the peak of the curve shifts to the right with increasing $[IP_3]$. Both of these features are observed experimentally.

4.7 Michaelis–Menten Kinetics

One of the most important reductions of a complex reaction is associated with the names of Michaelis and Menten, and in fact, this reduction is used in several places in this book. In many texts this reduction is used to introduce the ideas and methods of time scale reduction. However, the reduction of Michaelis and Menten is slightly different from the reductions discussed so far in this chapter, because it does not rely on large differences in reaction rates. Instead, the difference in time scale of reaction comes from an entirely different source, which we now discuss.

Working in the early part of the twentieth century, Michaelis and Menten set about to explain several key experimental facts regarding the conversion of substrate to product catalyzed by simple enzymes. Figure 4.12 illustrates their results for the enzyme invertase, which converts sucrose to glucose and fructose. These graphs give the time course of accumulation of product (measured as the change in optical rotation of the solution) for a fixed total enzyme concentration $[E]_T$. In the three curves the initial concentration of sucrose, $[S]_T$, is increased from 5.2 mM to 10.4 mM to 20.8 mM. The initial rate of increase of product, given by the slope of the three curves ($V(0)$), is plotted for these and similiar experiments in Figure 4.12. As the concentration of sucrose increases, the initial rate saturates at the value V_{max}. This function is *hyperbolic* and can be fit with the expression:

$$V(0) = V_{max}[S]_T/([S]_T + K_m). \tag{4.60}$$

The concentration of substrate at which $V(0) = V_{max}/2$ is called the *Michaelis constant* or K_m for the enzyme. In further experiments Michaelis and Menten established that for a fixed concentration of sucrose, $V(0)$ increased linearly with $[E]_T$. This hyperbolic dependence of the rate on $[S]_T$ and linear dependence on $[E]_T$ has been established for a number of enzymes, and Michaelis and Menten proposed a kinetic model to explain these facts.

Their work was motivated by Victor Henri, who had suggested earlier that enzyme and substrate might form a complex. Using the notation E to represent the free enzyme in solution, S the substrate, P the product, and ES the enzyme–substrate complex, Michaelis and Menten proposed that the kinetics could be described by the two chemical reactions

$$E + S \underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}} ES,$$

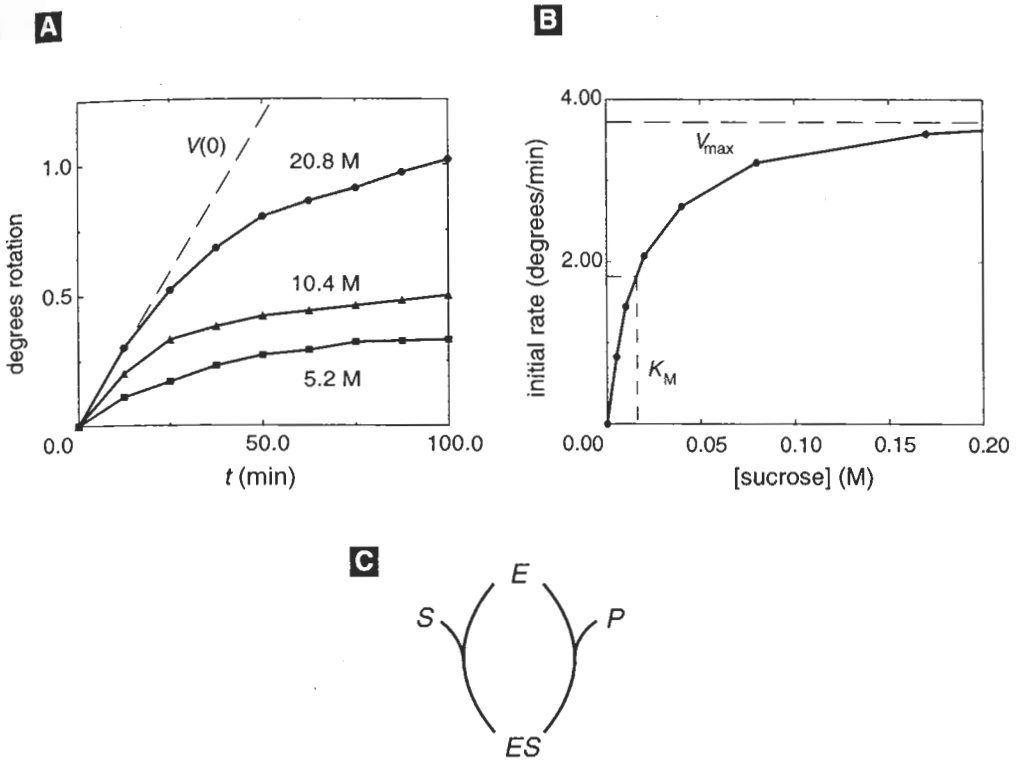

Figure 4.12 (A) Experimental rate of loss of optical activity of sucrose for three initial concentrations of sucrose and fixed concentrations of the enzyme. Data of Michaelis and Menten, replotted from Wong (1975). (B) The initial rate $V(0)$ in (A) of the invertase catalyzed reaction plotted as a function of sucrose concentration. (C) Two–state diagram for the Michaelis–Menten model.

$$ES \xrightarrow{k_2^+} P + E. \tag{4.61}$$

This scheme can be recast easily as a diagram for enzyme states like that used in Figure 3.2 for the GLUT transporter. This is done in Figure 4.12C, where the two states of the enzyme are the free form (E) and the complex (ES). This two–state model actually involves four variables, because S and P also vary. However, the total concentration of enzyme ($[E]_T = [E] + [ES]$) and of substrate ($[S]_T = [S] + [ES] + P_C$) are conserved, so that only two of the concentrations change independently.

In this analysis we choose the concentration of substrate [S] and enzyme–substrate complex [ES] as variables and eliminate the concentration of enzyme using the conservation condition $[E] = [E]_T - [ES]$. Because the catalytic process is irreversible, the concentration of product P_C does not appear in the kinetic equations for [S] and [ES],

which can be obtained from the mass action laws applied to (4.61):

$$d[\mathrm{S}]/dt = -k_1^+[\mathrm{E}]_\mathrm{T}[\mathrm{S}] + (k_1^- + k_1^+[\mathrm{S}])[\mathrm{ES}], \tag{4.62}$$

$$d[\mathrm{ES}]/dt = k_1^+[\mathrm{E}]_\mathrm{T}[\mathrm{S}] - (k_1^- + k_2^+ + k_1^+[\mathrm{S}])[\mathrm{ES}]. \tag{4.63}$$

The two important time scales in the Michaelis–Menten model are the time that it takes for substrate to be converted into product, and the time scale on which enzyme–substrate complex forms. Thus, the important rates are $k_1^+[\mathrm{E}]_\mathrm{T}$ and $k_1^+[\mathrm{S}]$. Now if we assume, as did Michaelis and Menten, that there is very little enzyme compared to substrate, then we also expect there to be very little complex compared to substrate. This means that the rate $k_1^+[\mathrm{E}]_\mathrm{T}$ is much smaller than the rate $k_1^+[\mathrm{S}]$, at least initially before a lot of product has been made. Thus, it is the small ratio of the concentration of catalyst to total concentration of substrate ($[\mathrm{S}]_\mathrm{T}$), i.e., $\epsilon = [\mathrm{E}]_\mathrm{T}/[\mathrm{S}]_\mathrm{T}$, that makes the two time scales widely different. The two natural time scales in the model are therefore $\tau_\mathrm{s} = 1/k_1^+[\mathrm{E}]_\mathrm{T}$ (slow) and $\tau_\mathrm{f} = 1/k_1^+[\mathrm{S}]_\mathrm{T}$ (fast).

Having identified the fast and slow time scales, we proceed to *nondimensionalize* all of the variables in (4.62) and (4.63), including the time. We define the nondimensional dependent variables $\hat{s} = [\mathrm{S}]/[\mathrm{S}]_\mathrm{T}$ and $\hat{es} = [\mathrm{ES}]/[\mathrm{E}]_\mathrm{T}$. The choice of a nondimensional time then determines whether our analysis focuses on the fast or the slow time scale.

If we nondimensionalize time using the slow time scale, then $\hat{t} = t/\tau_\mathrm{s} = tk_1^+[\mathrm{E}]_\mathrm{T}$, and we restrict ourselves to slow changes. Substituting these nondimensional variables into (4.62) and (4.63) gives

$$d\hat{s}/d\hat{t} = -\hat{s} + \left(\frac{k_1^-}{k_1^+[\mathrm{S}]_\mathrm{T}} + \hat{s}\right)\hat{es}, \tag{4.64}$$

$$\epsilon d\hat{es}/d\hat{t} = \hat{s} - \left(\frac{k_1^- + k_2^+}{k_1^+[\mathrm{S}]_\mathrm{T}} + \hat{s}\right)\hat{es}. \tag{4.65}$$

The small dimensionless parameter ϵ appears as a multiplicative factor on the left–hand side of (4.65). If we think of the asymptotic limit as $\epsilon \to 0$, then we can set the right–hand side of (4.64) equal to zero. This is the lowest–order term in the asymptotic analysis on the slow time scale, which allows us to solve explicitly for $\hat{es}$ and obtain

$$\hat{es} = \frac{\hat{s}}{(k_1^- + k_2^+)/(k_1^+[\mathrm{S}]_\mathrm{T}) + \hat{s}}. \tag{4.66}$$

Substituting this expression into the right–hand side of (4.64) gives the rate of change of substrate

$$d\hat{s}/d\hat{t} = -\frac{\hat{V}_\mathrm{max}\hat{s}}{\hat{s} + \hat{K}_\mathrm{m}}, \tag{4.67}$$

with

$$\hat{V}_\mathrm{max} = \frac{k_2^+}{k_1^+[\mathrm{S}]_\mathrm{T}}, \qquad \hat{K}_\mathrm{m} = \frac{k_1^- + k_2^+}{k_1^+[\mathrm{S}]_\mathrm{T}}. \tag{4.68}$$

Equation (4.67) can be written in terms of the original dimensional variables as

$$d[\mathrm{S}]/dt = -\frac{V_{\max}[\mathrm{S}]}{[\mathrm{S}] + K_{\mathrm{m}}}, \tag{4.69}$$

with $V_{\max} = k_2^+[\mathrm{E}]_{\mathrm{T}}$ and $K_{\mathrm{m}} = (k_1^- + k_2^+)/k_1^+$. This is identical to the expression obtained by Michaelis and Menten for the initial rate of product formation (4.60). However, time–scale analysis suggests that this expression is valid on the slow time–scale $\tau_{\mathrm{s}} = 1/k_1^+[\mathrm{E}]_{\mathrm{T}}$ and is not restricted to the initial period of the catalytic process.

To see how the model simplifies on the fast time–scale $\tau_{\mathrm{f}} = 1/k_1^+[\mathrm{S}]_{\mathrm{T}}$, we nondimensionalize the dependent variables in the same fashion, but now introduce the rescaled time $\tilde{t} = tk_1^+[\mathrm{S}]_{\mathrm{T}}$. This leads to the equations

$$d\hat{s}/d\tilde{t} = -\epsilon\left(\hat{s} - \left(\frac{k_1^-}{k_1^+[\mathrm{S}]_{\mathrm{T}}} + \hat{s}\right)\hat{es}\right), \tag{4.70}$$

$$d\hat{es}/d\tilde{t} = \hat{s} + \left(\frac{k_1^- + k_2^+}{k_1^+[\mathrm{S}]_{\mathrm{T}}} + \hat{s}\right)\hat{es}. \tag{4.71}$$

On this time scale when $\epsilon \to 0$, the left–hand side of (4.70) vanishes. Thus, $d\hat{s}/d\tilde{t} = 0$, and $\hat{s} = [\mathrm{S}]/[\mathrm{S}]_{\mathrm{T}}$ is constant. Using this result in (4.71) and reverting to dimensional variables gives, after a bit of algebra,

$$d[\mathrm{ES}]/dt = k_1^+[\mathrm{S}]([\mathrm{E}]_{\mathrm{T}} - [\mathrm{ES}]) - (k_1^- + k_2^+)[\mathrm{ES}]. \tag{4.72}$$

This equation describes the exponential increase of the enzyme–substrate complex concentration to its steady–state value $[\mathrm{ES}]^{\mathrm{ss}} = [\mathrm{E}]_{\mathrm{T}}[\mathrm{S}]/(K_{\mathrm{m}} + [\mathrm{S}])$. This is precisely the dimensional form of the expression for $\hat{es}$ on the slow time scale in (4.66). For this reason, this approximation is often referred to as the *quasi-steady-state* approximation, where "quasi" emphasizes that the value of $[\mathrm{ES}]^{\mathrm{ss}}$ changes in time, but only on a slow time scale.

Suggestions for Further Reading

- *Mathematical Models in Biology*, Leah Edelstein-Keshet. This is a great introductory textbook on general mathematical biology. Chapter 7 contains material on reduction of scale and molecular events (Edelstein-Keshet 1988).
- *Principles of Applied Mathematics*, James Keener. This book contains a good treatment of perturbation theory as well as other approximation techniques (Keener 1999).
- *Applied Mathematics*, J. David Logan. This book also contains a good treatment of perturbation theory (Logan 1997).