Enzyme Kinetics

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Enzymes are biological catalysts

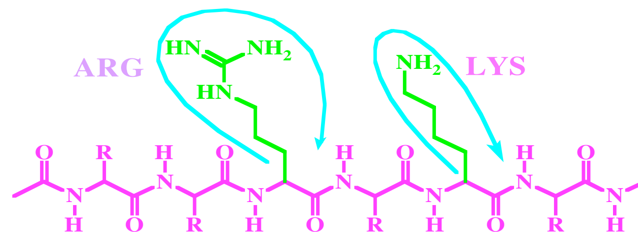
- Typically they are protein (exception : ribozymes - enzymatically active RNA)
- They permit reactions to occur at normal temperature and pressure in the body.
- They process several million molecules per minute.
- They are highly specific with regard to their substrates.

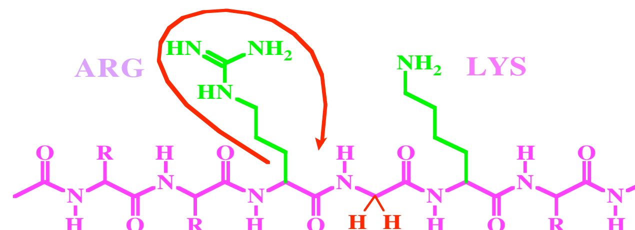
Enzymes are highly specific

Enzymes are able to distinguish between homologues (succinate and malonate)

- Enzymes attacking D-amino acid will not touch L-amino acid (Chiral specificity).
- Enzymes are even able to distinguish prochiral centers (Prochiral specific).
- Even nonspecific enzymes show certain specific requirement in their substrate structure.
- Some may be group or linkage specific. So they exhibit broad specificity.

Specificity of enzymes





Enzyme classification

Old way: Naming the enzymes with "ase".

Example: enzyme acting on tyrosine is called tyrosinase.

This kind of naming does not convey much about what the enzyme does in the cell. So Enzyme Commission formulated a set of rules for naming the enzymes.

- **Enzymes are classified into six categories:**
  1. **Oxidoreductases** (Catalyzing oxidation, reduction reactions).
  2. **Transferases** (Responsible for group transfer reactions).
  3. **Hydrolases** (Hydrolyze substrates into two parts).
  4. **Lyases** (Cleave the substrate molecule).
  5. **Isomerases** (Convert one isomer to the other).
  6. **Ligases** (Join two or more molecules together)

The first step of an enzymatic reaction is the formation of enzyme substrate complex

$$E + S \longrightarrow ES$$

This is simply a physical phenomenon of binding. It usually involves weak forces between E and S.

No covalent bond is formed at this stage.

In general, it is an easy and fast step in any enzyme action.

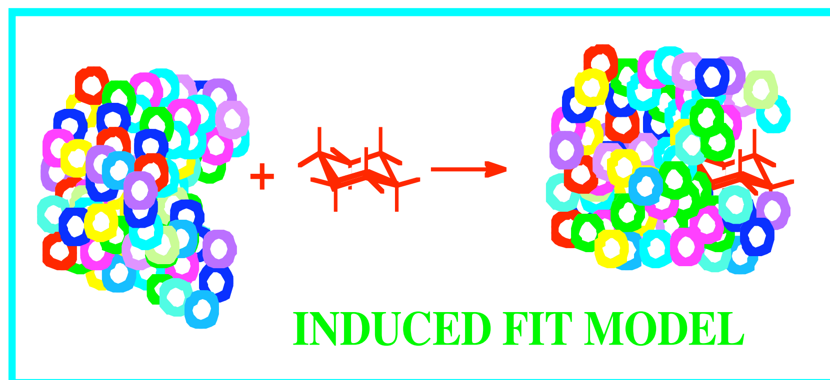
Enzyme uses a special site called active site to form ES complex.

Some characteristics of active site are:

- Active site consists of a small volume of the enzyme.
- Active site is a 3-D entity.
- Ligand binding to active site involves a number of weak forces and precise arrangement of atoms around the active site.
- Active site consists of clefts and crevices.

Koshland's Induced Fit Theory

Binding of substrate causes conformational changes on the enzyme to accommodate the substrate structure at the active site. The conformational changes could be as large as that is shown below or as small as possible.



Only a small portion of the enzyme forms the active site

- Enzymes are typically very huge.
- But only a small part it is in fact responsible for catalysis.
- The active site has two basic functions to perform: to serve as a binding site and to perform catalysis.

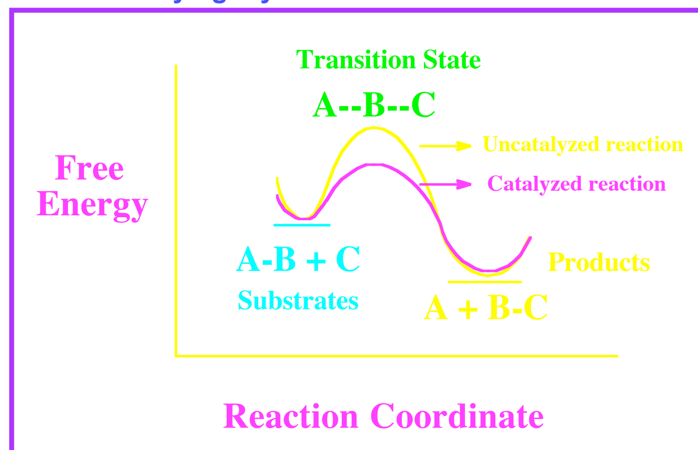
Characteristics of active site

- Catalytic site: The site at which actual catalysis takes place.
- Binding site: The place where the substrates are held together using weak noncovalent forces. Usually this site is complementary to the substrate.

How does an enzyme reaction occur?

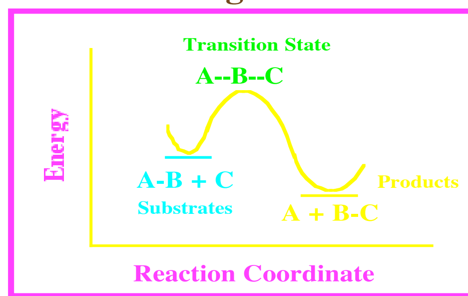
- Enzyme and substrate bind to form ES complex
- ES Complex is converted to a transition state complex .
- Transition state is neither the substrate nor the product.
- E-TS complex breaks down to enzyme product complex.
- Product is release from the EP complex.

Enzymes accelerate the reaction by stabilizing the transition state.
Enzymes are usually complementary to the transition state.
So they bind to it very tightly.

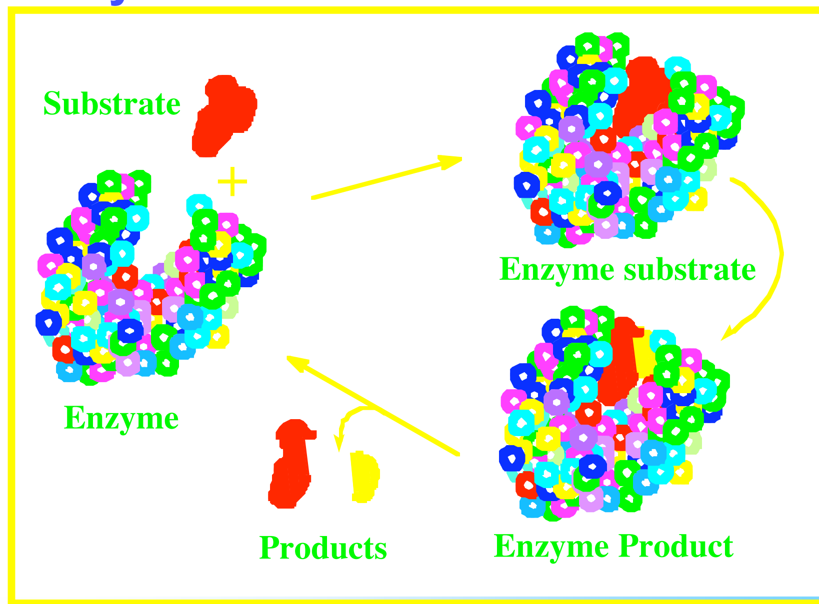


Enzymes show very high affinity to transition state.
They force substrates to go to transition state for binding tightly

The transition state formation involves bond breaking and/or bond making process.
Hence this is the slowest step.
Transition state is highly unstable and hence either it has to go forward to produce products (thus completing a cycle of reaction) or revert back to regenerate substrates.

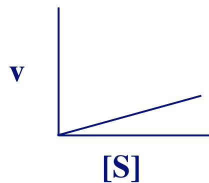


Enzyme reaction

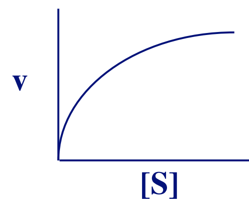


Effect of substrate concentration

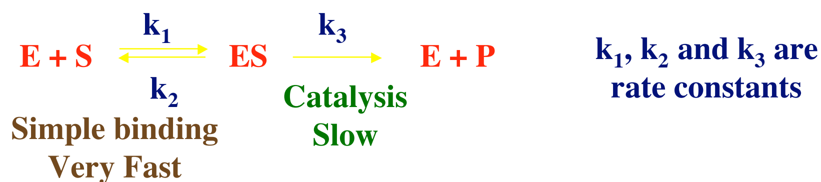
- For a non enzymatic reaction the rate of the reaction increase with the substrate concentration.



- For enzyme also, it increases; but only to a certain point. beyond that the velocity reaches constant, Vmax



Derivation of Michaelis Menten equation



k_1, k_2 and k_3 are rate constants

Velocity of the reaction is determined by slowest step

Therefore, $v = k_3[ES]$

When all the Enzyme $[E_t]$ is complexed with S and producing P at maximum

$$V_{max} = k_3[E_t] = k_3\{[E_o] + [ES]\}$$

Dividing v by V_{max} we get, $\dfrac{v}{V_{max}} = \dfrac{[ES]}{[E_o] + [ES]}$

According to equilibrium hypothesis, E and S are in rapid equilibrium with ES complex. So We could ignore the slow step ES --> E + P.

At equilibrium, the rate of forward reaction is equal to the rate of backward reaction $k_1 [E_o] [S] = k_2 [ES]$

or $[E_o] [S] / [ES] = k_2 / k_1 = K_s$

where K_s is the dissociation constant (Michaelis constant)

Therefore, $[ES] = [E_o] [S] / K_s$

Substituting in $\frac{v}{V_{max}} = \frac{[ES]}{[E_o] + [ES]} = \frac{[E_o] [S] / K_s}{[E_o] + [E_o] [S] / K_s}$

Dividing Numerator and Denominator by $K_S / [E_o]$, we get:

$$\frac{v}{V_{max}} = \frac{[S]}{K_s + [S]}$$

Michaelis Menten equation $v = \frac{V_{max} [S]}{K_s + [S]}$

For Michaelis Menten equation, $K_s = k_2/k_1$

Briggs -Haldane Approximation

Briggs and Haldane reasoned out that in some reactions k_3 could be fast enough to affect the E --> ES equilibrium. So they formulated what is known as the Steady State Approximation. The rate of formation of ES complex is approximately equal to its rate of decomposition at steady state.

or, $k_1[E_o][S] \approx k_2 [ES] + k_3[ES]$

but since $[E_o] = [E_t] - [ES]$

we get, $k_1[E_t][S] - k_1[ES] [S] = k_2 [ES] + k_3[ES]$

Grouping for [ES], $k_1[E_t][S] = [ES] \{k_1[S] + (k_2 + k_3)\}$

$[ES] = k_1[E_t][S] / \{k_1[S] + (k_2 + k_3)\}$

Substituting the value of [ES] in $v = k_3 [ES]$, we get

$v = k_3k_1[E_t][S] / \{k_1[S] + (k_2 + k_3)\}$

$v = k_3k_1[E_t][S]/ \{k_1[S] + (k_2 + k_3)\}$

Dividing numerator and denominator by k_1 we get

$v = k_3[E_t][S]/ \{[S] + (k_2 + k_3)/k_1\}$

Or $v = V_{max} [S]/ K_m + [S]$

Thus, we get the same equation irrespective of using equilibrium hypothesis or steady state assumption. However, the value of K_m is different.

$K_m = (k_2 + k_3)/k_1$ and not dissociation constant, k_2/k_1

K_m is defined as the substrate concentration required to produce half the maximum velocity.

V_{max} is same $= k_3 [E_t]$

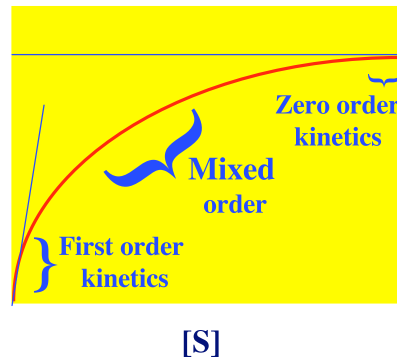
Michaelis Menten equation describes a Hyperbola

$v = V_{max} [S]/ K_m +[S]$

At very low [S], K_m>>[S],
So, $v \approx V_{max} [S]/ K_m$

Under this condition, velocity increases linearly with substrate concentration. This is called first order kinetics.

At very high [S], [S]>>K_m,
So, $v \approx V_{max}$



At high [S], increasing the concentration of S further, does not result in increased velocity. Velocity becomes constant (V_{max}). Velocity is independent of [S]. It is called zero order kinetics.

In between, we observe mixed order kinetics.

Values of K_m and V_{max} in $v = V_{max} [S]/ K_m + [S]$

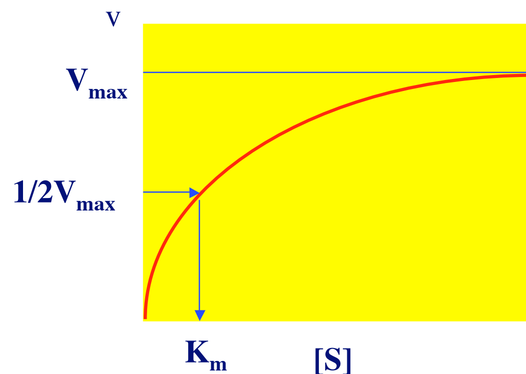
The v at very high [S] becomes V_{max}

When [S] = Km, Michaelis Menten gets reduced to

$v = V_{max} [S]/ 2[S]$

or $v = V_{max}/2$

Thus concentration of S required to produce $1/2V_{max}$ gives the value of K_m



Michaelis Menten equation describes a hyperbola.
A plot of V versus [S] gives a hyperbolic graph

Meaning of V_{max}

1. V_{max} = velocity at very high [S] conc.
2. $V_{max} = k_3[E_t]$

V_{max} is the velocity of the reaction when all the enzyme molecules are full saturated with S and producing product at maximum capacity.

Units of Vmax: µmoles of P formed/min/mg of enzyme.

$V_{max} = k_3[E_t]$, from this we can write,

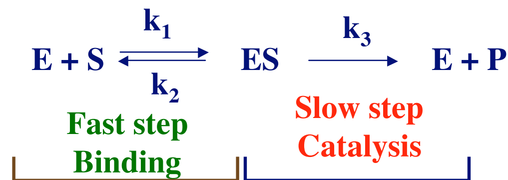
$k_3 = V_{max}/[E_t]$

k_3 is defined as the turnover number -
Number of S molecules converted into P per active site of the enzyme molecule in one minute (or second).

Meaning of K_m :

$K_m = (k_2 + k_3)/k_1$

1. 1. It is simply a group of rate constants = $(k_2 + k_3)/k_1$
   2. If k_3 is much smaller than k_2, K_m becomes = k_2/k_1 or Km is the dissociation constant of ES complex.
   3. The correct definition of Km is : It is the substrate concentration required to produce half the maximum velocity.

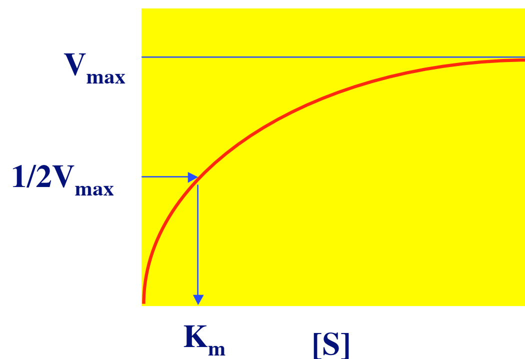


K_m talks about how Tight the ES complex is

Vmax talks about How fast the enzyme works

Thus both K_m and V_{max} are two important parameters of any enzyme catalyzed reaction

K_m and V_{max} values from Michaelis - Menten graph are approximate.



We can get V_{max} value only at infinite [S]. This is not possible. In some cases, even high concentration is not possible to obtain due to solubility problems. Therefore, we get only an estimate of V_{max}. Since V_{max} is approximate, we also make an error in the estimation of K_m (since it is [S] at 1/2 V_{max})

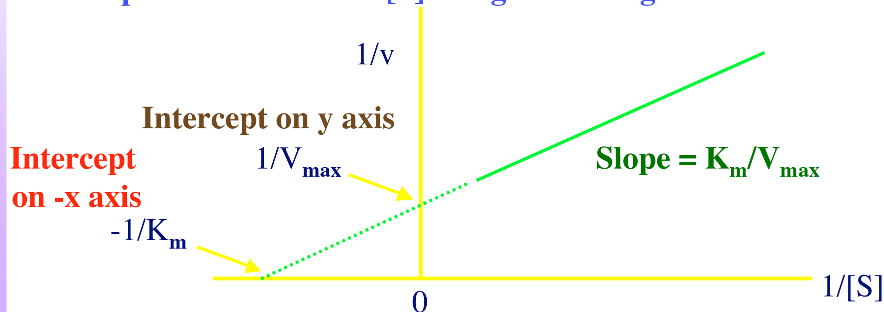
Lineweaver Burk Plot

Lineweaver - Burk solved this problem by inverting the Michaelis Menten equation $v = V_{max}[S] / K_m + [S]$

$$1/v = K_m/V_{max}[S] + [S]/V_{max}[S]$$

$$\text{or } 1/v = K_m/V_{max}[S] + 1/V_{max}$$

This equation describes a straight line: $y = mx + c$
A plot of 1/v versus 1/[S] will give a straight line.



ENZYME UNITS

One International Unit of Enzyme Activity :
Amount of Enzyme required
to produce 1µmole of product per minute.

Specific activity = units per mg of protein

New Enzyme Unit: Katal (kat)
Amount of enzyme causing the conversion of one mole of substrate into product per second.

Turnover number of some enzymes

Enzyme	Turnover number
Carbonic anhydrase	600,000
Acetylcholine esterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Lysozyme	0.5

Some enzymes act very fast and some are very slow.

Value of K_m for some enzymes

Enzyme (Substrate)	K_m value (in mM)
Catalase (H_2O_2)	1100
Hexokinase (glucose)	0.15
Chymotrypsin (N-benzoyltyrosinamide)	2.5
Aspartate transaminase (Asp)	0.9
Fumarase (fumaric acid)	0.005

Some enzymes bind to their substrate very tightly, and some enzyme bind very weakly (like catalase).
Higher the K_m weaker the binding
Lower the K_m stronger the binding.

Catalytic efficiency

$v = k_3[E_t][S]/K_m + [S]$

In cell, most often, [S] is << than K_m

Hence, the equation simplifies to: $v = k_3[E_t][S]/K_m$

The ratio, k_3/K_m, talks about the catalytic efficiency.
For example, chymotrypsin, an endoprotease that hydrolyses preferably at aromatic amino acids, has the following k_3/K_m.

Amino acid ester	Side chain	k_3/K_m ($s^{-1}M^{-1}$)
Glycine	- H	0.13
Valine	$-CH(CH_3)_2$	2.0
Norvaline	$-CH_2CH_2CH_3$	360
Norleucine	$-CH_2CH_2CH_2CH_3$	3000
Phenylalanine	$-CH_2Ph$	10,000

Thus phenylalanyl ester is an excellent substrate for chymotrypsin.

Upper limit of catalytic potential $k_3/K_m = \dfrac{k_3}{(k_2 + k_3)/k_1}$

If k_3 is much much faster than the rate of decomposition of ES complex (k_2), the above equation reduces to $k_3/K_m \approx k_1$

Thus the ultimate limit of k_3/K_m is set forth by the rate of formation of ES complex (k_1). Obviously this can not be greater than the rate of diffusion of substrate into the active site of the enzyme.

The normal diffusion rate is 10^8 to 10^9 $s^{-1}M^{-1}$.

Therefore, no enzyme can act faster than this.

Some enzymes have reached this limit:

Superoxide dismutase = 7×10^9 $s^{-1}M^{-1}$.

Triose phosphate isomerase = 2.4×10^8 $s^{-1}M^{-1}$.

Carbonic anhydrase = 8.3×10^7 $s^{-1}M^{-1}$.

Other enzymes can be improved by mutation and chemical modifications

References

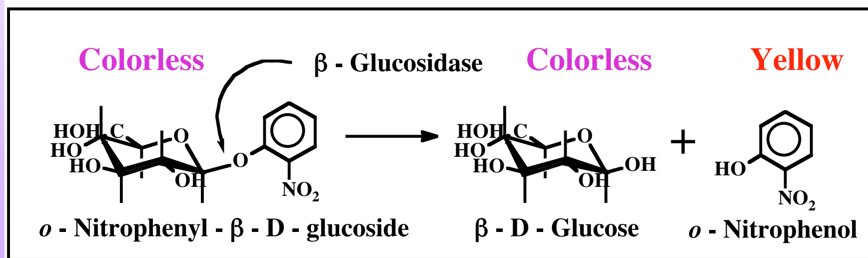
- Enzyme kinetics, Kent M. Plowman, McGraw Hill Book Co., New York. (1972).
- H. Lineweaver and D. Burke. J. Amer. Chem. Soc., 58, 658 (1934).
- L. Michaelis and M. L. Menten Biochem. Z., 49, 333 (1913).
- C. S. Hanes. Biochem. J., 26, 1406 (1932).
- B. H. J. Hofstee. Nature, 184, 1296 (1959).
- W. W. Cleveland. Biochim. Biophys. Acta 67, 104 (1963).
- J. B. S. Haldane. Enzymes. Longmans Publishing Co. , London (1930).
- A. R. Fersht. A guide to enzyme catalysis and protein folding. W. H. Freeman and Co. NY, 1999

β - glucosidase

- In this laboratory exercise, you will determine some kinetic properties of the enzyme β-glucosidase (E.C. 3.2.1.21).
- β-glucosidase hydrolyzes β-1,4-linked units. So it belongs to hydrolase class.
- It is active around pH 5 and is inhibited by heavy metal ions such as Hg. It shows wide substrate specificity.

Enzyme assay

- We will use an artificial substrate and assay the activity of glucosidase. The reason for using the artificial substrate is to conveniently monitor the reaction in a spectrophotometer.



Prior to normal assay you need to make sure the enzymatic reaction is linear with time.

- **For three different reasons, the product formation in an enzyme catalyzed reaction does not show linear relationship with time. These are:**
- **1. Substrate depletion.**
- **2. Product inhibition.**
- **3. Enzyme inactivation.**
- **Therefore, you need to make sure that all your measurements are in the linear range and the above factors do not interfere with the assay.**
- **Hence in the first part of the experiment, you will determine whether the enzyme works linearly for the given duration of the time or not.**

Second part - Determine the Michaelis constants

- In the second part of the experiment, you will determine the velocity of the reaction at different concentrations of substrate and calculate the K_m and V_{max} for β-glucosidase.

How to perform the experiment

Step. 1. Take increasing concentration of substrate in different tubes. Add buffer and other needed chemicals.
Step. 2. Add fixed catalytic amount of enzyme to each tube to start the reaction.
3. Monitor the product (in this case, formation of yellow color) for fixed amount of time.
4. Calculate the velocity. (Velocity is μmoles of product formed per min).
5. Plot the velocity against substrate concentration to get the Michaelis Menten plot.