Chapter 7

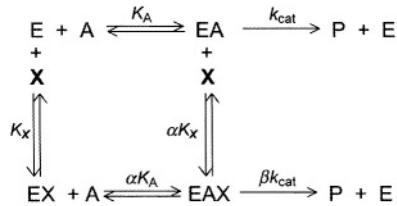
Enzyme Activation

In enzymology, *activators* are the molecules that increase the velocity of an enzyme-catalyzed reaction due to reversible binding to the enzyme. In *nonessential activation,* the reaction can occur in the absence of the activator, as well as in its presence. In an *essential activation,* the reaction will not take place in the absence of an activator (Reinhold, 1969; Segel, 1975; Dixon & Webb, 1979).

7.1 NONESSENTIAL ACTIVATION

Kinetically, the nonessential activation can be treated in the same manner as a general nonlinear inhibition; however, this time the changes are in the opposite direction: wherever we have had an inhibition, now we have an activation (Segel, 1975).

For a monosubstrate reaction, the kinetic model is analogous to a general model for a nonlinear hyperbolic inhibition, described in Chapter 6 (Section 6.1):



(7.1)

where $V_1 = k_{cat}E_0$, X is the activator molecule, and K_X an *activator dissociation constant,* with a dimension [concentration].

A rate equation for this general case may be derived from rapid equilibrium assumptions to obtain a Michaelis-Menten form:

$$v_0 = \frac{V_1 A}{K_A\left(\frac{1+\frac{X}{K_X}}{1+\frac{\beta X}{\alpha K_X}}\right) + A\left(\frac{1+\frac{X}{\alpha K_X}}{1+\frac{\beta X}{\alpha K_X}}\right)} \tag{7.2}$$

Equation (7.2) has the same form as Eq. (6.2) (Chapter 6) for the hyperbolic inhibition in monosubstrate reactions. In enzyme activation, however, contrary to inhibition, $\beta > 1$ and $\alpha < 1$.

Equation (7.2) can be rearranged in the Lineweaver–Burk manner:

$$\frac{1}{v_0} = \frac{1}{V_1}\left(\frac{\alpha K_X + X}{\alpha K_X + \beta X}\right) + \frac{\alpha K_A}{V_1}\left(\frac{K_X + X}{\alpha K_X + \beta X}\right)\frac{1}{A} \tag{7.3}$$

and presented graphically (Fig. 1).

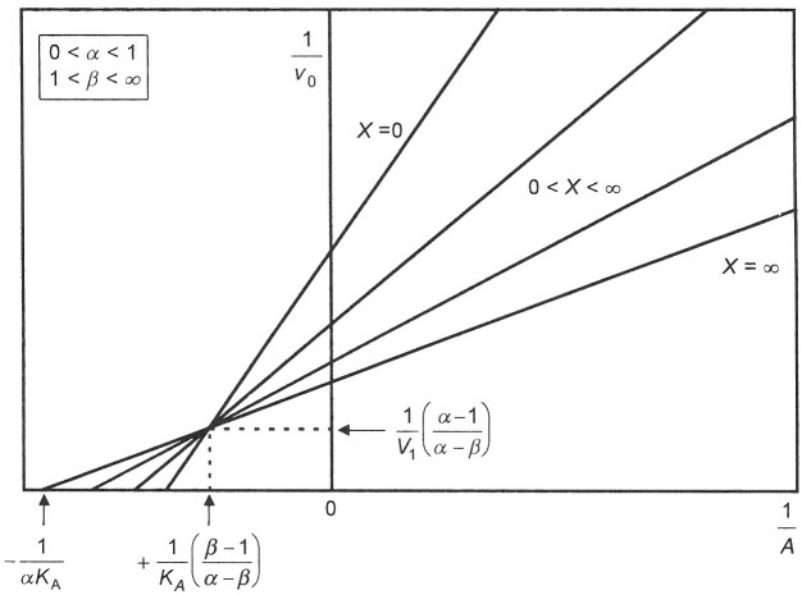


Figure 1. Nonessential activation. Graphical presentation of Eq. (7.3).

Compare Fig. 1 for the nonlinear inhibition of Chapter 6, and notice that the double reciprocal plot in both figures is a family of straight lines with a common intersection point; this intersection point has different coordinates in activation and inhibition systems.

Similarly to nonlinear inhibition, at infinitely high X, Eq. (7.2) reduces to

$$v_0 = \frac{\beta V_1 A}{\alpha K_A + A} \tag{7.4}$$

Thus, at infinitely high activator concentration, the apparent K_A is equal to αK_A and the apparent maximal velocity is equal to βV_1.

The slopes and intercepts for the plot shown in Fig. 1 are equal:

$$\text{Slope}_{1/A} = \frac{\alpha K_A}{V_1}\left(\frac{K_X + X}{\alpha K_X + \beta X}\right) \tag{7.5}$$

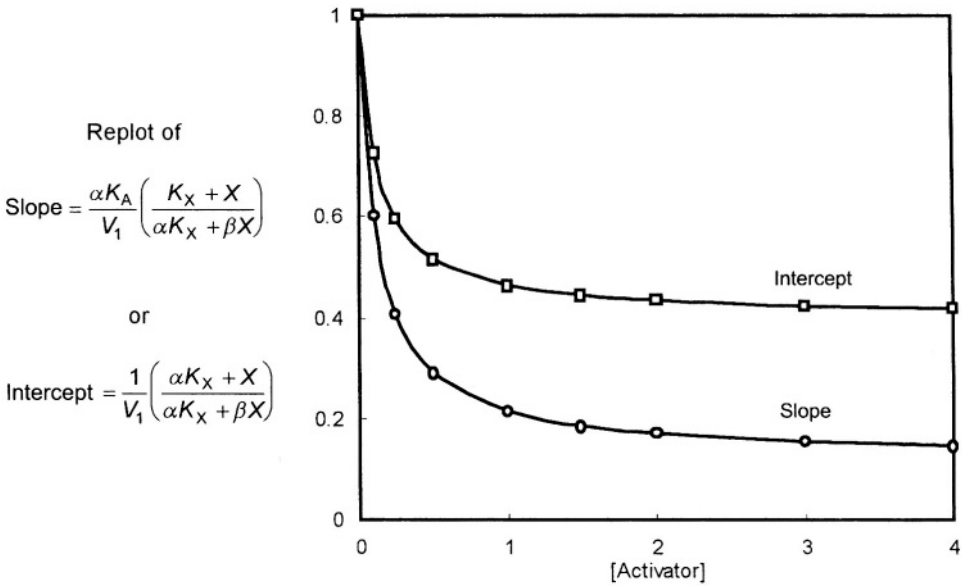


Figure 2. Replot of slopes and intercepts drawn according to the general rate Eq. (7.3). The data points are calculated assuming that $\alpha = 0.3$ and $\beta = 2.5$; $V_1 = K_A = K_X = 1$.

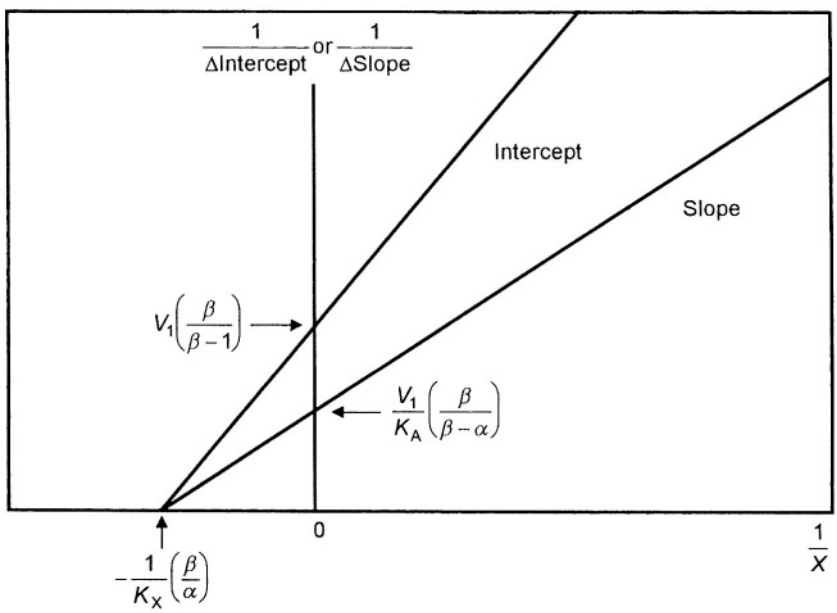


Figure 3. Nonessential activation. Analysis of Eqs. (7.5) and (7.6) by the differential method.

$$\text{Intercept}_{1/A} = \frac{1}{V_1}\left(\frac{\alpha K_X + X}{\alpha K_X + \beta X}\right) \tag{7.6}$$

Figure 2 shows a replot of slopes and intercepts from Fig.1 as a function of increasing concentrations of an activator. From Fig. 2, it is clear that the nonessential activation, in the general case, is a *nonlinear activation,* as the replots of slopes and intercepts are hyperbolas.

In this case, since both functions are nonlinear, it is possible to determine the kinetic constants α and β by the application of the differential method, similarly as described for the nonlinear inhibition in Chapter 6 (Section 6.2) (Fig. 3).

The nonessential activation, in the general case, is a hyperbolic nonlinear activation. Analogous to hyperbolic inhibition, we can derive a number of different activation mechanisms, by inserting different values for α and β into Eq. (7.2), as was described in Chapter 6 (Section 6.3), for different types of hyperbolic inhibitions. However, in activation processes, it is always $\beta > 1$.

7.2 ESSENTIAL ACTIVATION

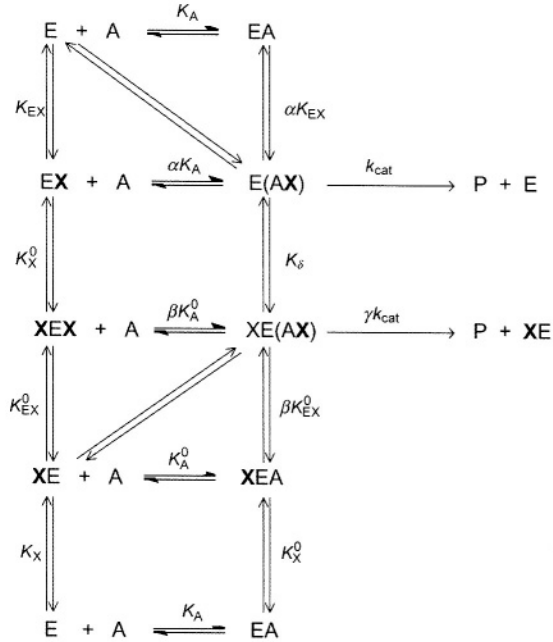
7.2.1 Activation by Substrates

According to our definition of essential activators in Section 7.1, the reaction will not take place in the absence of an activator. Thus, according to this definition, we may treat all the bisubstrate and trisubstrate reactions as essential activations in which both substrates in turn may be regarded as an activator for other substrates (Purich & Allison, 2000). This topic, however, is described in detail in Chapters 8 and 9 (bisubstrate reactions), and in Chapter 12 (trisubstrate reactions).

7.2.2 Complex Activation by Metal Ions

All enzymatic reactions involving ATP require Mg^{2+} ion as an activator. These types of reactions are very common in nature, especially with kinases. In such cases, the true substrate is $Mg \cdot ATP^{2-}$ complex, that is, a *substrate–activator complex,* and free ATP molecules are not the active substrates of enzymes. In addition to forming an active complex with substrate, metal ions may also combine with the enzyme at an additional specific *activation site*; this additional binding site may be *essential* or *nonessential.* Thus, the metal ions may be treated as true substrates of enzymes.

London and Steck (1969) have developed a general model, based on rapid equilibrium assumptions, for a monosubstrate enzyme that combines with substrate, activator, and a substrate–activator complex. The kinetic model for this type of activation is rather complex (Reaction (7.7)).



(7.7)

Additional equilibria:

$$\mathrm{E} + \mathrm{AX} \underset{}{\overset{K_{AX}}{\rightleftharpoons}} \mathrm{E(AX)}$$

$$\mathrm{XE} + \mathrm{AX} \overset{K_{AX}^0}{\rightleftharpoons} \mathrm{XE(AX)}$$

$$K_\delta = \frac{\beta K_A^0 K_X^0}{\alpha K_A}$$

The complex reaction (7.7) may be drawn in two dimensions as a cube, whereby the eight corners of the cube are represented by eight enzyme forms: E, EA, EX, E(AX), XEX, XE(AX), XE, and XEA.

The metal activator (X) not only combines with a free enzyme to form an enzyme–activator complex (XE), but also combines with EA to form E(AX), with an EX complex to form XEX, and with E(AX) complex to form XE(AX). If the activation is nonessential, both E(AX) and XE(AX) are catalytically active. Since $\mathrm{AX}\,(\mathrm{Mg \cdot ATP^{2-}})$ is a true substrate of enzyme, EA and XEA are inactive. The general velocity equation may be derived from the rapid equilibrium assumptions, in the following form:

$$\frac{v_o}{E_o} = \frac{k_{cat}\left(\frac{[AX]}{K_{AX}}\right) + \gamma k_{cat}\left(\frac{[X][AX]}{K_X K_{AX}^0}\right)}{1 + \frac{[A]}{K_A} + \frac{[X]}{K_{EX}} + \frac{[AX]}{K_{AX}} + \frac{[X]}{K_X} + \frac{[A][X]}{K_A^0 K_X} + \frac{[X]^2}{K_X K_{EX}} + \frac{[X][AX]}{K_X K_{AX}^0}} \tag{7.8}$$

Equation (7.8) can be rearranged as:

$$\frac{v_0}{E_0} = \frac{k_{cat}\left(\frac{[AX]}{K_{AX}}\right) + \gamma k_{cat}\left(\frac{[X][AX]}{K_X K^o_{AX}}\right)}{\left(1 + \frac{[A]}{K_A} + \frac{[X]}{K_{EX}} + \frac{[AX]}{K_{AX}}\right) + \frac{1}{K_X}\left(1 + \frac{[A]}{K^o_A} + \frac{[X]}{K_{EX}} + \frac{[AX]}{K^o_{AX}}\right)\cdot[X]} \tag{7.9}$$

If the activation by a metal ion is *essential,* the first numerator term AX/K_{AX} is omitted from Eq. (7.9).

The situation shown in reaction (7.7) may be further complicated if the metal ion combines with the substrate; this is often the case in reactions catalyzed by kinases, where the metal ion (usually Mg^{2+}) combines with ATP, thus affording an additional equilibrium:

$$Mg^{2+} + ATP^{4-} \overset{K_0}{\rightleftharpoons} (Mg \cdot ATP)^{2-} \tag{7.10}$$

The model shown in reaction (7.7) is a general model, very similar to the model for an inhibition by a mixture of two inhibitors in monosubstrate reactions, described in Chapter 5 (Section 5.7). From the model in reaction (7.7), one can derive several special cases that comprise only the parts of the general model shown in the reaction (Morrison, 1979). The derivation of rate equations for enzyme activation is analogous to the derivation of rate equations for enzyme inhibition; the main difference is that the binding of activators increases, whereas the binding of inhibitors decreases the activity of enzymes. In the general model (Reaction (7.7)), that is, described by Eq. (7.8), the dependence of initial velocity, v_o, on the concentration of activator X does not provide a hyperbola; that is, the response of v_0 to increasing concentrations of X is sygmoid. Therefore, this model will not be discussed further in this chapter; the nonhyperbolic kinetic systems are discussed in Chapter 13.

London and Steck (1969) have extended their work with activators by describing a useful graphical method for analyzing enzyme activation, particularly by metal ions.

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