method for enhancing the sulfur-containing amino acid level of soybean protein. J. Agric. Food Chem. *19*, 1151 (1971)

Yamashita, M., Arai, S., Kokubo, S., Aso, K., Fujimaki, M.: Synthesis and characterization of a glutamic acid enriched plastein with greater solubility. J. Agric. Food Chem. *23*, 27 (1975)

Yoshikawa, M., Tamaki, M., Sugimoto, E., Chiba, H.: Effect of dephosphorylation on the self-association and the precipitation of β-Casein. Agric. Biol. Chem. *38*, 2051 (1974)