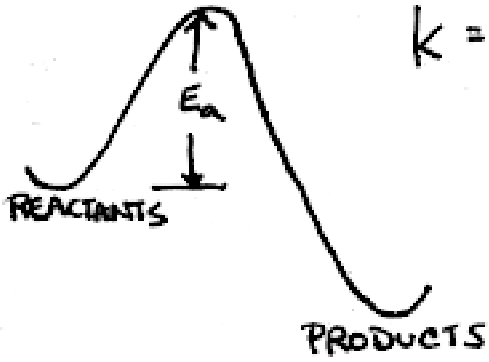
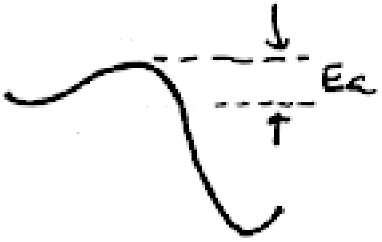
11/16/01

Chapter 8 - Enzyme Kinetics - Catalysis



$$k = Ae^{-E_a/RT}$$



SLOW

FAST

THERMO IS IDENTICAL!

Forward rate increases,

⇒ A catalyst lowers the energy of the transition state.

Q: Can a catalyst speed up the forward reaction, but not the reverse?

A: No. Thermo does not change

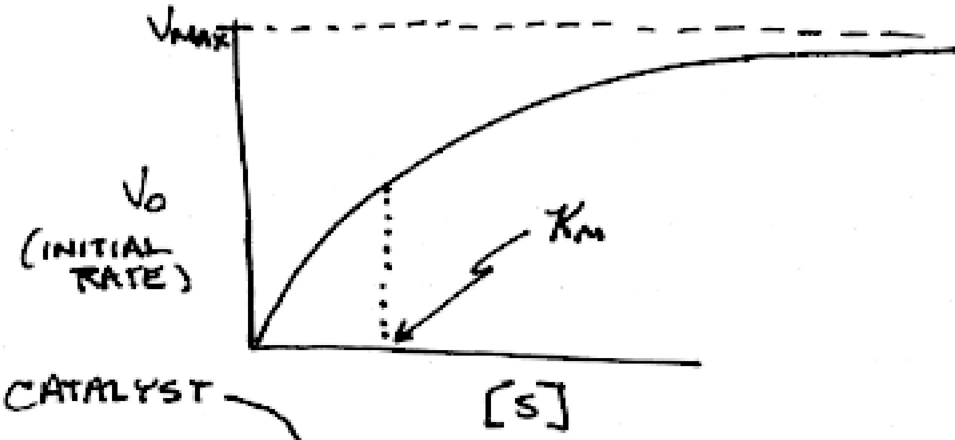
Enzyme is a type of catalyst. <u>Period</u>.

Enzymes/catalysts <u>cannot change thermodynamics</u>.

<u>N.B.</u>

Enzymes can speed reactions by 10^{11} or more. Wow!

Michaelis-Menten Kinetics



HOLD THE BOAT! LET'S <u>UNDERSTAND</u>

Kinetic Mechanism

CATALYST

$$\underbrace{E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES}_{\text{substrate must bind first}} \underbrace{\xrightarrow{k_2} E + P}_{\text{Reaction occurs and product is released.}}$$

DEFINE CATALYST

At <u>very</u> early time, no P is produced

[ES] increases. As it does, some gets converted to E + P. The more [ES], the faster P is produced.

Soon production of ES = consumption of ES

STEADY STATE

$$\frac{d[ES]}{dt} = 0 = k_1[E][S] - k_{-1}[ES] + k_2[ES]$$

Leads to: $$\frac{k_{-1}+k_2}{k_1} = \frac{[E][S]}{[ES]}$$

Looks like an equilibrium, but it's not.
It's steady state

Note that IF k_{-1} and $k_1 \gg k_2$
then

$$\frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]} = K_d$$ EQUILIBRIUM

In any case,

$$\frac{dP}{dt} = k_2[ES]$$ so we need [ES].

How to solve? [E], [S], [ES] are changing

MASS BALANCE

$$[E]_0 = [E] + [ES] \Rightarrow [E] = [E]_0 - [ES]$$

$$[S]_0 = [S] + [ES] \Rightarrow [S] = [S]_0 - [ES]$$

$$\frac{k_{-1}+k_2}{k_1} = K_M = \frac{([E]_0 - [ES])([S]_0 - [ES])}{[ES]}$$

$$= \frac{(E_0 - x)(S_0 - x)}{x}$$

SOLVE FOR x

Traditional treatment:

If [S] is in large excess

THEN $[S] \approx [S]_0$ $\quad ([ES] \ll [S]_0)$

$$\therefore \quad K_M \approx \frac{(E_0 - x)\,S}{x}$$

SOLVE FOR x

Do IT! AT HOME

$$x = [ES] = \frac{1}{1 + \frac{K_M}{[S]}}\,[E]_0$$

$$\therefore \quad v_0 = \frac{dP}{dt} = k_2[ES] = \frac{k_2[E]_0}{1 + \frac{K_M}{[S]}}$$

$k_2[E_0]$ is the velocity you would expect if ALL "E" were bound by substrate.

ie. $[E)_0$ is the maximum value of $[ES]$

So

$$v_0 = \frac{V_{MAX}}{1 + \frac{K_M}{S}}$$

initial rate
BEFORE MUCH SUBSTRATE CONSUMED

CLASSIC EQUATION
ONLY VALID
WHILE [S] in excess over [ES]

See p. 408-409 — Integrated Michaelis-Menten

RULER BEST-FIT APPROACHES

Re-arrange to yield:

$$\frac{1}{v_0} = \frac{1}{V_{MAX}} + \frac{K_M}{V_{MAX}}\frac{1}{[S]}$$

Line-Weaver Burk

$$[y = b + mx]$$

SIMILAR TO Scatchard analyses

$$\frac{S}{v_0} = \frac{S}{V_{MAX}} + \frac{K_M}{V_{MAX}}$$

Dixon

$$v_0 = -K_M\frac{v_0}{S} + V_{MAX}$$

Eadie-Hofstee

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} ES' \xrightarrow{k_3} ES'' \xrightarrow{k_4} E + P$$

More complex, but still has same form.

K and K_M are now complex functions of k_1 k_{-1} k_2 k_3 k_4

BE AWARE!!

$\frac{k_{cat}}{K_M} \Rightarrow$ specificity constant

relates rate to $[E]$ ← <u>free</u> enzyme

$$v_0 = \boxed{\frac{k_{cat}}{K_M}}[E][S]$$

← <u>Free</u> enzyme, <u>not</u> total

∴ Comparing two substrates A and B

$$\frac{v_A}{v_B} = \frac{\left(\frac{k_{cat}}{K_M}\right)_A [A]\cancel{[S]}}{\left(\frac{k_{cat}}{K_M}\right)_B [B]\cancel{[S]}}$$

FOR $[A] = [B]$ $\qquad \frac{v_A}{v_B} = \frac{\left(\frac{k_{cat}}{K_M}\right)_A}{\left(\frac{k_{cat}}{K_M}\right)_B}$

<u>INHIBITION</u> - SEE TEXT