

ANSWERS PROJECT #2 - Math 250b (Spring 2008)

Section 1, Professor J. M. Cushing

One of the most basic enzymatic reactions, first proposed by Michaelis and Menten¹, involves a substrate S reacting with an enzyme E to form a complex SE which in turn is converted into a product P and the enzyme. This is represented schematically by



Here k_1 , k_{-1} , and k_2 are rates of reaction about which we say more below. The double arrow symbol $\rightleftharpoons$ indicates that the reaction is reversible and the single arrow $\rightarrow$ indicates the reaction can go only one way. The overall result is a conversion of the substrate S , via the enzyme catalyst E , into a product P . In detail this says that one molecule of S combines with one molecule of E to form one of SE , which then produces one molecule of P and one molecule of E .

The *Law of Mass Action* says that a rate at which a reaction occurs is proportional to the product of the concentrations of the reactants. Denote the concentrations of the reactants by

$$s = [S], \quad e = [E], \quad c = [SE], \quad p = [P].$$

($[\cdot]$ traditionally denotes concentration, say moles/liter or molar.) The Law of Mass Action and the basic rate balance law allows us to derive differential equations for these four concentrations.

For example, the Law of Mass Action says that substrate concentration decreases (due to reaction with the enzyme) at a rate proportional to the product se , i.e., at a rate $-k_1 se$. The notational scheme above indicates that the constant of proportionality for this reaction is k_1 . The schematic (1) indicates that the intermediate complex SE concentration decreases in two ways: at a rate $-k_2 c$ due to the production of P and E and at a rate $-k_{-1} c$ due to the reverse reaction that produces S and E . Accounting in this way for all the reactions indicated in (1), the rate balance law gives us the following four differential equations:

$$\begin{aligned} s' &= -k_1 se + k_{-1} c \\ e' &= -k_1 es + (k_{-1} + k_2) c \\ c' &= k_1 es - (k_{-1} + k_2) c \\ p' &= k_2 c \end{aligned}$$

for the four state variables s, e, c , and p .

Notice that (in order to have units the same on both sides of all equations) k_{-1} and k_2 have units of $1/\text{time}$ while k_1 has units of $1/(\text{time})(\text{molar})$. (*molar = mole/liter*.)

Notice that p does not appear in the three differential equations for s, e, c . Thus, we can consider just the first three equations for the three unknowns s, e, c (and calculating p by integrating $k_2 c$).

In this project we will assume there is not a reversible reaction, i.e., we take $k_{-1} = 0$. This leaves us with the following system of three equations for the three state variables s, e , and c :

$$\begin{aligned} s' &= -k_1 se \\ e' &= -k_1 es + k_2 c \\ c' &= k_1 es - k_2 c. \end{aligned}$$

¹L. Michaelis and M. I. Menten, Die kinetik der invertinwirkung, *Biochem. Z.* 49 (1913), 333-369

We assign initial conditions

$$s(0) = s_0, \quad e(0) = e_0, \quad c(0) = 0$$

under the assumption that the intermediate compound SE is initially absent.

Problems

(1) Show that $(e + c)' = 0$. This tells us that $e(t) + c(t)$ is constant in time and therefore, in fact, $e(t) + c(t) = e_0$ for all time t . Give an interpretation of this fact in terms of the concentrations of reactants.

ANSWER:

$$(e + c)' = e' + c' = (-k_1 e s + k_2 c) + (k_1 e s - k_2 c) = 0.$$

This means any decrease in the enzyme concentration is compensated for by an increase in the concentration of the intermediate compound. This makes sense since no molecule of enzyme is created or lost, but only appears either in the free state or bound with the substrate.

(2) Use the fact that $e(t) + c(t) = e_0$ for all time to show that s and c satisfy the following first order system

$$\begin{aligned} s' &= -k_1 e_0 s + k_1 s c \\ c' &= k_1 e_0 s - (k_1 s + k_2) c. \end{aligned} \tag{2}$$

(Hint: Use $e(t) = e_0 - c(t)$.) Notice that mathematically we have reduced the system of differential equation we need to analyze from three to two.

ANSWER: *If we substitute $e_0 - c$ for e in the differential equations for s and c we obtain*

$$\begin{aligned} s' &= -k_1 s (e_0 - c) \\ c' &= k_1 (e_0 - c) s - k_2 c, \end{aligned}$$

the right sides of which are easily rearranged algebraically to give the desired result.

(3) Show that $s = c = 0$ is the only equilibrium of this nonlinear system (2).

ANSWER: The equilibrium equations are

$$\begin{aligned} -k_1 e_0 s + k_1 s c &= 0 \\ k_1 e_0 s - (k_1 s + k_2) c &= 0. \end{aligned}$$

There are several algebraic ways to solve these nonlinear equations for s and c . One way is to add the equations to get $-k_2 c = 0$ for which it follows that $c = 0$. This result, placed in either equation, in turn implies $s = 0$.

Another way is to factor the first equation $(-k_1 e_0 + k_1 c) s = 0$ and deduce that either $c = e_0$ or $s = 0$. In the first case, the second equilibrium equation implies $k_1 e_0 s - (k_1 s + k_2) e_0 = 0$. This leads to the contradiction $-k_2 e_0 = 0$. (since neither k_2 nor e_0 is 0). Thus, we are left with the second case $s = 0$, which when placed into the second equilibrium equation implies $-k_2 c = 0$ or $c = 0$ (since k_2 is not 0). In summary, the only possible solutions of the equilibrium equations are $s = 0$ and $c = 0$.

(4) Calculate the Jacobian of system and evaluate it at the equilibrium $s = c = 0$.

ANSWER: The Jacobian of (2) is

$$\begin{pmatrix} \frac{d(-k_1 e_0 s + k_1 s c)}{ds} & \frac{d(-k_1 e_0 s + k_1 s c)}{dc} \\ \frac{d(k_1 e_0 s - (k_1 s + k_2) c)}{ds} & \frac{d(k_1 e_0 s - (k_1 s + k_2) c)}{dc} \end{pmatrix} = \begin{pmatrix} k_1 (c - e_0) & k_1 s \\ k_1 (e_0 - c) & -(k_2 + s k_1) \end{pmatrix}$$

which when evaluated at $s = c = 0$ is the matrix

$$\begin{pmatrix} -k_1 e_0 & 0 \\ k_1 e_0 & -k_2 \end{pmatrix}.$$

(5) Identify the type of the phase portrait of the linearization at $s = c = 0$. (Recall that both rates $k_i > 0$ are positive and assume that the initial amount of substrate $e_0 > 0$ is not zero.)

ANSWER: The eigenvalues of the Jacobian in (4) are $\lambda_1 = -k_1 e_0$ and $\lambda_2 = -k_2$. (The easiest way to see this is to notice that the Jacobian calculated in (4) is triangular, and therefore the eigenvalues appear along the diagonal.) Both eigenvalues are negative real numbers and therefore the linearized phase portrait is a stable node.

A widespread and remarkable fact about enzymes in biology is that only small concentrations are needed (compared to the concentration of the substrate) for them to be effective in catalyzing reactions. (For example, typical ratios e_0/s_0 lie in the range 10^{-2} to 10^{-7} .) In the following problems we focus on the case when e_0 is small.

(6) Assume e_0 is small. Specifically, assume that the dimension-less ratio $k_1 e_0/k_2$ is less than 1. Sketch the phase portrait of the linearization at $s = c = 0$ that you obtain from your answer in (4).

ANSWER: To sketch the stable node, we need to have a look at the associated eigenvectors. The components v_1, v_2 of an eigenvector satisfy the equations

$$\begin{aligned} (-k_1 e_0 - \lambda) v_1 &= 0 \\ (k_1 e_0) v_1 + (-k_2 - \lambda) v_2 &= 0 \end{aligned}$$

For eigenvalue $\lambda_1 = -k_1 e_0$ the first equation reduces to $0 = 0$ and the second to $(k_1 e_0) v_1 + (-k_2 + k_1 e_0) v_2 = 0$. An eigenvector is

$$\hat{v} = \begin{pmatrix} k_2 - k_1 e_0 \\ k_1 e_0 \end{pmatrix}. \quad (3)$$

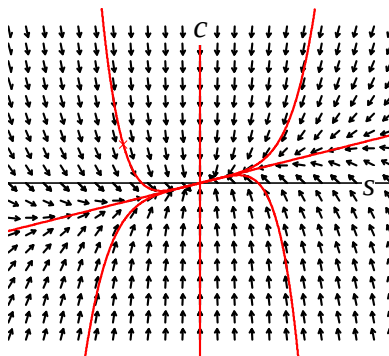
For eigenvalue $\lambda_2 = -k_2$ the equations become

$$\begin{aligned} (-k_1 e_0 + k_2) w_1 &= 0 \\ (k_1 e_0) w_1 &= 0 \end{aligned}$$

the second of which tells us that $w_1 = 0$, in which case both equations are satisfied for any value of w_2 . If we take, for example, $w_2 = 1$, we get an eigenvector is

$$\hat{w} = \begin{pmatrix} 0 \\ 1 \end{pmatrix}.$$

To sketch the phase portrait we make the following observations that follow from the given assumption $k_1 e_0 / k_2 < 1$. First, this assumption implies $\lambda_2 < \lambda_1 < 1$. Secondly, the assumption implies the components of the eigenvector (3) are positive, i.e., that it points into the first quadrant. These facts give a phase portrait like that sketched below.



(7) What does the linearization (for small e_0) imply about the phase portrait of the nonlinear system (2) for the substrate-enzyme reaction (i.e., about the nonlinear system (2))? Interpret your answer in terms of the concentrations of each chemical reactant s , c , and e . (Recall that $c(0) = 0$.) In particular, what happens to the concentrations of substrate S and enzyme E as $t \rightarrow +\infty$?

ANSWER: The Linearization Principle implies (at least in the neighborhood of $s = c = 0$) that the phase plane portrait of is a stable node that resembles that of the linearization in (6). This means that $s(t)$ and $c(t)$ (at least when $s(0) = s_0$ is small) both tend to 0 as $t \rightarrow +\infty$. Since $e(t) = e_0 - c(t)$ it follows that the enzyme concentration approach e_0 (its initial concentration!) as $t \rightarrow +\infty$.

(8) Calculate the limits of the eigenvalues and eigenvectors of the Jacobian in (4) as e_0 approaches 0. Use your answer to explain why it is reasonable to conclude, when the initial enzyme concentration e_0 is small, that $c'(t)$ is close to 0 after a short period of time has elapsed.

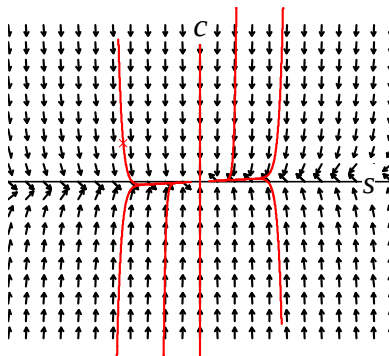
ANSWER: As $e_0 \rightarrow 0$

$$\lambda_1 = -k_1 e_0 \rightarrow 0 \quad \text{and} \quad \hat{v} = \begin{pmatrix} k_2 - k_1 e_0 \\ k_1 e_0 \end{pmatrix} \rightarrow \begin{pmatrix} k_2 \\ 0 \end{pmatrix}$$

while

$$\lambda_2 = -k_2 \quad \text{and} \quad \hat{w} = \begin{pmatrix} 0 \\ 1 \end{pmatrix}$$

remain unchanged. This means that for e_0 small the orbits move very slowly in the direction $\begin{pmatrix} k_2 \\ 0 \end{pmatrix}$ (i.e., in the direction of the horizontal s -axis), because λ_1 is nearly equal to 0, and rapidly in the direction $\begin{pmatrix} 0 \\ 1 \end{pmatrix}$ (i.e., in the direction of the vertical c -axis). The phase portrait looks like



It follows that after a short time the orbit is nearly on the s -axis, so that c' is nearly equal to 0

(9) Motivated by the conclusion in (8), when e_0 is very small we set $c'(t) = 0$ in the system (2). (This is called the “pseudo-state state assumption”.) Show then that s satisfies a differential equation of the form

$$s' = -\frac{V_{\max}s}{K_m + s}.$$

Give formulas for $V_{\max}$ and K_m in terms of k_1, k_2 and e_0 .

ANSWER: $k_1 e_0 s - (k_1 s + k_2) c = 0$ implies

$$c = \frac{k_1 e_0 s}{k_1 s + k_2}$$

which when substituted into the equation $s' = -k_1 e_0 s + k_1 s c$ yields

$$s' = -k_1 e_0 s + k_1 s \frac{k_1 e_0 s}{k_1 s + k_2} \quad \text{or} \quad s' = -\frac{k_2 e_0 s}{k_2 k_1^{-1} + s}.$$

Thus, $V_{\max} = k_2 e_0$ and $K_m = k_2 k_1^{-1}$.

Problems (1)-(9) show that when the initial enzyme concentration e_0 is small, then (after a short transient period of time) the reaction rate s' for the substrate concentration is related to the concentration s of the substrate according to the Michaelis-Menten Law $-\frac{V_{\max}s}{K_m + s}$. In other words, we have derived the famous Michaelis-Menten Law from the Law of Mass Action.