Basic Enzyme Kinetics

October 16, 2008 – Sauro

The vast majority of chemical transformations inside cells are carried out by proteins called enzymes. Enzymes accelerate the rate of chemical reactions (both forward and backward) without being consumed in the process and tend to be very selective, with a particular enzyme accelerating only a specific reaction. The model for enzyme action, first elucidated by Michaelis and Menten, suggests the binding of free enzyme to the reactant forming a enzyme-reactant complex. This complex undergoes a transformation, releasing product and free enzyme. The free enzyme is then available for another round of binding to new reactant. Traditionally, the reactant molecule that binds to the enzyme is termed the **substrate**, S, and the mechanism is often written as:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \tag{1}$$

This mechanism illustrates the binding of substrate and release of product, P. E is the free enzyme and ES the enzyme substrate complex. Note that substrate binding is reversible but product release is not. In a real mechanism there will always be some degree of reversibility in product formation so that a more realistic mechanism would be

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Steady State Equilibrium If we assume that the enzyme substrate complex rapidly reaches steady steady the rate of change of the enzyme substrate complex (1) can be written down using the laws of mass-action:

$$\frac{dES}{dt} = k_1\ E\ .\ S - k_{-1}\ ES - k_2\ ES$$

The concentration of enzyme substrate complex is assumed to rapidly reach steady-state (Fig. 2) so that the above equation can be set to zero:

$$0 = k_1\ E\ .\ S - k_{-1}\ ES - k_2\ ES$$

We also note that the total concentration of enzyme, E_t, is the sum of free enzyme, E and enzyme substrate complex, ES:

$$E_t = E + ES$$

From these relationships, the steady-state concentration of enzyme substrate complex can be derived:

$$ES = \frac{E_t\ S}{(k_{-1} + k_2)/k_1 + S}$$

By assuming that the rate of reaction is given by $v = k_2\ ES$, and combining constants we obtain:

$$v = \frac{Vmax\ S}{K_m + S} \tag{2}$$

where K_m is termed the Michaelis constant and Vmax the maximum steady-state reaction velocity. The Vmax can be expressed as the total enzyme concentration times the rate constant for the product formation, $E_t\ k_2$. The K_m is $(k_{-1} + k_2)/k_1$.

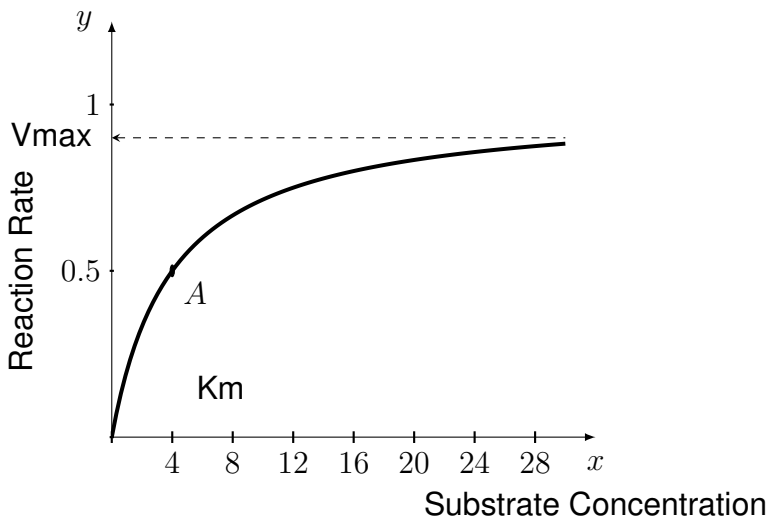

Figure 1: Relationship between the rate of reaction for a simple Michaelis-Menten rate law. The reaction rate reaches a limiting value (saturates) called the Vmax. Km is set to 4.0 and Vmax to 1.0. Note that the value of the Km is the substrate concentration that gives half the maximal rate.

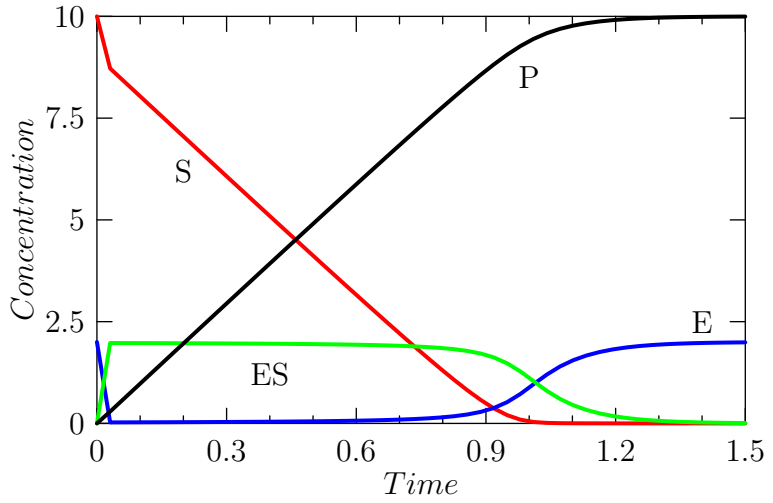

Figure 2: Progress curves for a simple irreversible enzyme catalyzed reaction. Initial substrate concentration is set at 10 units. The enzyme concentration is set to an initial concentration of 1 unit (E and ES curves have been scale by two on the graph). In the central portion of the plots one can observe the relatively steady concentrations of ES and E ($dES/dt \approx 0$). At the same time, the rate of change of S and P are constant over this period. $k_1 = 100$; $k_2 = 1$; $k_3 = 10$

The derivation of the irreversible Michaelis-Menten is an instructive exercise, however it is not a particularly realistic model for building certain kinds of models such as metabolic models. It is much better to consider the reversible Michaelis-Menten rate law. The derivation of the reversible form is very similar to the derivation of the irreversible rate law. The main difference is that the steady-state rate is given by an expression that incorporates both the forward and reverse rates for the product:

$$v = k_2\ ES - k_{-2}\ E\ .\ P$$

The expression that describes the steady-state concentration of the enzyme substrate complex also has an additional term from the product binding ($k_{-2}\ EP$). Taking these into consideration leads to the general reversible rate expression:

$$v = \frac{V_f\ S/K_S - V_r\ P/K_P}{1 + S/K_S + P/K_P}$$

At equilibrium the rate of the reversible reaction is zero. When positive, the reaction goes in the forward direction and in the reverse direction when negative. At equilibrium the equation reduces to

$$0 = V_f\ S_{eq}/K_S - V_r\ P_{eq}/K_P$$

where S_{eq} and P_{eq} represent the equilibrium concentrations for substrate and product. Rearrangement yields

$$K_{eq} = \frac{P_{eq}}{S_{eq}} = \frac{V_f\ K_P}{V_r\ K_S}$$

This expression is known as the **Haldane relationship** and shows that the four kinetic constants are not independent. The relationship can be used to eliminate one of the kinetic constants and substitute the equilibrium constants in its place. This is useful because equilibrium constants tend to be better known that kinetic constants. Incorporating the Haldane relationship yields the equation

$$v = \frac{V_f/K_S(S - P/K_{eq})}{1 + S/K_S + P/K_P}$$

Separating out the terms makes it easier to see that the equation has a thermodynamic term $(S - P/K_{eq})$ and a kinetic term as shown in the following expression:

$$v = (S - P/K_{eq})\frac{V_f/K_S}{1 + S/K_S + P/K_P}$$

The fact that the equilibrium constant appears are a constant factor in the expression suggests that enzymes *do not* change the equilibrium ratio, but simply accelerate the approach to equilibrium.

Advanced Kinetics

Generalized Cooperative Kinetics

One of the characteristics of regulated enzymes is that their kinetic response is often sigmoidal rather than hyperbolic.

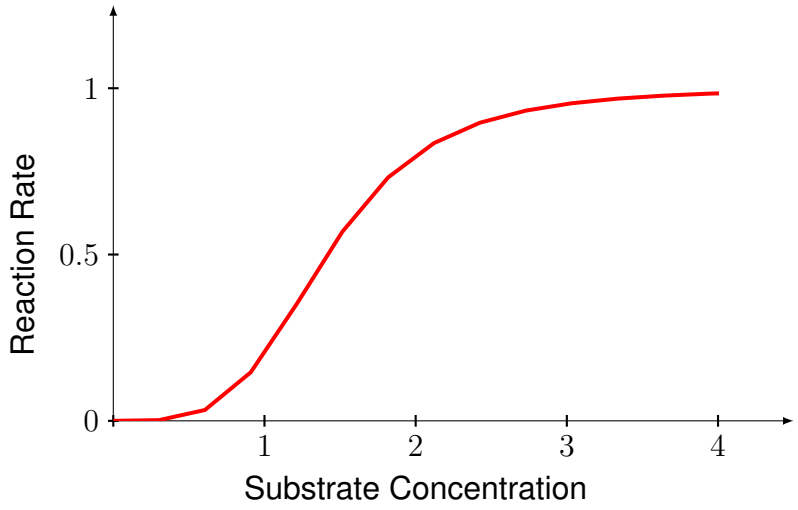

Figure 3: Relationship between the rate of reaction for a cooperative enzyme.

The reasons for this behavior is often related to the multimeric (multiple subunits) nature of regulated enzymes. Thus when a substrate binds to one of the subunits, a conformational change occurs which increases the binding affinity for the remaining vacant bindings on the other subunits. There are a number of mechanistic models which can account for cooperative behavior but for the purposes of this course only generalized rate laws will be given. The following equation represents a generalized reversible rate law that can account for sigmoidal behavior. In this equation, the term, h, can be used to change the degree of cooperativity.

$$v = \frac{Vm\ S/\mathrm{Km}_f\,(1+\Gamma/Keq)\,(S/\mathrm{Km}_f + P/\mathrm{Km}_r)^{h-1}}{1+(S/\mathrm{Km}_f + P/\mathrm{Km}_r)^{h}}$$

where Km_f and Km_r are the forward and reverse Michaelian constants respectively. Γ is the mass-action ration (P/S). Values of h greater than one indicate positive cooperativity, a value of one yields simple hyperbolic behavior, and values less than one lead to a property called negative cooperativity.

If we set h to unity (i.e. no cooperativity) and eliminate the reversible components the equation reduces to the simple irreversible Michaelis-Menten equation. If we eliminate the reversible components only, the equation reduces to the well known Hill equation:

$$v = \frac{\mathrm{Vmax}\ S^n}{(\mathrm{Km}+S)^n}$$

If a cooperative enzyme has modifiers that can change its behavior then the above generalized equation can be modified to:

$$v = \frac{Vm\ S/\mathrm{Km}_f\,(1+\Gamma/Keq)\,(S/\mathrm{Km}_f + P/\mathrm{Km}_r)^{h-1}}{\left(\frac{1+(M/Ka)^h}{1+\alpha(M/Ka)^h}\right) + (S/\mathrm{Km}_f + P/\mathrm{Km}_r)^{h}}$$

where M is the concentration of the modifier and α the degree of inhibition, if > 1 then

M acts as an activator, if < 1, then M acts as an inhibitor. Ka is the modifier Michaelian constant.

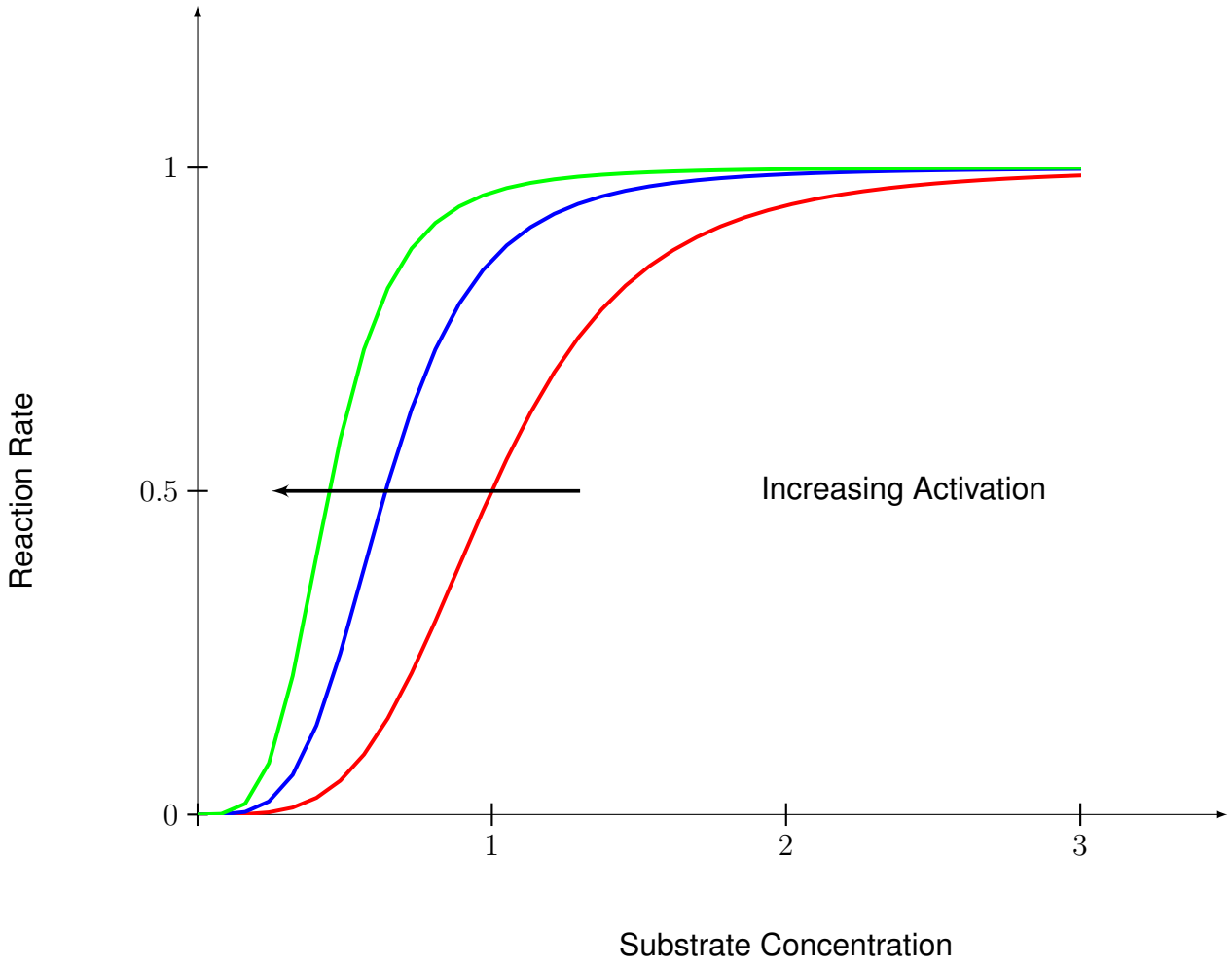

Figure 4: Effect of an activator on a cooperative enzyme.