Enzyme Inhibition

Competitive inhibition:

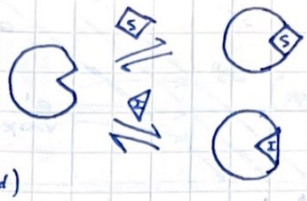
$E + S \rightleftharpoons ES \rightarrow E + P$

$+$

I "I" is an inhibitor

$\Updownarrow K_I$

EI $\quad K_I = \dfrac{[E][I]}{[EI]}$ (similar to K_d)



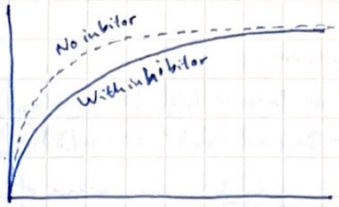
→ To calculate rate after inhibition, we can use a modified form of the Michaelis-Menten equation:

$$v_o = \frac{V_{max}[S]}{K_m\left(1 + \frac{[I]}{K_I}\right) + [S]} \qquad \left(a = 1 + \frac{[I]}{K_I}\right)$$

- Note that if $[I] = 0$, then it is just the MM function.
- As $[I]\uparrow$, $v_o \downarrow$. As $K_I \downarrow$, $v_o \downarrow$.

If the affinity of enzyme to inhibitor increases ($K_I \downarrow$), v_o would also decrease.

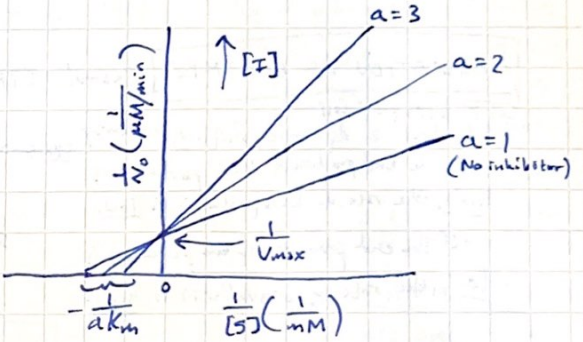
v_o with & without inhibitor (at fixed $[I]$)



- Note from the graph that the effect which the inhibitor has on the reaction rate decreases as $[S]$ increases. Therefore $[S]$ is also key for inhibition of rate, assuming $[I]$ is constant.

Double reciprocal plot of inhibition function (competitive)

$v_o = \dfrac{V_{max}[S]}{K_m(a) + [S]}$, then $\dfrac{1}{v_o} = \left(\dfrac{aK_m}{V_{max}}\right)\dfrac{1}{[S]} + \dfrac{1}{V_{max}}$

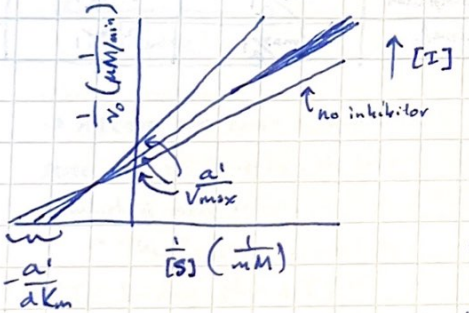


The slope of each double reciprocal plot is $\dfrac{aK_m}{V_{max}}$, as modeled by the double reciprocal equation function:

$$\frac{1}{v_o} = \underbrace{\frac{aK_m}{V_{max}}}_{m}\underbrace{\left(\frac{1}{[S]}\right)}_{x} + \underbrace{\frac{1}{V_{max}}}_{b}$$

- Note that the slope increases as $a = 1 + \frac{[I]}{K_I}$ increases. The steepest line would have the most inhibition.
- V_{max} is the same for all values because competitive inhibitors have no effect at high $[S]$, or V_{max}.
- K_m (apparent K_m) is greater in the presence of an inhibitor.

Inhibitors may also give other kinds of reciprocal plots. For example:



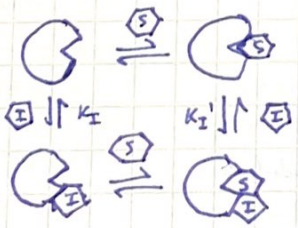
The left graph is an example of an inhibitor that inhibits at both a low $[S]$ and a high $[S]$ (V_{max}).

V_{max} is reduced with inhibitor, and the K_m is greater with the inhibitor.

This is known as "mixed inhibition"

Mixed inhibition: I binds to both E and E·S:

When the inhibitor is bound to E·S, it prevents the catalysis of E·S → E·P.



Equation for mixed inhibition:

$$\frac{1}{v_o} = \left(\frac{aK_m}{V_{max}}\right)\frac{1}{[S]} + \frac{a'}{V_{max}}, \text{ where } a' = \left(1 + \frac{[I]}{K_I'}\right)$$

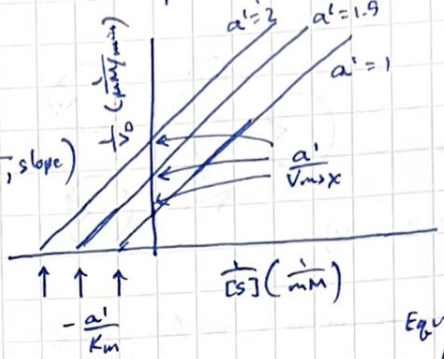
and $a = \left(1 + \frac{[I]}{K_I}\right)$

Uncompetitive Inhibition

→ Key points:
- Inhibits at high [S] (V_{max})
- Does not inhibit at low [S] ($\frac{V_{max}}{K_m}$, slope)

- V_{max} is reduced in the presence of an inhibitor
- K_m also is reduced in the presence of an inhibitor.

Double reciprocal plot for uncompetitive inhibition:

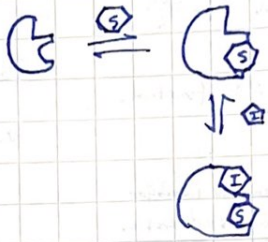


→ Notice that the slopes for this function are the same. This indicates that the value of $\frac{V_{max}}{K_m}$ is not impacted by the inhibition process.

→ In uncompetitive inhibition, I only binds to E•S:

$$E + S \rightleftharpoons ES \rightarrow E + P$$
$$ES + I \rightleftharpoons ESI \quad (K_I')$$

or



Equation for uncompetitive inhibition:

$$\frac{1}{v_0} = \frac{K_m}{V_{max}}\left(\frac{1}{[S]}\right) + \frac{a'}{V_{max}}$$

where $a' = 1 + \frac{[I]}{K_I'}$

In summary:

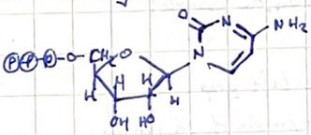
Effects of Reversible Inhibitors on apparent V_{max} & K_m

Inhibitor Type	Apparent V_{max}	Apparent K_m
None	V_{max}	K_m
Competitive	V_{max}	aK_m
Uncompetitive	V_{max}/a'	K_m/a'
Mixed	V_{max}/a'	aK_m/a'

REGULATION OF ENZYMES

Example: CTP regulation

CTP biosynthesis
→ Involves a dynamic, shifting pathway
- If the end product of the pathway is high, the rate of biosynthesis is low.
- If the end product of the pathway is low, the rate of biosynthesis is high.



5 CTP structure

Q: What factors affect the rate of an enzyme catalyzed reaction?

A: Consider the equation (expanded MM equation):

$$v_0 = \frac{k_2[E]_t[S]}{\left(\frac{k_{1r} + k_2}{k_1}\right) + [S]}$$

- $[E]_t$: Total enzyme concentration
- $[S]$: Substrate concentration
- k_2: How active the enzyme is.
- Fraction of enzyme that is active

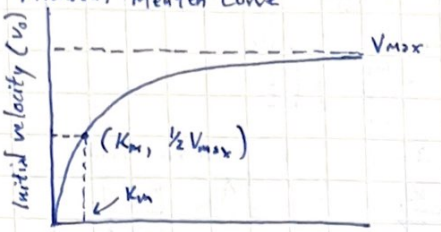
Therefore, some mechanisms of enzyme-catalyzed reactions include:
1) Substrate availability
2) Enzyme concentration in the cell (regulation of gene expression)
3) Presence of inhibitors.
4) Allostery *

*New.

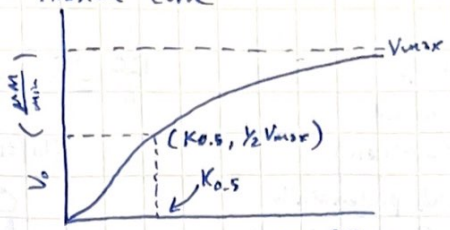
Allostery & Enzyme Kinetics

Compare the graphs of an allosteric curve & a Michaelis-Menten (non cooperative) curve:

Michaelis-Menten Curve



Allosteric Curve



- The MM- Curve is hyperbolic whereas the Allosteric curve is sigmoidal.
- We can adjust the MWC model to model the behavior of a cooperative enzyme.

$v_0 = \frac{V_{max}[S]}{K_m + [S]}$ $\quad E+S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\cdot S \xrightarrow{k_2} E+P$ $\quad K_{0.5} = C_{0.5}$

Some terminology:

→ Allosteric effectors: a ligand that binds at one site in a protein and influences ligand binding at another site.

→ Positive allosteric effector: a ligand whose binding increases the binding affinity at another site (opposite of neg. allo. eff.)

→ Negative allosteric effector: a ligand whose binding decreases the binding affinity at another site. (opposite of pos. allo. eff)

→ Homotropic allosteric effector: A ligand whose binding affects binding of the same ligand at another site. ex.) oxygen for hemoglobin - sigmoidal pattern

→ Heterotropic allosteric effector: A ligand whose binding affects binding of a different ligand at another site

Application of Allostery to Aspartate transcarbamylase (ATCase)

→ ATCase's rate does not follow Michaelis-Menten kinetics.

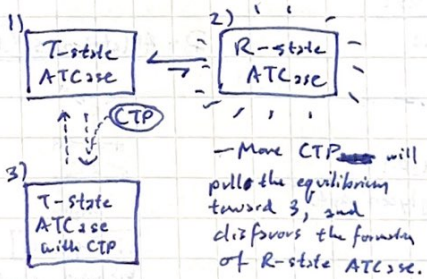
→ ATCase therefore has two states and two levels of activity, very much like the MWC model:

→ ATCase can come in both a T-state and an R-state, the latter of which is more active.

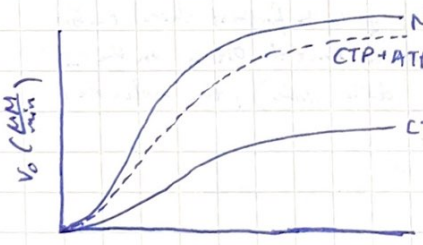
- The T-state is favored by CTP binding: that is, CTP preferentially binds to the inactive state of ATCase.
- The R-state is preferentially bound to the Aspartate substrate.

→ CTP and ATP are heterotropic allosteric effectors* for ATCase.

- Recall that CTP is the end product for the enzymatic pathway catalysis of aspartate.
- Therefore CTP is a negative heterotropic effector for ATCase, and acts as an inhibitor for ATCase.



- More CTP will pull the equilibrium toward 3, and disfavors the formation of R-state ATCase.



→ Experimentally, it has been shown that ATP in the presence of CTP actually reverses the inhibitive allosteric properties of CTP, as shown in the experimental graph to the left.

- ATP binds in the same site as CTP. They are competitors for the same spot, but ATP has very little effect on the ability of ATCase to swap to an R-state. In fact, it is thought that ATP actually stabilizes the R-state.

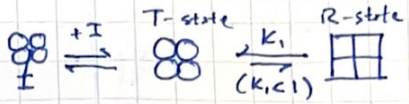
The Biological Significance of Allostery

→ The rate of reaction in a cell can change in response to changes in:

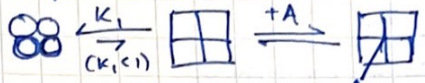
1) Substrate concentration
   - (e.g. Aspartate, for ATCase)
2) Concentration of other metabolites
   - (e.g. CTP & ATP, for ATCase)

→ Allosteric effectors may bind preferentially to either the T-state or the R-state, and would consequently shift the equilibrium in that direction.

Example diagram for a negative heterotropic effector:



- Shifts equilibrium to T-state (allosteric inhibition)



- Shifts equilibrium to R-state (allosteric activation)

(A for activator)

CARBOHYDRATES

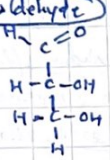
→ Carbohydrates have a general formula of $(C(H_2O))_n$, for hydrated carbons. (For this course $3 \leq n \leq 6$)

→ Monosaccharides are the simplest ~~monosaccharides~~ building block, or monomer, of carbohydrates.

- An example of a simple monosaccharide, glyceraldehyde:

Structural isomers form different carbohydrates:

Glyceraldehyde



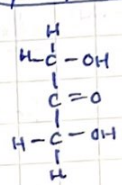
- This molecule is an aldotriose:
  - Aldo - aldehyde
  - Tri - 3 carbons
  - Ose - sugar

$C_3H_6O_3$

$(C(H_2O))_3$

- Example of an aldose

Dihydroxyacetone



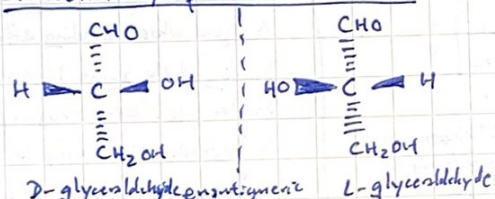
- This molecule is a dihydroxyacetone a ketotriose.

- Example of ketose

$C_3H_6O_3$

$(C(H_2O))_3$

Stereochemistry of Carbohydrates*

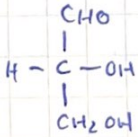


D-glyceraldehyde — enantiomeric set — L-glyceraldehyde

~~Glyceraldehyde~~

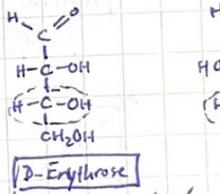
- In nature, D- enantiomers are the most commonly found form.

*Note: D-glyceraldehydes are usually drawn as a fischer projection:



D-glyceraldehyde.

D- Aldetetroses (Four carbons, $C_4H_8O_4$, or $(C(H_2O))_4$)



D-Erythrose
biologically significant

D - Threose

- Note that these two are diastereomers, and not enantiomers.
  - There for they are different molecules with different properties.

- To determine D or L, go to the furthest chiral carbon from the carboxyl carbon. Since the OH is on the right side (as marked by dotted circle), the molecule is in the D-orientation.