Chapter 5
The Regulation of Metabolisms

In a cell, the fluxes of matter and energy are highly controlled so that cells can maintain their organization and multiply when needed. We have discussed in the previous chapter that consecutive reactions can be driven through coupling favorable reactions to unfavorable reactions, many of those benefiting from the ΔG^{o} of ATP hydrolysis. Synthesizing ATP involves using energy associated to the chemical processing of nutrients or molecules stored for this purpose (catabolic metabolism). When in excess, the nutrients tend to engage a series of reactions whose end products are the storage molecules (anabolic metabolism) for later use. This shift implies a complex network of metabolisms that must be inhibited or activated. Inhibition and activation occur selectively at specific reactions which in turn occur in specific locations and precise timings inside the cells. In addition, the shift requires that different cells in the same tissue or in different tissues operate coordinately. Liver, adipose tissue, muscle, and brain, for instance, need to be coordinated so that when the brain and muscle require specific nutrients to operate, this process does not conflict with processes in other organs and no failure of the body function as a whole occurs.

Only specific selected sets of reactions take place at a given time in each organelle of a cell. Chemical entities (hormones) circulate in the body and are captured by cell receptors that trigger short chemical reaction sequences, generically named "signal transduction pathways" (see Box 5.1 and Fig. 5.1) upon binding. In the end, these short sequences of chemical reactions modify enzymes stimulating or inhibiting the catalysis of metabolic reactions (Fig. 5.1). The same hormone may be sensed by different cells in different organs. The metabolic events triggered in different organs are not necessarily the same but are coordinated. For instance, during prolonged aerobic exercise, muscles are consumers of fatty acids, which must be mobilized from the adipose tissues; in this situation both the muscle and the adipose tissue are not activating the same metabolic pathways, but they are certainly coordinated. A drop in glycemia (glucose concentration in blood) leads to the release of glucagon, a peptide hormone synthesized in the pancreas that binds to receptors in hepatocytes (liver cells) and triggers events that activate reactions leading to the

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production of glucose (see Chap. 9). Glucose is then released in the blood through the optimized mesh of capillaries throughout the whole liver. This matter will be revisited later in Box 8.1.

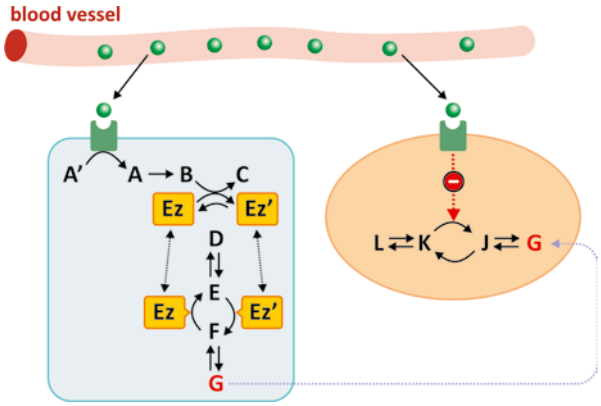


Fig. 5.1 When the hormone (green sphere) binds to its receptor, which may be located on the membrane or may be intracellular, this translates into a short series of chemical reactions (exemplified as compounds A′ to C) named "signal transduction." One of the consequences may be that one enzyme is modified (Ez to Ez′ in the picture). This transformation may be phosphorylation, for instance (Ez′ being in this case Ez with a covalently bound phosphate group). In this hypothetical situation in which Ez and Ez′ catalyze opposing reactions irreversibly, the practical effect of the hormone is to dictate the sense of metabolic reactions. In the case depicted in the figure, the presence of the hormone in blood generates metabolite G into the cell on the left. The same hormone may be acting at the same time in different cells (colored light blue and salmon) in different organs triggering different metabolic pathways. The hormone may stimulate G-consuming pathways in cells from other organs (e.g., leading to inhibition of the enzyme that converts K in J)

Box 5.1: Biosignaling, the Communication Among Cells and Inside Cells

In a complex organism, such as the human body, having specialized systems, with specialized organs, specialized tissues, and specialized cells, coordination requires fine-tuning and reliability. The homeostasis of the human body requires that the action of different organs is not conflicting. Imagine a situation such as prolonged starvation. The liver synthesizes glucose and releases glucose in blood to keep the glycemia within safe levels for the brain to operate. What would happen if other organs such as striated muscle were subtracting glucose from the blood to synthesize glycogen, for instance? This conflict between liver action and striated muscle action would be fatal or, at least, result in a paramount waste of energy and matter. There are mechanisms that prevent conflicts of this kind. These mechanisms coordinate the action of cells that may be in contact with each other in the same tissue or in very remote

(continued)

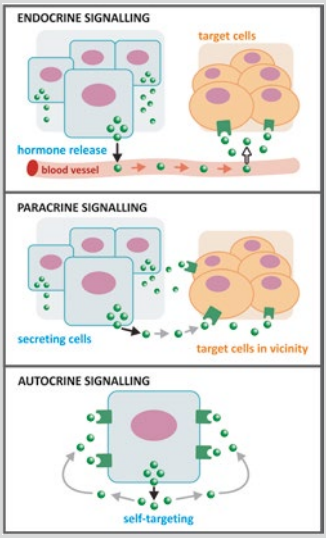
Box 5.1 (continued)
locations relative to each other, such as different organs. There are molecules that serve as "signals" that are released and trigger synchronized and compatible events in different cells. This is known as cellular communication or biosignaling.

Naturally, biosignaling, such as any communication process, requires that there is a source for the signal (e.g., hormone), the means for the signal to disseminate, and a receptor, or receptors. The signal is a molecule that is synthesized, so the steps of biosignaling are (1) synthesis of the molecule that will serve as signal; (2) release of the signal molecule; (3) transport/dissemination to the target cells, i.e., cells with the receptors that bind and are responsive to the signal molecule; (4) interaction with the receptor, usually a protein, in the target cell; (5) triggering intracellular chemical or physical events that result directly from signal–receptor interaction ("signal transduction"); and (6) generation of other events, frequently a series of events ("cascade" or "signaling pathway"), which constitutes the final response of the cell to the signal.

Hormones, neurotransmitters, prostaglandins, growth factors, and cytokines, such as interleukins and interferons, serve as biochemical communication signals. These signals may act at short range or long range (see figure). Short-range communication involves diffusion of signals in the extracellular medium in the immediate vicinity of the cell that secretes the signal molecule. This is named paracrine signaling. Synaptic signaling is an example of paracrine signaling: neuronal termini release neurotransmitters that bind to receptors in the postsynaptic cells. Long-range signaling is known as endocrine signaling. In this case, specialized cells in specialized organs synthesize hormones, which are usually released into the bloodstream and distributed throughout the body. Hydrophilic hormones are soluble in blood and are easily distributed; hydrophobic hormones might need transporters and/or have severe limitations in the concentrations they can achieve in blood. Steroid hormones, for instance, are hydrophobic hormones derived from cholesterol, and they need to be associated to carrier proteins to be distributed in the body through the blood.

(continued)

Box 5.1: (continued)



A third form of signaling exists in addition to endocrine and paracrine: autocrine. However, this signaling is mainly found in pathological conditions such as cancers. Tumor cells release growth factors that bind to receptors in the surface of the same cell that releases them, stimulating cell growth and cell division. These events become unbalanced and the tumors grow uncontrolled.

Most molecular signals have very high selectivity and affinity for their receptors. High selectivity means that the receptor is responsive only for a very precise structure of the ligand, i.e., the molecule that serves as signal. High affinity means that the binding equilibrium of the ligand signal to the receptor is very extensively shifted toward the bound ligand–receptor complex, which in practice means that very low concentrations of the ligand generate an integrated high response from the receptor. Nanomolar (10^{-9} M) concentration of hormones usually suffices to trigger physiological responses because the high affinity for the receptor compensates the low concentration of the ligand.

Depending on whether the hormones may translocate through the plasma membrane or not, the receptors may be located on the surface of cells, or inside the cells, in the cytoplasm or nucleus. Adrenalin, insulin, and glucagon, for instance, have surface receptors; in contrast, testosterone and progesterone, for instance, bind to receptors intracellularly.

(continued)

Box 5.1: (continued)
Upon binding of the ligand to receptor, the signal transduction process may be of three main different types depending on the functionality of the receptor:

1. When the receptor is an ionic channel, the ligand may activate or inhibit the flux of ions through the channel. This is the case of the receptor of acetylcholine in the neuromuscular junction.
2. When the receptor is coupled to G protein (guanosine nucleotide-binding proteins), binding of the ligand causes conformational changes in the receptor that indirectly activate the G protein, which detaches it from the receptor and binds to adenylate cyclase (also known as adenylyl cyclase) or a phospholipase or another enzyme that catalyzes the formation of the molecules so-called second messengers. Second messengers then initiate series of reactions that will interfere with metabolic processes. Cyclic AMP, cyclic GMP, inositol triphosphate, and diacylglycerol are examples of second messengers, and the receptors of adrenaline and serotonin are examples of G protein-coupled receptors.
3. When the receptor has catalytic tyrosine kinase activity, binding of the ligand causes conformational changes that make the enzyme active and thus able to phosphorylate proteins in Tyr residues using ATP as a source of phosphate. The insulin receptor is of this kind.

The intracellular signaling pathways that follow the binding of the signal molecule to the receptor are diverse. The main signaling pathways associated to the regulation of metabolism are addressed individually in the main text.

Glycemia is a key factor in energetic metabolism as the brain uses exclusively glucose as a source of ATP production (the only known exception occurs in long-term starvation; see Sect. 9.3.4), and there is no mechanism to keep a higher concentration of glucose in the central nervous system than in blood because glucose transporters across the blood–brain barrier do not operate against a concentration gradient. Therefore, the coordination of metabolisms occurring in different organs simultaneously is such that glucose concentration in the blood is kept higher than a specific threshold. Values under this threshold produce loss of conscience, coma and death may occur (Fig. 5.2). Given the importance of glycemia control and the investment represented by regulatory pathways it is not surprising that biochemists tend to overemphasize the importance of glucose as a nutrient compared to amino acids and lipids. Yet, lipids and proteins are the most important energetic reserves in the human body (see Table 9.1). Energetic metabolisms are often but erroneously associated to carbohydrate metabolism only. In reality, fatty acid, amino acids, and glucose metabolisms are interconnected. But despite the connections, the metabolites pertaining to each pathway are not necessarily interconvertible. Lipids are typically storage molecules so they cannot be converted to glucose or proteins; it can only be converted in metabolites that in principle will follow reaction routes leading to ATP synthesis (Fig. 5.2).

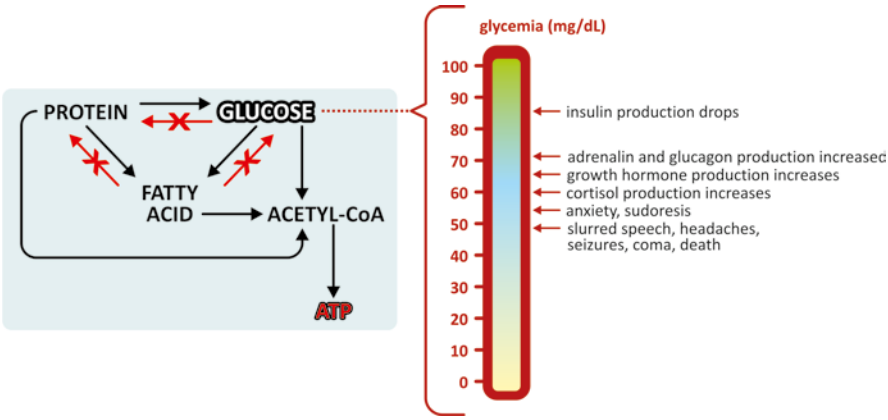


Fig. 5.2 Allowed and forbidden conversion routes in human metabolism. Glucose and fatty acids cannot be converted to proteins. Fatty acids cannot be converted to proteins or glucose. This implies that all excess nutrients from food intake end in lipids, stored in adipose tissue. Glycemia (the concentration of glucose in blood) needs to be kept above a certain threshold, even in the absence of food intake. In this case, synthesis of glucose is possible using amino acids from muscle protein degradation

In summary, metabolisms need regulation both inside the cells and in different tissues, frequently in different organs. Therefore, different levels of regulation exist. They are associated to mechanisms with different efficacies, time scales, and areas of impact.

5.1 Levels of Regulation: Impact and Time Scale

Think about your simplest daily routines, like eating, moving, and sleeping. They seem extremely banal and simple, almost unnoticeable in our lives, but they are demanding challenges from the metabolic point of view. They involve relatively fast alternation between states of nutrient abundance (meals) and nutrient absence (fasting), rest (e.g., sleeping), moderate exercise (e.g., walking), exercise bursts (e.g., short run to catch a bus) or intense enduring exercise (e.g., athletic running or swimming), and all possible combinations of feeding state with exercise. At the same time, there are processes that constantly consume energy such as brain activity (any basal activity, not only mental work), heartbeat, or keeping body temperature. Metabolic adaptation to fluctuating conditions on top of basal permanent activities requires mechanisms of metabolic regulation that are (1) fast, (2) efficient, and (3) robust. In fact these factors are not independent: robustness comes from redundancy of mechanisms—having more than one mechanism to assure the same effect decreases the chances of failure; redundancy is a cooperative combination of similar mechanisms—a fast one, albeit not so efficient, and a very efficient one, albeit not very fast. Usually fast regulation mechanisms consists in controlling the availability of substrates and/or the activity of enzymes, while very efficient regulation

mechanisms consist in controlling the presence of enzymes, i.e., their genetic expression, which is naturally a slower process. Genetic expression is usually dependent on hormones, which guarantee coordination among the metabolism in different organs, all under the influence of the same circulating hormones. This interplay between mechanisms is illustrated in Fig. 5.3.

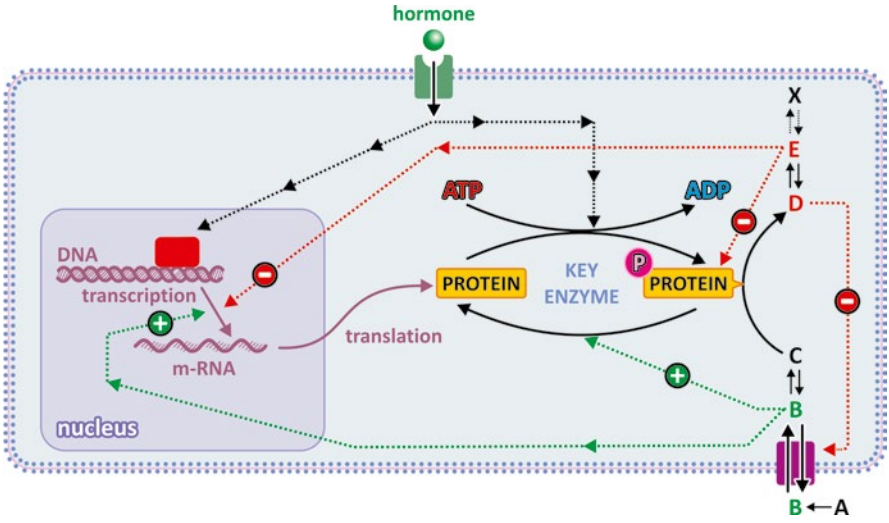


Fig. 5.3 The different levels of regulation are (1) substrate availability through control of transport across membranes (metabolite B); (2) enzyme activation by upstream metabolites (B) in the metabolic pathway and/or enzyme inhibition by the product of downstream metabolites (E) in the metabolic pathway; (3) activation or inactivation of enzymes by covalent attachment of a phosphate group (P), which is usually the end effect of a cascade of events caused by the binding of a hormone to its receptor (signal transduction); and (4) regulatory enzymes' expression/translation controlled by hormones through signal transduction inside cells. Hormone-dependent mechanisms are slower; the direct effect of metabolites on enzymes (activation or inhibition) is faster because enzymes coexist with regulatory metabolites (named effectors) in the same cell compartment

5.2 Inhibition and Activation of Enzymes by Ligands

As mentioned in the previous section, controlling the activity of enzymes (i.e., changing their kinetic characteristics such as K_M or V_{max}) is a fast way to influence the rate and course of metabolic pathways. Physical factors such as temperature and pH have a direct effect on the structure of proteins because they alter the intramolecular forces that stabilize protein folding. Therefore, they affect the activity of enzymes. Enzymes have optimal temperature and pH ranges to operate (Fig. 5.4). Below and above the optimal ranges, the reaction velocity decreases. Human enzymes are adapted to body temperature and have optimal activity around 37 °C. They are also adapted to the pH of their micro-environments. Pepsin and other stomach enzymes, for instance, have optimal activity at acidic pH.

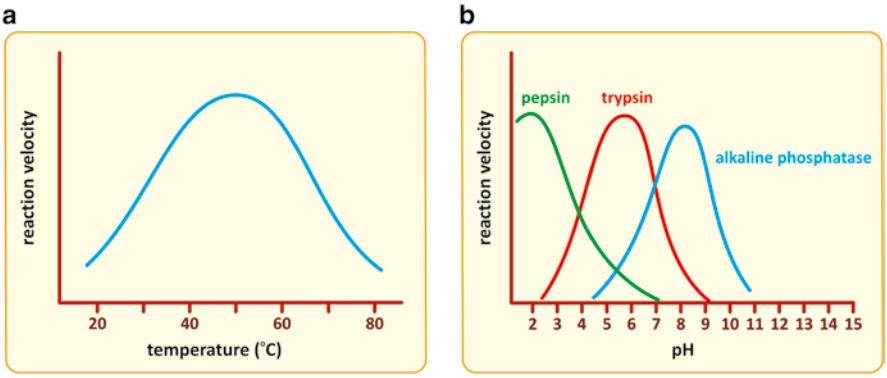


Fig. 5.4 Enzymes suffer structural alteration in their folding when physical factors such as temperature (**a**) or pH (**b**) change, which in turn cause alterations in the kinetics of catalysis. The reaction velocity is maximal in a limited interval of temperature or pH, decreasing for higher or lower values. Enzymes are adapted to the local pH of the different micro-environments of the human body from very acidic (such as pepsin) to alkaline (such as alkaline phosphatase), as illustrated in panel (**b**)

There are microorganisms living in extreme environments such as hot springs, at low pH and high temperatures. They are called extremophiles for this reason. Nevertheless, even in these cases, there is adaptation of the enzymes to the local pH and temperature. These molecules are very appealing to the biotechnological industry because they can be used in industrial processes that combine extreme pHs with high temperature; however, they are not in the realm of human biochemistry and will not be further discussed here.

In principle enzymatic activity could be modulated by changing pH or temperature, but this is not an option as these factors do not affect specifically a single enzyme in a specific metabolic pathway. The inhibition or activation of an enzyme has to be very selective. Shutting down a metabolic pathway demands inhibition and/or activation of very specific enzymes (recall Sect. 4.2.1), which is not compatible with the manipulation of pH or temperature. Instead, having small molecules that bind specifically to unique binding sites of enzymes, affect their conformation, and influence their kinetics is a much better way to specifically modulate the activities of selected enzymes. These small molecules are called ligands, and they can slow (inhibitors) or accelerate (activators) the catalytic process.

In the case of an enzyme obeying Michaelis–Menten kinetics, ligands may in principle affect V_{max}, K_M, or both. The molecular mechanisms behind the influence of ligands on V_{max} or K_M may be very diverse, even if the end result is the same. Two different inhibitors may impact on V_{max}, for instance, binding to different sites of the same enzyme and producing different effects on enzyme structure. In other words, the alterations ligands cause on kinetic parameters tell nothing about *how* ligands and enzymes interact. To relate kinetics with mechanism of interaction, one has to resort to models, i.e., hypothetical arrangements and postulated events, and deduce on the final in kinetics.

This means that the examples in Box 4.4 can be extended to the case in which an additional molecule, the inhibitor or the activator, interacts with the enzyme E_z, in

addition to S (the substrate) and P (the product). Let's work on a simple example. Postulating that there is a ligand that binds to E_z and prevents S from binding to E_z (in this case the ligand acts as an inhibitor and will be represented by I), the reaction scheme is composed of

$$E_z + S \rightleftharpoons E_zS \rightarrow E_z + P \quad \left(\text{The unperturbed catalysis itself}\right) \tag{5.1}$$

$$E_z + I \rightleftharpoons E_zI \quad \left(\text{The action of the inhibitor}\right) \tag{5.2}$$

The action of I is to prevent part of E_z to take part in the reaction. The effective concentration of E_z available to interact with S is decreased, and naturally, K_M appears to be increased. Having less extensive binding of E_z to S appears to be a decreased affinity of E_z to S. In reality the intrinsic binding of E_z to S is not affected in its nature; it is the "sequestration" of E_z by I that causes the alteration in K_M. For very high S concentration, S is so abundant relative to I that, in practice, the influence of I on binding of E_z to S is not relevant and so V_{max} remains constant. V_{max} is $k_2.[E_z]_0$ (Box 4.4) and I do not change k_2 or $[E_z]_0$. The reaction velocity vs. [S] plot shows a decreased slope at small [S] but the same asymptotic value (V_{max}) in the presence of I when compared to the plot in the absence of I (Fig. 5.5).

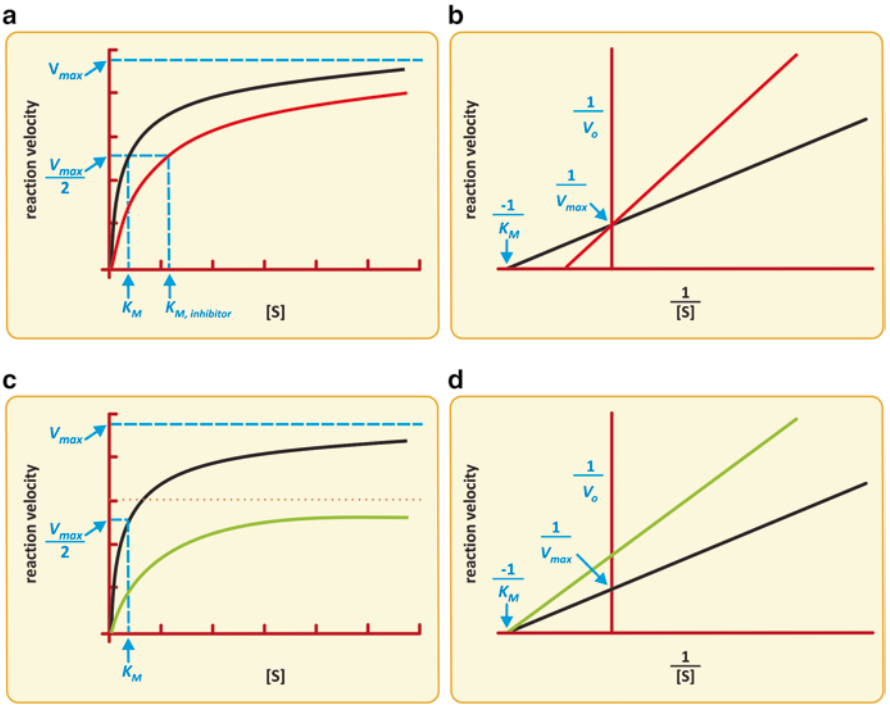


Fig. 5.5 Two possible effects of an inhibitor on the Michaelis–Menten kinetics of an enzyme. (**a**, **b**) K_M is increased with no alteration in V_{max}. (**c**, **d**) V_{max} is decreased with no alteration of K_M. Simultaneous alterations on V_{max} and K_m are also possible (not shown), which are the most frequent situations in practice

If I binds to E_z irreversibly $\left(E_z + I \rightarrow E_z I\right)$, part of enzyme population is permanently blocked by I, so the effective concentration of E_z free to interact with S is decreased even at high concentrations of S. In this case, V_{max} is decreased (recall again $V_{max} = k_2 \cdot [E_z]_0$—Box 4.4).

In the reaction scheme (5.1) and (5.2) shown above, E_z was assumed to bind to S or I but not to both simultaneously. This is expected to occur in cases in which S and I compete for the active catalytic site of the enzyme. This mechanism is thus called "competitive." There are other reaction schemes that consider simultaneous binding of I and S to E_z (which implies that they are not competing for the same binding site—"non-competitive" inhibition):

$$E_z + S \rightleftharpoons E_z S \rightarrow E_z + P \quad \left(\text{The unperturbed catalysis itself}\right) \tag{5.3}$$

$$E_z + I \rightleftharpoons E_z I \quad \left(\text{The action of the inhibitor}\right) \tag{5.4}$$

$$E_z I + S \rightleftharpoons E_z SI \quad \left(\text{Enzyme binding S after binding I}\right) \tag{5.5}$$

$$E_z S + I \rightleftharpoons E_z SI \quad \left(\text{Enzyme binding I after binding S}\right) \tag{5.6}$$

In this case, binding of I to E_z hinders the catalytic process but not the binding of S to the catalytic site itself, so V_{max} is decreased but K_M is not altered (Fig. 5.5). Even at high [S], a fraction of E_z is bound to I. The total amount of E_z available for catalysis is decreased and V_{max} is also decreased (again recall that $V_{max} = k_2 \cdot [E_z]_o$—Box 4.4). In practice, it is not frequent to find inhibitors that perturb the structures of proteins with a selective effect on V_{max} leaving K_M unchanged but non-competitive inhibition retains didactic interest.

It is interesting to note that V_{max} is also decreased in cases in which k_2 is decreased. This may happen when E_zSI in reactions 3 and 4 above retains catalytic activity $\left(E_z SI \rightleftarrows E_z S + P\right)$ but at slower rate when compared to free E_z. The catalytic rate constant (k_2) is decreased in the fraction of enzyme associated to I (E_zI) and V_{max} decreases.

It may also happen that E_z bind ligands that have the opposite effect of inhibitors on E_z: to increase k_2, thus increasing the velocity of the catalytic process. In this case the ligand is called an "activator," as opposed to "inhibitor." There are also activators that decrease K_M. The binding of S to E_z may be affected when the structure of the catalytic site of E_z is altered by the conformational changes caused by the binding of the activator to its specific binding site in the protein.

Depending on the characteristics of the reaction schemes, namely, stoichiometries of interactions, diversity of interacting molecules, reversibility of the reactions, etc., the expected kinetics may vary greatly. Figure 5.6 has simple illustrative examples based on Michaelis–Menten kinetics.

When performing work on enzymology, one has to bear in mind that there is no direct unequivocal correspondence between the experimental kinetics, reaction schemes, and molecular mechanisms of action. One may conceive mechanisms,

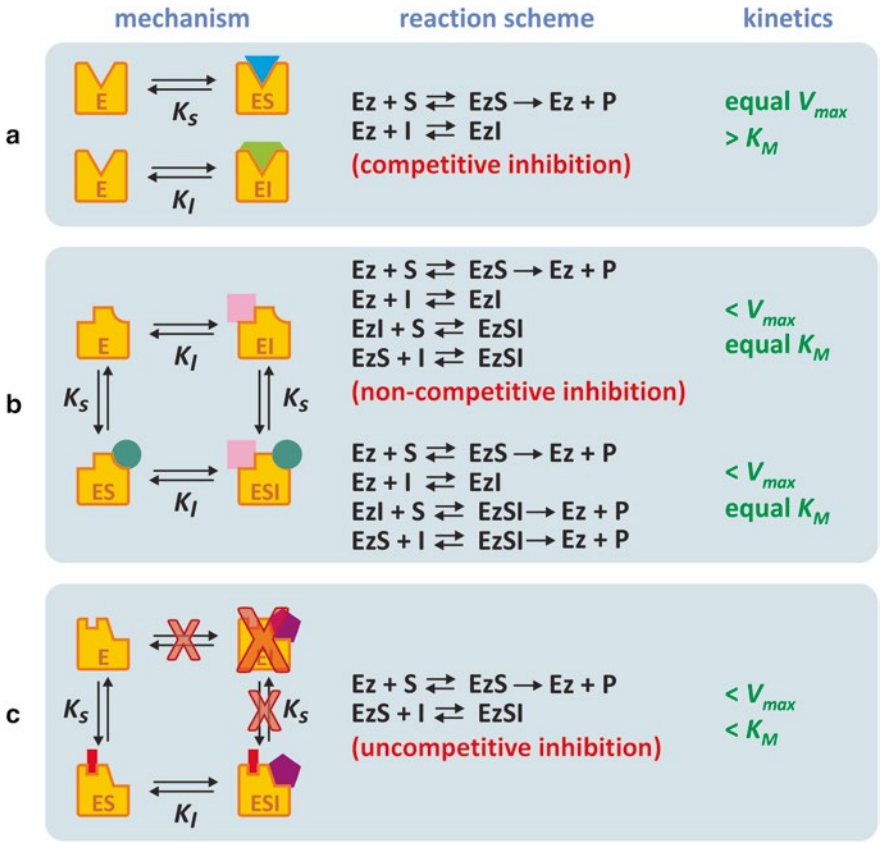


Fig. 5.6 Four examples of reaction mechanisms and associated kinetic alteration in Michaelis–Menten or Michaelis–Menten-like enzymes. Depending on the mechanisms and respective reaction scheme, the resulting apparent V_{max} and K_M may differ or not from V_{max} and K_M in the absence of the inhibitor, I. The first mechanism is named "competitive inhibition," but the other mechanisms bear names that are not always consensual. The second and third mechanisms are usually referred to as "non-competitive" and the fourth as "uncompetitive"

infer the corresponding underlying reaction scheme, deduce mathematically the associated kinetics, and study the match of the deduced kinetics to the experimental data, but the inverse is not possible. It is not possible to deduce unambiguously mechanism from experimental kinetic curves. In this way, it is premature to deduce a competitive mechanism in a situation in which experimental data show higher K_M and equal V_{max} as other mechanisms may result in similar kinetics. However, for most practical purposes in the health sciences, the exact mechanisms of action are not all that relevant, and this reasoning of enzymology research practice will not be further developed.

It is worth stressing that all that has been mentioned about enzymes having a Michaelis–Menten (or a Michaelis–Menten-like) kinetics is valid with adaptations for enzymes having more complex kinetics such as sigmoidal (Fig. 5.7). Thus, the

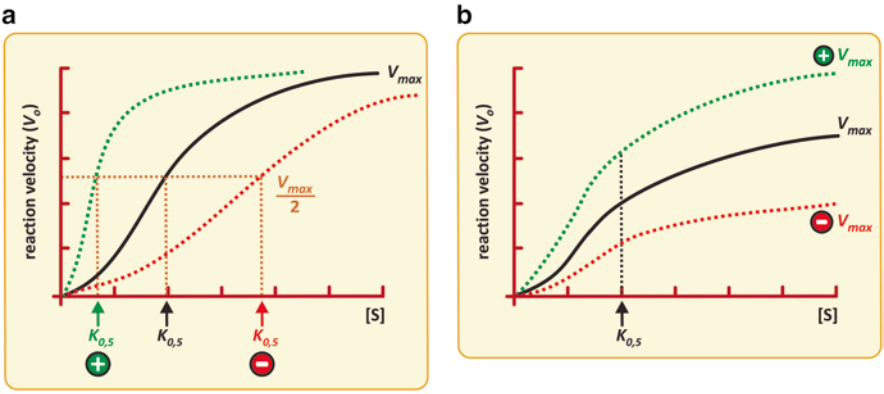


Fig. 5.7 Effect of activation and inhibition in sigmoidal kinetics of catalysis. The impact may be on the [S] needed to achieve 50 % of the maximal velocity ($K_{0.5}$) (**a**) or the maximal attained velocity, V_{max} (**b**)

general concept of inhibiting or activating enzymes using small molecules as ligands may potentially apply to all enzymes in metabolism.

The importance of enzyme inhibitors in drug discovery and development is huge. Many of the drugs in current medicines target enzymes (Table 5.1) and were conceived to block processes in which those enzymes are essential. Inhibiting

Table 5.1 Examples of drugs, targeted enzymes, and field of therapy

Drug	Target enzyme	Field of therapy
Aspirin	Cyclooxygenase	Anti-inflammatory
Captopril and enalapril	Angiotensin-converting enzyme (ACE)	Antihypertension
Simvastatin	HMG-CoA reductase	Lowering of cholesterol levels
Desipramine	Monoamine oxidase	Antidepression
Clorgyline	Morpramine oxidase A	Antidepression
Selegiline	Morpramine oxidase B	Treatment of Parkinson's disease
Methotrexate	Dihydrofolate reductase	Anticancer
5-Fluorouracil	Thymidylate synthase	Anticancer
Viagra	Phosphodiesterase enzyme	Treatment of male erectile dysfunction
Allopurinol	Xanthine oxidase	Treatment of gout
U75875	HIV protease	AIDS therapy
Ro41-0960	Catechol-O-methyltransferase	Treatment of Parkinson's disease
Omeprazole	H^+/K^+ ATPase proton pump	Ulcer therapy
Organophosphates	Acetylcholinesterase	Treatment of myasthenia gravis, glaucoma, and Alzheimer's disease
Acetazolamide	Carbonic anhydrase	Diuretic
Zileuton	5-Lipoxygenase	Anti-asthmatic

HMG-CoA reductase, for instance, results in the inhibition of cholesterol synthesis. One of the most common strategies to create inhibitors is to synthesize molecules that are similar enough to the substrate to bind the catalytic site but incapable of suffering catalysis. Figure 5.8 shows several examples of natural substrates and similar molecules that act as inhibitors of the enzymes. In some cases binding of the inhibitor to the enzymes is reversible, but in other cases, inhibitors react covalently with the enzyme and the binding is thus irreversible in practice.

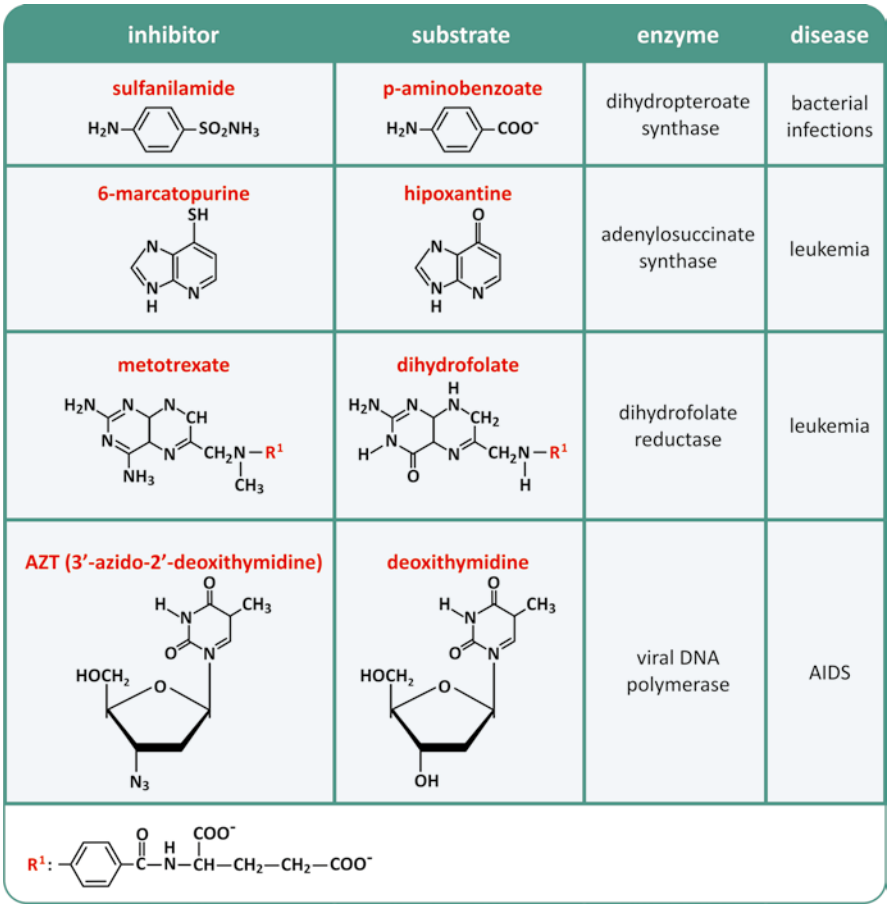


Fig. 5.8 Examples of enzyme inhibitors that are very similar in chemical structure to the natural substrates

5.2.1 Nomenclature of Ligands

Ligands other than the substrates that have a significant effect on the velocity of catalysis are generally called effectors. They can be activators (speed catalysis up) or inhibitors (slow down catalysis). In principle, effectors bind and dissociate from

enzymes without undergoing chemical modification, in contrast to substrates. For historical reasons, some ubiquitous substrates, such as NADH or NADPH, are often named separately as coenzymes. These so-called coenzymes are small molecules that transfer or accept groups from another substrate. This should not be confused with prosthetic groups. Prosthetic groups are chemical entities other than amino acid residues covalently attached to enzymes, such as heme groups of iron–sulfur centers (Fig. 5.9). They may have a direct role in catalysis, but they are not considered substrates as they are part of the enzyme itself in chemical terms.

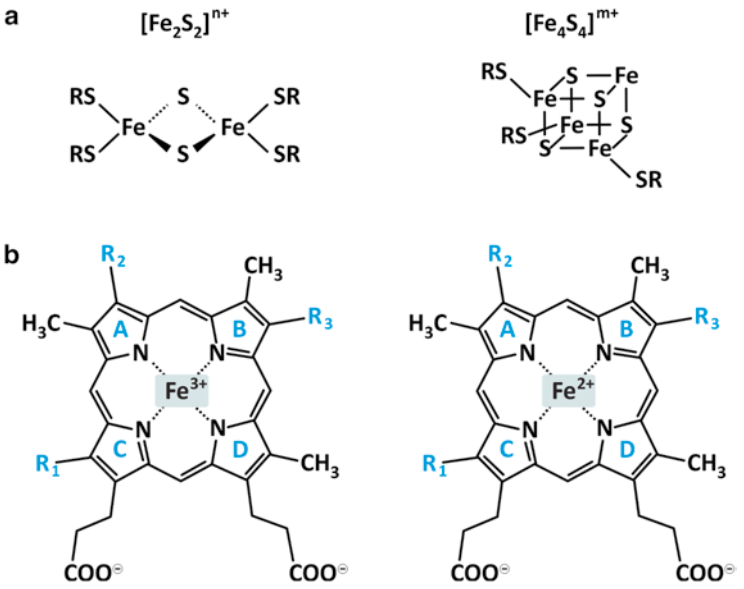


Fig. 5.9 Iron–sulfur centers (**a**) and heme groups (**b**) are examples of prosthetic groups as they are not amino acid residues and bind covalently to enzymes. The A–D multiring structure (tetrapyrrole) in panel (**b**) is the basic structure of a family of molecules named porphyrins. The central metal ion varies among porphyrins. Porphyrins conjugating iron ions are hemes. Both iron oxidation states (+3, *left*, or +2, *right*) are accommodated by the tetrapyrrole, meaning the central iron ion can engage redox reactions while inserted in the heme. The organic groups R_1–R_3 vary among hemes from different sources

The rules of nomenclature are not well defined. ATP for instance is often referred to as a coenzyme, but this is not a general rule. The same happens with CoA. NADH and NADPH are more consensually classified as coenzymes. FADH is often referred to as coenzyme, but it occurs in nature covalently bound to proteins so it is in reality a prosthetic group. While it is important to have precise nomenclature for the purpose of efficient and unbiased communication, one should not overemphasize name over action and one shall not further discuss semantics.

5.3 The Availability of Primary Precursors in a Metabolic Pathway

In a metabolic pathway such as the one depicted in Fig. 5.1, the velocity of reactions is not only controlled by the kinetic characteristics of the enzymes involved but also by the accumulation of G in the cell where it appears as the end product and availability of G in the cell where it is the primary precursor. To leave one cell and enter another, metabolite G needs to cross the membranes of both cells. Assuming that G is polar, it will not diffuse across the lipid bilayer of the membrane. It will need to be carried by specialized molecules or molecular assemblies. Take glucose as an example. It may be produced by hepatocytes in a metabolic pathway called gluconeogenesis and be consumed in neurons. Glucose is a polar molecule, soluble in plasma. To leave the hepatocyte and enter the neurons, glucose uses transmembrane proteins that assist in the process of translocating glucose across membranes.

Given the importance of transport across membranes to metabolisms, we shall address this issue in some detail in the next section.

5.3.1 Transport of Metabolites and Effectors Across Membranes

Figure 5.10 represents the six typical alternative routes molecules use to cross membranes. Small relatively hydrophobic molecules such as ethanol may not need transporters as they are able to simply diffuse across the lipid bilayer. Small ions have hindered diffusion across the bilayer due to their polarity, but they are able to cross

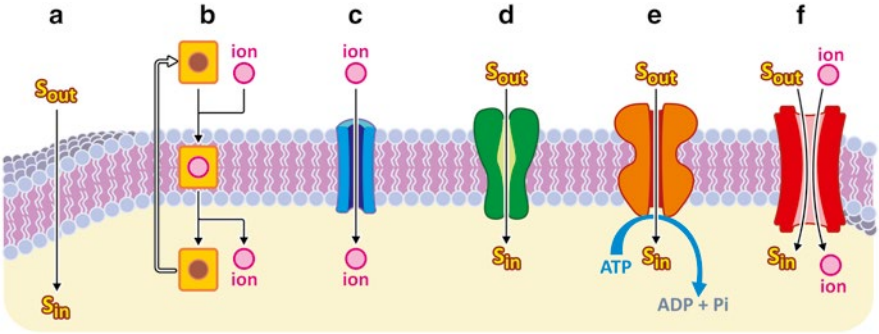


Fig. 5.10 The six different routes that molecules and ions may use to translocate lipid membranes: simple diffusion (**a**), ionophore mediated (**b**), ion channels (**c**), facilitated diffusion (**d**), primary active transport (**e**), and secondary active transport (**f**). Routes (**a**)–(**c**) are driven by electrochemical potential gradient (difference of concentration and charge distribution between both sides of the membrane)—passive transport. Routes (**e**)–(**f**) use ATP hydrolysis or the dissipation of an ion gradient, respectively, as sources of energy—active transport

membranes if encapsulated by chelating agents that are soluble in lipids. Ions can also cross membranes through ion channels, proteins that connect both sides of the membranes and are selectively permeable to specific ions. Similar proteins constitute channels for small molecules, which have thus a facilitated diffusion across the membrane. All these processes, from simple diffusion to facilitated diffusion, operate with net translocation of molecules in one direction, from the higher electrochemical gradient to lower electrochemical gradient. If the concentration and charge distribution is the same on both sides of the membrane, there is no net mass movement across the membrane, as the velocity of transfer on one sense equals the velocity of the opposite sense.

Figure 5.11 focuses on the molecular structure of a K^+-specific channel and aquaporin, a water channel across membranes.

Transporting molecules against electrochemical gradients is not energetically favorable. Thus, to transport molecules against an electrochemical gradient, an external source of energy has to be used. Some use ATP (primary active transport—Fig. 5.10d); others couple the transport of the solute to the transport of an ion along its electrochemical gradient (secondary active transport—Fig. 5.10e). The coupling may be such that both ion and solute are co-transported in the same direction (symport) or opposed directions (antiport). Primary and secondary transport mechanisms are coordinated when they transport common ions or solutes (Fig. 5.12). An example of simple diffusion and antiport is shown in Fig. 5.13. Glucose transport in intestinal epithelial cells is depicted in Fig. 5.14 as example of transport assembly that can be found in human epithelia.

Channels and transporters operate under the same thermodynamic principles as enzymes. Diffusion through membranes mediated by transporters has a decreased activation energy (Fig. 5.15). Moreover, transporters are proteins whose actions shares similarities with enzymes. Assuming that the transport of a solute, S, from the outer to the inner side of a membrane by the transporter, T, is described by

$$S_{out} + T \rightleftharpoons ST \rightarrow S_{in} + T$$

the associated kinetics of transport is hyperbolic, like a Michaelis–Menten process (Fig. 5.16). Likewise, there are inhibitors and activators of transporters. Thus, transporters may also be key points of regulation of metabolism and therefore drug targets.

Another way to control the action of transporters is to control their availability on the surface of cells. Transporters are embedded in the bilayer matrix so that they can be removed from the surface of the cell by vesiculation. This happens with glucose transporters such as glucose transport 4 (GLUT4). GLUT4 is responsible for insulin-stimulated glucose uptake in muscle and adipose tissue. Binding of insulin to its receptor on the surface of membranes leads to the fusion of intracellular vesicles having membranes loaded with GLUT4 with the cell

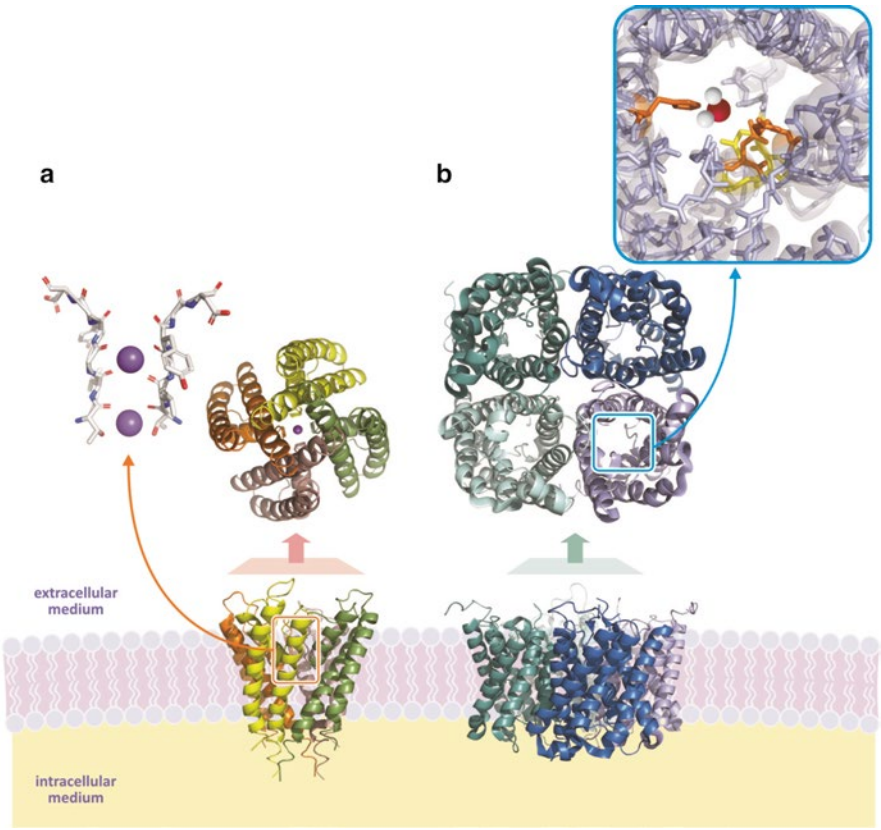


Fig. 5.11 (**a**) Molecular structure of the K^+ channel of *Streptomyces lividans* (PDB 1BL8) in longitudinal (*bottom*) and perpendicular (*top*) views in relation to the membrane. K^+ channels are the most widely distributed ion channel in living organisms. Four identical subunits, containing two transmembrane helices each, arranged in a conic structure in which the central backbone carbonyl oxygens form K^+-selective pores that fit the ion precisely (see detail in the *left*, PDB 1J95), removing the hydration shell from the ion when it enters the channel. The channel accommodates four K^+ sites that are occupied alternately. (**b**) Structure of the spinach aquaporin (PDB 2B5F) in longitudinal (*bottom*) and perpendicular (*top*) views in relation to the membrane. Aquaporin selectively transports water molecules in and out of the cells. The protein is a tetramer of identical subunits, each of them containing a transmembrane pore (see detail in the *right*). All aquaporins contain a conserved sequence of Asn-Pro-Ala (highlighted in *yellow*) as part of the water channel. Water channel also contains a conserved His that narrows pore diameter limiting the passage of molecules larger than water and a conserved Arg that repels cations, including H_3O^+ (highlighted in *orange*). The positioning of the water molecule is tentative for illustrative purposes only

membrane, exposing the transporters (see Sect. 8.4.3). Glucose transport may thus occur, and this molecule becomes available inside the cells (Fig. 5.17). From that moment on, metabolic pathways having glucose as precursor, such as glycolysis, may initiate.

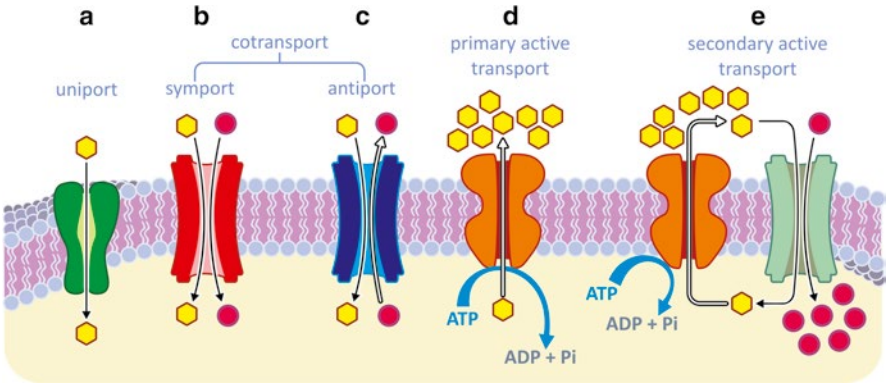


Fig. 5.12 Unidirectional transport of a single solute is called uniport (**a**). Co-transport implies that two solutes (or a solute and an ion) are transported simultaneously, either in the same direction (symport—**b**) or in opposite directions (antiport—**c**). Primary active transport (**d**) is sometimes coupled to a co-transport (**e**) by the creation of an ionic or molecular gradient (*yellow molecule*) that is used to transport the red solute independent of its gradient

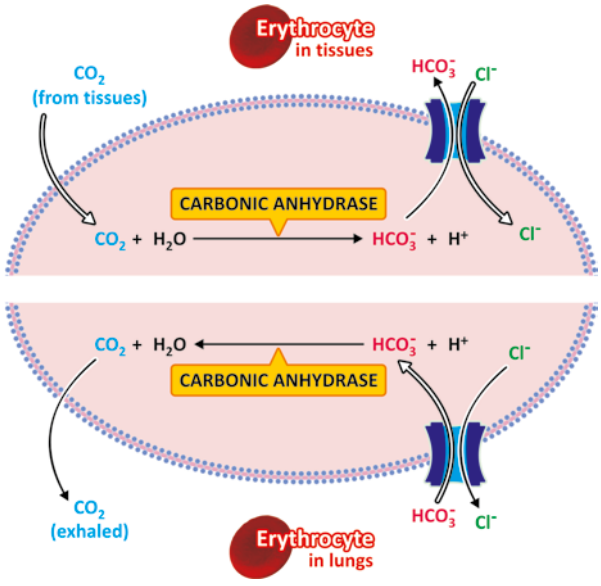


Fig. 5.13 Transport of ions and solutes across the erythrocyte membrane. CO_2 is a small molecule able to diffuse directly across the membrane according to its concentration gradient (in lungs: in → out; in respiring tissues: out → in). Hydrogen carbonate (bicarbonate) is co-transported with chloride. The direction of transport depends on CO_2 concentration because CO_2 equilibrates with HCO^-_3

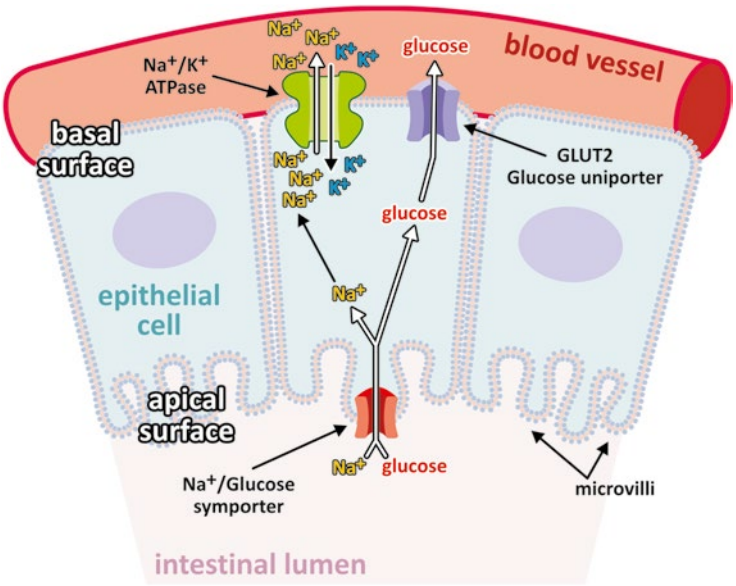


Fig. 5.14 The system of glucose transport in intestinal epithelial cells is complex and includes transport from the intestinal lumen to the cytoplasm and from the cytoplasm to blood. Na^+ has a central role by coupling both steps

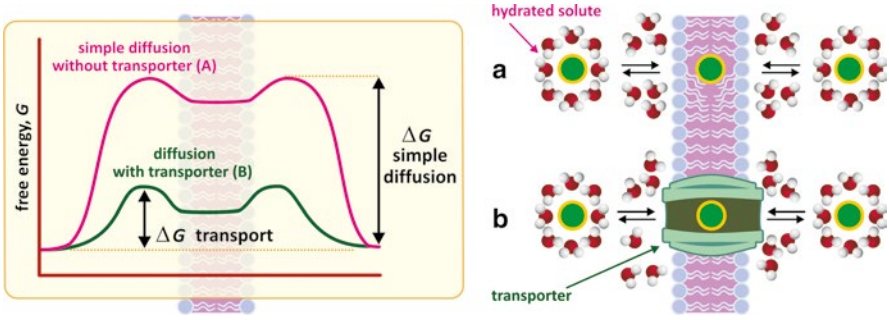


Fig. 5.15 Thermodynamics of the passage of a polar molecule or ion (*green circle*) across a lipid membrane. Simplified diffusion (**a**) implies a high energetic cost to remove the hydration shell around the solute. The action of a transporter (**b**) leads to the reduction of the activation energy (the ΔG represented in the *left panel*) of the membrane translocation by the molecule or ion. The transporter replaces water by forming hydrogen bonds with the solute

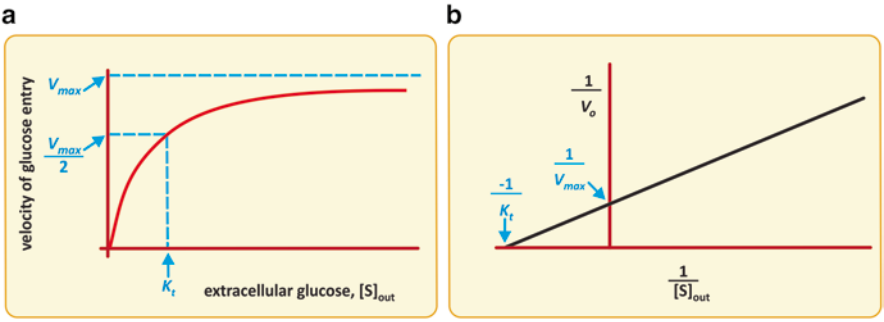


Fig. 5.16 Michaelis–Menten-like kinetics in solute transport across membranes. K_t is equivalent to K_M, except that it refers to transport, not catalysis

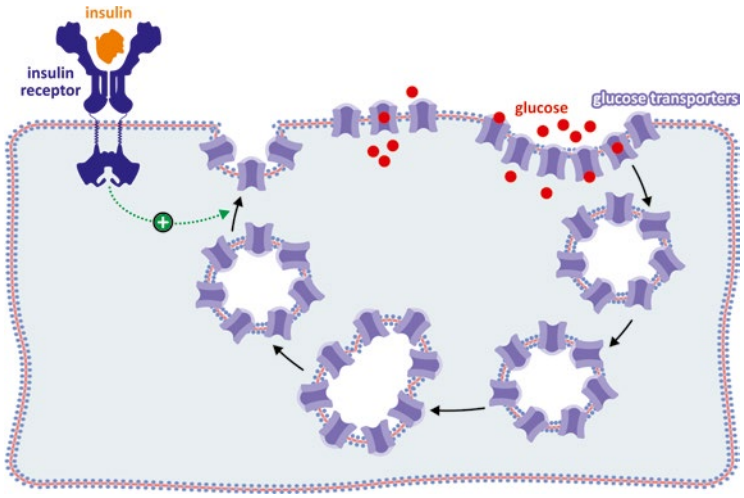


Fig. 5.17 Insulin-stimulated exposure of GLUT4 on the surface of muscle and adipose cells. Vesicles bearing GLUT4 are recruited and fused with the plasma membrane. GLUT4 is automatically exposed. In the absence of insulin, the process is reversed

5.4 Slow (But Efficient!) Mechanisms of Controlling Enzyme Action

Enzyme response to binding of effectors is very fast. Concomitant to the effector's accumulation or depletion, the binding or dissociation, respectively, of the effector is nearly immediate. Yet, the efficacy of the process of inhibition or activation is concentration dependent. At extreme concentrations, the effect is also extreme, but at intermediate concentrations of the effector, the inhibition or activation is only partial. In contrast to non-covalent binding of effectors, there are two mechanisms

of enzyme action that are highly efficient, although not immediate. These are the covalent modification of enzymes using phosphates, which may take up to minutes, and the synthesis or degradation of the enzymes themselves, which may take up to hours or days.

Phosphorylation of enzymes occurs at –OH groups; thus, it is a process that occurs in exposed Ser, Thr, or Tyr residues. This process frequently involves ATP and is catalyzed by a second enzyme, a kinase. The inverse process, dephosphorylation, is also catalyzed by enzymes, called phosphatases (Fig. 5.18). Phosphate is the ubiquitous group used for covalent modification of enzymes in nature, but there is no rule on whether the active forms of the enzymes are the ones phosphorylated or not. Table 5.2 shows examples of enzymes that are active or inactive when phosphorylated.

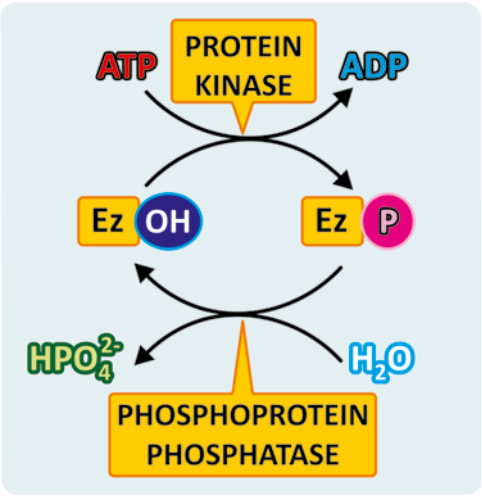


Fig. 5.18 Typical reactions for phosphorylation or dephosphorylation of enzymes. The process involves the action of the two other enzymes: a kinase and a phosphatase

The presence or absence of a phosphate group on the protein may have a high impact on the domain where the reaction takes place. Local changes in structure may propagate through the 3D architecture of the protein and trigger or block enzyme activity. The reason why phosphates are the only choice of nature to perform this task is intriguing. This is believed to be related to the abundance of phosphates and its chemical nature. Phosphates bind to organic molecules, forming esters, keeping their anionic charge, which protects them from hydrolysis. Nucleophiles, such as the hydroxide ion, are repelled by negative charges and therefore react less rapidly with anions than with neutral substrates. Furthermore, the same is generally true with respect to electrically neutral nucleophiles such as water.

The rate constant for the attack of hydroxide to the dimethyl phosphate anion is less than that for the attack of hydroxide ion to the trimethyl phosphate by a factor of more than 10^5 (Table 5.3).

Table 5.2 Some enzymes are active when phosphorylated; others are inactive. There is no general rule in this domain

Metabolic pathway	Enzyme	Phosphorylated form	
		Active	Inactive
Glycogenolysis	Glycogen phosphorylase kinase	✓	
	Glycogen phosphorylase	✓	
Glycogenogenesis	Glycogen synthase		✓
Glycolysis and gluconeogenesis	6-phosphofructo-2-kinase		✓
	Fructose 2,6-bisphosphatase	✓	
	Pyruvate kinase		✓
Lipolysis (conversion of triacylglycerols to glycerol and fatty acids)	Lipase	✓	
Lipogenesis	Citrate lyase		✓
	Acetyl-CoA carboxylase		✓
	3-Hidroxy-3-methylglutaryl-CoA reductase		✓

Table 5.3 Rates of reaction of esters with OH^- at 35 °C

Ester	k (M^{-1} s^{-1})	Fold increase in k[a]
$(CH_3O)_2PO^-{}_2$	2×10^{-9}	1.0
$(CH_3O)_3P{=}O$	3.4×10^{-4}	1.7×10^{5}
$CH_3CO^3{}_2C_2H_5$	1.0×10^{-2}	5.0×10^{6}

[a]Relative to $(CH_3O)_2PO^-{}_2$

The phosphorylation/dephosphorylation switch to turn on or turn off enzymes is found for key enzymes of metabolisms. The primary triggering of the events that result in phosphorylation or dephosphorylation of enzymes is the interaction of a hormone with its receptor, as illustrated before in Fig. 5.1. The figure shows that binding of H to its receptor triggers a series or transformations whose end result is the phosphorylation/dephosphorylation of the key enzyme. These intermediary steps are generally referred to as signal transduction and the chemical species involved as second messengers. We shall not address the theme of signal transduction in detail because in this section, we intend to remain focused on the basics of metabolic regulation (signal transduction triggered by specific hormones will be discussed in detail in the chapters that explore the regulation of metabolism in different physiological situations—Chaps. 8–11). Nonetheless, it is worth mentioning that there are two main classes of receptors that trigger signal transduction: G protein-coupled receptor and kinase-linked receptors. Binding of the ligand (usually named "agonist" in pharmacology) to the receptors of the first kind causes conversion of GDP into GTP and the release of a complex of proteins associated to the receptor that will associate to other molecular targets, such as adenylyl cyclase, which converts ATP into cyclic AMP (cAMP). The increase in cAMP concentration may in turn stimulate other chemical reactions in a cascade-like manner. Besides adenylyl cyclase-coupled receptors (Fig. 5.19), there are other cases, such as phospholipase C-coupled receptors. In this

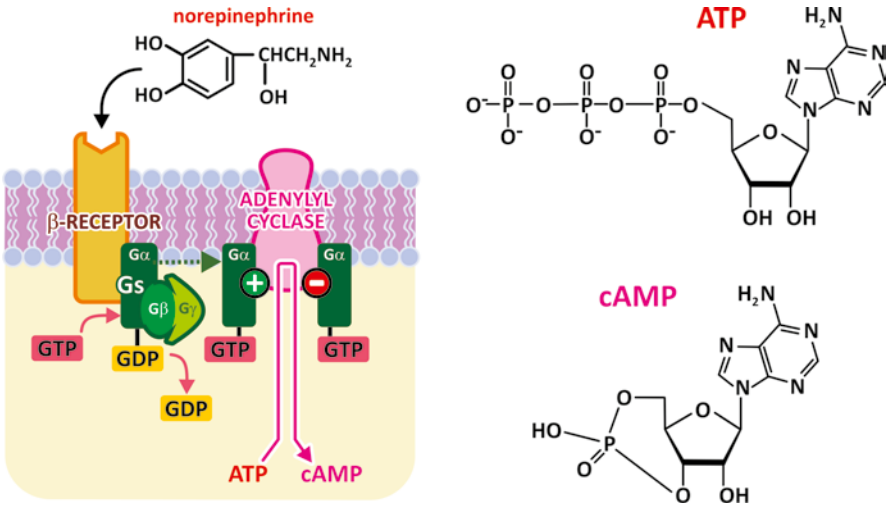


Fig. 5.19 Generic representation of a G protein-coupled receptor. Upon binding of the agonist ligand to the receptor (e.g., norepinephrine), adenylyl cyclase is activated and cAMP is produced

case the GTP-associated released complex of proteins associate to phospholipase C and leads to hydrolysis of the phosphatidylinositol bisphosphate in the membranes into diacylglycerol and inositol triphosphate, the latter serving as second messenger.

Kinase-linked receptors are transmembrane proteins with extracellular domains that bind agonist ligands, including hormones, and intracellular domains having amino acid residues that undergo phosphorylation. A cascade of events is initiated ("kinase cascade").

The most important signal transduction systems for metabolic dynamics and regulation will be shown throughout the following chapters, together with the metabolic pathway for which they are relevant. However, it is important to highlight that the end effect of some hormones that bind to cell surface receptors may take place in the nucleus, like the recruitment of transcription factors (Fig. 5.20). This enables hormones to control the activity of enzymes at a higher level: enzyme biosynthesis. Not only hormones may activate/inhibit enzymes by phosphorylation or dephosphorylation, but they can also control the availability of the enzymes through the control of their biosynthesis. This redundancy assures high efficacy in a wide time range as covalent modification of enzymes is operational in the minute to minute time scale and the control of synthesis is operational in hours to days. Cholesterol synthesis is shown as example in Fig. 5.21.

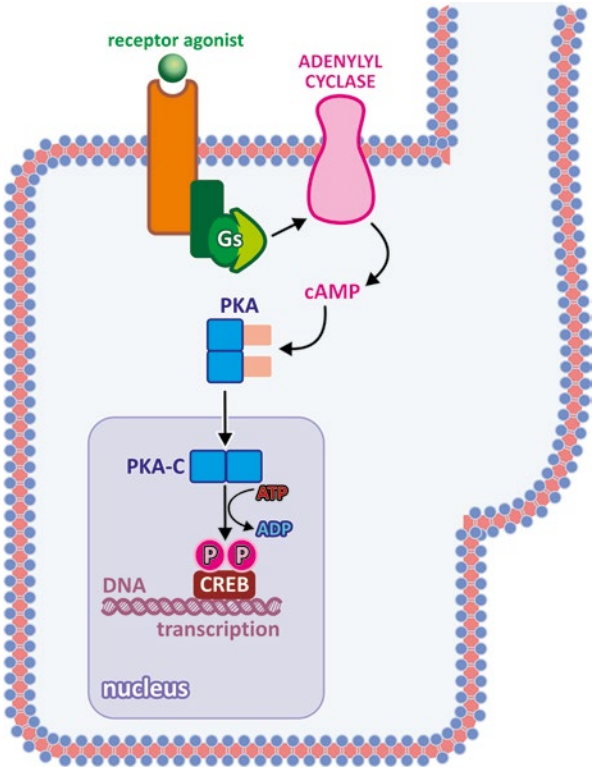


Fig. 5.20 Impact of a receptor agonist on transcription via adenylyl cyclase. Hormones may interfere in enzyme biosynthesis using similar mechanisms. Gs - stimulative regulative G-protein, PKA - Protein kinase A, PKA-C - PKA catalytic subunit, CREB - cAMP response element-binding protein

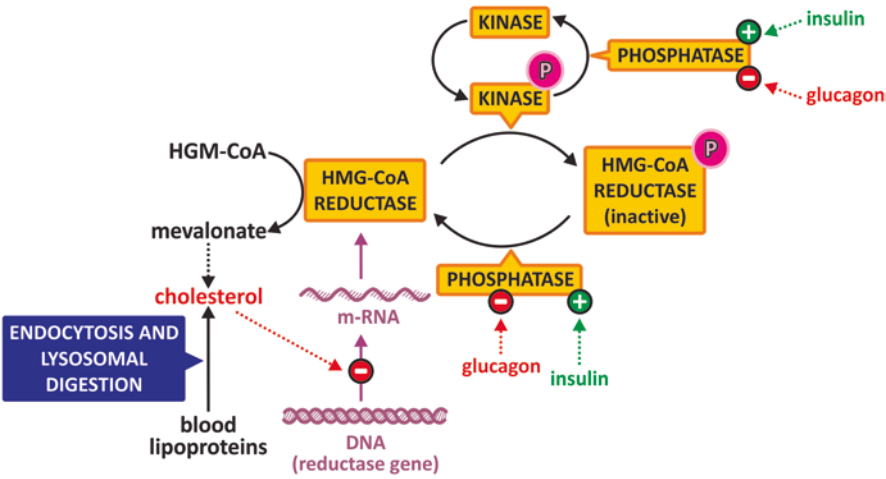


Fig. 5.21 HMG-CoA reductase is the key enzyme in the biosynthesis of cholesterol. It is regulated by slow mechanisms: covalent modification (inactivation by phosphorylation) and transcription inhibition by the end product, cholesterol. The hormones insulin and glucagon have opposite effects: insulin stimulates cholesterol synthesis and glucagon stimulates the inhibition of HMG-CoA reductase, shutting down the synthetic pathway

5.5 Key Molecules in Energy Metabolism

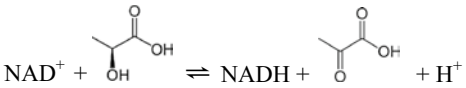
In the same way ATP is frequently used in metabolism because its favorable hydrolysis coupled to unfavorable reaction makes the whole set of chemical reactions favorable, NADH (see NADH chemical structure in Fig. 3.23) is also a molecule frequently used to drive redox processes. The process can be represented by

$$NAD^+ + 2H \rightleftharpoons NADH + H^+$$

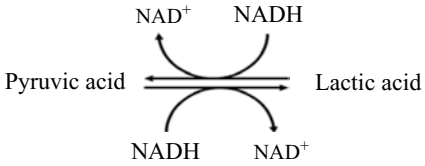
but this is not elucidative for its role in redox reactions. A better representation is

$$NAD^+ + RH_2 \rightleftharpoons NADH + RH^+ \left(\text{or } NADH + R + H^+\right)$$

in which RH_2 is a generic molecule. In this reaction the reduction of NAD^+ is coupled to the oxidation of RH_2. This is the case of



In biochemical literature, the reaction stoichiometries and the involvement of H_2O of H^+ (or H_3O^+) are not always represented for the sake of simplicity, and this process can simply be written as



but one must not forget the chemical details implied albeit not explicit.

NADH is so ubiquitous in cells that it can be considered as a "universal" electron carrier. NADH is used in cells due to its strong reducing (electron-donating) power; NAD^+ is converted back to NADH by oxidation of other molecules. Most reactions involving NADH/NAD^+ in metabolism are catalyzed. The enzymes that catalyze the oxidation of a substrate with the transfer of one or more hydrides (H^-) to an electron acceptor such as NAD^+ (or equivalent molecules such as $NADP^+$, or FAD, or FMN; see Fig. 3.23) are named dehydrogenases.

There are other examples of ubiquitous molecules in metabolism, although not as striking as NADH or ATP. Biotin, for instance, is a non-proteic "prosthetic" (not an amino acid residue) group that exists in carboxylases and participates in the addition of CO_2 groups from HCO_3^- to organic molecules, as exemplified in Fig. 5.22 (in which E_z is the enzyme):

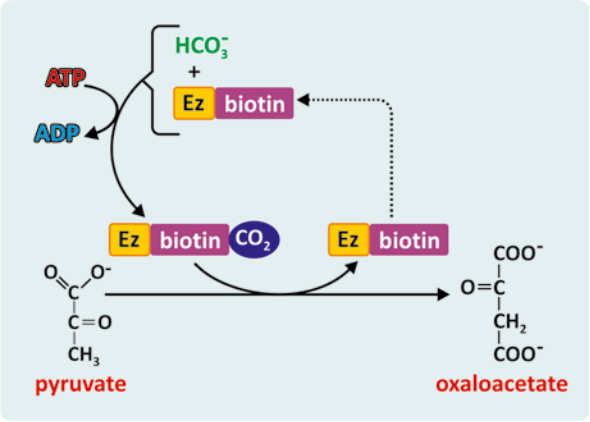
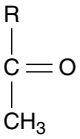


Fig. 5.22 Biotin occurs in carboxylases (E_z-biotin) and participates in the addition of CO_2 groups from HCO_3^- to organic molecules such as pyruvate

Acetyl-CoA (acetyl bound to coenzyme A, CoA—Fig. 5.24) is an amazing molecule as it is common metabolite. Acetyl-CoA is important in glucose metabolism, fatty acid degradation, amino acids metabolism, and cholesterol synthesis. Its ubiquity is not as simple to explain as the universality of ATP (hydrolyses with $\Delta G^{\circ} \ll 0$), NADH (strong reducing power; $\Delta G^{\circ} \ll 0$ in reduction), or biotin (specific functionality in carboxylation processes). Acetyl groups seem to be the universal "carbon transfer unit" and CoA their carrier for this purpose. Acetyl (or ethanoyl) is a two-carbon group of the kind:



In this formula R represents the generic molecule to which acetyl group is attached to (i.e., the acetylated molecule). In acetyl-CoA, R is CoA. Degradation of glucose, fatty acids or amino acids results in acetyl groups being attached to CoA. The acetyl groups can then be used to generate NADH from NAD^+, which in turn will help in transforming ADP to ATP. Yet, under different circumstances, acetyl groups in acetyl-CoA may also be used to synthesize molecules, serving as a carbon supplier for the growth of the carbon backbones. CoA concurs with biotin-containing enzymes for this purpose.

A generic scheme of the metabolisms built around the "universal molecules" ATP, NADH, and acetyl-CoA is presented in Fig. 5.23).

It is very curious that the fraction of the structure of the ATP, NADH, or acetyl-CoA molecules involved in chemical reactivity itself is very small when compared to the whole molecule (Fig. 5.24). Moreover, at first glance it may seem puzzling that in some reactions in metabolism, ATP is replaced by GTP or UTP, and NADH is replaced by $FADH_2$ or NADPH (Fig. 5.24). GTP or UTP do not differ from ATP in the phosphodiester bond broken to form ADP (which is analogous to GDP or UDP). Phosphorylation of NAD to form NADPH occurs at a site that does not affect the electron donor group. What is then the advantage of having relatively large molecules to perform simple chemical reactions that involve only small groups in their structures? What is the advantage in having analogous molecules with same reactivity that differ only in the "inert" part of these molecules? The answers are simple: having molecules of a significant dimension facilitates recognition and specificity of the binding sites of enzymes, and small variations in the chemical structure of these molecules determine that other enzymes should be used instead. In reactions in which NADPH intervenes, the associated enzyme is specific for NADPH, not NADH. The occurence of these reactions is an advantage in the sense of allowing the donation of electrons without affecting NADH pools in the cell. The reaction only occurs if NADPH is present. Therefore, the use of NADPH in addition to NADH enables that

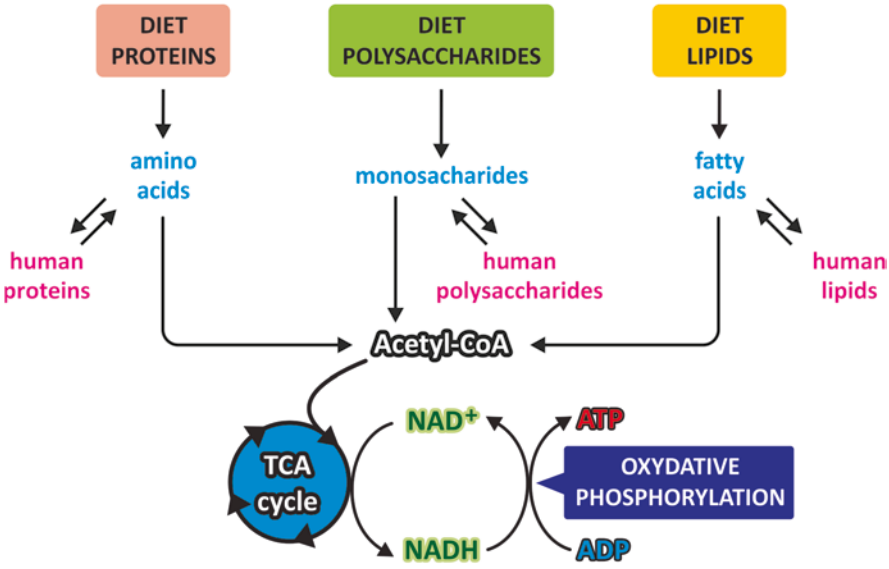


Fig. 5.23 Very broad and abbreviated view of human energy metabolism. Intermediary metabolites are not shown for the sake of simplicity. The main interplay of NADH, acetyl-CoA, and ATP is highlighted. TCA - Tricarboxylic acids cycle (also known as citrate cycle or Krebs cycle)

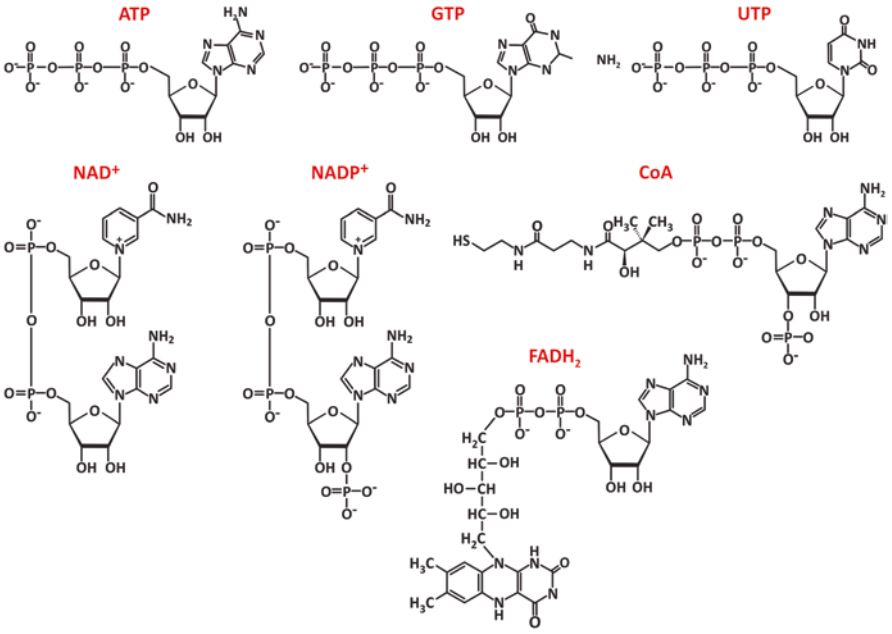


Fig. 5.24 The reactive groups of ATP, NAD^+, or CoA are relatively small compared to the whole molecule. Analogous molecules to ATP or NAD^+ differ in the "non-reactive" part of the molecule. Although not involved directly in reactivity, these parts of the molecules are important to grant specificity to different enzymes

specific redox reactions in the cell may be regulated regardless of the metabolic state of cell at the moment, i.e., regardless of NADH levels. Protective mechanisms against reactive oxygen species (ROS), for instance, are very much dependent on the reductive action of NADPH. The capacity to repair the chemical damage imposed by ROS is thus mantained regardless of low concentrations of NADH (see Sect. 6.2.5). Likewise, the use of GTP or UTP in some reactions instead of ATP may have the same advantage.

In the following chapters we will devote our attention to the synthesis of ATP, which in turn is dependent of the NADH production, which in turn is dependent on acetyl-CoA that is obtained from nutrients: fatty acids, monosaccharides, and amino acids.

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