Chapter 4
Introduction to Metabolism

Cells are made up of molecules, but cells are not simple mixtures or assemblies of molecules. If thrown to a test tube separately or in a random mixture, the whole set of molecules of a cell would interact physically and react chemically, but would not spontaneously form a cell. Cells form and exist because the molecular events that create and maintain cells are highly ordered. The sequence and specific place of events and the flows of matter and energy are such that the cell is able to preserve stability and evolve by adaptation to a certain extent, as addressed in Sect. 1.1. In Part II, we will focus on some of the most important chemical reactions occurring in different tissues of the human body and their coordination so that one understands how the changes of matter and transfer of energy enable the human body to exist, move, adapt to external challenges, and reproduce. This ensemble of chemical reactions is called metabolism.

Because the whole metabolism is such a complex array of chemical reactions, biochemists tend to study and concentrate on subsets of reactions separately. The easiest form to understand and explain metabolism is to divide it in parts depending on the chemical nature of the molecules involved, i.e., according to the families of molecules addressed in Part I. So, for practical reasons and for the sake of simplicity, the whole metabolism, which is a single highly complex network of chemical reactions and physical events, is regarded as a sum of "metabolisms": the metabolism of saccharides, the metabolism of amino acids, the metabolism of lipids, etc.

The part of metabolism more directly related to nutrient absorption and ATP production is the "energy metabolism." The human body continuously transforms molecules, sometimes forming higher molar mass products, others breaking down molecules into smaller molar mass entities. Typically, these situations correspond to incorporate mass and energy from nutrients, or consuming such mass and energy in the absence of nutrient intake, respectively. The "metabolisms" of the first kind are known as anabolic (construction), the latter being known as catabolic (degrading). So, "amino acid catabolism," for instance, refers to the breakdown of amino acids into smaller molecules, as opposed to amino acid synthesis (amino acids anabolism).

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Amino acids, in turn, may polymerize and form proteins: protein anabolism, in contrast to the breakdown of proteins, amino acids resulting therefrom (protein catabolism). Subsets of reactions that are active in both catabolic and anabolic conditions are "amphibolic" (the prefix "amphi" referring to its dual nature). This is the case of the tricarboxylic acid (TCA) cycle, also known as Krebs cycle (see Sect. 7.2).

An example of generic metabolism is schematized in Fig. 4.1. No matter how complex the metabolism may seem at first glance, the interpretation of a scheme of consecutive reactions is simple and depends on the identification of five key factors:

1. The reactants that originate the process
2. The end products, regardless of being formed in intermediate reactions or the final reaction
3. The branching points, i.e., steps where the sequence of reactions may follow different courses
4. The irreversible reactions
5. The specific reactions that are catalyzed by enzymes that are finely regulated and so have the ability to highly accelerate segments of the metabolism, or not

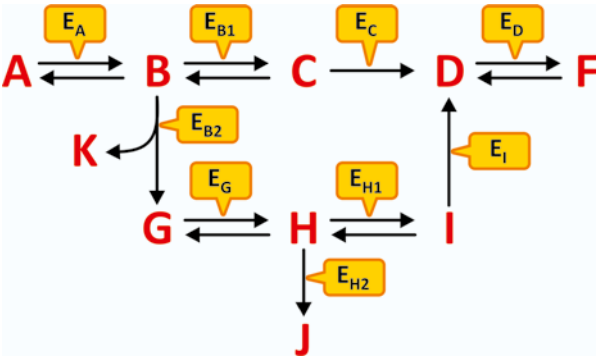


Fig. 4.1 Hypothetical schematic metabolism involving metabolites A to K and enzymes E_x (subscript X in E_x represents the substrate, X = A to K). The metabolism is "fed" by A and has K, F, and J as "end products." An external source of G may also lead to the formation of J and F, but not K. An external source of D can only lead to the formation of F. However, if E_D is not present or not operative, F will not be formed in any circumstance. Likewise, if E_{B2} is not present or not operative, K, G, H, J, and I will not be formed even in the presence of high concentration of B. If E_{B1} and E_{B2} are never active or inactive at the same time, F is always formed but not J; the reactions' scheme assures the permanent formation of F but the selective formation of J when the control of the reaction course is performed by alternate states of activity of E_{B1} and E_{B2}

These five key factors in the interpretation of a metabolism will help the reader not to look to a metabolism as a chaotic ensemble of chemicals, enzymes, and reaction arrows. Metabolism is an appealing and richly informative text on the organization of life, should we be able to interpret it.

Enzymes are key protagonists of metabolisms together with metabolites (i.e., the intermediate reactants/products of the reactions of metabolisms). Not only enzymes

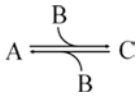
greatly accelerate reactions, they are the entities that make metabolisms happen in specific directions. Their importance and action is better illustrated if one considers first a hypothetical metabolism without enzymes (described in Sect. 4.1) and the same set of reactions with enzymes (described in Sect. 4.2).

4.1 Consecutive Reactions Without Enzymes

Consider the simple chemical reaction:

$$A + B \rightleftharpoons C$$

which in terms of a more amenable representation to biochemists may be written as:



to highlight the conversion of A into C with the intervention of B. Nevertheless, one should not underestimate the importance of B in the balance of energy and mass of the reaction, even if B is H_2O or a small ion, for instance, and A and C are big complex molecules. A, B, and C are all equally important to study the course and velocity of the reaction, i.e., the thermodynamics of the reaction. To better understand the basic principles that govern the chemical reaction, we will start with very simplistic formulations. One will assume that A and B are mixed at a specific instant in time ($t=0$) and the reaction initiates forming C. As soon as C is formed, it will also start converting to A + B, but in the beginning only few molecules of C exist, so the velocity of the conversion of A + B into C is larger than the opposite conversion, C to A + B. Naturally, in this condition, the concentration of C will increase until the point in which the velocities of both reactions will match: the velocity with which C is formed equals the velocity with which it is consumed. The reaction takes place because A and B are continuously transformed into C and vice versa, but the concentrations of A, B, and C present in solution do not change. This is the point of equilibrium. At this stage it is important to dissociate the extent of the reaction (i.e., the fraction of A and B that was transformed to reach the equilibrium) and the velocity of the reaction, which is related to the time needed to reach the equilibrium. A very extensive reaction is represented by:

$$A + B \rightarrow C$$

and named "irreversible" (therefore written with a one-way arrow), but this tells us absolutely nothing about the velocity of the reaction. In fact, the conversion of A and B into C may be so slow that in practical terms C is not formed. So, to study chemical reactions, one needs to address both the extent and the velocity of reactions. A favorable reaction has a high degree of conversion of reactants in products;

a fast reaction reaches the equilibrium in a short time. Both aspects are independent of each other, and so it is not surprising that they can be modulated by separate means in metabolism.

The extent of a reaction is described by the equilibrium constant, which in practical terms is calculated from the concentrations at equilibrium:

$$K_{eq} = \frac{[\mathrm{C}]_{eq}}{[\mathrm{A}]_{eq}[\mathrm{B}]_{eq}}$$

It is obvious that unfavorable reactions have low K_{eq} and favorable reactions have high K_{eq}. K_{eq} varies from nil to infinity. The determinants that contribute to the value of K_{eq} for a given reaction (i.e., how favorable it is) relate to the balance of both released energy and order of the whole environment in which the reaction takes place. Neither is intuitive, but both can be experimentally demonstrated. Chemical reactions tend to consume more reactants as the products are more stable ("less energetic") than reactants and the environment becomes more disordered with the presence of the products (i.e., closer to a random organization). The degree of disorder is generically referred to as entropy. The balance of energy and entropy are so deeply rooted in thermodynamics that they are the subject of the first and second laws of thermodynamics, respectively, as the founding principles of this discipline. The exact quantitative weighting between the balance of released energy as heat (the enthalpy, ΔH) and balance of entropy (ΔS) is provided by the parameter ΔG, the difference of the Gibbs energy during the chemical reaction:

$$\Delta G = \Delta H - T\Delta S$$

Assuming that the temperature, T, does not change in the process, ΔG is related to the concentration of reactants and products:

$$\Delta G = \Delta G^0 + RT \ln \frac{[C]}{[A][B]}$$

In equilibrium $\Delta G = 0$; therefore,

$$\Delta G^0 = -RT \ln K_{eq}$$

While thermodynamics may reach complex formulations to explain the interaction of energy with matter, we shall stick to those most important in medical biochemistry. Previous equations on ΔG are useful because they provide information about the spontaneity of reactions. If $\Delta G < 0$ (exergonic reaction), the reaction will proceed spontaneously until equilibrium; if $\Delta G > 0$ (endergonic reaction), the reaction will not be spontaneous; if $\Delta G = 0$, the reaction is at equilibrium. Whether ΔG is positive, negative of nil depends largely on the concentrations of reactants and products, and the reaction will continue until these concentrations reach equilibrium

($\Delta G=0$). $\Delta G_{eq}{}^{0}$ is related to the concentrations at equilibrium specifically and so provides information on the final extent of reaction (i.e., how far the reaction goes until equilibrium):

$$\Delta G^0 \ll 0 - high\ extent\ of\ reaction\ \left(high\ \ K_{eq}\right)$$
$$\Delta G^0 \gg 0 - low\ \ extent\ of\ reactions\ \left(low\ \ K_{eq}\right)$$

Box 4.1 illustrates these concepts for a simple reaction. Figure 4.2 shows two examples of how ΔH may dominate over ΔS and vice versa and be determinant for the extent of the reaction, i.e., for ΔG. However, in most biological processes related to the use of nutrients, ΔH is large compared to $T\Delta S$, which makes ΔH, the caloric "value" of food, an approximate measure of the total energy that can be used by the human body.

Isolated reactions such as the ones considered before are rarely relevant in metabolism as metabolism consists of consecutive reactions. So let us now consider the reaction

$$\mathrm{C} \rightleftharpoons \mathrm{D} + \mathrm{E}$$

Box 4.1: The Basic Thermodynamics of the Simplest Reaction

In the very simple case of one single reactant (R) being transformed in one single product (P):

$$R \rightleftharpoons P$$

the equilibrium constant is:

$$K_{eq} = \frac{[P]_{eq}}{[R]_{eq}}$$

and

$$\Delta G^0 = -RT \ln K_{eq} = -RT \ln \frac{[P]_{eq}}{[R]_{eq}}$$

It is obvious that:

$$\Delta G^o < 0 \quad \text{if} [P]_{eq} > [R]_{eq} \left(K_{eq} > 1\right)$$
$$\Delta G^o > 0 \quad \text{if} [P]_{eq} < [R]_{eq} \left(K_{eq} < 1\right)$$

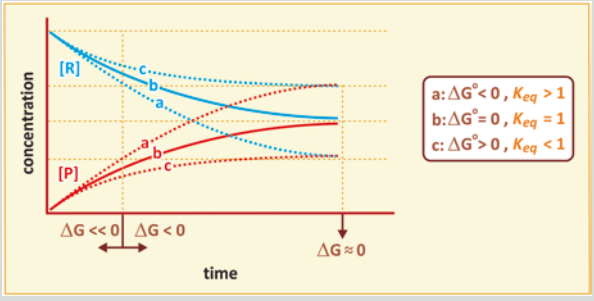
(continued)

Box 4.1 (continued)
The reactions with highest K_{eq} have the most negative ΔG^0_{eq}. If the equilibrium is not yet attained, two situations are possible:

1. $$[R] > [R]_{eq} \left(i.e. [P] < [P]_{eq}\right)$$
$$\Delta G = \Delta G^0 + RT \ln \frac{[P]}{[R]} = +RT \ln \left(\frac{[P]}{[R]} \frac{[R]_{eq}}{[P]_{eq}} \right)$$
which is obviously negative. The reactions will proceed with transformation of R in P until equilibrium, i.e., $\Delta G < 0$ implies spontaneous transformation of reactants in products.

2. $$[R] < [R]_{eq} \left(i.e. [P] > [P]_{eq}\right)$$
This is the opposite case, in which $\Delta G > 0$ and the spontaneous process is the reverse reaction, i.e., the transformation of the product P, in the reagent R.

The relationship between ΔG, ΔG^0, and the course of reaction is represented in the figure below.



coupled to the first reaction, $A + B \rightleftharpoons C$, to form:

$$A + B \rightleftharpoons C \rightleftharpoons D + E$$

At equilibrium, it is obvious that the concentrations of A and B are related to the concentrations of D and E because C takes part in both reactions. In term of equilibrium constants:

$$K_{eq,1} = \frac{[C]_{eq}}{[A]_{eq}[B]_{eq}}$$
$$K_{eq,2} = \frac{[A]_{eq}[B]_{eq}}{[C]_{eq}}$$

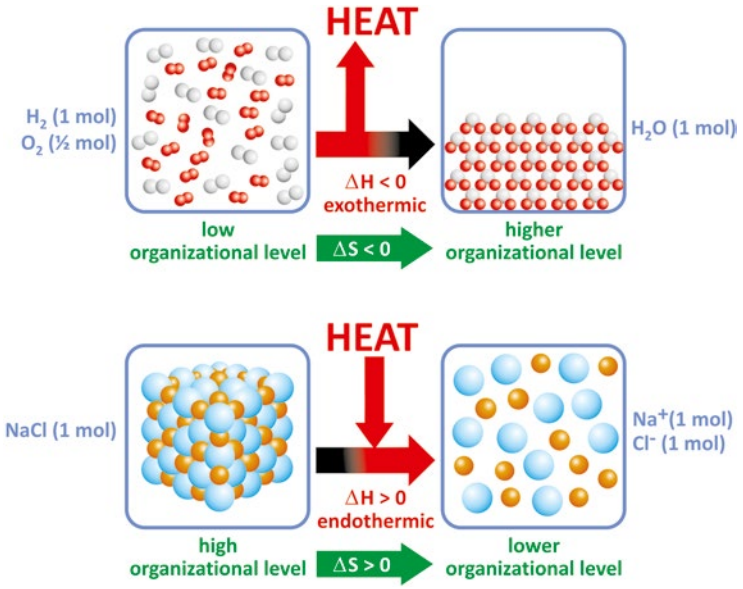


Fig. 4.2 Some reactions occur because the released energy, ΔH, compensates for the decrease in ΔS (**a**), or the increase in disorder compensates for the heat consumption (**b**)

(1 and 2 refer to the first and second reactions in the sequence).
Therefore,

$$K_{eq,1}K_{eq,2} = \frac{[\mathrm{D}]_{eq}[\mathrm{E}]_{eq}}{[\mathrm{A}]_{eq}[\mathrm{B}]_{eq}}$$

which is the apparent equilibrium constant of the abbreviated form ("sum of reactions") of the reactions $\mathrm{A}+\mathrm{B} \rightleftharpoons \mathrm{C}$ and $\mathrm{C} \rightleftharpoons \mathrm{D}+\mathrm{E}$ above:

$$\mathrm{A}+\mathrm{B} \rightleftharpoons \mathrm{D}+\mathrm{E}$$
$$K_{eq,app,1,2} = K_{eq,1}.K_{eq,2}$$

The amazing consequence is that $\Delta G°$ of the global process is simply the sum of ΔG of the two consecutive reactions:

$$\Delta G^0_{eq,1,2} = -\ln K_{eq,app,1,2} = -\ln\left(K_{eq,1}K_{eq,2}\right) = -\ln K_{eq,1} - \ln K_{eq,2} = \Delta G^0_{eq,1} + \Delta G^0_{eq,2}$$

The consequences of metabolism are immense because it is implied that the conversion of C in D+E may be extremely unfavorable, but the extent to which A+B is converted to C "pushes" the reaction toward the formation of D+E through the increase in the concentration of C. This can be generalized to any set of consecutive reactions, and metabolisms are generally composed by favorable and unfavorable

reactions influencing each other. In the extreme case where an irreversible reaction follows one or more reversible reactions, such as

$$A + B \rightleftharpoons C \rightleftharpoons D + E \rightarrow F$$

it is obvious that regardless of how unfavorable the first and second reactions may be, the end result is the total depletion of A and/or B and the formation of F. The same would happen in

$$A + B \rightleftharpoons C \rightleftharpoons D + E \begin{array}{l} \nearrow F \\ \searrow G \end{array}$$

but not in

$$A + B \rightleftharpoons C \rightleftharpoons D + E \begin{array}{l} \nearrow F \\ \searrow G \end{array}$$

in which the end products would be both F and G. Although both the formation of F and G are irreversible, they are competing reactions. Although they both occur to the total depletion of at least one of the reagents, the product of the fastest reaction dominates. One of the reactions may be much faster than the other, in which case only one product forms in practice.

In a branched chain of reactions with an irreversible conversion such as, for instance,

$$A + B \rightleftharpoons C \rightleftharpoons D + E \begin{array}{l} \nearrow F \rightleftharpoons J + K \rightleftharpoons L \\ \searrow G \rightleftharpoons H \rightleftharpoons I \end{array}$$

the final outcome critically depends on the irreversible steps and the branching points. Thus, the result may be complex.

Given the importance of the velocity of reactions to the course of chain reactions, we shall now address kinetics in more detail as ΔG^0 only accounts for how far the reaction goes in the degree of conversion of reactants into products, not kinetics.

4.2 Consecutive Reactions With Enzymes

The chain of reactions above is dominated by the irreversibility of reactions $D + E \rightarrow F$ and $D + E \rightarrow G$ ($\Delta G^0 \ll 0$ for both). If the latter, for instance, is much faster than the first, the metabolites G, H, and L will dominate. In the opposite case, F, J, K, and I will dominate.

Imagine now that (i) E is a key reactant of high reactivity that turns most reactions it enters irreversible ($\Delta G^0 \ll 0$ —in nature ATP fulfills this role, which makes metabolism critically dependent on this molecule, Box 4.2), and (ii) both reactions are catalyzed by enzymes. The combination of these two factors has a high impact in the variation of the concentrations of the metabolites over time (see an example in Fig. 4.3). Importantly, the concentration of E and the regulation of the activity of the enzymes are ways to control the course of reactions. The following typical situations are possible:

1. Low "level" (concentration) of E results in "accumulation" (increased concentration) of D.
2. A highly active catalysis of $D + E \rightarrow F$ with simultaneous absence of an enzyme to catalyze $D + E \rightarrow G$, or the presence of an enzyme that is inhibited, results in the production of F at a high rate. If $F \rightleftharpoons J + K$ is not catalyzed, a transient accumulation of F occurs.

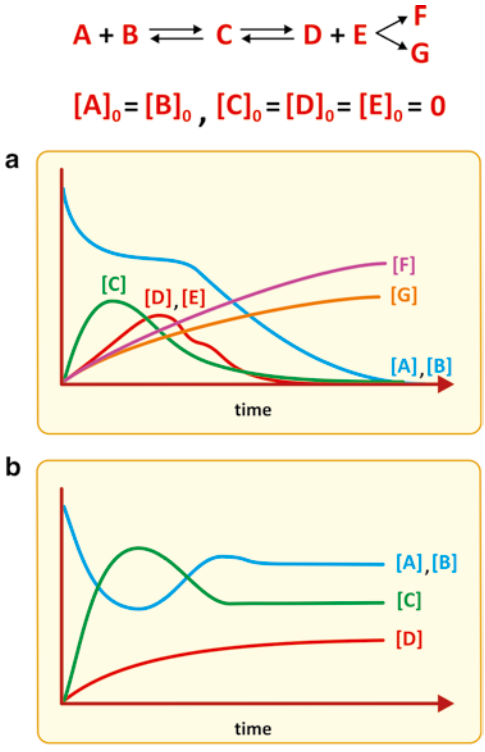


Fig. 4.3 Evolution of the concentration of metabolites in a hypothetical reaction. Depending on the reversibility of reactions and their velocity, the concentration of the metabolites following the addition of A and B at equal concentrations changes dramatically over time, in case reactions $D+E \rightarrow F$ and $D+E \rightarrow G$ are occurring (a) or not (panel b). In panel a the velocity of the formation of F is assumed to surpass the velocity of the formation of G. In panel b, [E] equals [D]

In most metabolic sequence of reactions ("pathways"), all reactions are catalyzed by enzymes, which generates a situation in which the concentration of metabolites may change very rapidly in high amplitude intervals, depending on the thermodynamics of the reactions involved, the enzymes present, and the eventual

degree to which their activity is affected by other molecules or physical conditions such as temperature.

4.2.1 The Basis of Enzymatic Catalysis and Its Impact in Metabolism

As addressed in previous sections and Box 4.2, ATP or related nucleotide triphosphate is a quasi-universal driver of metabolism for its hydrolytic dephosphorylation with $\Delta G^o \ll 0$. Coupling this reaction to others in a metabolic pathway grants the ability to perform reactions that would otherwise occur to a very low level. As long as ATP is available in the cell, the process is assured. This simplicity contrasts with the apparent complexity of the control of enzyme activity in the cellular environment. We say apparent because this complexity is founded on simple basic

Box 4.2: ATP—The Quasi-Universal Driver of Metabolism

ATP is a fascinating molecule for its ubiquity. ATP hydrolysis into $ADP + PO_4^{3-}$ or $AMP + PO_3^{2-} - O - PO_3^{2-}$ has $\Delta G^o \ll 0$, and these reactions are present in several metabolisms, coupled to unfavorable reactions. Any chemical bond splitting involves expenditure of energy, and ATP is no exception. The idea that ATP has "high-energy bonds" between phosphoryl groups whose energy is liberated and used to force the occurrence of unfavorable reactions is misleading albeit widely disseminated. The complete reaction of ATP hydrolysis is:

$$ATP + 3H_2O \rightarrow ADP + PO_4^{3-} + 2H_3O^+$$

but the intervention of water is frequently overlooked. The net balance of the bonds disrupted and created in this reaction is such that energy is liberated, yet the chemical bonds themselves are not energy depots that release energy when broken. It is the balance of bond dynamics involved the reaction that matters. Part of this misunderstanding comes from the fact that one tends to oversimplify the writing of chemical reactions. When written like

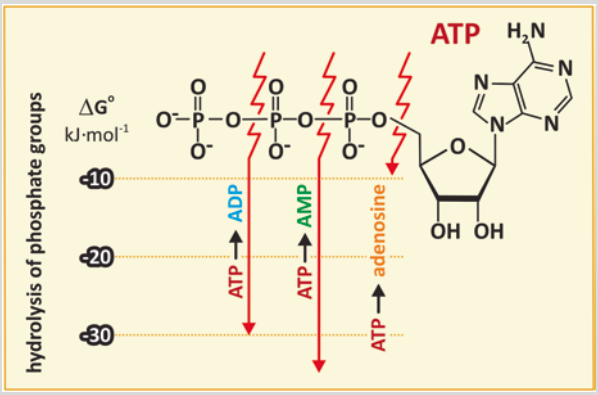
$$ATP \rightarrow ADP + P$$

the involvement of water is implicit but frequently overlooked. Only one bond fission is shown, leaving the idea that the released energy is contained in the chemical bond itself, which is illusory.

Figure 3.23 shows the structure of ATP. At first glance it may seem intriguing why the hydrolysis of ATP into ADP or AMP involves different ΔG^0.

(continued)

Box 4.2 (continued)
This happens because the phosphoryl groups are not equivalent. The electronic distribution in the molecule, for instance, is different, as shown in the following figure.



Having a common highly endergonic hydrolysis reaction to couple to other reactions is a big advantage for cells as only one molecule needs to be synthesized to drive metabolism, and the complexity and diversity of enzymes involved are very much decreased compared to a situation where many and diverse endergonic reactions would be used. To power metabolism, the cells use almost exclusively ATP and enzymes that catalyze its hydrolysis, coupling it to a simultaneous unfavorable reaction. Assuring that ATP is always present and never completely depleted is vital for the cell.

Specific processes that need to be driven thermodynamically by the use of endergonic reactions but should not be dependent on ATP make use of similar molecules such as GTP or UTP.

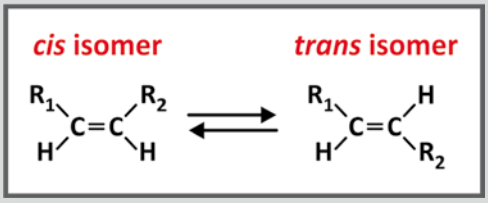
It is interesting that hydrolysis of $\mathrm{ATP} \rightarrow \mathrm{ADP}$, $\mathrm{ATP} \rightarrow \mathrm{AMP}$, and $\mathrm{P_2O_7} \rightarrow 2\mathrm{PO_4^{3-}}$ involve $\Delta G^o \ll 0$ in any case. Nevertheless, the frequency of $\mathrm{ATP} \rightarrow \mathrm{ADP}$ reaction over the others in metabolic processes is much higher. The eventual advantage may have been that, in parallel to the dedicated mechanisms cells have to convert ADP back to ATP using energy from nutrients, ATP can be readily obtained from $\mathrm{ADP} + \mathrm{ADP} \rightarrow \mathrm{ATP} + \mathrm{AMP}$ (see Sect. 8.4). The appearance of AMP may signal a state of ATP depletion that triggers regulatory events in the cell that favor ATP synthesis. This matter is still controversial and will be readdressed in Chap. 8 in connection to the use of ATP for muscle contraction.

principles and is not difficult to understand. The factors affecting enzyme activity are basically the ones affecting protein structure, as the essence of enzyme catalysis is the conformational dynamics of the protein with the bound substrate. Environmental factors affecting catalysis, such as pH or temperature, are not very important in regulating the activity of enzymes because most organelles in cell have buffered pH and constant temperature. The activity of enzymes changes depending on these conditions, but it is not feasible for the cells to change pH or temperature to influence the course of metabolic reactions. So, enzymes have a structure that fits optimal activity at specific temperature and pH ranges (recall the pH of different human tissues in Fig. 2.8), and their activity depends most on ligands, called effectors, that can improve activity ("activators") or decrease it ("inhibitors"). The exact mechanism these ligands use to act as activators or inhibitors of enzymes depends a lot on the structure of the enzyme and how catalysis takes places. The thermodynamics and performance of enzymes as catalysts can be studied under simple but rather abstract principles that describe generally how the velocity of catalysis is independent from the extent of reaction and how it changes with the concentration of substrate and effectors, but do not provide information on how a specific enzyme binds the substrate and distorts its electronic structure to facilitate catalysis (see Sect. 3.3.4).

The simplest thermodynamic description of catalysis is explained in Box 4.3. It illustrates that the velocity of reactions is independent of ΔG^{o}. The simplest

Box 4.3: The Thermodynamics of a Simple Catalyzed Reaction

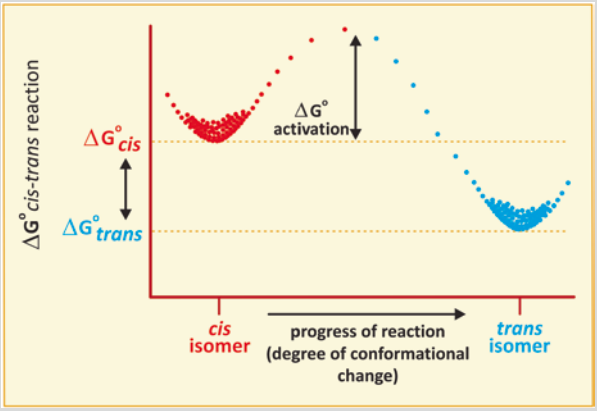
Isomerization is a simple process that we will use to illustrate the thermodynamics associated to the kinetics of reactions and the effect of enzymes on both. *Cis–trans* isomerization, for instance, can be represented by a simplified scheme:



in which R_1 and R_2 are any group of the molecule different from H. Depending on the temperature, solvent, and exact chemical nature of R_1 and R_2, the conformational dynamics of these molecules varies, but there is always a certain degree of flexibility that makes some molecules more distorted than others at any given instant. Some of these molecules acquire a structure that is intermediate between the *cis* and the *trans*-isomer, R_1, R_2, and

(continued)

Box 4.3 (continued)
the H being located in unstable positions that make the molecule more energetic. Higher energy states associated to intermediary conformations are very unstable, and few molecules acquire these conformations, although it may happen occasionally. Plotting a point for each molecule in an energy (of the total molecule) vs. degree of structural conversion plot, one would obtain at a given instant, in equilibrium, the distribution depicted in the following figure. Many molecules are in the *cis* and *trans* states because they correspond to energy minima, but few molecules reach conformations in between these states because these conformations correspond to electronic distributions and nuclei localizations that are not optimal in the balance of charge distributions. The difference between the energy of the reactant (*cis* isomer in this case) and the energy associated to the molecule in the most unlikely state (energy maximum) is the so-called activation energy because evolution from this point to the products corresponds to loss of energy (decrease in ΔG), i.e., the molecule spontaneously progresses to the product (*trans*-isomer) after this point.

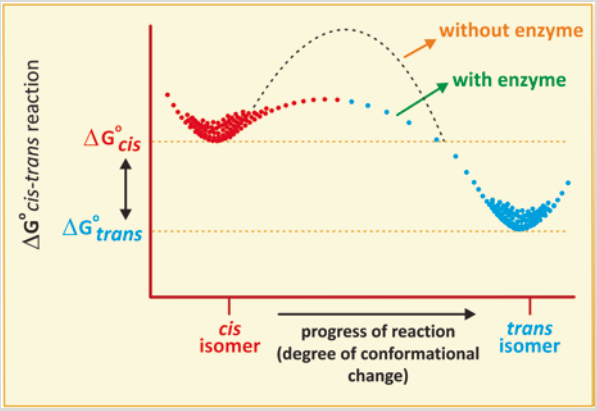


Energy of each molecule according to its degree of conversion between *cis* and *trans* conformations. Each point represents a molecule. This is a hypothetical distribution of a population of molecules in equilibrium at a certain given instant in time. Most molecules are in the cis or trans isomers conformation, which correspond to local minima of energy. The intermediate conformations correspond to molecules in high-energy states, therefore less populated. In practice this implies that very few molecules spontaneously convert between conformations

(continued)

Box 4.3 (continued)

When the activation energy is high, it is very rare that a molecule acquires the energy necessary to adopt the intermediate state that enables spontaneous conversion to products. In other words, the reaction progresses very slowly. Enzymes bind to substrates and distort the structure of the molecules in a way that the electronic distribution and nuclei interactions are more favorable. The energy of activation assigned to the reactant is decreased, and the consequence is that more molecules reach the maxima of energy and progress to products. This means that the velocity of the reaction increases (see figure). ΔG_{cis}° and ΔG_{trans}° remain unchanged, which means that the catalyzed reactions reach a state of equilibrium faster, but the extent of reaction (fraction of reactants converted to products) is not altered by the action of the enzyme.



Enzymes distort the molecular structure of substrates turning the intermediary conformations not so energetic, therefore more likely. Because more molecules reach the intermediary conformations, a higher velocity of product formation is achieved

formulation for an enzymatic reaction was devised mainly by Leonor Michaelis and Maud Menten (Box 4.4). In their abstract model of catalysis, the generic enzyme E_z would bind the generic substrate, S, to form a complex of undetermined nature from which the generic product, P, was formed irreversibly:

$$E_z + S \rightleftharpoons E_zS \rightarrow E_z + P$$

The mathematical deduction in Box 4.4 shows that in this case the velocity of the reaction (i.e., the variation in [P] per time unit) is:

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

Box 4.4: Fundamentals of Enzyme Kinetics of Simple Reactions
Enzymes (E_z) associate to substrates (S) through specific amino acid residues that distort the substrate and make the conversion to products (P) faster. The enzyme interacts actively with the S molecule in the course of catalysis, but the end of reaction results in the release of P with E_z being in the original state as before binding S. In abstract, this situation can be represented by:

$$E_z + S \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} E_zS \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} E_z + P \quad (1)$$

E_zS is the transitory complex in which E_z and S are in contact, S being distorted. k_x are the kinetic rate constants of each conversion ($x = 1, 2, -1, -2$), which are proportionality factors, between velocity and concentration of reactants. In broad terms, it may be said that k_x is the intrinsic propensity of step x to occur (e.g., k_{-1} is the intrinsic propensity for the fast unbinding of E_z and S after E_zS is formed, while k_2 is the intrinsic propensity for the fast release of P after E_zS is formed; once E_zS is formed, the processes to which k_{-1} and k_2 are associated compete with each other). The velocities of each of the four events of the reaction are:

$$v_1 = k_1[E_z][S], \quad v_{-1} = k_{-1}[E_zS], \quad v_2 = k_2[E_zS], \quad v_{-z} = k_{-2}[E_z][S] \quad (2)$$

Assuming that the reaction proceeds in a steady-state condition, the concentration of E_zS does not change with time, i.e., the velocity of creation of E_zS equals the velocity of consumption of E_zS:

$$v_1 + v_{-2} = v_2 + v_{-1} \quad (3)$$

$$\left(k_1[S] + k_{-2}[P]\right)[E_z] = \left(k_{-1} + k_2\right)[E_zS] \quad (4)$$

$[E_z]$ and $[E_zS]$ are not practical variables to work with as is the concentration of total enzyme ($[E_z]_o = [E_zS] + [E_z]$) that is known or more readily measurable in an experiment. At the same time, the velocity of the reaction is the velocity at which P is produced, v:

$$v = k_2[E_zS] - k_{-2}[E_z][P] \quad (5)$$

Combining all equations, it can be deduced that the velocity of product creation is:

$$v = [E_z]_0 \frac{k_2k_1[S] - k_{-2}k_{-1}[P]}{k_{-1} + k_2 + k_1[S] + k_{-2}[P]} \quad (6)$$

(continued)

Box 4.4 (continued)
This is an interesting equation that describes how the velocity of a catalyzed reaction varies with the total concentration of the enzyme, the concentration of the substrate, and the concentration of the product. It also describes how the velocity changes depending on the intrinsic kinetic properties of each step (k_1, k_2, k_{-1}, k_{-2}).

Regardless of the particular [S], [P], or kinetic constants, the equation shows that v is always proportional to $[E_z]_o$. The total concentration of enzyme has a direct impact on the velocity of the reaction. In fact, in cells, increasing or diminishing the expression of enzymes is a way to directly interfere with the rate of critical steps of metabolism.

It is important to further explore the equation in particular situations that may be of significance:

1. $[P] \approx 0$ and/or $k_{-2} \approx 0$ or any other conditions in which $k_{-2}[E_z][P] \ll k_2[E_zS]$ (Michaelis–Menten condition)

   In this case the reaction simplifies to

$$E_z + S \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} E_zS \xrightarrow{k_2} E_z + P \tag{7}$$

   and v simplifies to ($[P] \approx 0$ and/or $k_{-2} \approx 0$ in Eq. (6) of this box):

$$v = \frac{k_2 \left[E_2\right]_0 \left[S\right]}{\frac{k_{-1} + k_2}{k_1} + \left[S\right]} \tag{8}$$

$$v = \frac{V_{max} \left[S\right]}{K_M + \left[S\right]} \tag{9}$$

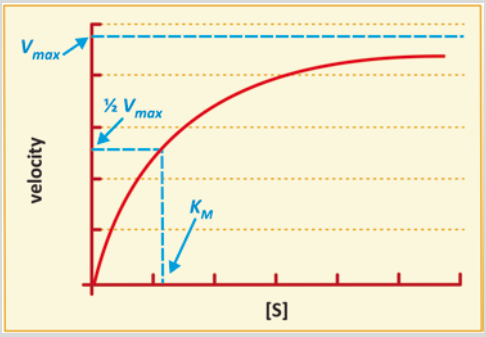
   $k_2[E_z]_o$ corresponds to the velocity of reaction when all the enzyme molecules are bound to S, which is the maximal possible velocity, V_{max}; $(k_{-1} + k_2)/k_1$ corresponds to the equilibrium constant of the dissociation of E_zS and is named Michaelis constant. As K_M^{-1} relates to the extent of the association between E_z and S in equilibrium, it is frequently taken as a measure of the enzyme–substrate affinity.

   Michaelis–Menten conditions lead to a dependence of v on [S] that is hyperbolic (see figure). Although corresponding to a very particular condition of a very simple reaction scheme, the Michaelis–Menten equation [Eq. (9)] has great historical importance, and most enzymes having

(continued)

Box 4.4 (continued)

hyperbolic-like kinetics are described by apparent K_M and V_{max}, even though the reaction scheme they follow is not always exactly the one in Eq. (7).



Dependence of v on [S], as predicted by Eq. (9) (Michaelis–Menten equation). This dependence is a particular case of a rectangular hyperbole (i.e., having asymptotes perpendicular to x and y axes; rectangular hyperbola may be expressed as $(x-h)(y-t)=m$, in which h, t, and m are constants that can be rearranged to the format of a Michaelis–Menten equation for particular cases of h, t, and m

2. $[S] \approx 0$ and/or $k_1 \approx 0$ or any other condition in which $k_1[E_z][S] \ll k_{-1}[E_zS]$

This is a situation similar to the one of Michaelis–Menten but in the reversed sense:

$$E_z + S \underset{k_{-1}}{\leftarrow} E_zS \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} E_z + P \qquad (10)$$

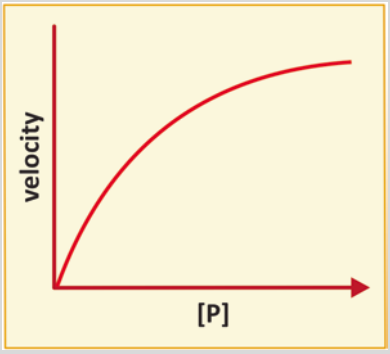
and the velocity dependence on [P] is similar ($[S]=0$ or $k_1=0$ in Eq. (6)):

$$v = \frac{V_{-\max}[P]}{K_{-M} + [P]} \qquad (11)$$

(The subscripts "–" are used to refer the inverse sense of the reaction, i.e., negative velocities.)

(continued)

Box 4.4 (continued)



3. Intermediate values of [S] and [P]
   Because we are working with an example in which the conversion between S and P follows a 1:1 stoichiometry, one may consider a situation where the reaction starts with S being at concentration $[S]_o$ in the absence of P:

$$[S] = [S]_o - [P] \tag{12}$$

   Combining Eqs. (12) and (6):

$$v = [E_z]_0 \frac{k_2 k_1 - (k_2 k_1 + k_{-2} k_{-1}) \frac{[P]}{[S]_0}}{\frac{k_2 + k_{-1}}{[S]_0} + k_1 + (k_{-2} - k_1) \frac{[P]}{[S]_0}} \tag{13}$$

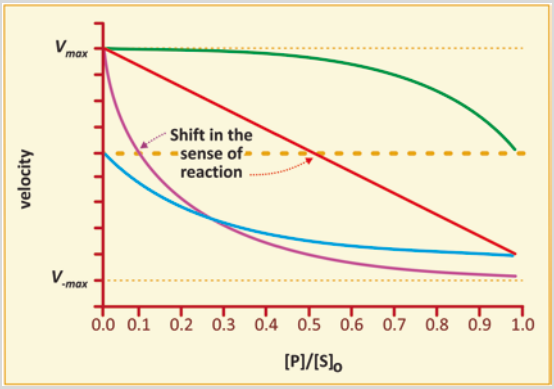
   For a given initial concentration of S, the velocity of reaction changes as P is being produced, as depicted in the figure below. The exact curves depend on the specific values of k_1, k_2, k_{-1}, and k_{-2} for the reactions, but the pictorial examples in the figure show that when [S] dominates over [P] ($[P]/[S]_o \approx 0$), the reaction proceeds with net transformation of S into P with a velocity proportional to [S]. When [P] dominates over [S] ($[P]/[S]_o \approx 1$), the reaction proceeds with net conversion of P to S with a velocity that is proportional to [P]. The negative values of v in the graph reflect the net conversion of P into S (i.e., an overall course of reaction in the reverse direction). The $[P]/[S]_o$ point at which $v = 0$ (i.e., the velocity of formation of P and consumption of P is equal) is obtained setting $v = 0$ in Eq. (13):

$$\left(\frac{[P]}{[S]_0} \right)_{v=0} = \frac{k_2 k_1}{k_2 k_1 + k_{-2} k_{-1}} \tag{14}$$

(continued)

Box 4.4 (continued)

This shows that the shifting point at which the enzyme action changes the sense of the reaction is in fact a balance between the conjugated parameters of the forward and reverse sense steps.



The velocity of a reversible reaction catalyzed by a single enzyme in both senses shifts from positive (net consumption of substrate and production of P) to negative (net consumption of P and production of the substrate) depending on the balance of the kinetic constants involved and the concentration of the substrate and product. The shifting point ($v=0$) is attained when [P] relative to $[S]_0$ is $\frac{[P]}{[S]_0} = \frac{k_2 k_1}{k_2 k_1 + k_{-2} k_{-1}}$.

The *green line* corresponds to $k_{-2}=0$ (Michaelis–Menten condition), $k_1=k_{-1}=k_2$; the *blue line* corresponds to $k_1=0$, $k_{-1}=k_{-2}=k_2$; the *red line* corresponds to $k_1=k_{-1}=k_{-2}=k_2$; the *magenta line* corresponds to $k_1=k_{-1}=k_2=k_{-2}/10$

The practical implication of Eq. (13) is immense. It shows that enzymes catalyzing the simple reaction described by equation 1 are able to increase the velocity of the reaction in both senses depending whether S or P is accumulating, always favoring the consumption of the metabolite (S or P) that is accumulating. This is vital to understand metabolic regulation.

in which V_{max} is the maximal possible velocity for the reaction at a given concentration of enzyme and K_m is the dissociation constant of E_zS (if the first reaction reaches equilibrium). K_m is named Michaelis constant and K_m^{-1} reflects the extent to which E_z binds S, which is usually referred to as the "affinity" of E_z toward S, an intuitive but rather ambiguous concept.

The irreversible step makes the model simpler, but in many cases the second step is reversible, and the same enzyme can catalyze a reaction both ways, from S to P and vice versa. In the excess of S, the dominant reaction is $S \rightarrow P$, and when P accumulates, $P \rightarrow S$ dominates.

Box 4.4 shows that in a reversible reaction, the velocity of reaction is dependent on the relative concentrations of S and P. When the concentration of P reaches cer-

tain critical points, the velocity of the conversion of P to S surpasses the velocity of the opposite process, and the net result is the consumption of P and production of S (Fig. 4.4). This is very important in metabolism as most enzymes catalyze reactions in both directions and the dominant direction depends on the relative concentrations of the metabolites.

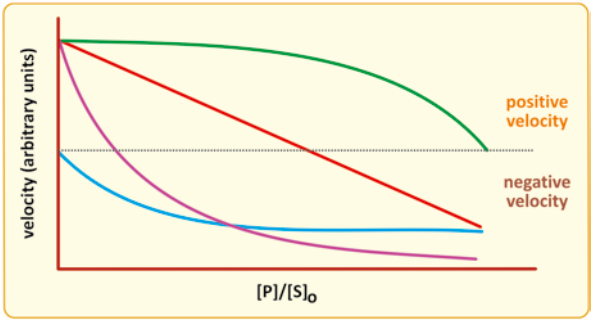
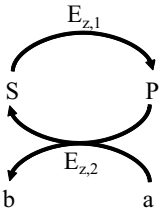


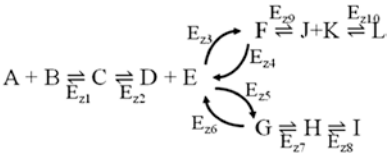
Fig. 4.4 Velocity of the reversible reaction $S \rightleftharpoons P$ catalyzed by an enzyme. $[S]_o$ is the initial concentration of the substrate; [P] equals $[S]_o$ ($[P]/[S]_o = 1$) when all S was converted to P. Positive values of velocity correspond to a net conversion of S to P; negative values correspond to net conversion of P to S. The exact $[P]/[S]_o$ at which $v = 0$ (no net production of P or S) depends on the specific enzymes and substrates to be considered, but the transition from positive to negative values of velocity is a generic trend among enzymes that catalyze reversible reactions. The *red line* corresponds to the variation in velocity for an enzyme that catalyzes the conversion of S to P and P to S equally. In this case, the turning point of velocity corresponds to half conversion of substrates ($[P]/[S]_o = 0.5$). The *magenta line* corresponds to an enzyme that is more efficient in converting P into S: a small concentration of P is enough to drive the net reaction in the direction of converting P into S. The *green line* corresponds to a Michaelis–Menten enzyme: catalysis only converts S into P (always positive velocities) with maximal velocity for maximal concentration of [S] ($[P] = 0$). The *blue line* corresponds to a Michaelis–Menten enzyme that only converts P into S: the velocity is always negative and maximal for maximal concentrations of P ($[S] = 0$)

It is important to stress that a certain enzyme may catalyze the conversion of S to P irreversibly, and a second enzyme may catalyze the inverse reaction also irreversibly. In this way S and P are interconvertible, but two enzymes are involved. Moreover, third molecules may be involved in one of the directions only. This is the case of



where two different enzymes are involved, and the conversion of P to S may also imply conversion of a to b, which may have $\Delta G^o \ll 0$ to make this particular reaction more favorable. It should be noted that with this reaction scheme, the conversion of S to P may be blocked (absent or inactive E_{z1}) without interfering in the conversion of P to S, or even while E_{z2} is activated. When the same enzyme catalyzes $S \rightleftharpoons P$, this possibility does not exist; this enzyme may be activated or inhibited, although both directions will be affected. In the reaction above catalyzed by $E_{z,1}$ and $E_{z,2}$, a fine-tuning of the direction of reaction is possible, but in $S \rightleftharpoons P$ is not.

Returning to the last metabolic scheme in Sect. 4.1, now modified to include enzyme-catalyzed reactions:



It is clear that E_{z3}, E_{z4}, E_{z5}, and E_{z6} are the ones that determine the direction of the flux of the metabolism. There are mechanisms that prevent cells from having E_{z5} and E_{z6}, and E_{z3} and E_{z4} simultaneously activated, so that depending exclusively on which of these key enzymes are active or inactive, this metabolic pathway may be producing L or I, or both, at the expense of consuming A and B, or it can be producing A and B at the expense of consuming I or L, or both. This is the key concept of metabolic regulation. The mechanisms used in cells to activate or inhibit enzymes are then an issue of critical importance and will be addressed in Chap. 5.

An additional factor improves the metabolic processes further. The velocities of reactions catalyzed by key enzymes such as E_{z3}, E_{z4}, E_{z5}, or E_{z6} usually do not follow equation. Whereas enzymes following Michaelis–Menten-like kinetics (Sect. 4.2.1 increase the velocity of reactions proportional to the substrate concentration when [S] is low, these other enzymes change the velocity of reaction abruptly around critical concentrations of the substrate. Velocity varies sigmoidly in response to [S] (Fig. 4.5), in the same way oxygen fixation efficiency by hemoglobin depends on oxygen concentration. This means that some reactions are triggered at high velocity only when the substrate accumulates to a certain level.

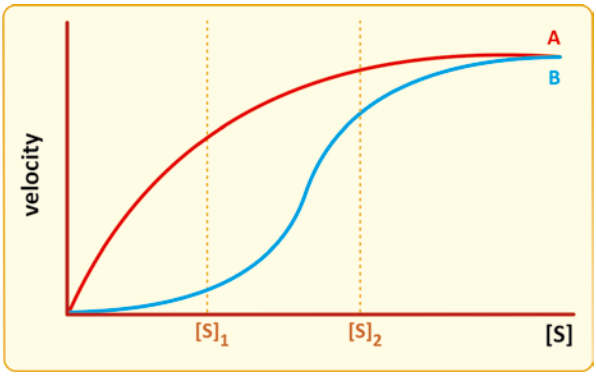


Fig. 4.5 Two types of dependencies of the velocities of reactions catalyzed by enzymes on the concentration of substrate. Enzymes following Michaelis–Menten kinetics (A) have a hyperbolic-like dependence of velocity on [S]. The initial trend (up to $[S]_1$) is linear. In this range, accumulation of the substrate is counteracted by a proportional increase in the velocity of its conversion to product. Enzymes with sigmoidal kinetics (B) have a lag initial regime in which the velocity is low until [S] reaches a critical value, $[S]_1$, after which the velocity increases abruptly. In both cases (A and B), high substrate concentrations ($[S] > [S]_2$) correspond to almost maximal velocity of the catalyzed reaction. In this situation, the enzymes do not modulate velocity in response to substrate concentration because they are already working at maximal possible capacity

Box 4.5: The Importance of K_M, the Michaelis Constant

In strict terms, K_M is only valid for an enzyme that catalyzes a reaction described by

$$E_z + S \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} E_zS \xrightarrow{k_2} E_z + P \qquad (1)$$

as explained in Box 4.4. In practice, most reactions cannot be described this way. For instance, reactions involving more than one substrate do not fit in this scheme. Yet K_M is a combination of all kinetic constants ($(k_2 + k_{-1})/k_1$) that reflects the extent of dissociation of E_zS when equilibrium conditions are reached ($k_2 \ll k_{-1}$). Thus K_M^{-1} reflects the "affinity" of S for E_z and is an extremely valuable tool to compare the "preference" of the same enzyme for different substrates. For this reason, K_M acquired great importance in the study of enzymes from the early days of enzymology, the discipline that is

(continued)

Box 4.5 (continued)
devoted to the study of the enzymes. Even enzymes that did not follow reaction scheme 1 but had hyperbolic-like dependences of catalytic velocity vs. [S] had apparent K_M^{-1} assigned. Other enzymes were studied in conditions in which the velocity would vary in a hyperbolic-like manner with [S] and apparent K_M^{-1} calculated. When more than one substrate is used, for instance, if all substrate concentrations are kept high and only one substrate concentration varies, the velocity of the reaction depends on the concentration of that substrate. The dependence is usually hyperbolic-like and an apparent K_M is estimated, which is valid for that specific substrate. Somewhat mistakenly, K_M became the key parameter to describe the kinetic properties of enzymes, and the quest for methods on how to calculate K_M for specific E_z–S pairs became an import part of biochemistry for many years.

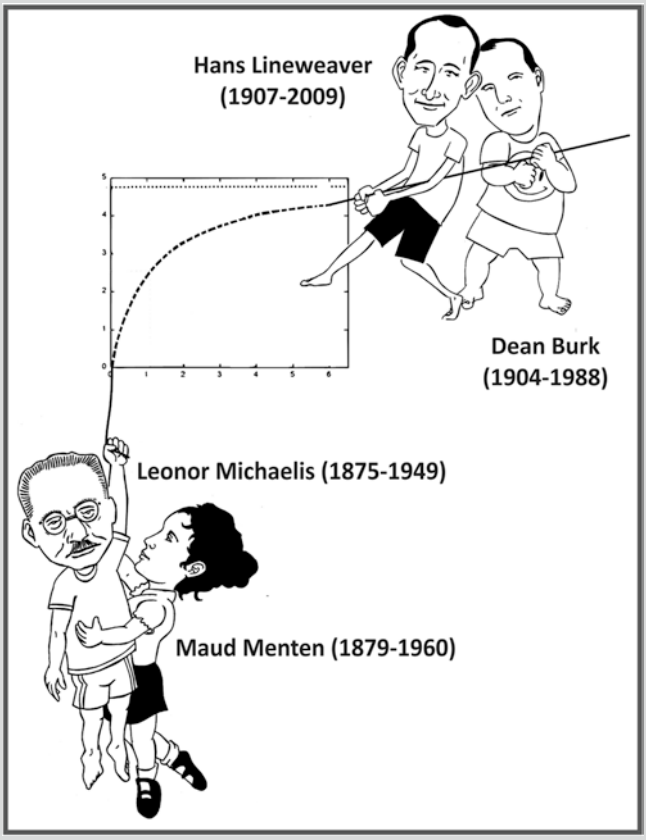
It is obvious from the Michaelis–Menten equation:

$$v = \frac{V_{\max[S]}}{K_M + [S]} \tag{2}$$

that when [S] equals K_M, $v = V_{max}/2$. The graphical interpretation is immediate, as depicted in the figure below. V_{max} is the asymptotic limit of v when [S] tends to infinity; K_M is the [S] in which v is half V_{max}. This is the reason why K_M is so popular among biochemists studying metabolism. When the substrate concentration drops below K_M, the velocity of the reaction is considerably below V_{max} and the process being catalyzed loses efficacy. So K_M is the reference value to estimate the metabolic impact of drops in the concentration of metabolites.

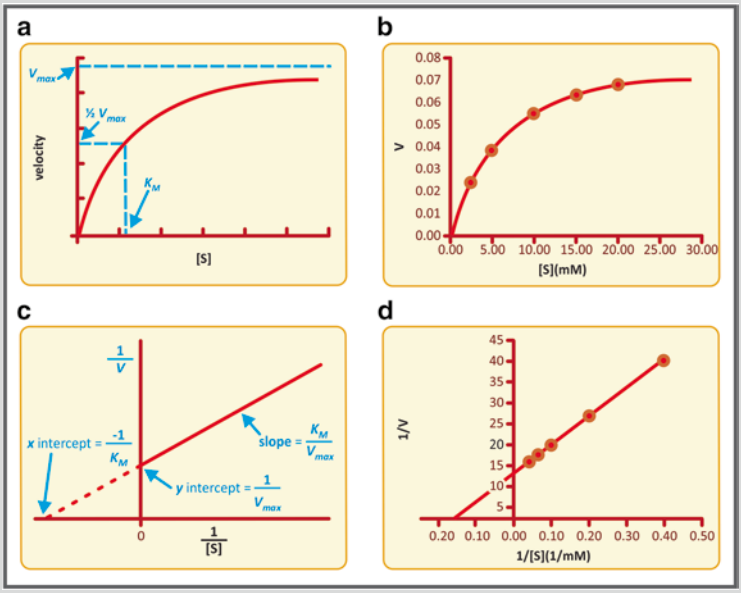
Box 4.5 (continued)
Calculation of K_M is thus of the uppermost importance. However, in an experimental plot of v vs. [S] with discrete data, V_{max} is not easy to identify as experimental data at very high [S] are difficult to attain (see figure below). Nowadays, a computational nonlinear regression fit of Eq. (2) to the experimental data selects the best statistical K_m and V_{max} easily, fastly, and accurately, but in the early twentieth century, when Leonor Michaelis and Maud Menten derived Eq. (1), this was not an option. In the 1930s Dean Burk and Hans Lineweaver overcame the limitation of V_{max} determination by linearizing the Michaelis–Menten equation. When rewritten in the form $1/v$ vs. $1/[S]$, Eq. (1) becomes:

$$\frac{1}{v} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}} \tag{3}$$



(continued)

Box 4.5 (continued)
which describes a straight line from which both K_M and V_{max} are readily calculated (see panels c and d below).



Graphical interpretation of the Michaelis–Menten rectangular hyperbole (**a**). K_M is the substrate concentration at which the reaction velocity is half of the maximal velocity, V_{max}, which is the asymptotic limit of the velocity for infinite [S]. In practice, with experimental data (**b**), V_{max} is very difficult to identify as [S] cannot be extended indefinitely due to solubility or cost limitations. Historically, the most common option to obtain K_M and V_{max} was to perform the linearization of the Michaelis–Menten plot to estimate K_M and V_{max} graphically (**c**): the y intercept is $1/V_{max}$ and the slope is K_M/V_{max}. K_M can also be estimated from the x intercept, which is $-1/K_m$. Panel (**d**) shows the linearization of the data in panel (**b**). For the data presented in the figure, $K_M = 6.39$ mM and $V_{max} = 0.085$ Ms^{-1}. The data were extracted from a 1951 study on the hydrolysis of carbobenzoxyglycyl-L-tryptophan using pancreatic carboxypeptidase (R. Lumry, E. L. Smith, and R. R. Glantz, 1951, J. Am. Chem. Soc. 73, 4330). The linearization of the Michaelis–Menten equation is owed to Hans Lineweaver and Dean Burk, who used the method for the first time in 1934. With modern computational techniques and statistical methods, linearization is not mandatory as V_{max} and K_M can be estimated by nonlinear regression methods that fit the Michaelis–Menten equation directly to experimental data [*red line* in panel (**b**)]

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