Project # 2
Enzyme Kinetics

Due Date 10/3/05

1 The Law of Mass Action

Suppose that two chemicals, say A and B combine to make a third, say C. We write this reaction in the form

$$A + B \xrightarrow{k} C$$

The simplest model of the rate at which the reactant C is produced is that the rate of production is proportional to the product of the concentrations of A and B. The constant of proportionality k is written in the equation describing the reaction.

If a is the concentration of A, b is the concentration of B, and c is the concentration of C, this then implies that

$$\frac{da}{dt} = -kab$$

with a similar equation for b; we also have

$$\frac{dc}{dt} = kab.$$

Taken together, these are called the *law of mass action*.

In most reactions, the reverse reaction also takes place, meaning that the product C will disassociate into the reactants A and B, however the forward and backward reaction occur at different rates, say k_+ and k_-. We write this reaction as

$$A + B \underset{k_-}{\overset{k_+}{\rightleftarrows}} C$$

Questions:

1. Write down the differential equations for the concentrations of A, B, and C in this case.
2. The system will be in *equilibrium* if the concentrations of the reactants and the product remain constant. This equation has an equilibrium solution; find it.
3. The number k_+/k_- is called the *equilibrium constant* of the reaction. What is its significance?

2 The Michaeils Menten Model (1913)

Most chemical reactions in biological systems take place with the assistance of an enzyme. An enzyme is a catalyst that speeds up a chemical reaction, but is not consumed by the reaction. Suppose that one chemical S, called the substrate, reacts with an enzyme E to form a complex C. This complex then disassociates into the product P together with the enzyme. We can represent this by the reaction

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_2} E + P.$$

Here we have assumed that the disassociation of the complex is irreversible. This model for enzymatic reactions was first proposed by Michaelis and Menten in 1913.

Questions:

1. Write down a set of four differential equations for the concentrations of the substrate s, complex c, product p, and enzyme e.
2. Show that $c' + e' = 0$. Explain its significance.
3. Reduce your original set of four equations to three equations for the concentrations of the substrate s, complex c, product p.
4. Explain why we can analyze the equations for s and c separately.

3 The Quasi-Steady Approximation of Briggs and Haldane (1925)

Let e_0 be the total amount of the enzyme available to the reaction, and let s_0 be the total amount of substrate available to the reaction. Then the new variables $\sigma = s/s_0$ and $x = c/e_0$ are dimensionless, meaning that they have no units.

Questions:

1. Write a pair of differnential equations for σ and x.
2. The pair of differential equations obtained above in 3 can be simplified further if we make the change of variables $\tau = k_1 e_0 t$. Do so, and obtain the system of equations

$$\frac{d\sigma}{d\tau} = -\sigma + x(\sigma + \alpha)$$
$$\epsilon \frac{dx}{d\tau} = \sigma - x(\sigma + \kappa)$$

   for appropriate choices of ϵ, α, and κ. Find these equations, and determine these choices.
3. The effectiveness of catalysts in general and enzymes in particular is the fact that the amount of an enzyme needed is much less than the amount of the substrate or product. This implies that one of the parameters described above is roughly zero. Which?
4. If we set the small parameter described previously to zero, we obtain the *quasi-steady approximation* of the original system. What is the quasi-steady approximation?
5. Solve the quasi-steady approximation. Include a representative graph.
6. In the quasi-steady approximation, how long will it be before $r\%$ of the original amount of the substrate is used? Will the substrate ever be completely used?
7. In the quasi-steady approximation, how rapidly is the product produced?
8. In the quasi-steady approximation, show that if V is the rate at which the product is produced, then

$$\frac{1}{V} = \frac{1}{V_{max}} + \left(\frac{K_M}{V_{max}}\right)\frac{1}{s}.$$

   What is the significance of K_M and V_{max}? This relationship is useful because chemists can create experiments that allow them to measure V as a function of s. As a consequence, they can use that information to estimate the parameters K_M and V_{max}.

4 Glycolosis and Glycolytic Oscillation

The model of a catalyzed reaction described in the previous section is a prototype for the more complicated chemical reactions that occur. One of the fundamental reactions for life is the chemical reaction that converts sugar and water into energy. This reaction has the form

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{energy}.$$

The actual reaction is much more complex than this indicates, and is composed of a sequence of enzyme based reactions. These reactions can be split into three major stages- glycolysis, the Krebs cycle, and the cytochrome system. We shall contruct a simple model of a portion of the glycolysis reaction, and shall study its behavior.

An important carrier of energy in the cell is adenosine triphosphate, or ATP. When one of the phosphates is removed from ATP to form adneosine diphosphate, or ADP, a large amount of energy is released. This reaction is catalyzed by the enzyme phosphofructokinase, or PFK1. We shall model the conversion of ATP to ADP catalyzed by PFK1.

We begin by assuming that ATP is introduced to the system at a constant rate, say ν_1, so that we have the reaction

$$\xrightarrow{\nu_1} \text{ATP}.$$

Unlike the previous models, here the enzyme PFK1 does not act singly; rather it must first be activated by combining with γ molecules of ADP; this reaction has the form

$$\gamma\text{ADP} + \text{PFK1} \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} (\text{PFK1})(\text{ADP})^{\gamma}.$$

This compound then reacts with ATP to form a complex $(\text{ATP})(\text{PFK1})(\text{ADP})^{\gamma}$ that dissasociates into $(\text{PFK1})(\text{ADP})^{\gamma}$ and ADP as follows

$$\text{ATP} + (\text{PFK1})(\text{ADP})^{\gamma} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} (\text{ATP})(\text{PFK1})(\text{ADP})^{\gamma} \xrightarrow{k_2} \text{ADP} + (\text{PFK1})(\text{ADP})^{\gamma}$$

The resulting ADP is then irreversibly removed at a rate proportional to the amount of ADP present, giving us the reaction

$$\text{ADP} \xrightarrow{\nu_2} .$$

This is the sequence of reactions we wish to analyze. In what follows, we shall use the following:

- s_1 is the concentration of ATP,
- s_2 is the concentration of ADP,
- e is the concentration of the enzyme PFK1,
- x_1 is the concentration of the compound $(\text{PFK1})(\text{ADP})^{\gamma}$, and
- x_2 is the concentration of the compond $(\text{ATP})(\text{PFK1})(\text{ADP})^{\gamma}$.

Questions:

1. Write down a set of five differential equations for s_1, s_2, e, x_1, and x_2.
2. Show that $e' + x_1' + x_2' = 0$. What conclusions can you draw?
3. Write down a set of four differential equations for the variables s_1, s_2, x_1, and x_2.
4. Let e_0 be the total amount of PFK1 available for this reaction, and consider the variables
   - $\sigma_1 = \frac{k_1 s_1}{k_2 + k_{-1}}$