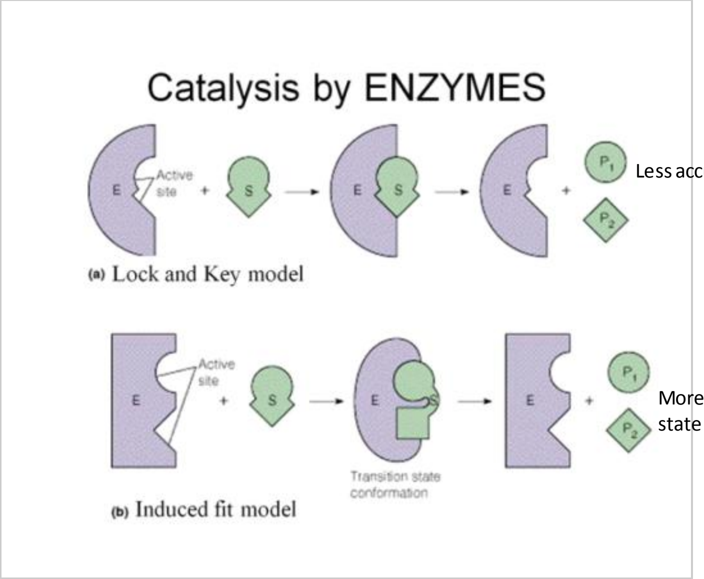
Notes 10/29

Monday, October 29, 2007
9:01 AM

Notes

Audio recording started: 9:01 AM Monday, October 29, 2007

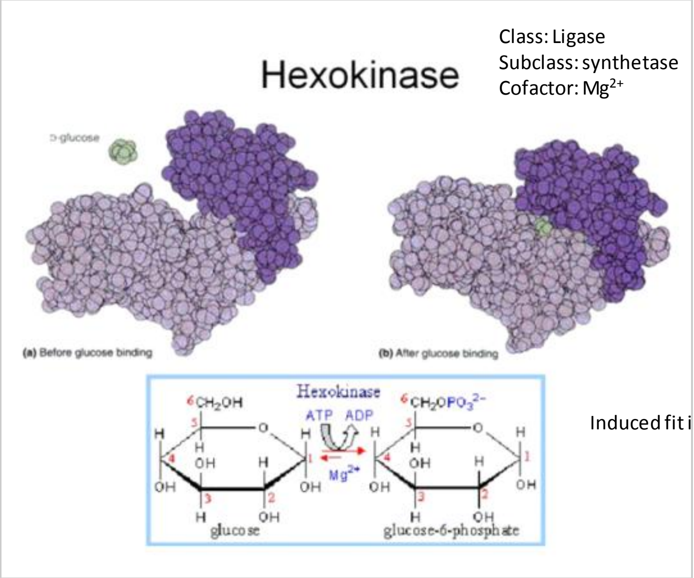
ΔG - 209K, 1atm, 1M
ΔG^0 (biochemical free energy change) - 209K, 1atm, pH 7.0



Catalysis by ENZYMES

Less accurate

More accurate. Resembles transition state



Hexokinase

Class: Ligase
Subclass: synthetase
Cofactor: Mg^{2+}

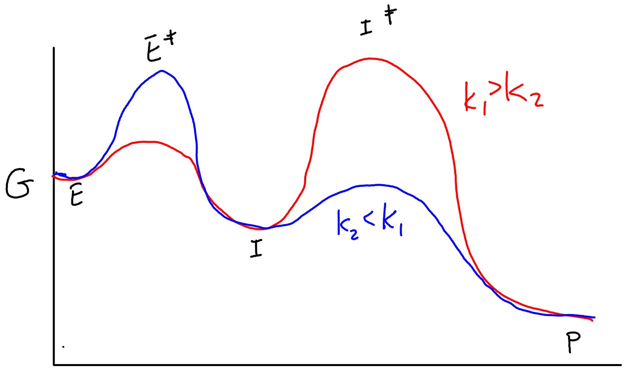
Induced fit in action

Contributors to $\Delta G^{\ddagger}$(Ea)

- Change in entropy
- Solvation shell of H-bonded water
- Distortion of substrates
- Alignment of catalytic functional groups

Benefits of binding to an E

- Entropy reduction
- Align substrates and reactive functional groups
- Desolvation



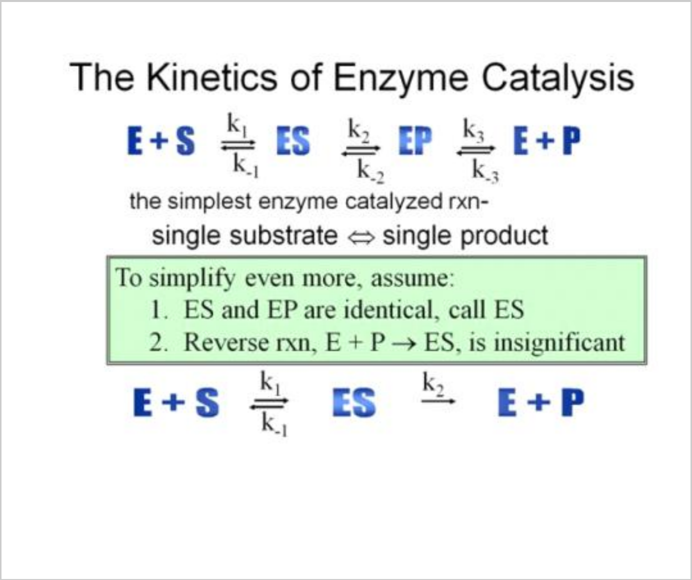
Mechaelis-Menten

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

V_0 = initial rate/velocity

Assume steady state

Steady state: refers to analysis of initial rates



The Kinetics of Enzyme Catalysis

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$$

the simplest enzyme catalyzed rxn-
single substrate ⇔ single product

To simplify even more, assume:
1. ES and EP are identical, call ES
2. Reverse rxn, E + P → ES, is insignificant

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

$V_0 = k_2[ES]$

$[E_t]$ = total enzyme concentration
Free enzyme $= [E_t] - [ES]$

Consider that at very high [S], [ES] is negligible to [S].
Rate of [ES] formation = $k_1([E_t]-[ES])[S]$
Rate of [ES] breakdown = $k_{-1}[ES] + k_2[ES]$
[ES] is constant since we are assuming steady state
$K_1([E_t]-[ES])[S] = k_{-1}[ES] + k_2[ES]$

Need to know how to derive this equation

Solve the equation
$K_1[E_t][S] - k_1[ES][S] = (k_{-1}+k_2)[ES]$
$K_1[E_t][S] = (k_{-1} + k_2)[ES] + k_1[ES][S]$
$K_1[E_t][S] = (k_1[S] + k_{-1} + k_2)[ES]$
SOLVE FOR [ES]

$$[ES] = \frac{k_1[E_t][S]}{k_1[S]+k_{-1}+k_2}$$

$$[ES] = \frac{[E_t][S]}{[S]+(k_2+k_{-1})/k_1}$$

$$k_m = (k_2+k_{-1})/k_1$$

$$[ES] = \frac{[E_t][S]}{[S]+k_m}$$

$$\frac{V_0}{k_2} = \frac{[E_t][S]}{[S]+k_m} \qquad V_0 = k_2[ES]$$

$$V_0 = \frac{k_2[E_T][S]}{K_m+[S]} \qquad V_{max} = k_2[E_t]$$

$$V_0 = \frac{V_{max}[S]}{K_m+[S]}$$

When $V_0 = \frac{V_{max}}{2}$

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m+[S]}$$

Limitations of michaelis-menten equation:

$$\overline{\;2\;} \qquad K_m+[S]$$

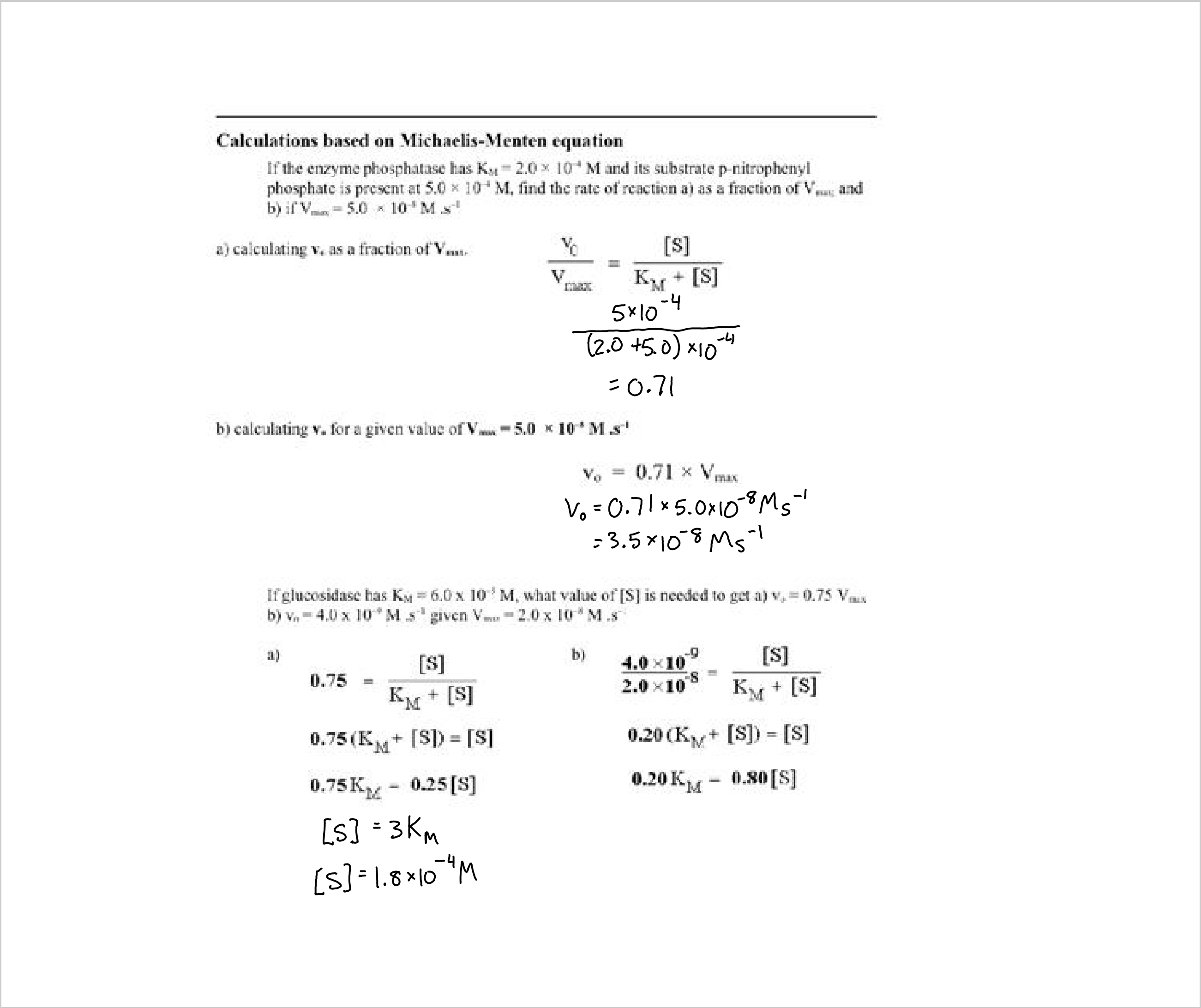
solve K_m

$K_m+[S]=2[S]$

$K_m=[S]$ when $V_0=\frac{1}{2}V_{max}$

Limitations of michaelis-menten equation:

1. Doesn't show us how many steps
2. Doesn't show us rates of each step
3. Doesn't show us what chemistry is involved



Calculations based on Michaelis-Menten equation

If the enzyme phosphatase has $K_M = 2.0 \times 10^{-4}$ M and its substrate p-nitrophenyl phosphate is present at 5.0×10^{-4} M, find the rate of reaction a) as a fraction of V_{max}; and b) if $V_{max} = 5.0 \times 10^{-8}$ M .s^{-1}

a) calculating **v_o** as a fraction of **V_{max}**.

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

$$\frac{5\times10^{-4}}{(2.0+5.0)\times10^{-4}}$$

$$=0.71$$

b) calculating **v_o** for a given value of **$V_{max} = 5.0 \times 10^{-8}$ M .s^{-1}**

$$v_o = 0.71 \times V_{max}$$

$$V_0 = 0.71 \times 5.0\times10^{-8} Ms^{-1}$$

$$= 3.5\times10^{-8} Ms^{-1}$$

If glucosidase has $K_M = 6.0 \times 10^{-5}$ M, what value of [S] is needed to get a) $v_o = 0.75\ V_{max}$ b) $v_o = 4.0 \times 10^{-9}$ M .s^{-1} given $V_{max} = 2.0 \times 10^{-8}$ M .s^{-}

a)

$$\mathbf{0.75} = \frac{[S]}{K_M + [S]}$$

$$\mathbf{0.75}\,(K_M + [S]) = [S]$$

$$\mathbf{0.75}\,K_M - \mathbf{0.25}\,[S]$$

$$[S] = 3K_M$$

$$[S] = 1.8\times10^{-4} M$$

b)

$$\frac{\mathbf{4.0 \times 10^{-9}}}{\mathbf{2.0 \times 10^{-8}}} = \frac{[S]}{K_M + [S]}$$

$$\mathbf{0.20}\,(K_M + [S]) = [S]$$

$$\mathbf{0.20}\,K_M - \mathbf{0.80}\,[S]$$

If [S] = 0.5 K_M, what is v_o, expressed as a fraction of V_{max}?

$$\frac{V_0}{V_{max}} = \frac{[S]}{K_m + [S]}$$

$$= \frac{.5K_m}{K_m + 0.5K_m}$$

$$0.33\,V_{max}$$

If v_o / V_{max} = 0.86, what is [S], expressed as a multiple of K_M?

$$0.86 = \frac{[S]}{K_m + [S]}$$

$$.86(K_m + [S]) = [S]$$

$$= 7.1K_m$$

Double reciprocal plot (Lineweaver-burk plot)

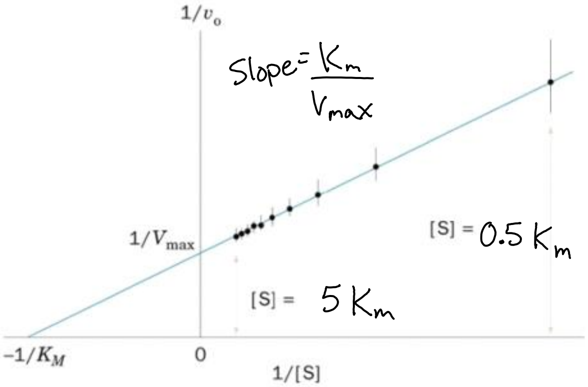


Figure 14-9 A double reciprocal (Lineweaver–Burk) plot.

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$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{1}{V_0} = \frac{K_m + [S]}{V_{max}[S]}$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

lineweaver-burk equation