Lecture 3

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(The odd numberred lectures are given by D. Ramakrishnan, and the even ones by R. Tanner.)

0.1 The basic equations of enzyme kinetics

If X is a substance, we will use x (or $[X]$) for its molar concentration in gram moles per liter. It is customary in Chemistry to write (X), but we will not use it here since we want to be able to write $f(X)$ to mean a function of X.

S: Substrate

E: Enzyme

C: Intermediate complex (denoted by Tanner and others by ES)

P: Product

t: Time

v: $\frac{dp}{dt}$

e_0: Initial concentration of Enzyme $(= E^*)$

V: $k_3 e_0 \quad (= k_3 E^*)$

s_0: Initial concentration of Substrate $(= S^*)$ $\quad =$ value of s at $t = 0$

The initial concentrations of C and P are zero.

At the end of Lecture 1, we came to the following *Reaction Kinetic Scheme*:

$$S + E \xrightarrow{k_1} C \xrightarrow{k_3} P + E$$
$$S + E \xleftarrow{k_2} C$$

Hypothesis: $\frac{ds}{dt} < 0$, for all positive t.

So s is a strictly decreasing function, and it goes from s_0 to 0. In particular, it is one-to-one as a function of t and admits an inverse. (Otherwise, as Calculus teaches us, $s'(t)$ would become zero at some point in the interval $(0, s_0)$.) Consequently, we may view all the basic quantities, which are *a priori* functions of t, as functions of s instead.

Assuming the *Law of Mass Action*, we obtained at the end of Lecture 1, the following four inter-related, inhomogeneous differential equations

$$\frac{ds}{dt} = -k_1 se + k_2 c \tag{1}$$

$$\frac{dc}{dt} = k_1 se - (k_2 + k_3)c \tag{2}$$

(Note that $\frac{dc}{dt}$ would be > 0 if k_3 were zero, since then $\frac{ds}{dt}$ would equal $-\frac{ds}{dt}$.)

$$\frac{de}{dt} = -k_1 se + (k_2 + k_3)c \tag{3}$$

$$v = \frac{dp}{dt} = k_3 c \tag{4}$$

Adding (2) and (3) and integrating, we get, using $c_0 = 0$,

$$c = e_0 - e, \tag{5}$$

while adding (1), (2) and (4), followed by integration, and using $p_0 = 0$, leads to

$$s + c + p = s_0. \tag{6}$$

Consequently, given all the initial parameters, the four quantities s, e, c, p are all determined by just the knowledge of any two of them.

0.2 The critical points

Thanks to the algebraic equations (4) and (5) above, we see that v is 0 at the start, i.e., when $s = s_0$, and it is again zero at the end, i.e., when $s = 0$. Thus the maximum value of v, written as v_{peak}, occurs in $(0, s_0)$.

Put

s_{peak}: the value of s where the absolute maximum value of v occurs.

We will see later (see Lemma 0.3.1) that s_{peak} is unique.

Lemma 0.2.1 *At any critical point of v as a function of s in $(0, s_0)$, we have*

$$s = \left(\frac{k_2 + k_3}{k_1}\right)\left(\frac{e_0 - e}{e}\right).$$

In particular, this happens at s_{peak}.

Proof. As $v = \frac{dp}{dt}$, we have

$$\frac{dv}{ds} = \frac{dt}{ds}\frac{d}{dt}\frac{dp}{dt}.$$

Recall that by our hypothesis, $\frac{ds}{dt}$ is strictly negative, so $\frac{dt}{ds}$ is well defined and non-zero (outside the end points). So we see that the critical points occur exactly when

$$\frac{dv}{dt} = \frac{d^2p}{dt^2} = 0.$$

Applying (4), since $k_3 > 0$, we have to solve

$$\frac{dc}{dt} = 0.$$

Thanks to (2), this condition becomes

$$k_1 se - (k_2 + k_3)c = 0.$$

Because of (5), the critical point for v occurs at s if and only if we have

$$k_1 se - (k_2 + k_3)(e_0 - e) = 0.$$

The Lemma now follows easily. □

We have implicitly assumed that $e_0 - e$ is positive except at the end points, where it is zero.

0.3 Convexity of $v = \frac{dp}{dt}$ as a function of s

Recall that s_{peak} is, by definition, where v attains its absolute maximum. Since v is a differentiable function of s, it attains its maximum at a critical point. On the other hand, by Lemma 0.2.1, there is a unique critical point of v.

Proposition 0.3.1 *We have*

(i) s_{peak} *is the unique critical point of* $v = \frac{dp}{dt}$ *on the open interval* $(0, s_0)$*;*

(ii) $\frac{d^2v}{ds^2}$ *is everywhere non-positive, hence the graph of* v *as a function of* s *is bell-shaped, meaning it is convex downwards. Moreover,* $\frac{d^2v}{ds^2}$ *does not vanish on any non-zero interval.*

We are assuming here that the function v of s is smooth, at least twice differentiable.

Proof. Since $v = dp/dt$ equals k_3c (with $k_3 > 0$), and since $c = e_0 - e$ is always non-negative, the unique critical point is, as observed earlier, the unique maximum at $s = s_{\text{peak}}$. Hence d^2v/ds^2 is < 0 at s_{peak}. Note that v must increae steadily from 0 to s_{peak} and then decrease to 0 at $s = s_0$. (In terms of time, this is reversed, as $t = t_0$ corresponds to $s = s_0$, and $s = 0$ at infinite time.) Thus dv/ds is ≥ 0 in the interval $[0, s_{\text{peak}}]$ and ≤ 0 in $[s_{\text{peak}}, s_0]$. Assertion (i) is evident.

Now we prove part (ii). Again, since by our hypothesis, ds/dt is everywhere negative on $(0, s_0)$, and as k_3 is positive, we are left (by equation (4) of section 1) to check that

$$\frac{d}{dt}\left(\frac{dc}{ds}\right) \geq 0.$$

This is clear from the behavior of dc/ds.

Applying equations (1) and (2) of section 1, we obtain

$$\frac{dc}{ds} = \frac{c'}{s'} = \frac{k_1se - (k_2 + k_3)c}{-k_1se + k_2c},$$

where s', resp. c', denotes $\frac{ds}{dt}$, resp. $\frac{dc}{dt}$. Comparing (1) and (2), we have

$$c' = -s' - k_3c,$$

which yields
$$\frac{dc}{ds} \;=\; -1 - k_3\frac{c}{s'}.$$
Taking derivatives with respect to t and multiplying both sides by $-k_3^{-1}(s')^2$, we obtain

$$(*) \qquad -k_3^{-1}(s')^2\frac{d}{dt}\left(\frac{dc}{ds}\right) \;=\; s'c' - s''c.$$

Now we *claim* that there is no non-empty interval I (contained in $(0, s_0)$) on which $\frac{d}{dt}\left(\frac{dc}{ds}\right)$ is identically zero. Indeed, by $(*)$, it can be zero if and only if we have
$$s'c' - s''c \;=\; 0 \;\text{ on }\; I.$$
In other words,
$$\frac{s''}{s'} \;=\; \frac{c'}{c}.$$
which integrates to give
$$\log s' \;=\; \log c + c,$$
for a real constant c. Exponentiating, we obtain
$$s' \;=\; e^c c.$$
Since $e^c > 0$ for any real number c, we deduce that, if the claim were false, s' and c must, in particular, have the same sign in $(0, s_0)$. This is patently false as s' is negative and c is ≥ 0. Hence the Claim.

Consequently, to prove the Proposition, we need only show that
$$s'c' - s''c \;<\; 0, \;\text{ for some }\; s \in I.$$
This is because the expression on the left is continuous (since c, s are repeatedly differentiable) and non-zero (by the claim above), and thanks to the intermediate value theorem, once it is positive somewhere, it will be so everywhere.

Since $s' < 0$ and $c \geq 0$, it suffices to prove that
$$\exists\, s \;\text{ such that }\; c' > 0 \;\text{ and }\; s'' > 0.$$

Differentiating (1) (with respect to t) yields
$$s'' \;=\; -k_1 s' e - k_1 s e' + k_2 c'.$$

From (2) and (3), we see that $e' = -c'$, implying

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

which is positive when $c' > 0$, since $s' < 0$, while e, s, k_1, k_2 are positive.

This finishes the proof of the Proposition.

□