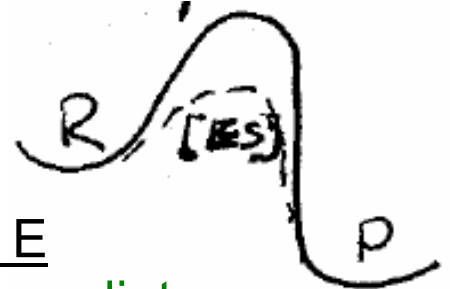
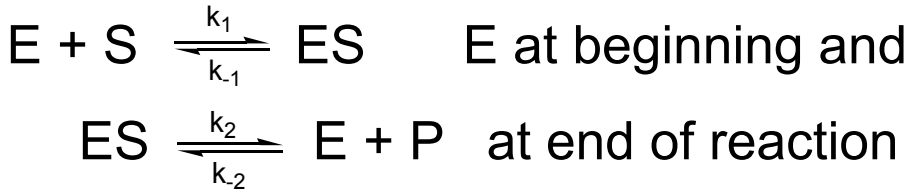


Enzyme Kinetics – modified 2009

Enzyme reaction – example of **Catalysis**, simplest form:



No consumption of E

(ES): **enzyme-substrate complex – Intermediate**

Enzyme stabilize (lower E_a) transition state by binding S

Can promote bond formation/breaking

same as catalyst – **efficient, selective** - usually not general
rate enhancements can be tremendous

Mechanism above looks like typical rapid equilibrium

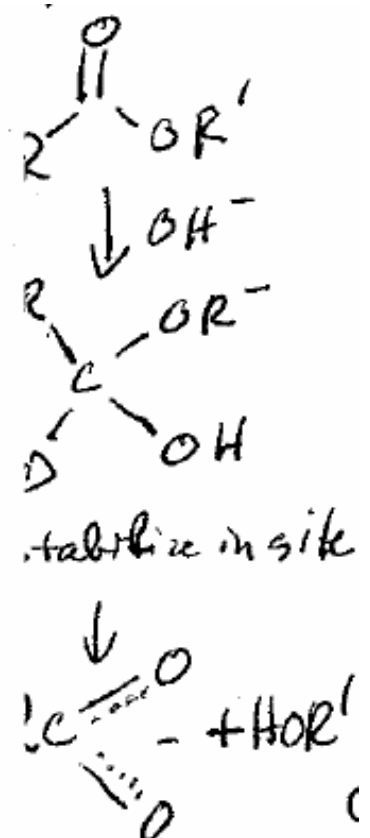
– but there may be more steps – especially reverse k_2

Note: typically think of enzymes as **proteins**
bind substrate (S) to specific site
can **modify S to “activate”** for reaction

Models: a) “Lock and key” – substrate fits
specific enzyme site

b) “Induced fit” – enzyme modifies
structure to fit substrate

Idea – **in site easier**: “stretch bond” /
exchange charge /deform / add atom/
rearrange electron density ...etc.



means transition state **lower E_a** than in solution
specific – dramatic efficiency

Other enzymes: Ribozyme – RNA catalyze
Others – carbohydrates ...

ex: $2\text{H}_2\text{O}_2 \xrightarrow{\text{cat}} 2\text{H}_2\text{O} + \frac{1}{2} \text{O}_2$ slow in solution

General catalyst:

1st order: $r \sim [\text{H}_2\text{O}_2] [\text{catalyst}]$ **typical- inorganic catalyst:**
Fe or HX, increase rate by $\sim 10^4 - 10^5$

Ezymatically—two behaviors (orders)

1st order: $r \sim [\text{H}_2\text{O}_2]^1$ – **low conc.** **catalase enhance**
 $r \sim k_1[\text{cat}][\text{H}_2\text{O}_2]$ **rate $\sim 10^{15}$**

0th order: $r \sim [\text{H}_2\text{O}_2]^0$ – **high conc.**
→ maximum rate (10^7 molecules $\text{sec}^{-1} \text{cat}^{-1}$)
 $r \sim k_0[\text{cat}]$

$\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \frac{1}{2} \text{O}_2$ (s)

exergonic:

$$\Delta G_{298}^0 = -103 \text{ k J mol}^{-1}$$

most of this is enthalpy

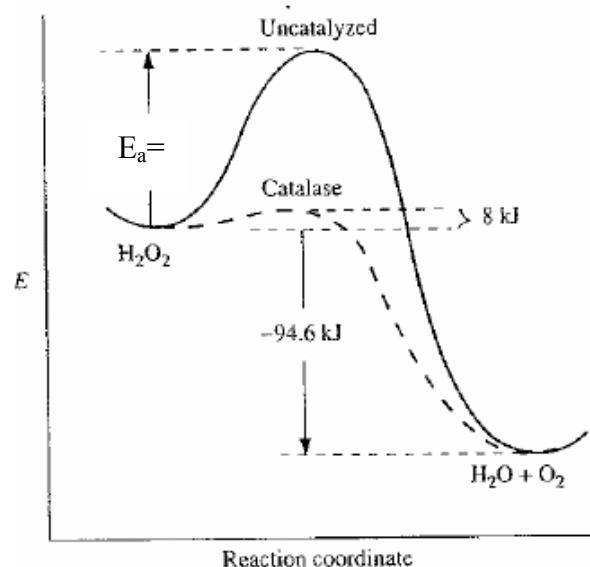
$$\Delta H^0 = -94.6 \text{ k J mol}^{-1}$$

Activation high:

$$E_a = 71 \text{ k J mol}^{-1}$$

slow solution reaction

since $r < 4 \times 10^{-8} \text{ Ms}^{-1}$ - 1st ord.



pre-exponential $A < 1 \times 10^5 \text{ s}^{-1}$

with Fe or HBr $E_a \sim 45 \text{ k J mol}^{-1}$

with Catalase $E_a \sim 8 \text{ k J mol}^{-1}$

$A \sim 1.6 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ -- entropy barrier also lower

Maximum reaction velocity v_{\max} with inc. $[S]$ $v_m = k [E_0]$
Catalytic constant – turnover number: $k = v_{\max}/[E_0] \text{ s}^{-1}$
 {# of active site limited} - v_{\max} depend on $[E_0]$

Michaelis-Menten – Analyze mechanism / get rate law
 Since enzyme so efficient – works very low concentration
 (actual value enzyme conc. may be unknown)

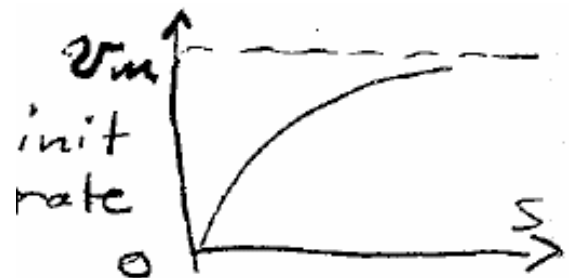
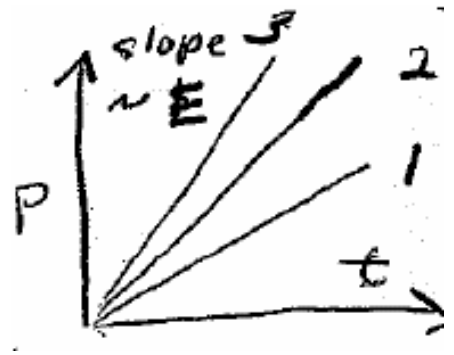
Goals—mechanism should fit these:

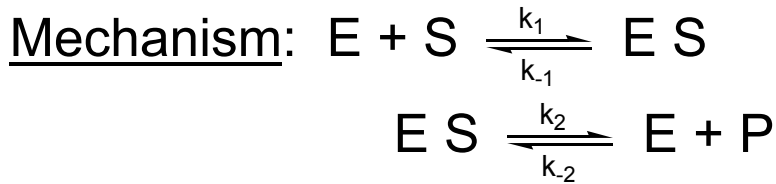
a) $v = -dS/dt = dP/dt$ inc. linear w/ $[E]$
 (double E—double v)

b) $v = k [S]$ if $[E]$ constant
 1^{st} order in substrate, low $[S]$

c) $v \rightarrow v_{\max}$ at high $[S]$
 (becomes zero order)

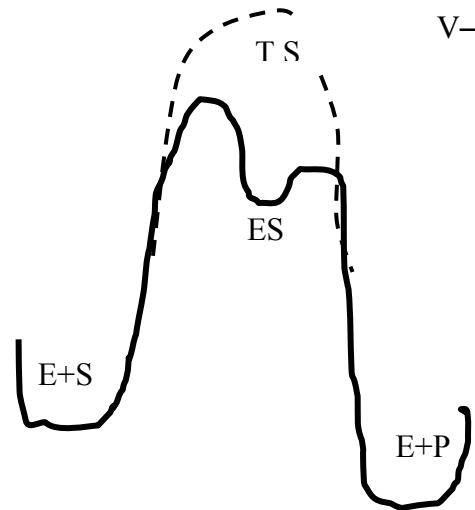
\Rightarrow goes through intermediate
 enzyme-substrate complex





initial rate – ignore k_{-2} – no P initially

$$v_0 = (dP/dt)_0 = k_2[ES]$$



if steady state: $d(ES)/dt = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0$
 $[ES] = k_1[E][S]/(k_{-1} + k_2)$

note: conc. are free E, S → not initial E_0, S_0

can also picture as **equilibrium**, conc. ratio is const.

$$K_M = (k_{-1} + k_2)/k_1 = [E][S]/[ES] \text{ --reflect } \underline{ES \text{ dissociation}}$$

(plug in – regular 2nd order)

$$v_0 = k_2 \{k_1 / (k_{-1} + k_2)\} [E][S] \sim k_2 [E][S] / K_M$$

Note 1: what if assume rapid equilibrium? Build up ES?

$$v_0 = k_2 K_e [E][S] \quad K_e = [ES]/[E][S] = k_1/k_{-1}$$

$$= k_2 (k_1/k_{-1}) [E][S] \quad \text{– miss denom. – balance 2 k's}$$

Note 2: **free enzyme** hard to determine (protein conc.?):

$$[E]_0 = [E] + [ES]$$

$$[S]_0 = [S] + [ES] \approx [S] \quad \text{initially, since } [ES] \text{ low}$$

→ works for initial rate: equate $[S] \sim [S_0]$ and $[P] \sim 0$

Substitute these into steady state result:

$$[ES] = \{k_1 / (k_{-1} + k_2)\} \{[E_0] - [ES]\} [S] \quad \text{recall: } K_M = (k_{-1} + k_2)/k_1$$

→ES on both sides, rearrange: $[ES]\{1 + [S]/K_M\} = [E_0][S]/K_M$
 $[ES] = \frac{\{k_1/(k_{-1} + k_2)\}[E_0][S]}{1 + \{k_1/(k_{-1} + k_2)\}[S]}$ let $K_M = (k_{-1} + k_2)/k_1$
 mult. top/bottom by K_M

$[ES] = [E_0][S]/\{K_M + [S]\}$ now [ES] indep. [E], just $[E_0]$

Product only in 2nd step, depend on [ES], substitute:

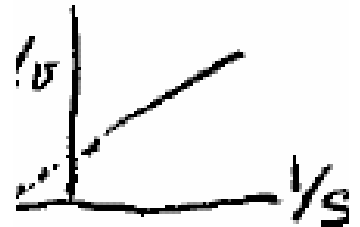
$v = k_2 [ES] = k_2[E_0][S]/\{K_M + [S]\}$ right form, low-high [S]
 $v = v_{max}/(\{K_M/[S]\} + 1)$ div. by [S], $v_{max} = k_2[E_0]$

Analysis invert rate equation: $1/v = (1/v_{max})(K_M/[S] + 1)$
 independent $[E_0]$

Lineweaver-Burk plot: $1/v$ vs. $1/[S]$

slope: K_M/v_{max}

x intercept: $-1/K_M$, y intercept: $1/v_{max}$



recall – $v_{max} = k_2[E_0]$

avoids expressing value for E_0

integrate M-M: $v = -d[S]/dt \rightarrow (K_M/[S] + 1)d[S] = -v_m dt$

$K_M \ln \{[S]/[S_0]\} + [S] - [S_0] = -v_m t \rightarrow$ complex t-dep.

Behavior:

a) low [S] $v \cong (v_{max}/K_M)[S]$ $K_M/[S] \gg 1$
 1st order in S

b) high [S] $v \cong v_{max}$ $K_M/[S] \ll 1$
 0th order in S - turnover: $k_{cat} = v_{max}/E_0 = k_2$

Interpret

if $[S] = K_M \Rightarrow v = v_{\max}/2$

K_M small \rightarrow E bind S tightly ($k_{-1} + k_2 \ll k_1$) or low $[E]$

\rightarrow not much $[S]$ needed to saturate $[E_0]$

S does not come off ES easily

How about Product – if P build up need consider k_{-2}
normally– since consider initial rates – could ignore

if include: $ES \xrightleftharpoons[k_{-2}]{k_2} P + E$ do a steady state:

$$d(ES)/dt = 0 = k_1[E][S] - k_{-1}[ES] - k_2[ES] + k_{-2}[E][P]$$

$$[ES] = (k_1[E][S] + k_{-2}[E][P]) / (k_{-1} + k_2)$$

$$\text{let: } K_M = (k_{-1} + k_2) / k_1 \text{ and } K'_M = (k_{-1} + k_2) / k_{-2}$$

Remember!

$$[ES] = [E] \{ [S]/K_M + [P]/K'_M \} \text{ was one term}$$

$$\text{Also recall } [E_0] = [E] + [ES]$$

$$[E_0] = [E] \{ 1 + [S]/K_M + [P]/K'_M \}$$

Substitute in rates:

$$v = k_2[ES] - k_{-2}[E][P] = [E] \{ k_2([S]/K_M + [P]/K'_M) - k_{-2}[P] \}$$

$$= [E_0] \{ k_2([S]/K_M + [P]/K'_M) - k_{-2}[P] \} / (1 + [S]/K_M + [P]/K'_M)$$

recall: $k_{-2}K'_m = (k_{-1} + k_2) \rightarrow k_{-2}K'_m [P]/K'_m = (k_{-1} + k_2)[P]/K'_m$ cancel 2nd term

$$v = [E_0] \{ (k_2[S]/K_M - k_{-1}[P]/K'_M) / (1 + [S]/K_M + [P]/K'_M) \}$$

$$\text{Now if } v_{\max} = k_2[E_0] \quad v'_{\max} = k_{-1}[E_0]$$

$$\text{then } v = (v_{\max}[S]/K_M - v'_{\max}[P]/K'_M) / (1 + [S]/K_M + [P]/K'_M)$$

(note: this took me many tries and help to get right form)

Point—if include the Product, still interpretable

a) **Beginning** of reaction $\rightarrow [P] = 0$

$$v = (v_m[S]/K_M)/(1 + [S]/K_M) = v_m/(K_M/[S] + 1) \quad \text{same as MM}$$

b) as **product** builds up – **reaction slows** ($-v'/K_M'$) will dom.

Lineweaver-Burk

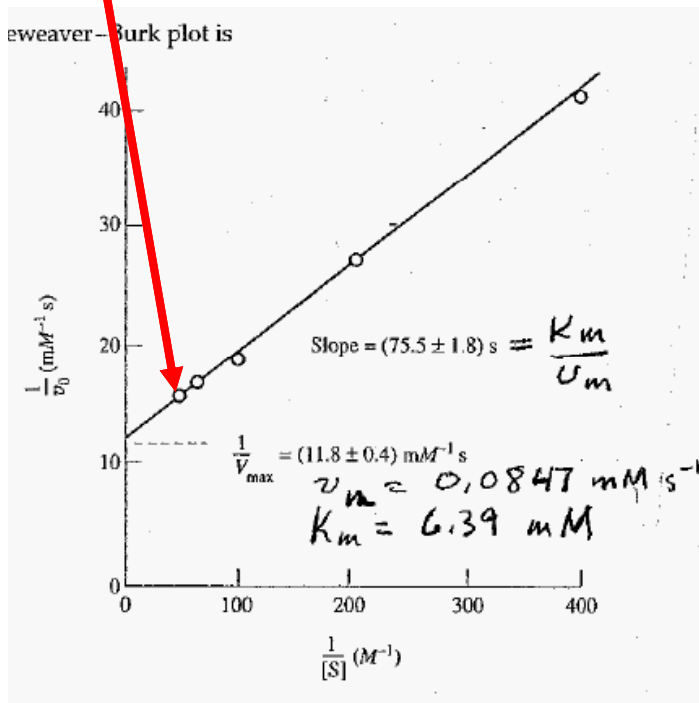
$$1/v_0 = K_M/v_m \cdot 1/[S] + 1/v_m$$

Plot $1/v$ vs. $1/S \rightarrow$

compress high S value

slope: K_M/v_m int: $1/v_m$

a. Lineweaver-Burk plot:



Eadie-Hofstee (mult. by $v_m v_0$)

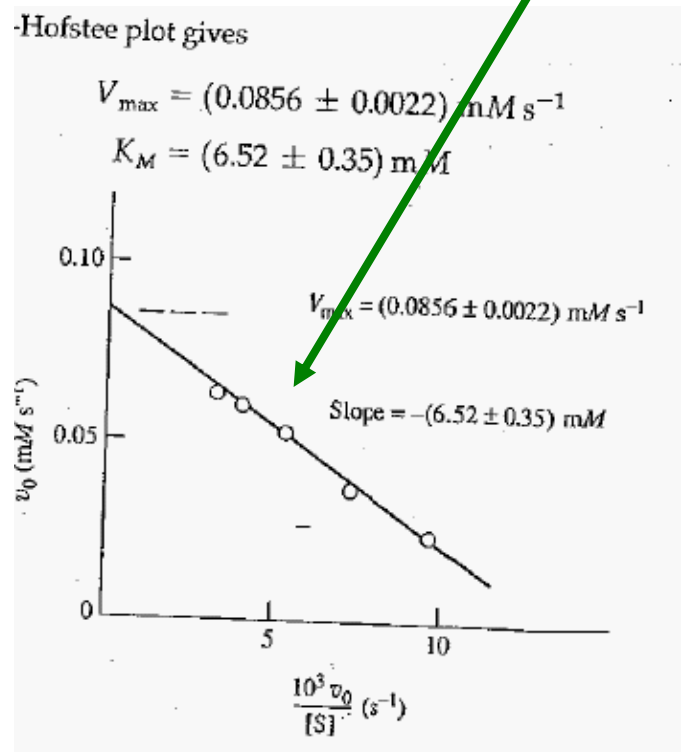
$$v_0 = -K_M\{v_0/[S]\} + v_m$$

Plot v_0 vs. $v_0/S \rightarrow$

spreads high S values

slope: $-K_M$, int: v_m

b. Eadie-Hofstee plot:



Two plots analyze same data set – **hydrolysis CBZ-Gly-Trp**
by **carboxypeptidase** \rightarrow vary $[S]$: 2.5 \rightarrow 20 mM