Chemical Reactivity and Molecular Interactions

29

Every step of metabolism is in essence a chemical reaction. The vast majority of these reactions in an organism are catalyzed. Enzymes act as catalysts to facilitate the bond forming and/or breaking steps of these chemical transformations. Enzyme catalyzed or not, chemical reactions proceed mainly through the formation and cleavage of chemical bonds. In some cases, the catalyst itself participates covalently in the overall chemical Scheme. A reaction is therefore best understood through an appreciation of chemical reactivity and how molecules interact with each other. The nature of chemical bonds and associated molecular interactions is reviewed in this chapter. A thorough understanding of these basic chemical mechanisms is essential to appreciate how enzymes facilitate chemical reactions.

29.1 Atoms, Molecules, and Chemical Bonding

All the elements in the periodic table (with the exception of inert gases!) display various degrees of reactivity. Consequently they occur in nature as their compounds. The reactivity of an element is the reflection of its atomic structure and electronic configuration (Table 29.1). Hydrogen, carbon, nitrogen, oxygen, phosphorus, and sulfur dominate the reactions in biochemistry and hence are the elements we most often encounter in enzyme chemistry. The valence electrons of these atoms participate in chemical reactions; they belong to the s and p orbitals of highest energy level. For instance, carbon has four electrons in its 2 s and 2p orbitals – defining its valency as four.

A chemical bond may be defined as the force that holds the atoms together within a molecule. The valence electrons are available for the formation of covalent bonds. When two atoms with appropriate electronic configuration approach each other at close enough range, they can enter into bond formation. Bonding between atoms leads to molecules.

N. S. Punekar, *ENZYMES: Catalysis, Kinetics and Mechanisms*,
https://doi.org/10.1007/978-981-13-0785-0_29

Table 29.1 Electronic structure of elements encountered in enzyme mechanisms

Element	Atomic number	Radius (Å)	Electronic configuration	Valency (most relevant)	Electronegativity (Pauling scale)
H	1	1.2	$1s^1$	1	2.20
C	6	2.0	$1s^2,2s^2 2p^2$	4	2.55
N	7	1.5	$1s^2,2s^2 2p^3$	3	3.04
O	8	1.4	$1s^2,2s^2 2p^4$	2	3.44
P	15	1.9	$1s^2,2s^2 2p^6,3s^2 3p^3$	5	2.19
S	16	1.9	$1s^2,2s^2 2p^6,3s^2 3p^4$	2	2.58

29.1.1 Covalent Bonds

Among the different explanations of chemical bonding, molecular orbital theory has found a wide acceptance. According to this theory when two atoms approach each other for bonding, their respective valence atomic orbitals (one from each atom) can combine to form two molecular orbitals. Distribution of valence electrons among these molecular orbitals (the bonding and antibonding molecular orbitals) is called the electronic configuration of the molecule. Valence electrons from atoms entering into bond formation are distributed between bonding and antibonding (shown with an asterisk) orbitals – according to their order of energy levels.

$$\sigma 1s < \sigma^* 1s < \sigma 2s < \sigma^* 2s < \pi 2p \,\text{etc.}$$

Since bonding orbitals occur at a lower potential energy, electron occupancy in them stabilizes the bond. Electrons occupying the antibonding orbitals destabilize the bond. As long as there are more number of valence electrons in the bonding orbital than in the antibonding orbital, the bond (and the molecule) is stable. We then define the bond order as follows:

$$\text{Bond order} = \frac{1}{2}\left(\text{number of bonding electrons} - \text{number of antibonding electrons}\right)$$

For instance, if the bonding molecular orbitals contain a pair of electrons more than the antibonding molecular orbitals, then the bond order is one. Integral bond order (**n**) values of 1, 2, or 3 correspond to single, double, or triple covalent bonds, respectively. A single bond thus means the bonding atoms share a pair of electrons between them. Fractional bond orders are possible and may be encountered in a resonance stabilized molecule (see below).

Two different atoms can approach each other to the extent permitted by their van der Waals radii (Fig. 29.1). This takes into account the overall size of the two atoms including their valence shells. Any closer approach is sterically not feasible. However when the two atoms enter into covalent bonding, their valence atomic orbitals overlap – then the two atoms are closer than their van der Waals radii can allow. The

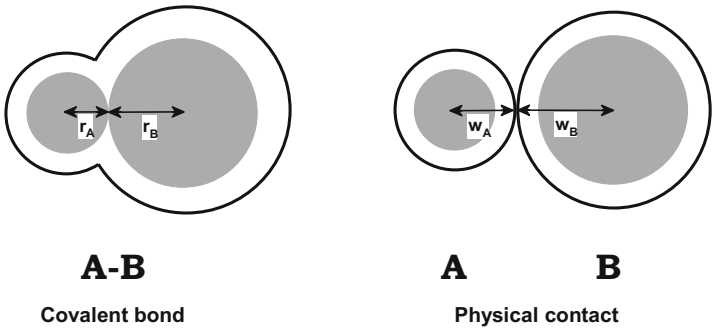


Fig. 29.1 Covalent radius, van der Waals radius, and bond length. In a covalent diatomic molecule A–B, the bond length is the sum of the two covalent radii ($R = r_A + r_B$). This distance is shorter than the sum of their respective van der Waals radii ($= w_A + w_B$)

Table 29.2 Average covalent bond lengths relevant to enzyme chemistry

Bond type	Bond length (Å)	Bond type	Bond length (Å)
C–H	1.14	C–O	1.43
C–C	1.54	C=O	1.24
C=C	1.34	C–N	1.51
C≡C	1.20	C=N	1.32

equilibrium internuclear separation distance of the covalently bonded atoms is called bond length. The bond length is the sum of the covalent radii of the two participating atoms (Fig. 29.1). The two atoms participating in a covalent bond cannot approach closer than the bond length due to repulsive forces between them. However their separation by a distance longer than the bond length leads to weakening of the covalent bond – due to less than optimal overlap of their atomic orbitals.

An empirical relation (according to Linus Pauling) between bond order (**n**) and bond length (R) may be given as shown.

$$R_n = R_1 - 0.3 \times \log \mathbf{n}$$

With increasing bond order, the bond length decreases. Accordingly, a carbon-carbon double bond is shorter than the corresponding single bond. A few bond lengths relevant in biochemical reactions are listed in Table 29.2. Bond lengths in a molecule can be measured by spectroscopy, X-ray, and electron diffraction methods.

While covalent bonds are quite stable (bond enthalpy of about 90 kcal $\times$ mol^{-1}; Table 29.3, below), electrons from these bonds can get displaced – leading to reactivity of a particular molecule. The atoms with larger electronegativity (Table 29.1) tend to pull more electron density toward them. A covalent bond may be affected and/or polarized due to different electronegativities of the participating atoms and their neighbors. These influences are variously ascribed to *inductive* effect, *electromeric* effect, *hyperconjugation*, and *resonance*. Some molecules cannot be represented by a single covalent structure. Instead they are better shown as

Table 29.3 Strengths of covalent and non-covalent chemical bonds

Bond type	Bond strength[a] (kcal × mol^{-1})
van der Waals attraction	0.1 (per atom)
Hydrogen bond	1–3
Ionic	3–80
Covalent	90

[a]Bond strength is given as the energy required for breaking it. Hydrogen bonds and ionic interactions are weakened in an aqueous environment as water competes in such interactions (kJ = 0.24 kcal)

equivalent to a combination of two or more simple structures. Such resonance structures tend to stabilize the overall molecular structure by delocalization of electron density. Examples include the peptide bond, the aromatic benzene nucleus, and the carboxylate group. One measurable outcome of resonance stabilization is the unusual bond lengths (and bond orders). The peptide bond has a partial (40%) double bond character. The C–N bond length of a peptide bond is shorter than a typical C–N bond but longer than the C=N bond (Table 29.2). The carbon-carbon bond length between the adjacent carbon atoms of benzene is 1.45 Å, which is intermediate between the expected lengths for single and double bonds. Similarly, the negative charge on the carboxylate group is equally shared between the two oxygen atoms – both the oxygens and the C–O bonds are equivalent.

When two atoms sharing electrons in a covalent bond are of equal electronegativity, the resulting bond is nonpolar. A carbon–carbon bond is one such example. However when the two participating atoms are of different electronegativities, the covalent bond is polarized. Covalent bonds acquire degrees of polar character depending on the electronegativities of the bonding atoms. Proportionately more electron density resides with the more electronegative partner of the covalent bond. For example, the carbon of a C–O covalent bond will carry a δ+ charge while the oxygen will carry a corresponding δ-charge.

29.1.2 Directional Property of Covalent Bonds

Biochemical reactions often involve establishing or cleaving covalent bonds at the carbon atom in a molecule. The electronic configuration of carbon atom either in its ground state ($1s^2,2s^2 2p^2$) or in the excited state ($1s^2,2s^1 2p_x^{\ 1} 2p_y^{\ 1} 2p_z^{\ 1}$) suggests that the four valence electrons are not identical and hence the four bonds should not be equivalent. However the four single bonds around a tetravalent carbon (such as in methane) are equivalent. This is due to hybridization of 2 s and 2p orbitals of carbon. The similar energies of the 2s and 2p orbitals can interact to form hybrid orbitals of equivalent energy and shape. In fact, three types of hybrid orbitals (Fig. 29.2) are possible with carbon: (a) Combination of one 2s orbital with three 2p orbitals yields four sp^3 orbitals. The four sp^3 orbitals are equivalent and are capable of forming four σ bonds along the apices of a regular tetrahedron. In this tetrahedral geometry, the

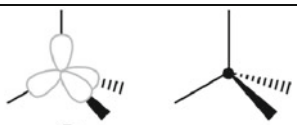
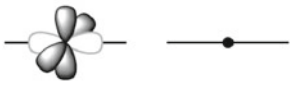
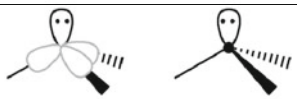
Hybridization	Orbitals and Geometry	Bond angle	Covalent bonds	Shape and example
sp^3 carbon; **tetrahedral**		109.5°	**4 σ** bonds	**Tetrahedral;** Methane
sp^2carbon; **trigonal**		120.0°	**3 σ** bonds and **1 π** bond	**Planar;** Ethylene
sp^1carbon; **linear**		180.0°	**2 σ** bonds and **2 π** bonds	**Linear:** Acetylene
sp^3 nitrogen; **tetrahedral**		107.3°	**3 σ** bonds (1 lone pair)	**Trigonal pyramid;** Ammonia
sp^3 oxygen; **tetrahedral**		104.5°	**2 σ** bonds (2 lone pairs)	**Bent;** Water

Fig. 29.2 Types of hybridization leading to directionality of covalent bonds. A covalent σ bond is formed due to maximal overlap of participating orbitals. A lateral overlap of p orbitals (dumbbell shaped, shaded gray) results in a π bond. A double bond is made of one σ bond and one π bond while a triple bond consists of one σ bond and two π bonds

four bonds on the carbon are equally separated from each other. (b) It is possible that one s and two p orbitals mix to give three new hybrid orbitals. In this sp^2 hybridization, the new hybrid orbitals allow for three trigonal planar σ bonds to be formed. The two lobes of the remaining p orbital are perpendicular to this plane (Fig. 29.2) and are capable of a π bond through a lateral overlap. A double bond at carbon thus is in effect a combination of a σ bond and a π bond. (c) When a single p orbital combines with the 2 s orbital, two equivalent sp. orbitals result. With this linear hybridization, the remaining two p orbitals are placed perpendicular to each other. They can enter into π bonds through lateral overlaps. A triple bond at carbon is therefore a combination of one σ bond and two π bonds.

Different hybridization modes of s and p orbitals clearly accounts for the directional property of covalent bonds around carbon. From the second row of the periodic table, nitrogen and oxygen are the other two elements of importance in enzyme reactions. Both of them can exist in sp^3 or sp^2 hybridization states (Fig. 29.2). The lone pair of nitrogen occupies one of the hybrid orbitals whereas oxygen has two such orbitals bearing a pair of electrons each. The bond angles in sp^3 hybridized nitrogen and oxygen are smaller than those in carbon due to lone pair repulsions.

29.1.3 Non–covalent Interactions and Intermolecular Forces

Apart from the strong covalent bonds discussed above, molecules can interact with each other through different non-covalent forces. They include van der Waals interactions, hydrogen bonding, and ionic interactions. These are generally weaker than the covalent bonds (Table 29.3). Both ionic and hydrogen bonds are further weakened in aqueous environments. These three weak attractive forces are very important in enzyme catalysis, particularly due to their readily reversible nature.

The fact that noble gases can be liquefied suggests that even they display molecular interactions. Weak van der Waals interactions do occur between all molecules. These are contributed by (a) dipole-dipole interactions, (b) dipole-induced dipole interactions, and (c) London dispersion forces. Although weak, a large number of them can produce significant cooperative interactions. They are important in protein structure, hydrophobic recognition, and enzyme catalysis.

A hydrogen atom bonded to an electronegative atom (like N or O) can interact with another electronegative atom (like N or O) bearing a lone pair of electrons. This weak charge interaction is called a hydrogen bond. The atom to which the hydrogen is covalently bonded is referred to as the hydrogen bond donor and the other electronegative atom is called the hydrogen bond acceptor. In a hydrogen bond (shown as dotted line) of the type "**>N–H·····O=C<,**" N is the donor and O is the acceptor. Typically the length of a hydrogen bond is longer than the corresponding covalent bond. Strength of a hydrogen bond depends on distance, direction (angle), and the nature of the participating electronegative atoms. The more nonlinear a hydrogen bond (bend at the H atom!), the weaker it gets – bond directionality matters. While Cl and N are of comparable electronegativity, chlorine cannot form a hydrogen bond due to its larger size. Hydrogen bonds are central to the structure and the catalytic apparatus of an enzyme molecule. A very strong hydrogen bond results when the H atom is equally shared and strongly bonded to both the donor and acceptor atoms. Such low-barrier hydrogen bonds (LBHBs) do occur and form part of the catalytic strategy of many enzymes (see Chap. 6).

That quintessential scientist, JBS Haldane, once famously said "even the Pope is 70% water!" Water is the universal protic solvent and most life processes have evolved around the unique properties of water (Table 29.4). It is the smallest and

Table 29.4 Properties of water

Property	H_2O	D_2O
Molecular mass	18.015	20.028
Melting point	273.0 K	276.8 K
Boiling point	373.0 K	374.4 K
Density at 298.0 K	1.000	1.106
Maximum density at	277.0 K	284.2 K
Viscosity (centipoise)	0.89	1.11
Dielectric constant	78.39	78.06
O-H/O-D bond length	0.958 Å	0.918 Å
$[H^+]$ measure (as $-\log [H^+]$)	pH	pD (=pH + 0.41)

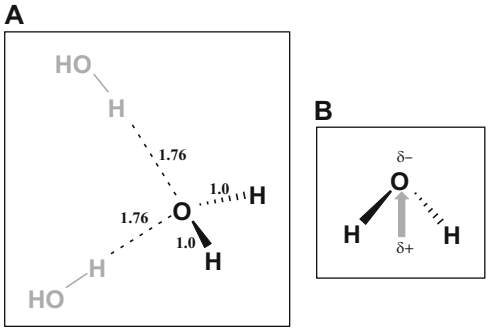


Fig. 29.3 Attributes of water molecule. (**A**) Hydrogen bonding and the structure of water. Dashed lines represent hydrogen bonds and all the bond lengths are in Å. (**B**) Dipole moment of water molecule

most abundant molecule in a cell. But for its ordered hydrogen bonded network, water would not be in liquid form under ambient conditions. All biological (and biochemical) processes (and reactions) are either directly or indirectly under the influence of characteristic features of water. That biological catalysis which has primarily/predominantly originated in an aqueous environment is consistent with this.

Hydrogen bonds play a major role in the structure and solvent properties of water. The unusually high boiling point, melting point, and density behavior are the manifestations of strong intermolecular hydrogen bonding in water (Fig. 29.3). Because of difference in the electronegativity of O and H, water also has a large dipole moment. A substitution of H by D (heavy isotope of hydrogen) significantly changes the solvent properties of water. Some of these differences are listed in Table 29.4. Most acids are 3–5 times weaker in D_2O than in H_2O. The shorter O–D bond (by about 0.04 Å or 0.004 nm) leads to changes in polarizability and solvent structure. Because of this, hydrogen bonds and hydrophobic interactions are also affected. Even proton transfers rates may be affected leading to what is known as solvent isotope effects.

Two oppositely charged atoms/groups attract each other – these electrostatic forces are called ionic interactions or salt bridges. The strength of this Coulombic attraction depends directly on the two charges involved ($\mathbf{Q_1}$ and $\mathbf{Q_2}$) and is inversely proportional to the square of the distance (**r**) between them.

$$\mathbf{F} = \frac{\mathbf{Q_1} \times \mathbf{Q_2}}{\mathbf{r^2} \times \mathbf{D}} \quad \text{where D is dielectric constant}$$

Ionic interactions also depend on the dielectric constant (D) of the medium. They are nearly as strong as a covalent bond in vacuum but are greatly weakened by the aqueous environment (with typical bond energies in water of the order 3–-5 kcal $\times$ mol^{-1}). Therefore salt bridges are stronger in the hydrophobic interior of a protein than on the solvent-exposed surface. Charged ligands often form salt bridges with their charged counterparts at the enzyme active site. For instance, negatively charged phosphate group is bound through positively charged active site arginine residue (the guanidinium group).

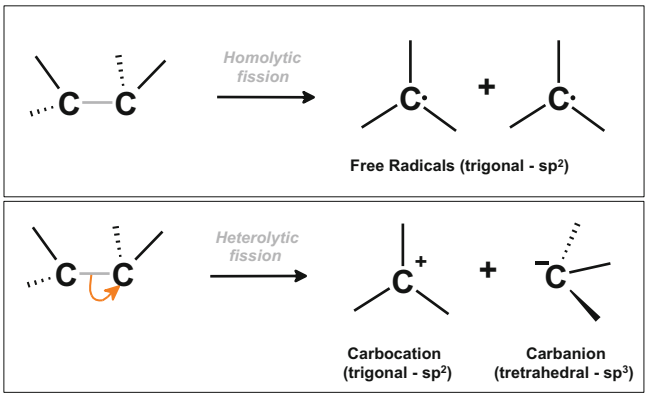


Fig. 29.4 **Homolytic versus heterolytic fission of a carbon-carbon covalent bond**

29.2 Chemical Reaction Mechanisms

Living beings depend on a myriad of chemical reactions – the sum total of metabolism. These chemical transformations are almost invariably brought about by enzyme catalysts. Many biochemical reactions may appear quite complex but are not. Their apparent complexities are largely due to the variety of reactant structures involved. However at the mechanistic level, these reactions are simply the elementary reactions of organic chemistry. Relevant description of some of these reaction types and the principles of underlying chemical mechanisms will follow.

29.2.1 Cleaving and Forming Covalent Bonds

Enzymes utilize many of the same mechanisms that are well known to chemists – for the synthesis and degradation of organic compounds. There are two possible ways of forming and cleaving a carbon-carbon single bond. Upon *homolytic cleavage*, the participating carbon atoms depart with one electron each from the C-C bond (of the two electrons shared by them). This type of fragmentation results in two radicals (Fig. 29.4). Two carbon atoms (with one unpaired electron each) come together to establish a covalent bond – in the reverse of homolytic fission. Free radicals are generally very reactive and unstable species. The larger the number of alkyl substituents on the carbon carrying the unpaired electron, the more stable is that free radical (due to hyperconjugation). The carbon atom (bearing the lone electron in a free radical) is sp^2 hybridized (trigonal) with the third p orbital bearing the lone electron. Although not common, enzymes catalyzing reactions with free radical reaction intermediates are known. Ribonucleotide reductase, involved in the biosynthesis of deoxyribonucleotide precursors of DNA, is an important example of this type.

The second mode of C–C bond cleavage is the heterolytic cleavage (Fig. 29.4). Fragmentation by *heterolytic cleavage* generates a pair of oppositely charged ionic species. The species carrying the positively charged carbon atom is *carbocation*. The other product of heterolytic cleavage is the *carbanion*, with a negatively charged carbon atom. The combination of a carbocation with a carbanion leads to the formation of a covalent C–C bond – which is the exact reverse of a heterolytic cleavage. Both forms of the charged carbon species, namely, carbocations and carbanions, are extremely unstable. The carbon bearing the positive charge in the carbocation is sp^2 hybridized (trigonal) with an empty unhybridized p orbital. The more the number of alkyl substituents on the carbon bearing the positive charge, the more stable the carbocation. The order of stability for carbanions is the exact reverse of carbocations. Furthermore, carbanion carbon is sp^3 hybridized with the lone pair occupying one of the vertices of a tetrahedron.

Enzymes make use of the ionic mechanism in most C–C bond cleavage and formation events. Since both carbocations and carbanions are unstable, how are they generated and stabilized during reaction? A carbocation is generated/stabilized in the context of the overall molecular structure. The positive charge may be distributed and stabilized through resonance. For instance, the carbonyl carbon (>C=O) can be a suitable carbocation for reaction. It has a large dipole moment with significant negative charge on its oxygen atom while the carbon atom bears an equal amount of positive charge. In effect the carbonyl group is a resonance hybrid of charged and uncharged structures, imparting a carbocation character to the carbon atom (Fig. 29.5). Similarly, any molecular arrangement that stabilizes the negative charge on carbon, in principle, supplies a carbanion. Quite often the carbanion is attached to a functional group that allows the negative charge to be delocalized by resonance. Distribution of negative charge over several atoms, in addition to the carbon atom in question, stabilizes the carbanion. An adjacent β-carbonyl group (and not an -α-carbonyl group!) often provides such an apparatus (Fig. 29.5). Due to such resonance stabilization, the reactivity of the bond β to carbonyl becomes $\sim 10^{33}$ times greater than a typical hydrocarbon bond. This unique reactivity underlies every C–C bond-breaking reaction of central metabolism (Rabinowitz and Vastag

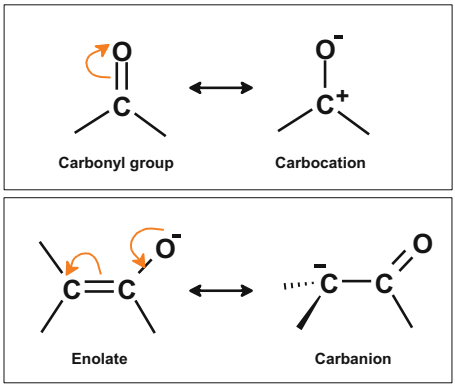


Fig. 29.5 Stabilization of carbocations and carbanions. The carbonyl group can act as a device to present carbocations (top) and carbanions (bottom) for reaction. The carbanion shown in effect is also an enolate

2012). Resonance delocalization of negative charge allows the carbon atom to react as a carbanion – without letting full formal negative charge to develop on that carbon. Chemistry involving enolates (such as with pyruvate) is an excellent example of this concept.

The unequal heterolytic cleavage of a covalent bond brings us to yet another important definition of electron-rich and electron-deficient groups/centers. A *nucleophile* (literally *nucleus lover*) is a species that forms a covalent bond to its reaction partner (the *electrophile – electron lover*) by donating both bonding electrons. All molecules or ions with a free pair of electrons can act as nucleophiles. Because nucleophiles donate electrons, they are by definition *Lewis bases* (see Chap. 30 Acid–Base Chemistry and Catalysis). A nucleophile is thus an electron-rich chemical reactant that is attracted by electron-deficient compounds. Examples of nucleophiles are anions such as COO^- or a compound with a lone pair of electrons such as $–NH_2$ (amine), NH_3 (ammonia), or H_2O (water). In the same sense, carbanions are nucleophiles and carbocations are electrophiles. The chemistry of formation of the C–C bond thus involves the nucleophilic attack by carbanion on to a carbocation. The carbonyl group in a molecule, for instance, often sets up the electrophilic (carbocation) or nucleophilic (carbanion) center required for many enzyme chemistries. A detailed discussion on nucleophiles, nucleophilic chemistry, and its role in catalysis is available in a subsequent chapter (Chap. 31).

29.2.2 Logic of Pushing Electrons and Moving Bonds

To appreciate how enzymes work, familiarity with two languages is necessary. One is the kinetic/thermodynamic description of reactions – that formed the significant thrust of earlier two sections (Part II and Part III). Second is the description of the reaction mechanism – which involves moving electrons and making/breaking of bonds. The first is physical and the second is chemical (largely organic chemistry!). The vast majority of organic reactions are polar in nature, where nucleophile and electrophiles participate. Electron flow is the key to molecular reactivity – nucleophiles donating electrons to the electrophiles. Curly arrows (shown in color in each reaction) are used to describe reaction mechanisms. A *curly arrow represents the movement of a pair of electrons* with the result that a bond is formed between a nucleophile and an electrophile. A simple example would be the association/dissociation equilibrium of a Bronsted acid. The lone pair electrons on the carboxylate oxygen of acetate are transferred to empty 1s orbital of H^+ (Fig. 29.6). Exact opposite of this electron pair movement occurs during dissociation of the acid – which is a bond cleaving event.

Charge is conserved in each step of a reaction – *net charge cannot be created or destroyed*. If we start with neutral molecules (like acetic acid) and make a cation (like H^+), we must make a corresponding anion (like acetate). Protonation of ammonia is yet another example of this conservation of charge concept (Fig. 29.6). There is one net positive charge on either side (H^+, reactant and NH_4^+, product). Curly arrows are

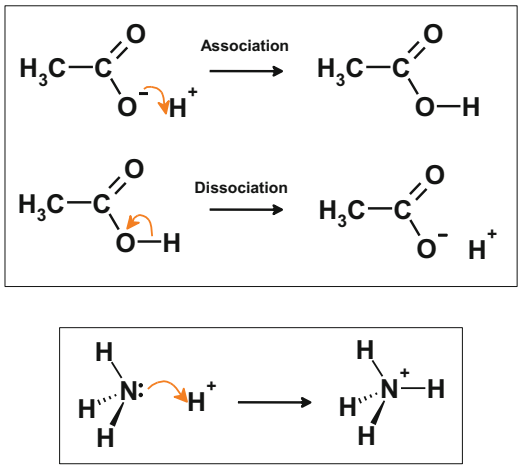


Fig. 29.6 Curly arrows are used to show the movement of electron pair. Ionization equilibrium of acetic acid (top) and protonation of ammonia (bottom) are shown as examples

also used to show movement of electrons within molecules. Enolization is an example where two lone pair shifts (two curly arrows; Fig. 29.5) occur.

Curved arrows are vital to understand reaction mechanisms. Curly arrows are important conceptual representation of electron pair movements in a chemical mechanism. Such arrows always represent the *movement of electrons and not atoms*. The tail of the arrow shows the source of electron pair – usually from a lone pair, a π bond or a σ bond. The arrowhead indicates the ultimate destination of the electron pair – often to an electronegative atom that can harbor a negative charge. Oxygen of a carbonyl group is one such atom (see Fig. 29.5).

A tricky question is to know where to begin the first arrow. It is usual to start pushing electrons (first curly arrow!) from the nucleophile, anion, or a lone pair. But some mechanisms are better understood as electron pulling – generally by a reagent (electrophile) such as a cation, an acid, or a Lewis acid. All the curly arrows in a given mechanism move in the same direction. However, we may a) either draw the first arrow from a nucleophile (electron pushing) or b) end the first arrow into an electrophile (electron puling).

The conventions and concepts used to write proper chemical mechanisms are summarized in the box below.

Guidelines to a Chemical Mechanism

In writing and understanding chemical reaction mechanisms, the following broad guidelines operate:

- Identify the nucleophilic and electrophilic atoms taking part in the reaction.
- Decide whether the mechanism involves electron pushing (arrow to begin from the nucleophile) or electron pulling (beginning at the electrophile).

(continued)

- Mark the lone pair of electrons on the nucleophilic atom.
- Draw curly arrow(s) to show the flow of electrons from an electron-rich center to an electron-deficient center.
- Multiple curly arrows always move in the same direction – they never meet head on or end up in a single atom.
- C, N, and O atoms can have a maximum of eight electrons in the outer valence shell (2s, $2p_x$, $2p_y$, $2p_z$) while H has two (1s). Carbon has a valency of 4.
- If you make a new bond to uncharged H, C, N, or O, you must break one of the existing bonds in the same step.
- Define the charges clearly and ensure that overall charge is conserved (before and after) in the mechanism.

Drawing curly arrows and writing reaction mechanisms are like learning swimming. Once you have mastered the skill (of course with some practice!), it is difficult to forget or make mistakes.

29.3 Stereochemical Course of Reaction

Majority of biomolecules are chiral compounds. The presence of a carbon atom bonded to four different groups (*abcd*) leads to chirality (asymmetry), and such atoms are termed *stereo-centers or chiral centers*. Consider alanine, for example. It is chiral because the α-carbon is bonded to four different groups, namely, –COOH, –NH_2, –CH_3, and –H. There can be two distinct three-dimensional arrangements (*configuration*) of the four groups around the α-carbon. The two nonsuperimposable mirror-image forms are called enantiomers – the L- and D-forms of alanine. In modern nomenclature, the two are designated as 2*S*-alanine and 2*R*-alanine, respectively. The L- and D-forms of alanine are identical in their physical properties except for their interaction with *plane-polarized* light. Hence such structural isomers are also commonly called *optical isomers*. It is possible that a molecule may have more than one chiral carbon in it. For instance, threonine has two asymmetric centers – one at the α-carbon and the other at β-carbon.

Glycine, unlike alanine, does not have a chiral α-carbon and is optically inactive. Apart from –COOH and –NH_2 groups, the other two substituents on its α-carbon are hydrogen atoms. However these two –H atoms are stereochemically distinct. Carbon centers that are surrounded by *aabc* groups are known as *prochiral* centers. For instance, the α-carbon of glycine or the C-1 of ethanol is prochiral. In general, a molecule is said to be prochiral if it can be converted from achiral (such as *aabc*) to chiral (*abcd*) in a single chemical step. Numerous biological reactions involve prochiral compounds. Nevertheless, the two identical substituents are selectively recognized by enzymes. The two H atoms attached to C-1 of ethanol are

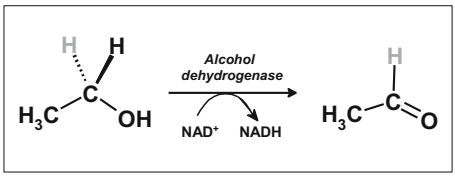


Fig. 29.7 Stereochemistry of alcohol dehydrogenase reaction. Only the *proR*-hydrogen of ethanol is selectively removed by the enzyme. The C-1 of ethanol is an example of prochiral carbon with *aabc* arrangement of groups around it. The *proR*-hydrogen is shown in black and *proS*-hydrogen is in gray

distinguished by alcohol dehydrogenase. In the chiral active site, environment ethanol is held in a fixed orientation – so that only *proR*-hydrogen is removed (Fig. 29.7). Other examples of such chiral discrimination at a prochiral center include aconitase (acting on citrate) and fumarase (acting on fumarate to form L-malate). Such discrimination is possible because by themselves enzymes are chiral catalysts. Enzyme active sites almost always provide a chiral environment and act in a stereo-specific manner. When this is not the case, then a nonenzymatic step may be involved.

The stereochemical course of an enzyme reaction may be determined by suitable isotopic labeling of substrate. The fate of this label is subsequently monitored to deduce the reaction path. For instance, the deuterium label in *proR* position of ethanol alone is removed by alcohol dehydrogenase – *proS*-hydrogen is retained in the product acetaldehyde (Fig. 29.7). In principle, one can construct chiral methyl groups with the three different isotopes (H, D, and T) of hydrogen. Similar stereochemical strategy is used to analyze the stereochemistry of phosphoryl transfer reactions. Notably, phosphates contain three apparently identical oxygen substituents and they can be labeled with ^{16}O, ^{17}O, and ^{18}O, the three isotopes of oxygen (we will have more on phosphate chemistry in a subsequent chapter).

It is important to know the exact chirality relationship between the reactant and the product. The absolute configuration of a compound (e.g., the reaction product) can be assigned by (a) converting it chemically into a compound of known stereochemistry and then measuring its optical activity, (b) complexing it with a known chiral reagent and then determining its relative configuration by NMR spectroscopy or X-ray crystallography, and (c) using an enzyme of known chiral specificity. For a more detailed treatment on stereochemistry, the reader is encouraged to consult elementary texts on organic chemistry.

29.4 Common Organic Reaction Types

Enzyme reactions involve breaking/making covalent bonds in their substrates. Such events involving covalent bonds are greatly influenced by the surrounding chemical environment and functional groups. A *functional group* is a group of atoms within a molecule that has a characteristic chemical behavior, such as a carboxylate

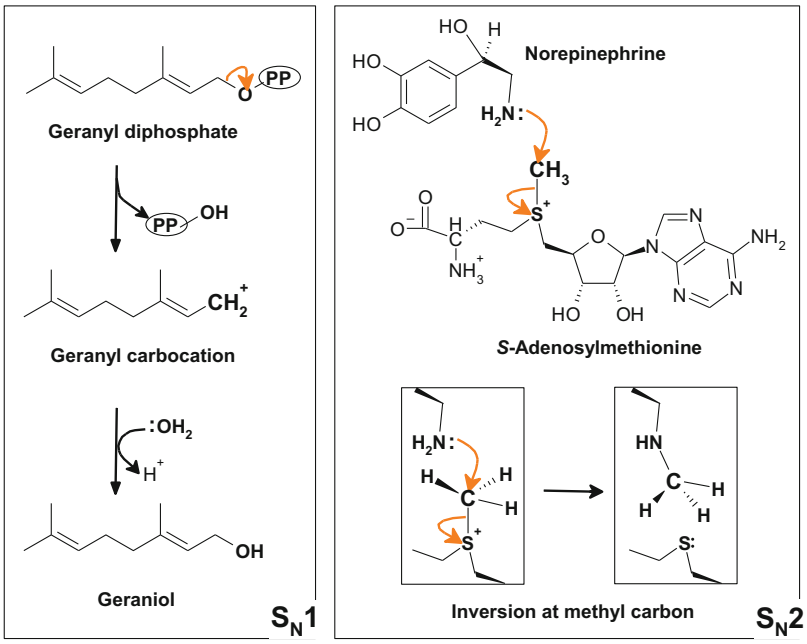


Fig. 29.8 **Nucleophilic substitution reaction mechanisms**. Examples of $\mathbf{S_N1}$ (geraniol synthase; left panel) and $\mathbf{S_N2}$ (phenylethanolamine N-methyltransferase; right panel) type reactions are shown

(–COOH) group in acetic acid or a thiol (–SH) in cysteine. A functional group that makes off with a pair of electrons (of the σ bond that is broken) is called an outgoing nucleophile or a *leaving group*. In a given reaction, we may come across an incoming nucleophile (^{in}Nu:) that replaces an outgoing nucleophile (^{out}Nu:). Although a vast variety exists, we will restrict ourselves to more common organic reaction types encountered in enzyme reactions here.

29.4.1 Nucleophilic Displacements

In a nucleophilic substitution (S_N type) reaction, one nucleophile (the leaving group) is substituted by another on a saturated sp^3-hybridized carbon atom. In an $\mathbf{S_N1}$ (**S**ubstitution, **N**ucleophilic, **1**st order) reaction, the substrate undergoes a spontaneous dissociation to generate a carbocation intermediate. After this rate-determining first-order event, the carbocation reacts with the substituting nucleophile to form the product. Thus S_N1 reactions occur in two steps and usually take place with tertiary or allylic carbon of the substrate. Conversion of geranyl diphosphate to geraniol is an example of S_N1 reaction (Fig. 29.8).

An $\mathbf{S_N2}$ (**S**ubstitution, **N**ucleophilic, **2nd** order) reaction takes place in a single step – where the incoming nucleophile attacks the electrophilic carbon with its electron pair. Because the incoming and outgoing nucleophiles are on opposite

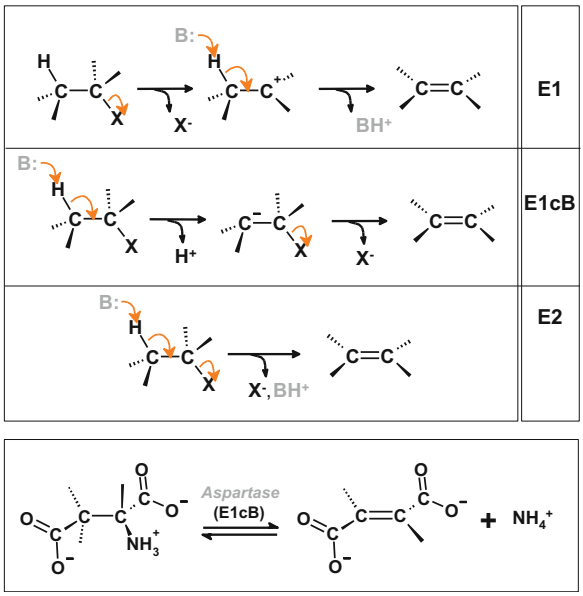


Fig. 29.9 Three possible elimination reaction mechanisms. While the **E1** reaction goes through a carbocation intermediate, the **E1cB** mechanism involves a carbanion intermediate. In the **E2** mechanism, breaking of C–H and C–X bonds is simultaneous. Aspartase reaction (shown below) follows **E1cB** mechanism where the C–N bond breaks to eliminate ammonium

sides (180° apart), the stereochemistry at the reacting center is inverted during an $\mathbf{S_N2}$ reaction. Methylation of norepinephrine to epinephrine by *S*-adenosylmethionine is one such reaction. The amine nitrogen of norepinephrine (incoming nucleophile) displaces *S*-adenosylhomocysteine (outgoing nucleophile or the leaving group) on the electrophilic methyl carbon atom (Fig. 29.8). Many other important examples of nucleophilic displacement include acyl, phosphoryl, and glycosyl transfer reactions. We will revisit few of them as specific cases in the later chapters.

29.4.2 Elimination Reactions

Reactions involving elimination of HX to yield an alkene are important in many biochemical pathways. Particularly common are dehydration (removal of H_2O) and deamination (removal of NH_3) reactions. An *elimination* reaction in the reverse direction simply describes an *addition* reaction. Addition and elimination reactions generally occur adjacent (at α,β position) to a carbonyl group. The substrates are normally thioesters, carboxylic acids, ketones, or aldehydes. Mechanistically elimination reactions are more complex and may be classified into E1, E2, or E1cB reaction types. The elimination of HX involves the cleavage of a C–H bond and the cleavage of C–X bond. The timing of C–H and C–X bond cleavages in the reactant determines the type of elimination reaction (Fig. 29.9). In **E1** (**E**limination **1**st order) mechanism, first the C-X bond breaks to generate a carbocation. This carbocation then undergoes base abstraction of H^+ (C-H bond break) to form the double bond. The **E1** reactions, found in organic chemistry, are rarely encountered in enzymology.

In the **E1cB** (**E**limination **1**st order, **c**onjugate **B**ase assisted) reaction, the C-H bond cleavage occurs first to give a carbanion intermediate. This carbanion in a subsequent step loses X^- to give the alkene. Eliminations with **E1cB** mechanism are quite common in biological chemistry – the carbanion intermediate being stabilized by the functional residues at the enzyme active site. A single-step **E2** (**E**limination **2**nd order) mechanism is followed when C–X and C–H bond cleavages are concerted, i.e., occur simultaneously (Fig. 29.9). When **E2** mechanism operates, the H and X are eliminated from opposite faces of the molecule (*anti* elimination). With **E1cB** reaction however, reaction stereochemistry may be *anti* or *syn* (same side) – depending on the active site geometry.

A well-documented case of **E1cB** mechanism is aspartase reaction (Fig. 29.9). Aspartases from different organisms show high sequence homology, and this homology extends to functionally related enzymes such as the class II fumarases, the argininosuccinate, and adenylosuccinate lyases. Others examples include dehydratases like 3-dehydroquinate dehydratase (shikimate pathway) and β-hydroxyacyl ACP dehydratase (fatty acid biosynthesis). Since addition/elimination reactions are reversible, a dehydratase performs hydration in the opposite direction. For instance, *trans*-2-enoyl CoA hydratase adds water across a double bond during fatty acid oxidation.

The dehydration of a β-hydroxycarboxylic acid by aconitase (citrate → *cis*-aconitate → isocitrate) is likely an example of **E2** elimination (Fig. 29.9).

29.4.3 Carbon–Carbon Bond Formation

Reactions that lead to formation or cleavage of C–C bonds are central to metabolic logic. They are the key steps in building (synthesis) and degradation (catabolism) of diverse cellular metabolites. Some of the most fundamental reactions of this type involve carbon dioxide as substrate (carboxylation) or product (decarboxylation). These reactions will be discussed a little later (Chapter 34). A few less widely distributed C–C bond formation reactions include steps in terpene synthesis (via carbocation intermediate) and lignin biosynthesis (via free radical intermediate). Besides carboxylation/decarboxylation, two other C–C bond-forming reactions are of great importance. Significantly both of them are carbonyl condensation reactions. A carbonyl condensation results in bond formation between the carbonyl carbon of one partner and the α carbon of the other carbonyl partner. This reactivity is because the α hydrogen of a carbonyl compound is weakly acidic and susceptible to base capture. The enolate so formed (in its carbanion form; see Fig. 29.5) functions as a nucleophile.

The *aldol reaction* is the condensation of two carbonyl compounds (aldehyde or ketone) via an enolate intermediate. It yields a β-hydroxy carbonyl compound from two molecules of aldehyde or ketone. This is an example of nucleophilic addition reaction. One molecule reacts with base to generate a nucleophilic enolate, which then attacks the carbonyl carbon of the second molecule (Fig. 29.10). Reverse of aldol condensation is also possible and constitutes a C–C bond cleavage reaction.

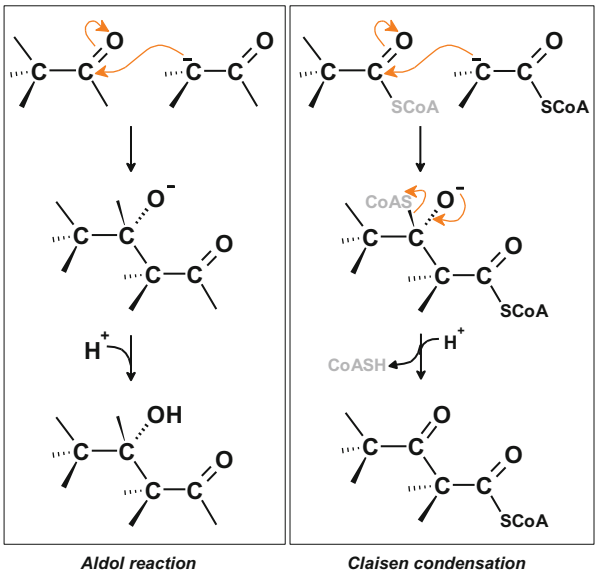


Fig. 29.10 Biochemically important carbon–carbon bond-forming reactions. Aldol reaction (left panel) yields a β-hydroxy carbonyl compound from two molecules of aldehyde or ketone. The key step is the nucleophilic addition of enolate (carbanion) to the other >C=O group. In Claisen condensation (right panel), two molecules of an ester combine to yield a β-keto-ester. The key event here is the nucleophilic acyl substitution by the enolate (carbanion). Example of a thioester (acetyl CoA) condensation is shown

The enzymes which catalyze aldol reactions are known as aldolases. Fructose-1,6-bisphosphate aldolase reaction from glycolysis is a prototype. Other examples include citrate synthase (aldol condensation) and ATP citrate lyase (aldol cleavage). A few amino acids can also undergo aldol-type cleavage. The carbanion formed from such C–C bond breaks is stabilized through a cofactor like pyridoxal phosphate (e.g., serine hydroxymethyltransferase).

Carboxylic esters can react to form β-keto-esters, via an ester enolate intermediate. Such reactions are known as *Claisen condensation* reactions. Claisen ester condensation is more difficult than an aldol reaction because the C–H bond adjacent to an ester is significantly less acidic than the proton next to a ketone. The carbonyl group of a thioester is more ketone-like and better suited for Claisen condensation. Therefore we often encounter thioesters of coenzyme A (CoA) in biological reactions. In principle, one molecule of ester reacts with base to give a nucleophilic enolate ion. This enolate adds to the second molecule in a nucleophilic acyl substitution reaction (Fig. 29.10). The initial alkoxide expels the leaving group (thiolate is a better leaving group – hence thioesters!) to regenerate a carbonyl group and form the β-keto-ester product. There are many examples of Claisen reactions involving acetyl CoA in biological systems. These include condensation reactions in the biosynthesis and assembly of fatty acids, polyketides, and steroids. Two acetyl CoA units condense to form 3-ketobutyryl CoA, which in turn condenses with another molecule of acetyl CoA to give hydroxymethylglutaryl CoA (HMG CoA – onward to cholesterol biosynthesis).

Claisen condensation reaction is reversible; a β-keto-ester can be cleaved by a suitable base to yield two ester molecules. It is worth noting that an essential reversal

of Claisen condensation reaction occurs in fatty acid catabolism. For example, thiolysis of β-ketoacyl CoA each time releases one molecule of acetyl CoA.

29.5 Summing Up

The variety of chemical reactions catalyzed by enzymes is vast. Indeed some of the chemistry – like the formation of a C–P bond – was novel even to organic chemists. Nature of the reaction catalyzed by enzymes forms the basis of EC classification. The six general reaction categories include oxidation–reduction reactions, group transfers, hydrolysis, lyase steps, isomerizations, and synthetic steps. At the mechanistic level, nucleophiles, nucleophilic attack, and general acid–base catalyzed proton transfers permeate most of bioorganic chemistry and enzymology. A basic understanding of their reactivity is essential to appreciate enzyme chemical mechanisms. These aspects will be elaborated in the two subsequent chapters.

Reference

Rabinowitz JD, Vastag L (2012) Teaching the design principles of metabolism. Nat Chem Biol 8:497–501

Suggested Reading

Clayden J, Greeves N, Warren S (2014) Organic chemistry, 2nd edn. Oxford University Press, Oxford ISBN:9780198728719

McMurry JE, Begley TP (2015) The organic chemistry of biological pathways, 2nd edn. WH Freeman, New York ISBN:10 193622156X, ISBN:13 978-1936221561