Concepts of Equilibrium and Steady State 10

The key to understand catalytic action of an enzyme is the study of reaction velocities and not equilibria. Nevertheless, equilibrium and steady state are two important states of any dynamic system. Both have much relevance to the understanding of enzyme mechanisms and hence metabolism. This chapter will elaborate on these concepts. There are many analogies/models to describe these states which include ponds and rivers. We will look at two simple setups to understand what is meant by equilibrium and steady state, before going into details.

Imagine two beakers connected via a stopcock. Suppose the stopcock is kept closed and water is filled into one of the beakers. What will happen if the stopcock is opened now? Water will move into the second beaker until the levels in the two beakers are the same (Fig. 10.1). Once the two water levels become equal, there is no net flow of water, and the system as a whole becomes stable (and attains *equilibrium*). While water molecules continue to diffuse from one beaker into the other – the water level in the two beakers remains same. This is an example of *dynamic equilibrium*. What happens if the stopcock is now closed? The water level on the two sides remains the same, but there is no free exchange of water molecules across the two beakers. This stable state is an example of static equilibrium. We can distinguish between the static and the dynamic equilibrium by a simple test. A dye introduced in any one beaker will diffuse into the other over time only in the case of dynamic equilibrium. This two-beaker setup is an excellent analogy to "glucose ⇄ fructose" isomerization. Equilibrium mixture of glucose and fructose defines a static equilibrium, as no interconversion occurs due to the prevailing activation energy barrier. Addition of glucose isomerase (enzyme catalyzing this interconversion, equivalent to opening the stopcock and open a path to mix the two compartments!) makes it a dynamic equilibrium.

Let us now consider another situation. Suppose we have a beaker fitted with an inlet and an outlet for water as shown (Fig. 10.1). We start filling the beaker by letting water in (through the inlet) at a constant rate. Initially water drains out

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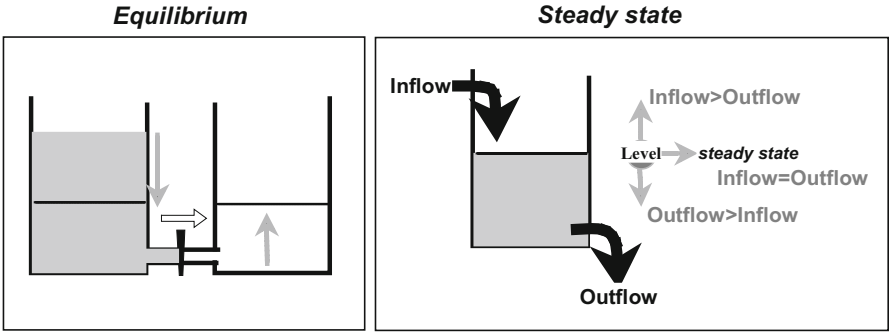


Fig. 10.1 Equilibrium and steady state. When the stopcock is opened, water flows into the empty beaker until the two levels become equal – equilibrium is attained (left panel). Water level is maintained as long as the inflow equals the outflow – steady state (right panel)

(through the outlet) more slowly than it enters because of lower water level (and lower hydrostatic pressure). However, this will cause water level to rise in the beaker – generate more pressure – consequently water will drain more quickly. When the inflow of water becomes equal to the outflow, the water level in the beaker is maintained – and it reaches a *steady state*. There is constant flow of matter (and/or energy) through the system in a steady state.

A thermodynamic equilibrium indicates total randomness (heat death), while living beings represent systems at steady state that are maintained away from equilibrium. Because biological systems are open systems, exchanging energy and matter with their surroundings, they are best represented by a steady-state model than an equilibrium one. Although an equilibrium assumption is simplistic, it is often invoked to approximate many living processes.

10.1 Chemical Reaction Equilibrium

All chemical reactions are reversible in principle. Consider the following equilibrium:

where k_1 and k_{-1} are rate constants for the forward and reverse reactions, respectively. The position of this equilibrium is defined by the equation

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

where K_{eq} is the *equilibrium constant*, while $[A]_{eq}$ and $[P]_{eq}$ represent the concentration of A and P at equilibrium, respectively. At any given instant, the overall rate of change in $[A]$ will be the sum of its rate of disappearance and the rate of formation. This can be written as

$$\frac{d[A]}{dt} = -k_1[A] + k_{-1}[P]$$

The reverse reaction rate becomes negligible when either $[P] = 0$ or k_{-1} is much smaller than k_1. Under these conditions essentially a unidirectional reaction ($A \rightarrow P$) is defined with the rate equation $-d[A]/dt = k_1[A]$. We have already come across a detailed kinetic treatment for such reactions (Chap. 9).

For a reversible reaction at equilibrium, the forward and the backward reactions cannot take different paths. This follows from the principle of detailed balance and *microscopic reversibility* of such phenomena. The forward and the reverse rates must be identical for a reaction at equilibrium. Accordingly

$$k_1[A]_{eq} = k_{-1}[P]_{eq}$$

(We note that, at equilibrium, the two rates are equal and not the rate constants!). Rearranging and by definition of K_{eq} (as above), we obtain

$$\boxed{K_{eq} = \frac{[P]_{eq}}{[A]_{eq}} = \frac{k_1}{k_{-1}}}$$

This equation links the equilibrium constant (K_{eq}, a thermodynamic parameter) with the corresponding rate constants (kinetic parameters) for a given reaction. "Haldane relationship" is one such equation that relates enzyme kinetic constants with the corresponding reaction equilibrium constant (Chap. 15).

ΔG and equilibrium: For any reaction to occur, it should be accompanied by a decrease in free energy. The change in free energy (ΔG) for a reaction at equilibrium is zero. During the course of a reaction, the composition of the reaction mixture changes with time (Fig. 9.2), and ΔG decreases. The actual ΔG is thus related to composition of the reaction mixture and the standard free energy (ΔG°) of the reaction by the following expression:

$$\mathbf{\Delta}G = \mathbf{\Delta}G^\circ + \mathbf{RT}\ln\mathbf{\Gamma}$$

where $\mathbf{\Gamma}$, the *mass action ratio*, is the ratio of product concentration to substrate concentration ($\Gamma = [P]/[A]$). At equilibrium, $\Gamma = K_{eq}$ and $\Delta G = 0$. Therefore

$$\Delta G^\circ = -RT\ln K_{eq} \quad \text{and} \quad K_{eq} = e^{-\frac{\Delta G^\circ}{RT}}$$

Table 10.1 Variation of K_{eq} with ΔG°

K_{eq} ($[P]_{eq}/[A]_{eq}$)	Percent [A] at equilibrium	ΔG° (at 25 °C and pH 7.0)	
		kcal/mol	kJ/mol
10^{-5}	99.99	+6.82	+28.5
10^{-3}	99.90	+4.09	+17.1
10^{-1}	90.91	+1.36	+05.7
10^{0}	50.00	00.00	00.0
10^{1}	09.09	−1.36	−05.7
10^{3}	00.09	−4.09	−17.1
10^{5}	0.001	−6.82	−28.5

Table 10.2 ΔG° and its relation to the position of $[P] \rightleftarrows [A]$ equilibrium

ΔG°	K_{eq}	At equilibrium	Reaction
Positive	<1.0	$[P]_{eq} < [A]_{eq}$	Reactant(s) favored; endergonic
Negative	>1.0	$[P]_{eq} > [A]_{eq}$	Product(s) favored; exergonic
Zero	=1 0	$[P]_{eq} = [A]_{eq}$	At equilibrium; no net change

This is an important relationship – we can determine the value of ΔG° for a reaction if its K_{eq} is known (or *vice versa*). A small difference in ΔG° makes a big difference in K_{eq} – this is because of the log term in the equation (Table 10.1). Thus for a given reaction with a favorable ΔG° of −4.0 kcal/mol, there will be 1000 times more molecules of *P* than *A* at equilibrium. Finally, ΔG° informs us about the position of equilibrium (Table 10.2).

In thermodynamic terms, ΔG of a given reaction is a *state function*. It is independent of the path (or molecular mechanism) of the reaction. For instance, ΔG for oxidation of glucose to CO_2 and water is the same regardless of whether it occurs by combustion in a bomb calorimeter or through cellular metabolism. Hence ΔG provides no information about the rate of a reaction. A negative ΔG simply indicates that the reaction can occur spontaneously.

Lastly, all the rate constants (e.g., k_1 for forward and k_{-1} for reverse) contributing to the equilibrium (and the reaction mechanism) vary independently with temperature. It follows that K_{eq} for a reaction need not be the same at different temperatures; for example, it is 1.00 at 55 °C and 1.17 at 80 °C for glucose isomerase reaction.

ΔG and ΔG°: It is important to recall that ΔG for a given reaction depends on the concentration of reactants and products. It can be numerically larger, smaller, or the same as ΔG°. We will illustrate this with two examples (Fig. 10.2). (1) Isomerization of glucose to fructose (Fig. 10.2) has a K_{eq} of one, i.e., $[\text{Glucose}]_{eq} = [\text{Fructose}]_{eq}$. The standard free energy change for this reaction can be calculated by substitution ($\Delta G^\circ = -RT \ln K_{eq} = 0$). Therefore at equilibrium no net reaction takes place.

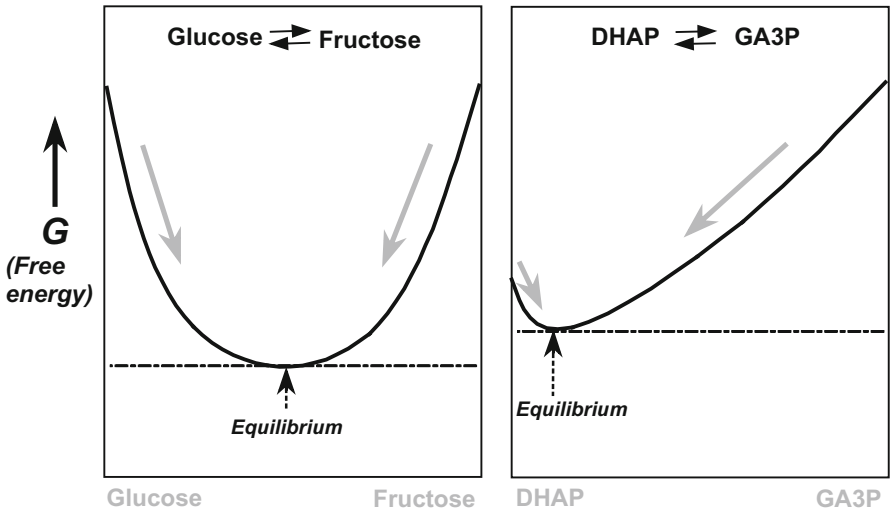


Fig. 10.2 Free energy and equilibrium constant of reaction. The equilibrium composition of reaction mixtures correspond to the lowest point on the curve for each reaction. Gray arrows indicate the direction of spontaneous reaction

However, with 1.0 M glucose and 0.1 M fructose as initial concentrations and at 25 °C, we obtain

$$\Delta G = \Delta G^\circ + RT \ln K_{eq} = 0 + RT \ln \frac{[\text{Fructose}]}{[\text{Glucose}]}$$

$$= 0 + \text{RT} \ln (0.1)$$

$$= 2.303 \times 1.987 \times 10^{-3} \times 298 \times (-1)$$

$$= -1363.67 \times 10^{-3} = -1.364\,\text{kcal/mol}$$

Since the ΔG is negative, under these conditions the reaction glucose → fructose is exergonic and can occur spontaneously. For initial concentrations of 0.1 M glucose and 1.0 M fructose, however, the ΔG will be +1.364 kcal/mol. Therefore, glucose → fructose is now endergonic, while fructose → glucose is exergonic and becomes spontaneous.

(2) Isomerization of dihydroxyacetone phosphate (DHAP) to glyceraldehyde 3-phosphate (GA3P) occurs in glycolysis and has a K_{eq} of 0.0475. Corresponding ΔG° for this reaction (at 25 °C) is +1.80 kcal/mol. Therefore, DHAP will not spontaneously convert to GA3P. However, when the initial concentration of DHAP is 200 μM and the initial concentration of G3P is 3 μM, ΔG becomes

−0.69 kcal/mol. At these concentrations (see Fig. 10.2) DHAP → GA3P becomes exergonic and can occur spontaneously.

The two examples drive home the message – *the criterion of spontaneity for a reaction is* ΔG *and not* ΔG°. Continuous depletion of GA3P maintains the ΔG negative for DHAP ⇄ GA3P reaction and feeds DHAP into glycolysis. Nature has exploited this principle to couple reactions of metabolic pathways; reactions are made spontaneous by adjusting the concentration of reactants and products. The direction of an equilibrium reaction is decided by suitably adjusting the mass action ratio (Γ).

10.2 Binding Equilibrium

Yet another type of equilibrium relevant to biological phenomena (including enzyme catalysis) is the binding equilibrium. To illustrate this let us consider the reversible interaction between an enzyme (E) and a small molecular ligand (L). Binding of L to E proceeds until an equilibrium is reached:

$$E + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} EL$$

As discussed before (for reaction equilibrium), at equilibrium the rates of formation of EL and dissociation of EL are equal:

$$\text{Association rate} = k_{on}[E]\,[L] \quad \text{and}$$
$$\text{Dissociation rate} = k_{off}[EL]$$

At equilibrium

$$\text{Association rate} = \text{Dissociation rate}$$
$$k_{on}[E]\,[L] = k_{off}[EL]$$
$$\frac{k_{on}}{k_{off}} = \frac{[EL]}{[E]\,[L]} = K_{eq}$$

This binding equilibrium is maintained by a balance between the two opposing reactions. The ratio of the rate constants for the association (k_{on}) and the dissociation (k_{off}) reactions is equal to the *equilibrium constant* ($\boldsymbol{K_{eq}}$). Molecules of E and L must collide with each other in order to produce EL. Hence this bimolecular association rate is proportional to the product of [E] and [L].

Traditionally, the equilibrium constant is so defined that the concentration of product (s) appear in the numerator and the concentration of the reactant(s) appear in the denominator. In this sense, K_{eq} for "$E + L \rightarrow EL$" reaction is an *association constant*

($\boldsymbol{K_A}$), also known as *affinity constant*. It has the units of M^{-1}. The larger the value of K_A, the stronger is the binding between E and L. We may also define the K_{eq} for "$EL \rightarrow E + L$" reaction" – accordingly called the *dissociation constant* ($\boldsymbol{K_D}$). The K_D is the reciprocal of K_A and has the units of M. Obviously the smaller the value of K_D, the stronger is the binding between E and L. This is illustrated with an example in the box below.

Suppose a fungal cell contains an enzyme with $[E] = 10^{-9}$ M and its ligand with $[L] = 10^{-6}$ M; suppose the K_D for their binding be 10^{-7} M. Then

$$\frac{[E]\,[L]}{[EL]} = K_D$$

The ratio of unbound to bound $[E]$ will be $[E]/[EL]$ and therefore

$$\frac{[E]}{[EL]} = \frac{K_D}{[L]} = \frac{10^{-7}\text{M}}{10^{-6}\text{M}} = \frac{1}{10}$$

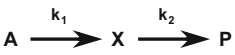
Inside the cell, we thus expect one molecule of E to be free for every 10 molecules of E in the bound (EL) form. What if for some reason (mutations or regulation!) the K_D changes to 10^{-4}M? We see that $[E]/[EL]$ will then be 100; only one molecule of E in a hundred is present as EL.

In most enzyme literature, the equilibrium constant is presented as dissociation constant (K_D). Unless otherwise required, we will follow this convention for K_D throughout this book.

10.3 Complex Reactions Involving Intermediates

If the reaction $A \rightarrow P$ goes through several steps, then it is described as a *complex reaction*. Complex reactions may include one or more reversible steps and intermediates. Therefore the kinetic investigation of such reactions is an analytical problem to determine the nature and number of constituent steps. Complex reactions are often described by complicated rate equations that are not amenable to direct analysis. It is useful to introduce certain assumptions in order to simplify them. We will explore some of these approximations through examples.

Consider a reaction involving two first-order consecutive steps:



An intermediate X is produced in the first step and is consumed in the next. In other words, A yields P not directly but through X. Representative concentration

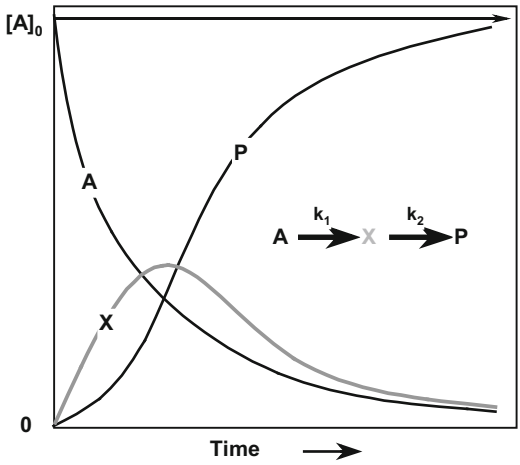


Fig. 10.3 Concentration versus time curves for a reaction involving two first-order consecutive steps

versus time curves for A, X, and P are shown in Fig. 10.3. As the reaction proceeds, we note that:

– [A] decreases to zero at completion.
– [P] increases from its initial value of zero.
– [X] builds up first, reaches a maximum, and then decreases to zero.

The position of the maximum in [X] – the extent of accumulation of X – depends on the relative magnitudes of k_1 and k_2. If $k_2 >> k_1$ then significant accumulation of intermediate X will not occur. However if $k_1 >> k_2$ then X is a relatively long-lived intermediate, an appreciable concentration of it may develop during the time course. This brings us to the concept of slow and fast steps of a complex reaction. We recall that rates and rate constants of an elementary step are not the same. Actual rates for the two consecutive reactions are as follows:

For $A \rightarrow$ X, rate $= k_1$ [A] and
For $X \rightarrow P$, rate $= k_2$ [X]

The actual rates of the two steps therefore depend both on the rate constants and the concentration of the reactant species. When compared, the actual rate of the first step may be slower than the second or *vice versa*. If the first step ($A \rightarrow X$) is slower than the second ($X \rightarrow P$), then the barrier for the first step must be higher than that for the second (Fig. 10.4, black curve) (Sudi 1991). The overall reaction $A \rightarrow P$ can only be as fast as the slowest step in the sequence. Hence the first slow step acts as a "bottleneck" and becomes the *rate-determining* (or *rate-limiting*) step of the reaction (see box below for a simple analogy). Consequently the overall rate equation in such cases gets simplified to the rate equation for the slowest step itself. In general, *the slowest step of a complex reaction mechanism will control the overall reaction rate*.

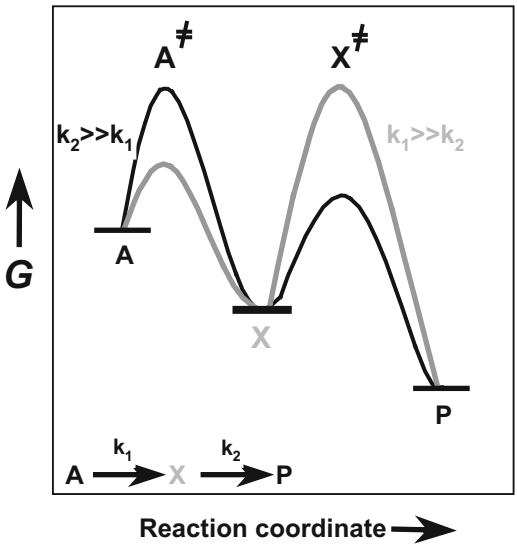


Fig. 10.4 Reaction profile for a sequential two-step overall process. When $k_2 >> k_1$ (the black curve), the first step is rate-limiting. But if $k_1 >> k_2$ (the gray curve), then the second step is rate-limiting

There are many interesting ways of illustrating the concept of *rate-limiting step* in a sequence of operations. These include passing the baton during a relay race, assembling cycles in factory, sequential steps in a laundry, liquid flow in pipes of different diameters, etc. We will use dish washing as an example (Last 1985). Consider two significant operations in washing of dirty dishes – scrubbing and rinsing.

Dirty Dishes → Scrubbed Dishes → Clean Dishes

In this sequence of operations, if rinsing (second step) is faster than scrubbing, then (a) there will be very few scrubbed dishes at any given time and (b) overall dish-washing activity cannot be faster than that of scrubbing. For every ten dishes scrubbed in 10 minutes, we cannot have more than ten dishes cleaned – even if rinsing is done much faster. On the other hand, if scrubbing (first step) is faster than rinsing, then (a) a pile of scrubbed dishes accumulates over time, and (b) overall dish-washing process cannot be faster than rinsing. For every ten dishes rinsed in 10 min, we cannot clean more than ten dishes – even if scrubbing is done much faster.

As reactions become more complicated, their exact kinetic solution becomes increasingly difficult. Some modifications to the reaction setup can simplify the situation to an extent. For instance, by taking large excess of one reactant, the working reaction order may be reduced (such as pseudo-first-order reactions). Introducing certain assumptions can also make the problem manageable. Two generally useful tools are the application of the *equilibrium assumption* and the

steady-state approximation. They are helpful in deriving rate equation for complex reactions that involve simultaneous changes in three (or more) concentrations and two (or more) rate constants. In the case of reaction $A \rightarrow X \rightarrow P$, deducing the rate equation is hampered by the fact that $[X]$ is a continuous variable and often difficult to measure directly. This is overcome either by assuming a rapid equilibrium between A and X (with a slow $X \rightarrow P$ step) or a steady state for $[X]$ (after an initial induction period, the rate of formation and rate of disappearance of X are just balanced). When either of these assumptions are valid, we can express $[X]$ in terms of initial $[A]$. The overall rate equation can then be deduced in terms of initial concentration of A, the two rate constants (k_1 and k_2), and the single independent variable, time.

Equilibrium and steady state are general concepts broadly applicable at various scales of biological systems. They help us appreciate/analyze multistep rate processes at the level of ecosystems, population growth, metabolic pathways, and enzyme forms along the reaction mechanism. We will revisit the two assumptions (equilibrium assumption and the steady-state approximation) and their utility in deriving the rate equation describing an enzyme catalyzed reaction (Chap. 15, Henri–Michaelis–Menten equation). The trick in employing these assumptions however is in appreciating their limitations and conditions under which they may not be used!

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