ENZYMES

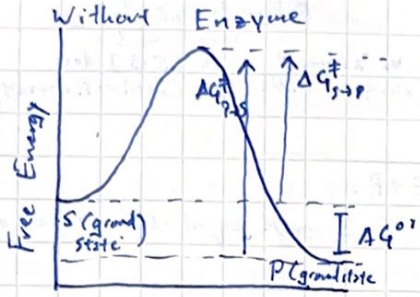
→ Enzymes are biological catalysts. They are slow under physiological conditions (37 °C, pH ~7, in aqueous solution)

How do enzymes work?

→ Enzymes have a site in which a substrate, S, binds, known as the "active site".

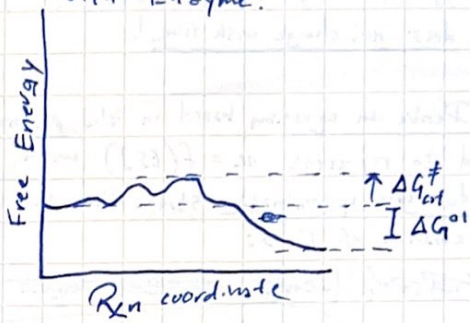
→ Enzymes lower the energy barrier between the ground state and the ground state:

Without Enzyme



Note in the diagram that the high $\Delta G^{\ddagger}_{P\to S}$ indicates that the reaction occurs much more slowly.

With Enzyme:



Properties of enzymes:

1. Enzymes make reactions faster
2. The enzyme is unchanged at the end of a reaction
3. Enzymes do not affect the equilibrium constant for a reaction.
4. Enzymes catalyze the reaction in both directions, S → P and P → S.
5. One enzyme can catalyze the reaction many times (i.e. one enzyme can catalyze many substrates to product)

Steady-state enzyme kinetics

→ Enzyme kinetics – experiment and analysis

→ Michaelis-Menten kinetics

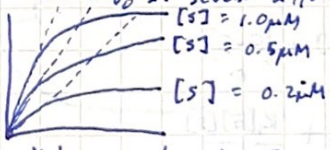
→ Meaning of Michaelis-Menten equation

The Enzyme Kinetics Experiment – "Steady State" or Michaelis-Menten Kinetics:

1) Mix E and S with no product in solution

2) Measure reaction rate, v, where $v = \frac{\Delta[P]}{\Delta t}$

- Specifically, v_0, or the initial reaction rate, is of the greatest interest
- Measure v_0 at several different starting [S] values.



The dotted lines represent $v_0 = \frac{d[P]}{dt}$, the initial rate of reaction.

- Make sure also that [E] is constant

3) Plot v_0 vs. [S]

→ Prediction: if the mechanism were E + S → E + P, v_0 vs. [S] would result in a straight line, as this is a 2nd order reaction (two reactants)

- However data actually turns out to be hyperbolic.

4) Write a new chemical mechanism

5) Derive equation based on that mechanism

6) Confirm that equation predicts behavior observed in the experiment.

The hyperbolic plot of rate vs [S] implies that there must have been a reaction intermediate:

$$E + S \underset{k_{1r}}{\overset{k_1}{\rightleftharpoons}} E \cdot S \underset{k_{2r}}{\overset{k_2}{\rightleftharpoons}} E + P$$

- The measuring of the initial rate at $t \approx 0$ results in $[P]_{initial} \approx 0$. Therefore the reverse reaction k_{2r} as constant may be safely ignored: $E + S \underset{k_{1r}}{\overset{k_1}{\rightleftharpoons}} E \cdot S \xrightarrow{k_2} E + P$
- Rate law for this reaction:

$$v_0 = \frac{d[P]}{dt} = k_2[E \cdot S]$$

→ This is very hard to determine

∴ Express [E · S] in terms of [S].

Step 4) Writing a new equation to model enzyme substrate activity

Proposed mechanism for enzyme catalyzed reaction:

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

$$v_o = \frac{d[P]}{dt} = k_2 \times [E\cdot S]$$

↓

Q: How does $[E\cdot S]$ change over time?

A: Let us "assume" that $[E\cdot S]$ does not change with time (spoiler: it doesn't)

In a steady-state enzyme equilibrium approximation, $[E\cdot S]$ does not change with time!

Step 5) Derive an equation based on the proposed mechanism

→ Need to represent $v_o = f([S])$ with $E + S \underset{k_{1r}}{\overset{k_1}{\rightleftharpoons}} E\cdot S \xrightarrow{k_2} E + P$.

→ Steady state approximation states that the rate of formation of $E\cdot S$ must equal the rate of disappearance of $E\cdot S$:

~~→ The rate of formation of E·S equals the rate of disappearance~~

$$\frac{d[E\cdot S]}{dt} = \underbrace{k_1[E][S]}_{\text{Rate of formation}} - \underbrace{(k_{1r}[E\cdot S] + k_2[E\cdot S])}_{\text{Rate of disappearance}} = 0$$

Conservation expressions:

$[S]_{total} = [S] + [E\cdot S]$

$[S] = [S]_{total}$, since $[S]_{total} >> [E]_{total}$

$[E]_{total} = [E] + [E\cdot S]$

Derive with algebra (work shown)

$$\frac{d[E\cdot S]}{dt} = k_1[E][S] - (k_{1r}[E\cdot S] + k_2[E\cdot S]) = 0$$

$$k_1[E][S] = k_{1r}[E\cdot S] + k_2[E\cdot S] = (k_{1r} + k_2)[E\cdot S]$$

Recall that $[S] = [S]_t$ since $[E]_t$ is negligible

$$\frac{k_1[E][S]}{k_{1r}+k_2} = [E\cdot S]$$ key term

$[E]_t = [E] + [E\cdot S]$

$$[E]_t = [E] + \frac{k_1[E][S]}{k_{1r}+k_2}$$

$$[E]_t = [E]\left(1 + \frac{k_1[S]}{k_{1r}+k_2}\right)$$

$$[E] = \frac{[E]_t}{1 + \frac{k_1[S]}{k_{1r}+k_2}}$$ key term

Recall that $v_o = k_2[E\cdot S] = \frac{d[P]}{dt}$

So $$v_o = k_2\left(\frac{k_1[E][S]}{k_{1r}+k_2}\right)$$

$$v_o = \frac{k_1k_2}{k_{1r}+k_2}[E]_t[S] \Big/ \left(1 + \frac{k_1[S]}{k_{1r}+k_2}\right)$$

Multiply by reciprocal

$$= \frac{k_1k_2}{k_{1r}+k_2}[E]_t[S] \Big/ \left(\frac{\frac{k_{1r}+k_2}{k_1}}{\frac{k_{1r}+k_2}{k_1}} + \frac{[S]}{\frac{k_{1r}+k_2}{k_1}}\right)$$

Define $\frac{k_{1r}+k_2}{k_1} = K_m$ and $k_{cat} = k_2$.

$$v_o = \frac{k_2[E]_t[S]}{\left(\frac{k_{1r}+k_2}{k_1}\right) + [S]}$$

↓ Define $k_{cat}[E]_t \equiv V_{max}$

$$v_o = \frac{k_{cat}[E]_t[S]}{K_m + [S]}$$

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

In summary after algebraic substitutions:

$$v_o = \frac{K_{cat}[E]_t[S]}{K_m + [S]} \quad \text{and} \quad v_o = \frac{V_{max}[S]}{K_m + [S]}$$

where

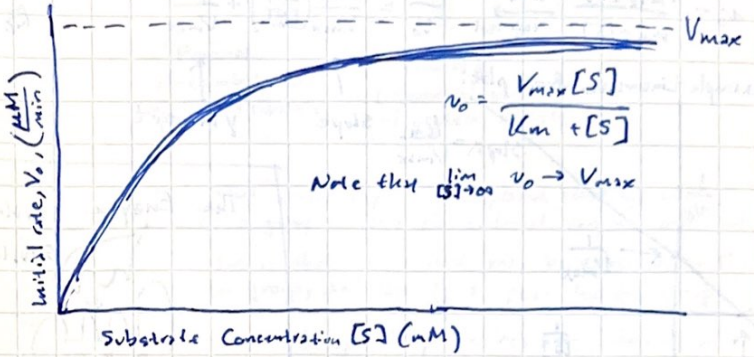
$$V_{max} \equiv K_{cat}[E]_t$$

$$K_m \equiv \left(\frac{K_{1r} + K_2}{K_1}\right)$$

$$K_{cat} \equiv K_2$$

Step 6) Does this equation model behavior of enzymes and substrates as seen in experiments?

It does. NLLS tests show that the A_{max} is the asymptote of the function $v_o = f([S])$



Connection to single site non-cooperative protein ligand binding:

Recall the graph for $P + L \rightleftharpoons P \cdot L$.

If you notice, this graph is very similar to the enzymatic catalysis graph

→ The key difference is that in the former, the thermodynamics of the binding is measured ($\Delta G°'$ values for binding of ligands to subunits) whereas the enzymatic catalysis graph is more of a kinetic approach.

→ $P + L \rightleftharpoons P \cdot L$ is measured at equilibrium, whereas $E + S \rightleftharpoons E \cdot S \to E + P$ is measured at the initiation of the rxn.

comparison of equations:

$$[P \cdot L] = \frac{[P]_t[L]_t}{(K_d + [L]_t)} \qquad v_o = \frac{V_{max}[S]}{K_m + [S]}$$

Note that in the above equation K_d is not an equilibrium constant whereas K_m is.

Similarly, derived equations also share a common structure:

$$Y = \frac{[P \cdot L]}{[P]_t} = \frac{[L]_t}{(K_d + [L]_t)} \quad \left(\frac{v_o}{V_{max}}\right)^* = \frac{[S]}{K_m + [S]}$$

*This value represents the rate of the reaction initially as a fraction of the maximum possible rate of reaction

- Takeaway points

→ If [S] is very high, then the value of K_1 must be much greater in $E + S \underset{K_{1r}}{\overset{K_1}{\rightleftharpoons}} E \cdot S$. That is to say that this step happens much faster.

- Since $K_m = \frac{K_{1r} + K_2}{K_1}$ and K_1 increases drastically with a copius amount of [S], K_m becomes very very small.

- So as [S] ↑, K_m ↓, and v_o ↑.

→ $K_{cat} \equiv K_2$ in $E \cdot S \xrightarrow{K_2} E + P$

- Why bother? In more complex reactions with enzymes, such as:

$$E + S \underset{K_{1r}}{\overset{K_1}{\rightleftharpoons}} E \cdot S \underset{K_{2r}}{\overset{K_2}{\rightleftharpoons}} \text{Intermediate} \underset{K_{3r}}{\overset{K_3}{\rightleftharpoons}} E \cdot P \xrightarrow{K_4} E + P$$

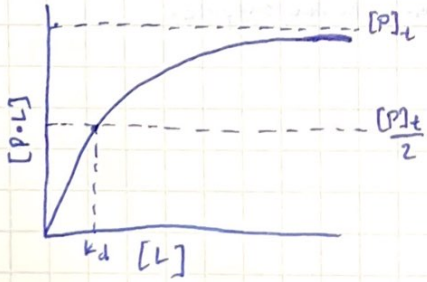
Realize that in this case $K_{cat} \neq K_2$, as K_{cat} is defined as: $E \cdot S \xrightarrow{K_{cat}} E + P$

where $K_{cat} = f(K_2, K_{2r}, K_3, K_{3r}, K_4)$

- K_{cat} is also referred to as the "turnover number" and is always in units of sec^{-1} (per second). Therefore this constant tells us the maximum number of reactions one enzyme molecule can catalyze per second (reactions/second)

- K_{cat} is always first order.

Remember that K_d may be estimated as the halfway point between the $[P]_{total}$ and 0 for $[P \cdot L]$ vs. $[L]$ graphs. K_m may also be estimated as the value at which the v_o is at $\frac{1}{2} V_{max}$:



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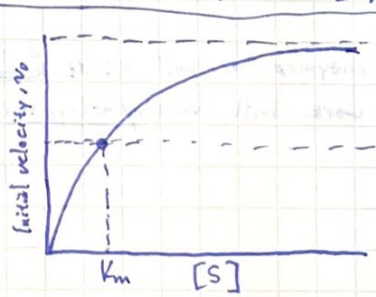
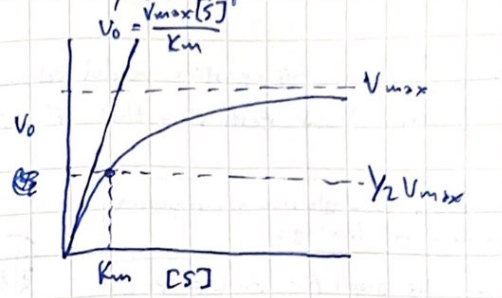


Q: What happens as $[S] \to 0$?

$$v_0 = \frac{V_{max}[S]}{K_m + [S]} \xrightarrow[[S]\to 0]{} v_0 \approx \left(\frac{V_{max}}{K_m}\right)[S]$$

→ Indicates that reaction rate depends almost linearly on $[S]$.

This value, $\frac{V_{max}}{K_m}$, represents the instantaneous slope at the very start of the reaction:

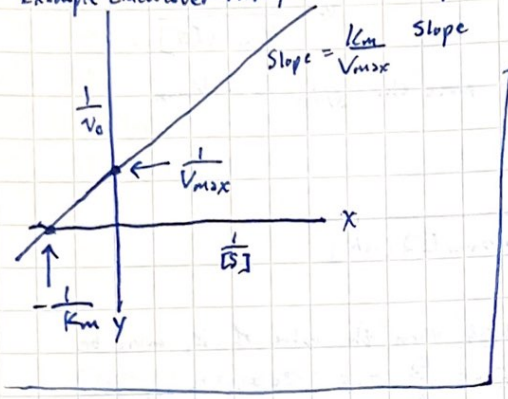


Lineweaver-Burke plot (Double reciprocal plot)

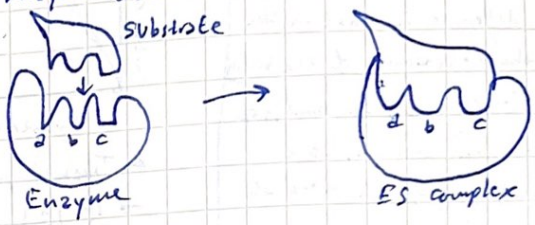
$$v_0 = \frac{V_{max}[S]}{K_m + [S]} \xrightarrow{\text{Take reciprocal}} \frac{1}{v_0} = \frac{K_m}{V_{max}}\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$

slope ↑ y intercept ↑

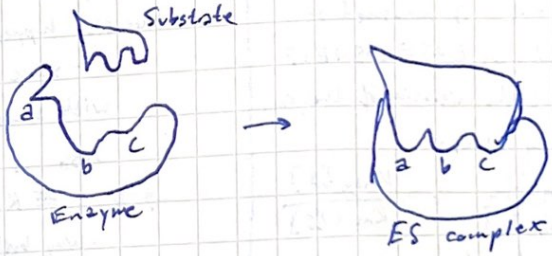
Example Lineweaver-Burke plot:



The Enzyme substrate Binding Model - Lock & Key



Induced fit model:



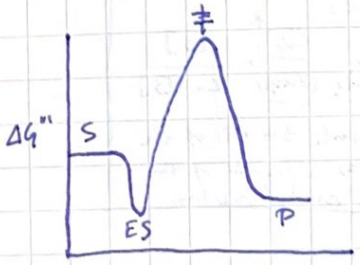
Hexokinase - example of induced fit enzyme. Without substrate, hexokinase is open, and with substrate, hexokinase "scrunches up".

How do Enzymes Work?

1) Enzymes bind to their substrate(s)
2) Enzymes bind to and stabilize the transition state by preferential binding to the transition state, compared to reactant or product binding.
3) Chemical catalysis in the active site
   → Acid base catalysis from acidic R-groups or basic R-groups
   → Metal ion catalysis (co-factors)
   → Covalent catalysis

In this class the enzyme we will use as a model for how enzymes work will be chymotrypsin.

Transition-state stabilization - It is possible for an enzyme to bind too well to a substrate.



This would be an example of an enzyme which binds too well to its substrate.

In this case, the reaction would actually be slower with the enzyme.