12/8/99

Chapter 8 is short (1/3 the length of Chapter 7!)

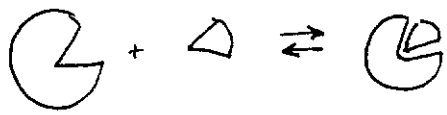
It covers largely classical approaches to kinetics, and is covered in most Biochemistry texts. But now we have a better background in which to study it.

Enzyme Reactions

($E + S \rightleftarrows S \rightarrow E + P$)

Classic

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



$$ES \xrightarrow{k_2} E + P$$

Look only at initial rates, so ignore k_2

Enzyme (E) binds substrate (S) to form an enzyme-substrate complex (ES)

The (ES) complex then reacts and (quickly) releases product.

At low conc's of S, the reaction is first order in E and first order in S

At high conc's of S, Le'Chatelier pushes the first reaction almost completely to the right. At this point, adding more S can't lead to more ES (E is limiting), so the ~~rate~~ velocity now becomes independent of S.

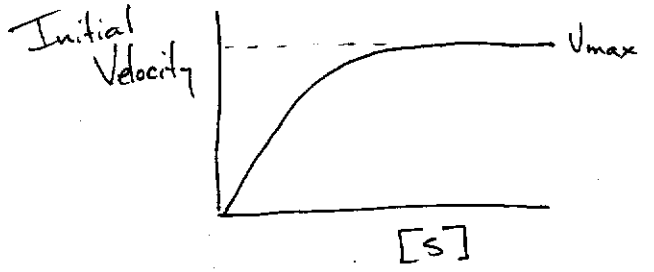
We say that this is "saturating concentrations" of S

The reaction is now first order in E only.

In other words, because of Le'Chatelier,
the first reaction is not rate-limiting.
the second reaction is rate-limiting.

Under these conditions, the velocity is maximal (V_{MAX})

We saw "initial velocity" in Chapter 7



$\frac{V_{MAX}}{E}$ gives the velocity per enzyme, or turnover number.

Michaelis-Menten (Briggs-Haldane) ← 1925

Assume steady-state [ES]

1) $\frac{\partial[ES]}{\partial t} = 0 = k_1 E \cdot S - k_1[ES] - k_2[ES]$

2) $v_o = \frac{\partial P}{\partial t} = k_2[ES]$

3) $E_o = E + [ES]$

$S_o = S + [ES] \approx S$ ← assumes substrate in large excess over enzyme.

From (1) $\Rightarrow$ $[ES] = \frac{k_1}{k_1 + k_2} E \cdot S = \frac{1}{K_M} E \cdot S$

But from (3) $E = E_0 - [ES]$

$S = S_0 - [ES] \approx S_0$

Therefore:

$$[ES] = \frac{1}{K_M}(E_0 - [ES])(S_0)$$

$$= \frac{S_0 E_0}{K_M} - \frac{[ES] S_0}{K_M}$$

$$K_M [ES] = E_0 S_0 - S_0 [ES]$$

$$(K_M + S_0)[ES] = E_0 S_0$$

$$[ES] = \frac{E_0 S_0}{K_M + S_0} = \frac{E_0}{\frac{K_M}{S_0} + 1}$$

$$K_M = \frac{k_1 + k_2}{k_1}$$

$\therefore$ $$\frac{dP}{dt} = \frac{k_2 E_0}{\frac{K_M}{S_0} + 1} = \frac{V_{MAX}}{\frac{K_M}{S} + 1}$$

From the above, you can see that at $S \gg K_M$

$$\frac{dP}{dt} = \frac{V_{MAX}}{\frac{K_M}{S} \to 0 + 1} \approx V_{MAX}$$

as discussed earlier (independent of S)

Can also show that when $S = K_M$ then $\frac{K_M}{S} = 1$

$$\frac{dP}{dt} = \frac{V_{MAX}}{1 + 1} = \frac{1}{2} V_{MAX}$$

This is one of the most widely used (and most widely abused) equations in Biochemistry.

Why abused?

Assumptions are not always valid.
Is substrate really in large enough excess that it can be safely ignored?

The equation is actually easy to deal with.

Back up $\Rightarrow$ let $S_0 = S + [ES]$ (not $S_0 \approx S$)

then $S = S_0 - [ES]$

then $[ES] = \frac{1}{K_M}[E_0 - [ES]][S_0 - [ES]]$

$$K_M[ES] = E_0S_0 - S_0[ES] - E_0[ES] + [ES]^2$$

$$[ES]^2 - (E_0 + S_0 + K_M)[ES] + E_0S_0 = 0$$

Quadratic $\longrightarrow$

$$[ES] = \frac{(E_0 + S_0 + K_M) - \sqrt{(E_0+S_0+K_M)^2 - 4E_0S_0}}{2}$$

Drawback:
Can't do simple Lineweaver-Burk plots, etc.

So what!?

Manipulation of data, taking $\frac{1}{v_0}$ and $\frac{1}{S_0}$
DISTORTS ERRORS
in these observables.

Points are no longer evenly weighted in fitting the data.

$\Longrightarrow$ BAD

Finally, Chapter 8 shows that

$$E + S \rightleftharpoons A \rightarrow B \rightarrow C \rightarrow P + E$$

is indistinguishable from

$$E + S \rightleftharpoons Q \rightarrow P + E$$

But again, Occam's Razor applies.
Just remember that the underlying mechanism MAY be more complex.