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

\[ E + S \rightarrow ES \]

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, this 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 the active site are:

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

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.
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.

The transition state formation involves bond breaking and/or bond making process.
Hence it is slow.
Once transition state is formed, the molecule 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.

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

An enzyme, typically shows a very high affinity to the transition state. Thus, it forces substrates to go to the transition state so that it can bind tightly to it.

Derivation of Michaelis Menten equation

\[
E + S \xrightarrow{k_1} ES \xrightarrow{k_{-1}} E + P \quad k_p, k_{-1} k_2
\]

- Fast step
- Slow step
- Binding
- Catalysis

Velocity is determined by the slowest step. So \( v = k_2 [ES] \)

\( V_{max} \) maximum velocity is obtained when all the enzyme \( E_t \) is bound to S and working at maximum capacity.

\[ V_{max} = k_2 [E_t] \]

Fractional velocity is \( Y = \frac{v}{V_{max}} \)

We have derived for myoglobin \( Y = \frac{[O_2]}{K + [O_2]} \)

Substituting \( [S] \) for \( [O_2] \) and \( v/V_{max} \) for \( Y \) we get Michaelis Menten equation

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

$K_m = (k_2 + k_{-1})/k_1$ rather than simple dissociation constant, $k_{-1}/k_1$

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

$V_{\text{max}}$ is maximum velocity of the reaction

When all the enzyme molecules are saturated

$V_{\text{max}} = k_2 [E_t]$

Michaelis Menten equation describes a hyperbola $v = V_{\text{max}} [S]/ K_m + [S]$

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

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

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

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

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

In between these two areas, we observe mixed order kinetics.

For the chemical catalyzed reaction, $S \rightarrow P$, velocity of the reaction is proportional to [S].

In the case of enzyme catalyzed reaction, $v$ versus [S] gives a hyperbola.

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

Velocity of the reaction $v = k_2 [ES]$
Meaning of $V_{\text{max}}$

1. $V_{\text{max}}$ = velocity at very high [S] conc.
2. $V_{\text{max}} = k_2[E_t]$

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

Units of $V_{\text{max}}$:

$V_{\text{max}} = k_2[E_t]$, from this we can write,

$k_2 = V_{\text{max}}/[E_t]$

$k_2$ 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 $K_m$:

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

1. It is simply a group of rate constants = $(k_2 + k_{-1})/k_1$
2. If $k_1$ is much smaller than $k_{-1}$, $K_m$ becomes $k_1/k_1$
   or $K_m$ is the dissociation constant of ES complex.
3. But the most correct definition of $K_m$ is “the substrate concentration required to produce half the maximum velocity”.

\[
\begin{align*}
E + S & \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ES & \overset{k_2}{\rightarrow} & E + P \\
\text{Fast step} & & \text{Slow step} & \\
\text{Binding} & & \text{Catalysis} & \\
K_m \text{ talks about how} & & V_{\text{max}} \text{ talks about} & \\
\text{Tight the ES complex is} & & \text{How fast the enzyme works} & \\
\text{Thus both $K_m$ and $V_{\text{max}}$ are two important parameters of any enzyme catalyzed reaction} & & & \\
\end{align*}
\]

Lineweaver Burk Plot

Lineweaver - Burk solved this problem by inverting the Michaelis Menten equation

\[
v = \frac{V_{\text{max}} [S]}{K_m + [S]} \quad \text{or} \quad \frac{1}{v} = \frac{1}{V_{\text{max}}} \frac{1}{K_m + [S]} + \frac{1}{V_{\text{max}}}
\]

This equation describes a straight line: $y = mx + c$

A plot of $1/v$ versus $1/[S]$ will give a straight line.

\[
\begin{align*}
\text{Intercept on y axis} & = \frac{1}{V_{\text{max}}} \quad \text{Slope} = \frac{1}{K_m V_{\text{max}}} \\
\text{Intercept on -x axis} & = \frac{-1}{K_m} \\
1/v & \quad 1/[S]
\end{align*}
\]
### Turnover number of some enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Turnover Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonic anhydrase</td>
<td>600,000</td>
</tr>
<tr>
<td>Acetylcholine esterase</td>
<td>25,000</td>
</tr>
<tr>
<td>Penicillinase</td>
<td>2,000</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>1,000</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>100</td>
</tr>
<tr>
<td>DNA polymerase I</td>
<td>15</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Thus some enzymes act very fast and some are very slow.

### Value of $K_m$ for some enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$K_m$ value (in mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase (H$_2$O$_2$)</td>
<td>1100</td>
</tr>
<tr>
<td>Hexokinase (glucose)</td>
<td>0.15</td>
</tr>
<tr>
<td>Chymotrypsin (N-benzoyltyrosinamide)</td>
<td>2.5</td>
</tr>
<tr>
<td>Aspartate transaminase (Asp)</td>
<td>0.9</td>
</tr>
<tr>
<td>Fumarase (fumaric acid)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

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

\[ v = \frac{k_2[E]}{[S]} \frac{[S]}{K_m + [S]} \]

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

Therefore, the equation can be simplified as,
\[ v = \frac{k_2[E]}{[S]} \frac{[S]}{K_m} \]

The ratio, $k_2/K_m$, is an important ratio. It talks about the catalytic efficiency.

For example, chymotrypsin, an endoproteinase that hydrolyses preferably at aromatic amino acids, have the following $k_2/K_m$ for its substrates.

<table>
<thead>
<tr>
<th>Amino acid ester</th>
<th>Side chain</th>
<th>$k_2/K_m$ (s$^{-1}$M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>-H</td>
<td>0.13</td>
</tr>
<tr>
<td>Valine</td>
<td>-CH(CH$_3$)$_2$</td>
<td>20</td>
</tr>
<tr>
<td>Norvaline</td>
<td>-CH$_2$CH$_2$CH$_3$</td>
<td>360</td>
</tr>
<tr>
<td>Norleucine</td>
<td>-CH$_2$CH$_2$CH$_3$</td>
<td>3000</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>-CH$_2$Ph</td>
<td>10,000</td>
</tr>
</tbody>
</table>

Thus phenylalanine ester is an excellent substrate for chymotrypsin.

### Upper limit of catalytic potential

\[ k_2K_m = \frac{k_2^2}{(k_1 + k_2^2)/k_1} \]

If $k_2$ is much much faster than the rate of decomposition of ES complex ($k_1$), the above equation reduces to $k_2K_m = k_1$

Thus the ultimate limit of $k_2/K_m$ 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 = $7 \times 10^9$ s$^{-1}$ M$^{-1}$
- Triose phosphate isomerase = $2.4 \times 10^9$ s$^{-1}$ M$^{-1}$
- Carbonic anhydrase = $8.3 \times 10^8$ s$^{-1}$ M$^{-1}$

Other enzymes can be improved by mutation and chemical modifications.
Enzyme classification

Naming the enzymes with “ase” ending does not convey much about what the enzyme does in the cell. So Enzyme Commission formulated a set of rules for naming the enzymes.

• Broadly, enzymes are classified into six categories:
  1. Oxidoreductases (Catalyzing oxidation, reduction reactions).
  2. Transferases (Responsible for group transfer reactions).
  3. Hydrolases (Hydroyze 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).

INHIBITORS - DIFFERENT TYPES

Inhibitors

  - Reversible
  - Irreversible
    1. Competitive
    2. Noncompetitive
    3. Uncompetitive

THREE DIFFERENT REVERSIBLE INHIBITORS

- Competitive
- Uncompetitive
- Noncompetitive

Competitive Inhibition

Both Substrate and Inhibitor compete for the same active site on the enzyme
A competitive inhibitor competes with the substrate for the same active site on the enzyme molecule. Therefore, to relieve this kind of inhibition, you need to add a large excess of substrate.

In other words, a competitive inhibitor increases the $K_m$ value for the substrate. But it does not affect the velocity maximum (eventually high concentrations of substrate restores the full maximum activity of the enzyme by relieving the inhibition).

**Use of Competitive Inhibitors**

In the U.S. alone, several deaths occur due to antifreeze consumption. Ethylene glycol (antifreeze) is oxidized by the liver alcohol dehydrogenase to glycoaldehyde. Subsequently, it is converted to oxalic acid which precipitates and causes renal failure.

If copious amount of alcohol is consumed, due to competition between the two substrates, ethanol gets metabolized faster than ethylene glycol. Unutilized, ethylene glycol safely excreted.

The same protocol is also used for methanol poisoning. In this case, formaldehyde is the oxidation product. It causes blindness initially in patients and eventual death.

Alternate use inhibitors of alcohol dehydrogenase, so neither of these compounds are oxidized to cause damage.

**Simple way to derive equations for inhibition**

In Michaelis-Menten equation,

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

$K_m$ term arises because of free $E$ by ES complex.

S term is contributed by $I$.

Any inhibitor that interacts with free Enzyme contributes a $(1+I/K_I)$ term to this part of the equation.

Therefore, a competitive inhibitor contributes a $(1+ I/K_I)$ term to $K_m$.

$$v = \frac{V_{max} [S]}{K_m (1+ I/K_I) + [S]}$$
Similarly uncompetitive Inhibitor adds a \( \frac{1+ [I]}{K_I} \) term to [S] because it interacts only with ES complex.

\[
v = \frac{V_{max} [S]}{K_m + [S] \left( 1 + \frac{[I]}{K_I} \right)}
\]

Non competitive inhibitor interacts with both the free enzyme and the ES complex. Therefore, it contributes \( \frac{1+ [I]}{K_I} \) term to both \( K_m \) and \( [S] \).

\[
v = \frac{V_{max} [S]}{K_m \left( 1 + \frac{[I]}{K_I} \right) + [S] \left( 1 + \frac{[I]}{K_I} \right)}
\]

**Uncompetitive Inhibitor**

- An uncompetitive inhibitor binds to the ES complex.
- Since, it affects both binding of the substrate and the velocity of the reaction, \( K_m \) as well as \( V_{max} \) are altered in this case.
- But, the ratio of \( K_m/V_{max} \) is constant.
- This type of inhibition occurs only at multisubstrate reactions.
Noncompetitive inhibitor

- A noncompetitive inhibitor binds at a site different from the substrate binding site on the enzyme.
- Therefore, it can react with both E and ES.
- Km value for substrate is unaffected by this binding.
- But Vmax is altered.

Two substrate reactions

Two substrate reactions are of two types:

Ping Pong  Sequential

Ping Pong Mechanism

One substrate reacts with the enzyme producing the first product. The enzyme holds a group from this substrate temporarily. Therefore, chemically it is a different form that can be isolated for characterization purposes. This second form of the enzyme reacts with the next substrate transferring the group and generating the second product.

Sequential Bi Bi -Lactate dehydrogenase

Ping Pong Bi Bi -Transaminases

Random Bi Bi mechanism

Example - Creatine kinase

Random Bi Bi Reaction Creatine Kinase

(Cr = Creatine; Cr - P = Phosphocreatine)