

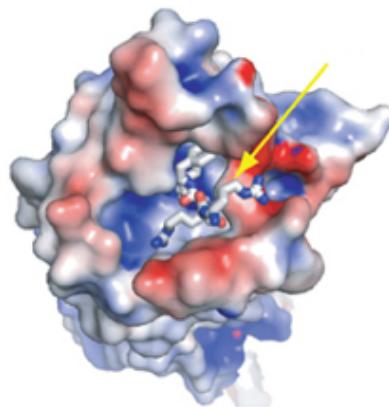
ENZYMOLOGY

Enzymes are catalyst, are protein molecule (with a specific 3d shape), very specific; their role is to increase the rate of reactions.

Enzyme : a protein molecule that accelerates a specific chemical reaction

Active Site: the region of an enzyme that binds the substrate during the reaction

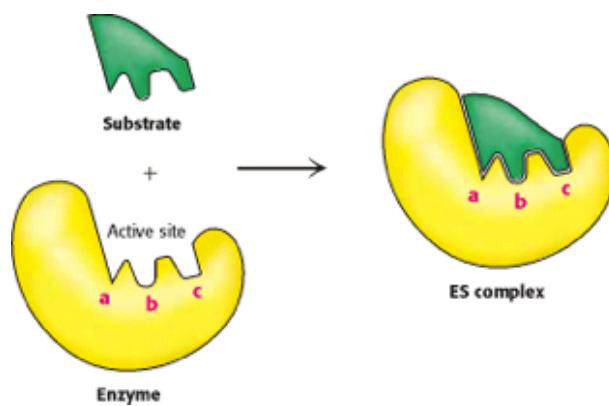
Substrate: the reactant(s) in an enzyme-catalysed reaction



- ACTIVE SITE:
 - Small relative to the total volume of the enzyme.
 - Usually occur in clefts and crevices in the protein. Excluding solvents which would otherwise reduce the catalytic activity of the enzyme.
 - Amino acids and cofactors are held in precise arrangement with respect to structure of the substrate.
 - Amino acids in active site define specificity
 - It is the active site that dictates the orientation of the molecule
- **Higher reaction rates** in the range of 10^6 - 10^{12} greater than uncatalysed ones
- **Milder reaction conditions:** neutral pH, atmospheric pressure and close to ambient T
- **Greater specificity:** greater specificity in terms of substrate and products. No byproducts
- **Capacity for control:** responds to concentration of substances (allosteric control, covalent modification etc)

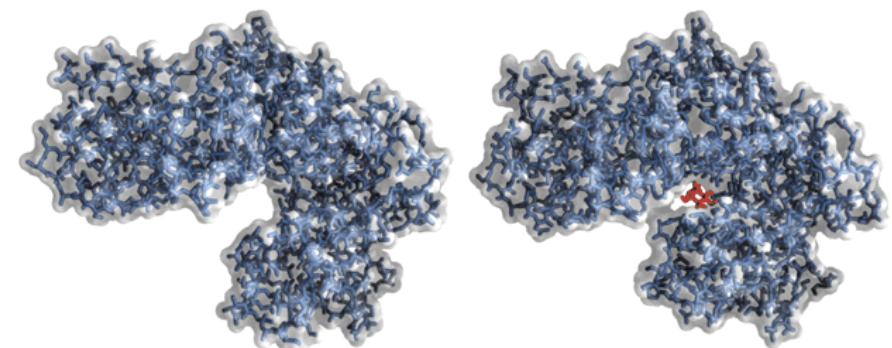
HOW DO ENZYMES BIND THE SUBSTRATES ?

- **Lock and key model**
 - Ligand binding site are rigid and complementary to the shape of the ligand
 - The structure of the active site is complementary to its substrate like a lock to its key
 - The shape of the active site is determined by the tertiary and quaternary structure of the protein.



- **Induce fit**

- Flexible interaction between the ligand and active site induces a conformational change
- Certain enzymes can catalyse similar reactions
- The induced fit model suggests that the active site will interact with the substrate and adapt to it to make a perfect fit (change its shape)

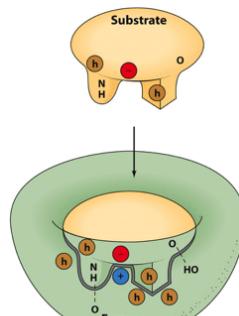


Hexokinase without and with glucose-6-phosphate (product)

- **Transition state stabilisation** (to be discussed later)

SUBSTRATE SPECIFICITY

- The active site is complementary to the structure of the substrate (geometric)
- The amino acids in the active site interact specifically with the substrate (electronic)
- Molecules that differ in shape cannot bind and form an enzyme-substrate complex; important for catalysis
- Lock and key and induce fit



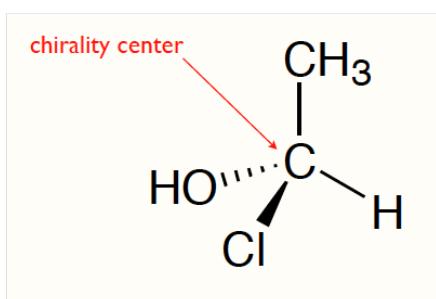
STEREOSPECIFICITY

- Enzymes are highly specific in binding **chiral** substrates and in catalysing their reactions
- Stereospecificity** is due to the enzymes active site
- Absolutely stereospecific

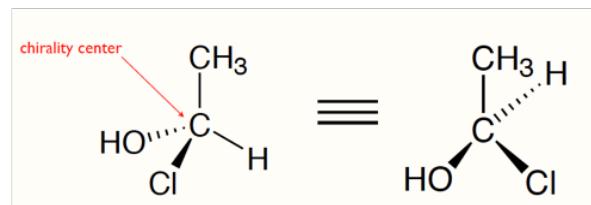
CHIRALITY

- A chiral molecule is one that is not superimposable on its mirror image
- Cahn-Ingold-Prelog (CIP) priority rules
- R/S notation

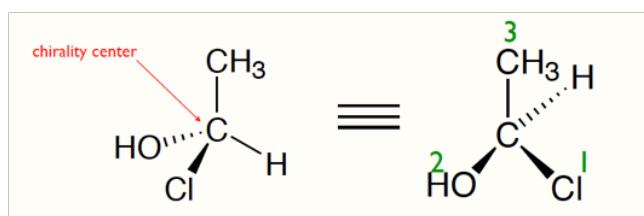
Locate chirality centre



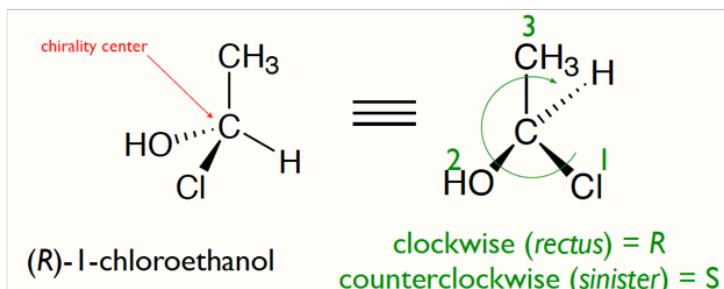
Orientate molecule that lowest priority group faces away



Number three groups in order of increasing priority



Determine rotation of groups in decreasing priority



YEAST ALCOHOL DEHYDROGENASE (YADH)

- Converts ethanol to acetaldehyde.
- Requires NAD⁺
- Ethanol is prochiral
- The enzyme's active site determines how the ethanol is bound
- The YADH transfers the *pro-R* hydrogen of ethanol to NAD⁺

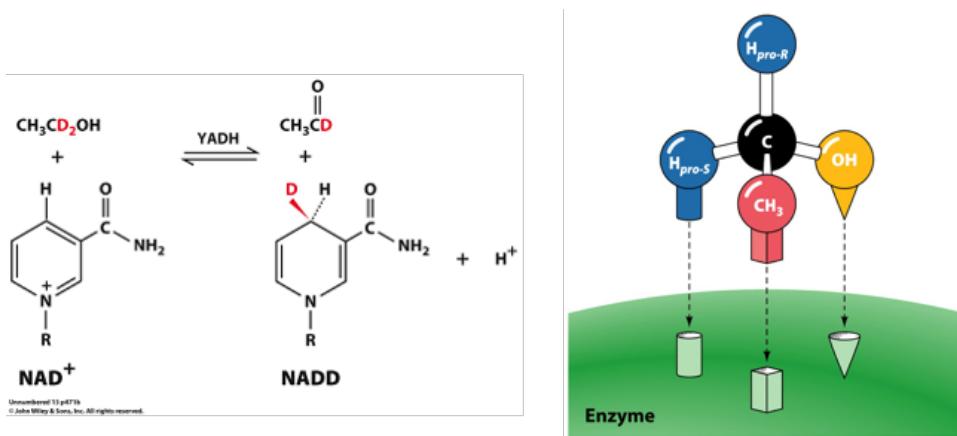
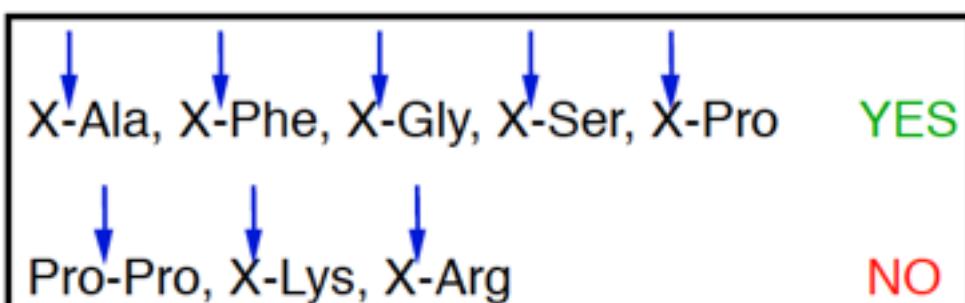


Figure 13-3
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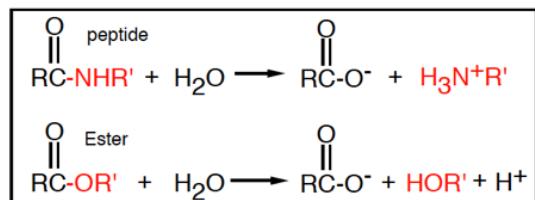
GEOMETRIC SPECIFICITY

- Selective about chemical groups of the substrate
- More stringent requirement
- Varying degrees of geometric specificity.
- A few enzymes are absolutely specific for one substrate.
- Some work on a group of related molecules
 - e.g. yeast ADH oxidizes primary and secondary alcohols, but ethanol is the most efficiently converted.
- Many enzymes are very permissive (e.g. digestive enzymes such as carboxypeptidase A, catalyses the hydrolysis of C-terminal peptide bond to all residues except Lys and Arg, and Pro (if the preceding residue is not Pro.)).



Some enzymes are not specific in the types of reaction catalysed (e.g. chymotrypsin can catalyse peptide bond hydrolysis and ester bond hydrolysis).

These examples of permissiveness are the exceptions rather than the rule



ENZYME NOMENCLATURE

- The first Enzyme Commission (EC) in 1961 devised a system for classification and coding of enzymes. It has 4 elements:
 - (1) First number = one of the 6 main divisions (classes)
 - (2) Second number = subclass
 - (3) Third number = sub-subclass
 - (4) Fourth number = serial number
- e.g. Tryptophan synthase (EC 4.2.1.20)

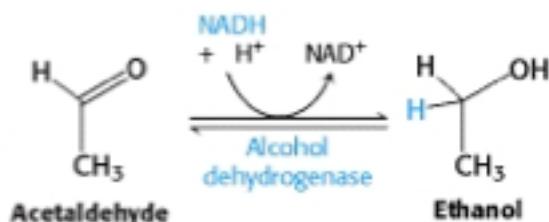
6 classes:

1. Oxidoreductases:

Oxidation/reduction reactions

Transfer of hydrogen and oxygen atoms or electrons from one substrate to another

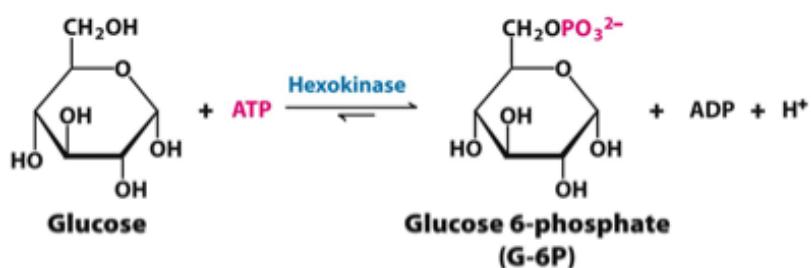
eg. Alcohol dehydrogenase (oxidases alcohols) EC1.1.1.1



2. Transferases

transfer of functional groups, e.g. a methyl group, from one compound to another

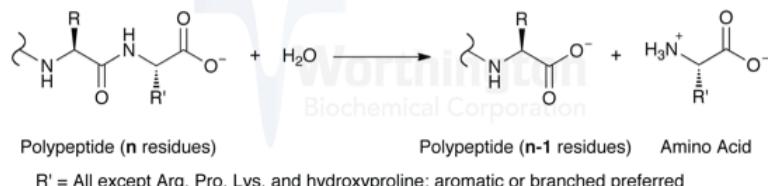
eg. hexokinase EC 2.7.1.2



3. Hydrolases

catalyse the hydrolytic cleavage of C-O, C-N, C-C and some other bonds, including phosphoric anhydride bonds

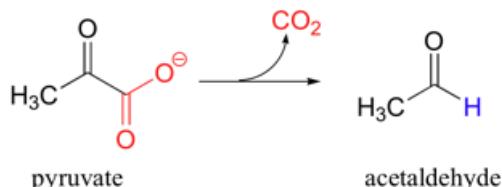
eg. carboxypeptidase A EC 3.4.17.1



4. Lyases:

Lyases are enzymes cleaving C-C, C-O, C-N, and other bonds by elimination, leaving double bonds or rings, or conversely adding groups to double bonds

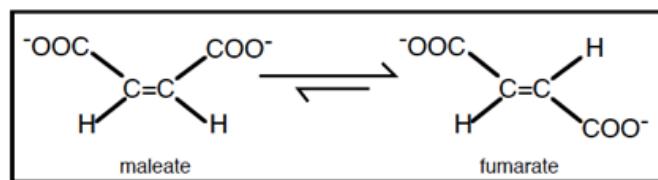
eg. pyruvate decarboxylase EC 4.1.1.1



5. Isomerases

catalyse geometric or structural changes within one molecule (isomerisation)

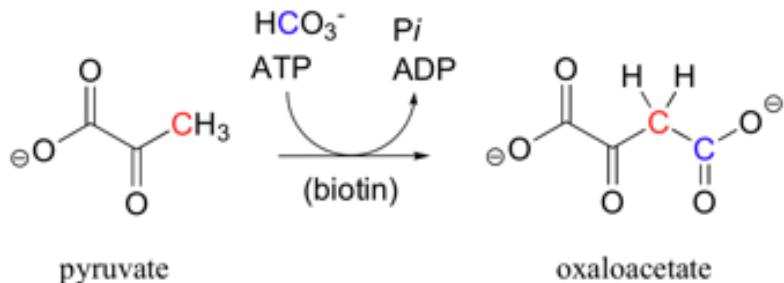
eg. maleate isomerase EC 5.2.1.1



6. Ligases:

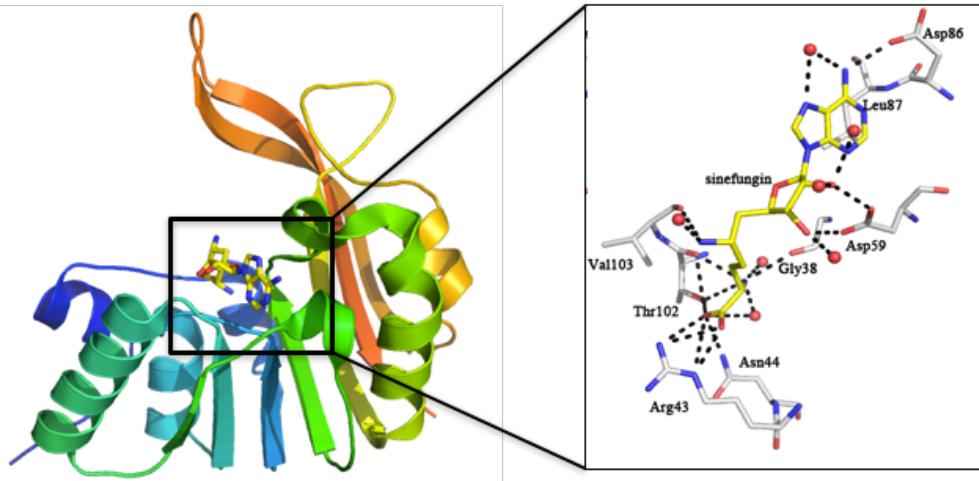
catalyse the joining together of two molecules coupled with the hydrolysis of a diphosphate bond

eg. pyruvate carboxylase EC 6.4.1.1

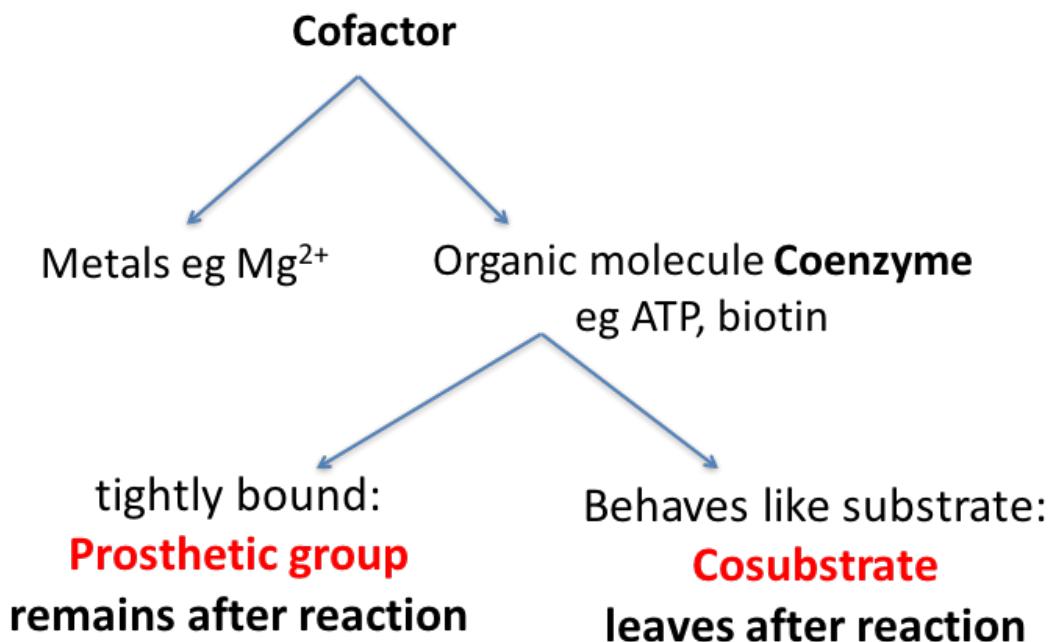


COFACTORS:

- Some enzymes require small molecules during catalysis: **cofactors**
- Cofactors can be: metal ions or organic molecules (**coenzymes**)
- Some coenzymes are transiently associated with the enzyme known as **cosubstrates**
- Cofactors associated with the enzyme are known as **prosthetic groups**



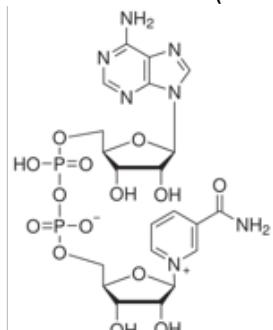
- A catalytically active enzyme-cofactor complex is called **holoenzyme**.
- The inactive protein (absence of the cofactor) is called an **apoenzyme**



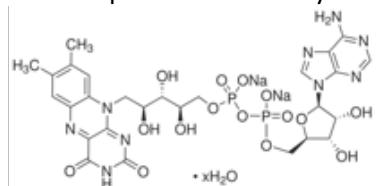
VITAMINS:

- Many organisms are unable to synthesise parts of essential cofactors
- These substrates are present in organisms diet

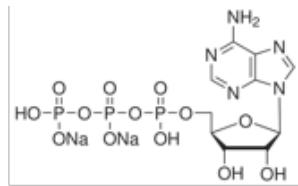
- Vitamins in human diet that are precursors are water-soluble
- Lipid soluble vitamins (A and D) are not components of coenzymes



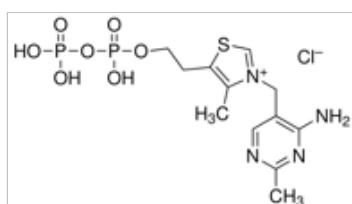
NAD



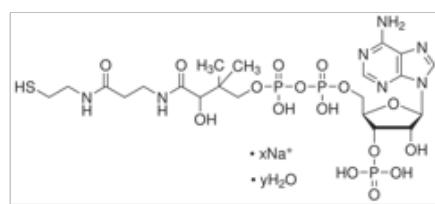
FAD



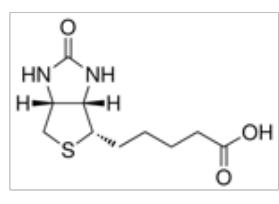
ATP



Thiamine pyrophosphate



Coenzyme A



Biotin

ENZYMES AS MARKERS:

Acid phosphatase: a tumour marker in prostatic carcinoma

Alanine aminotransferase: an indicator of hepatocellular damage

Alkaline phosphatase: increase in cholestatic liver disease and is a marker of osteoblast activity in bone disease.

Amylase: an indicator of cell damage in acute pancreatitis.

Aspartate aminotransferase: an indicator of hepatocellular damage, or as a marker of muscle damage, such as a myocardial infarction (MI).

Creatine kinase: a marker of muscle damage and acute MI.

γ -glutamyl transpeptidase: a sensitive marker of liver cell damage.

Lactate dehydrogenase: a marker of muscle damage.

RATE OF REACTIONS

Many biological reactions involve **energy transformations**:

- Conversion of chemical energy in nutrients to another form of chemical bond energy in ATP
 - conversion of bond energy in ATP to do cellular work
 - osmotic work (pumping ions and small molecules)
 - mechanical work (muscle contraction)
 - metabolic work (driving energy-requiring biosynthetic reactions) etc.
-
- Tells whether or not a process or a reaction can occur
 - Applicable to systems in stable or metastable equilibrium
 - Sufficient driving force to enforce a favorable reaction

CHEMICAL KINETICS

- It is the study of the chemical reaction rates
- Calculate binding affinities
- Influence of different conditions to the rates
- Elucidate enzymes mechanism

A simple reaction like $A \rightarrow B$ may proceed through several elementary reactions like



The characterization of elementary reactions comprising an overall reaction process constitutes its mechanistic description.

Rate Equations

Consider $aA + bB + \dots + zZ$. The rate of a reaction is proportional to the frequency with which the reacting molecules simultaneously bump into each other

$$\boxed{\text{Rate} = k[A]^a[B]^b \cdots [Z]^z}$$

The order of a reaction = the sum of exponents

Generally, the order means how many molecules have to bump into each other at one time for a reaction to occur.

A first order reaction one molecule changes to another



A second order reaction two molecules react



3rd order rates $A + B + C \rightarrow P + Q + R$ rarely occur

and higher orders are unknown.

- Let us look at a first order rate $A \rightarrow B$
- Few reactions in biochemistry are as simple as the first-order reaction described above. In most cases, reactions are reversible and equilibrium does not lie far to one side.



$$v = -\frac{d[A]}{dt} = \frac{d[P]}{dt}$$

v = velocity of the reaction

in Molar per min.

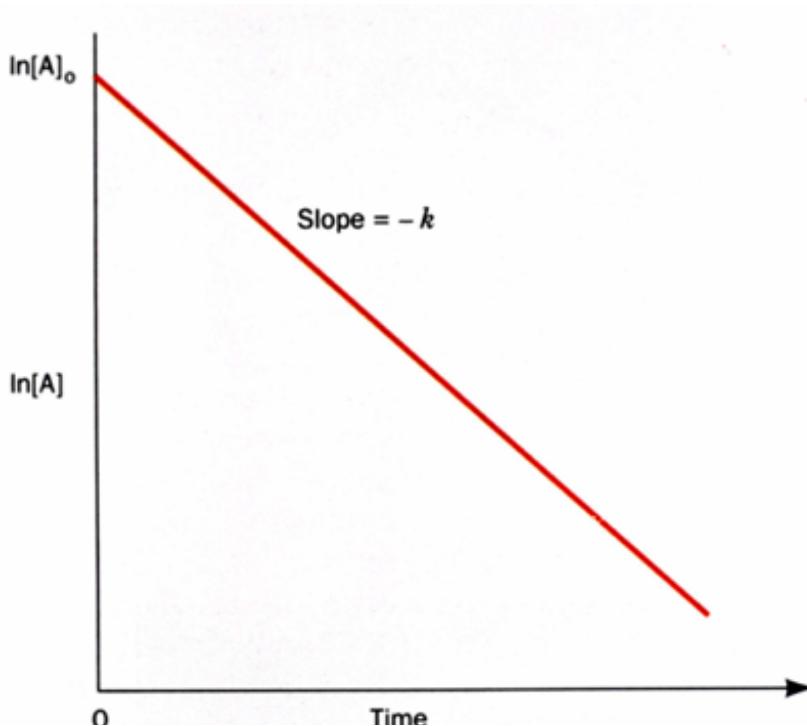
or

moles per min per volume

$$v = -\frac{d[A]}{dt} = k[A]$$

k = the rate constant of the reaction

$$v = -\frac{d[A]}{dt} = k_1[A] - k_{-1}[P]$$



The rate constant for the first order reaction has units of s^{-1} or min^{-1} since velocity = molar/sec and $v = k[A]$: $k = v/[A]$

Gather your data and plot $\ln[A]$ vs time.

However, the natural log of the concentration is directly proportional to the time.
for a first order reaction-

Instantaneous rate: the rate of reaction at any specified time point that is the definition of the derivative.

We can predict the shape of the curve if we know the order of the reaction.

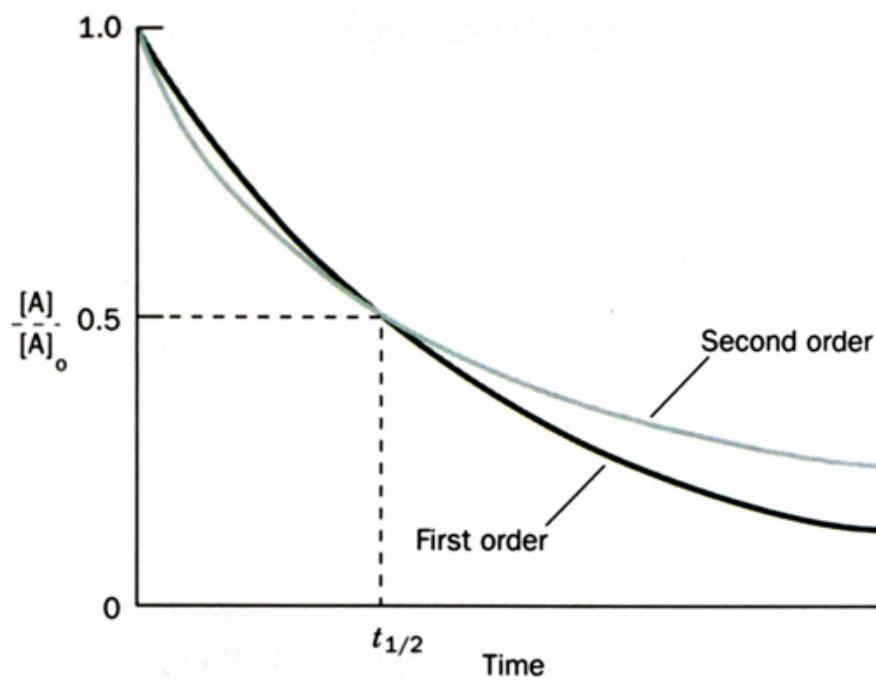
A second order reaction: $2A \rightarrow P$

$$v = -\frac{d[A]}{dt} = k[A]^2$$

Or for $A + B \rightarrow P + Q$

$$v = -\frac{d[A]}{dt} = -\frac{d[B]}{dt} = k[A][B]$$

Percent change in A (ratio) versus time in first and second order reactions



- It is difficult to determine if the reaction is either first or second order by directly plotting changes in concentration.

$$-\frac{d[A]}{dt} = k[A] \longrightarrow \frac{d[A]}{[A]} = -kdt$$

$$\int_{A_0}^A \frac{dA}{A} = -k \int_0^t dt$$

$$\int \frac{1}{x} = \ln(x)$$



$$\ln[A] = \ln[A]_0 - kt \longrightarrow [A] = [A]_0 e^{-kt}$$

- The half-life of a first order reaction

$$[A] = \frac{[A]_0}{2} \quad \text{Plugging in to rate equation}$$

$$\ln\left(\frac{\frac{[A]_0}{2}}{[A]_0}\right) = -kt_{\frac{1}{2}}$$

$$\star t_{\frac{1}{2}} = \frac{\ln 2}{k} = \frac{0.693}{k} \star$$

The half-life of a first order reaction can be used to determine the amount of material left after a length of time.

The time for half of the reactant which is initially present to decompose or change.

^{32}P , a common radioactive isotope, emits an energetic β particle and has a half-life of 14 days.
 ^{14}C has a half life of 5715 years.

- A second order reaction such like $2\text{A} \rightarrow \text{P}$

$$\int_{[A]_0}^{[A]} -\frac{d[A]_0}{[A]^2} = k \int_0^t dt$$

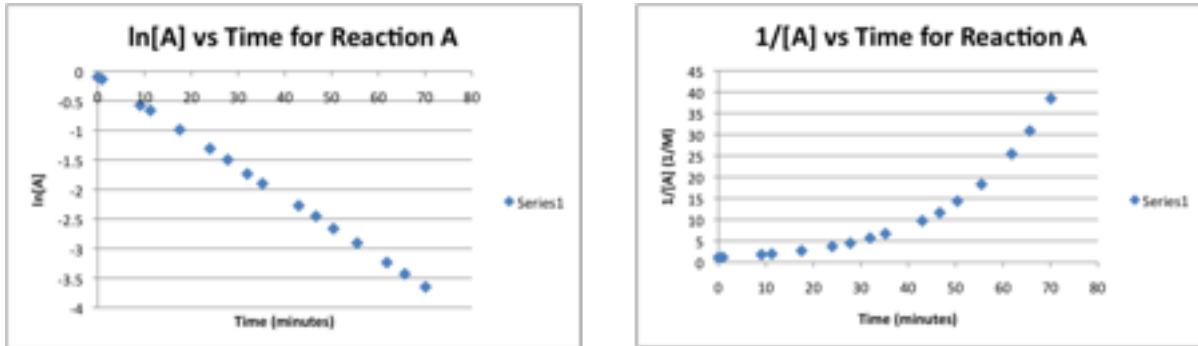
$$\frac{1}{[A]} = \frac{1}{[A]_0} + kt$$

When the reciprocal of the concentration is plotted versus time a second order reaction is characteristic of a straight line.

The half-life of a second order reaction is
and shows a dependence on the initial concentration

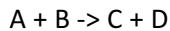
$$t_{\frac{1}{2}} = \frac{1}{k[A]}$$

plot $[A]$ versus time, $\ln[A]$ versus time, and $1/[A]$ versus time to see which plot yields a straight line. The reaction order will then be the order associated with the plot that gives a straight line.

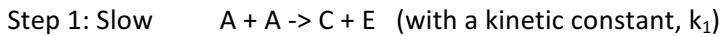


RATE DETERMINING STEP

The rate determining step is important in deriving the rate equation of a chemical reaction. For example, consider a multi-step reaction,



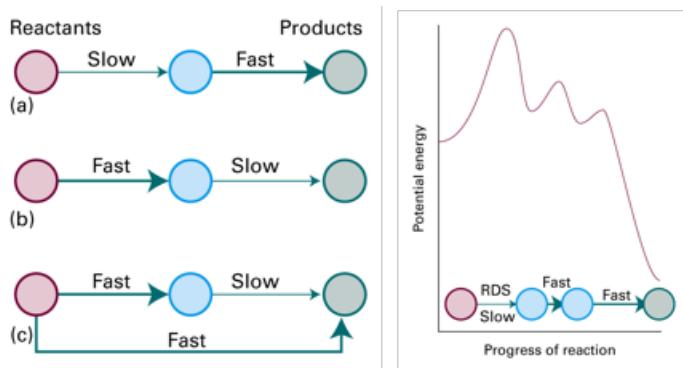
And assume the elementary steps for this reaction are:



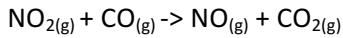
In the case of this hypothetical reaction, if step 1 is the slow step and step 2 is the fast step of the reaction, then the overall reaction rate will depend on step 1; this is because the slow step in a reaction is always the rate-limiting step, which is also called the rate determining step.

The rate equation will be:

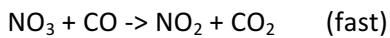
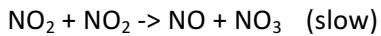
$$\text{rate} = k_1 [A][A] = k_1 [A]^2$$



Consider the reaction:



which occurs in two elementary steps:



Which is the rate determining step and what is the rate constant?

DETERMINING RATE LAWS:

Can be determined experimentally with these techniques:

- Absorption measurement (spectrophotometer)
- Conductivity (reactions between ions in solution)
- Polarimetry (if reactants/products are optically active, e.g. glucose)
- Aliquot method (titration)

COLISION THEORY

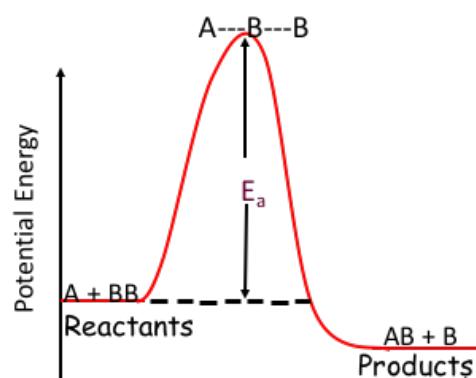
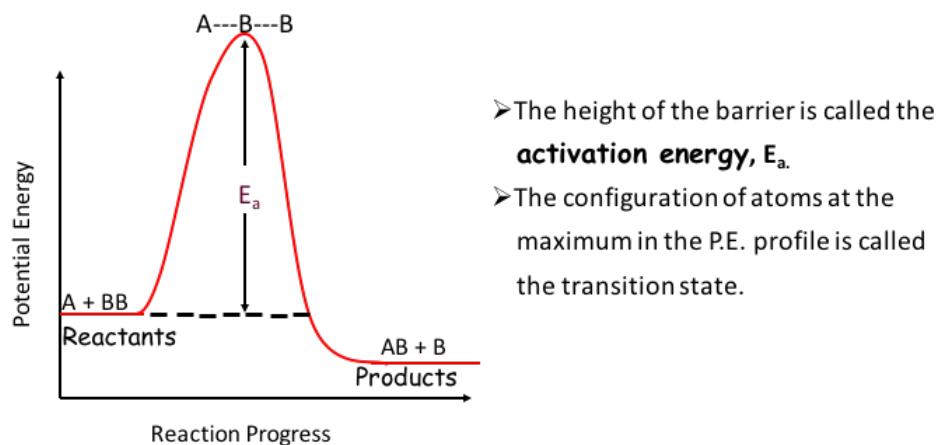
According to the Collision Theory Model: a bimolecular reaction occurs when two **properly oriented** reactant molecules come together in a **sufficiently energetic collision**

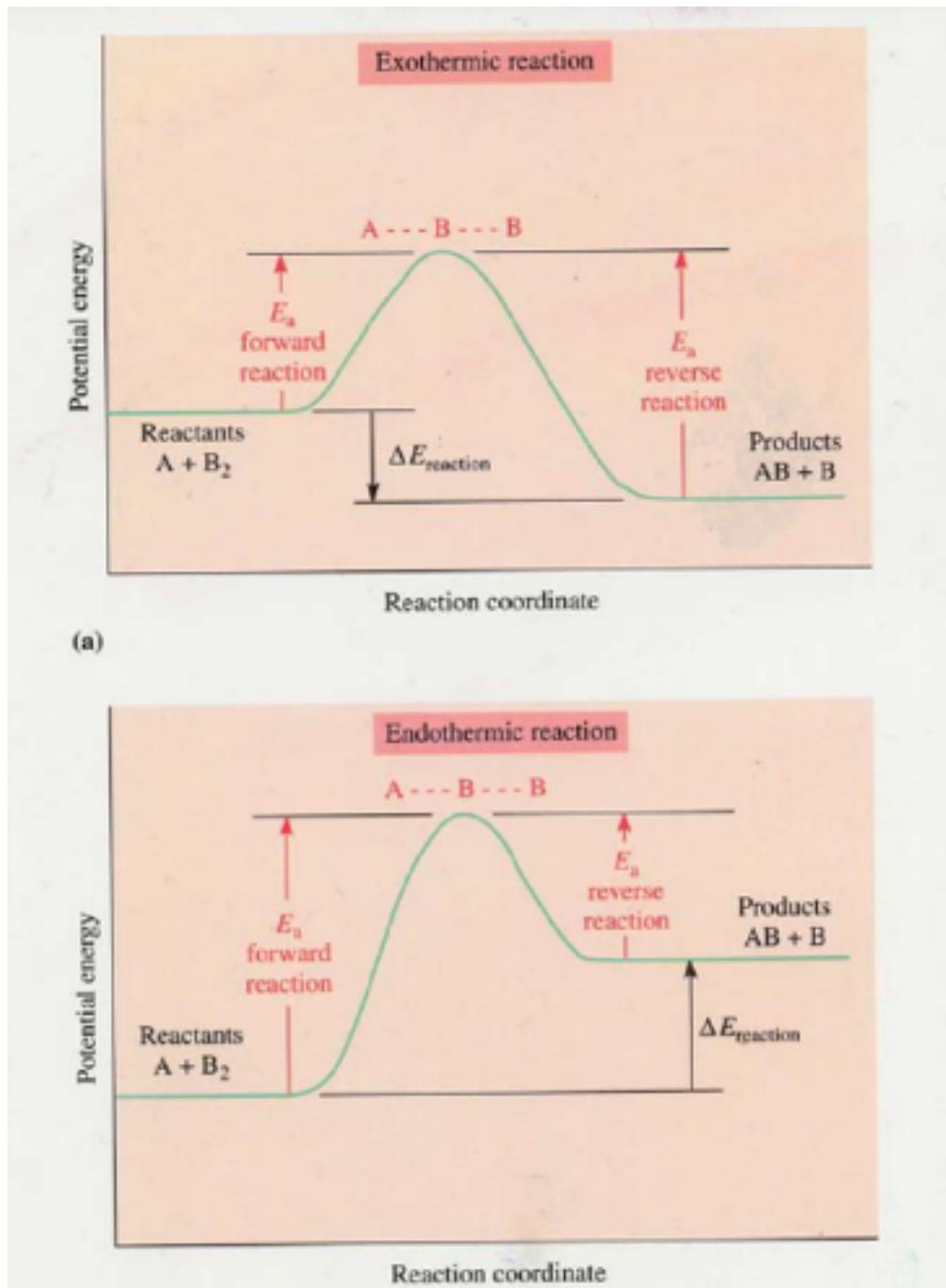
i.e. for a reaction to occur, molecules, atoms or ions must first collide.

POTENTIAL ENERGY PROFILE

If the collision energy $< E_a$, the reactant molecules cannot surmount the barrier and they simply bounce apart.

If the collision energy is $\geq E_a$, the reactants will be able to surmount the barrier and be converted to products.





Very few collisions are productive because very few occur with a collision energy as large as the activation energy. Proper orientation is necessary for product formation

There must be some effect by Temperature on reaction systems.

Temperature can result in an increase in energy.

The average kinetic energy of a collection of molecules is proportional to the absolute temperature.

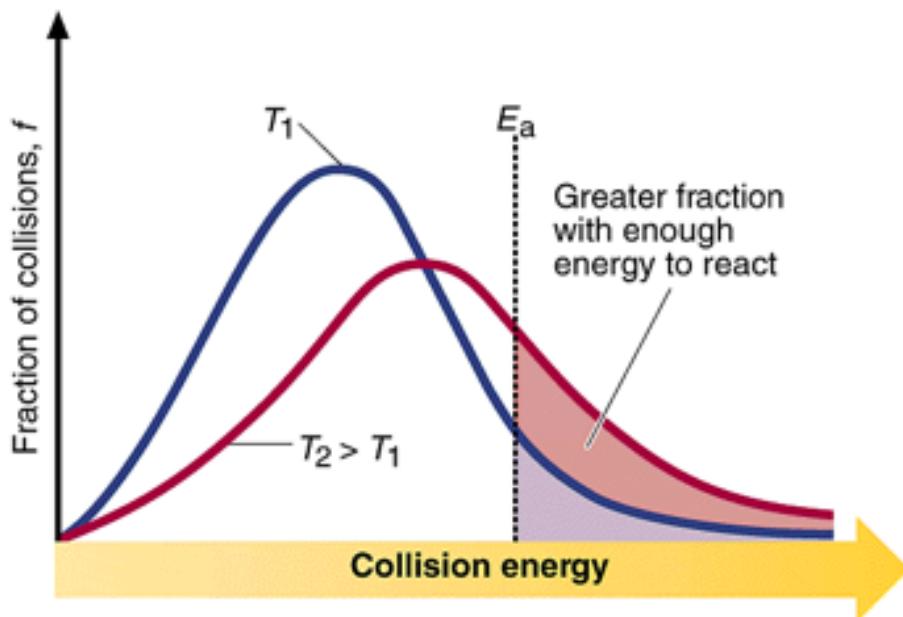
At a temperature T_1 , a certain fraction of the reactant molecules have sufficient K.E., i.e. $K.E. > E_a$.

At a higher temperature T_2 , a greater fraction of the molecules possess the necessary activation energy, and the reaction proceeds at a faster rate.

**In fact it has been found that reaction rates tend to double when the temperature is increased by 10°C .

MAXWELL-BOLTZMANN DISTRIBUTION CURVE

- The total area under the curve is proportional to the total # molecules present.
- Total area is the same at T_1 and T_2 .
- 3. The shaded areas represent the number of particles that exceed the energy of activation, E_a .



It was observed by Svante Arrhenius that almost all of the reaction rates (obtained from experiments) accumulated over a period showed similar dependence on temperature.

This observation led to the development of the **Arrhenius Equation**:

$$k = A e^{-E_a/RT}$$

Collectively, **A** and **E_a** are called the **Arrhenius parameters** of the reaction.

E_a = activation energy (kJ mol^{-1}), and is the minimum kinetic energy required to allow reaction to occur

The exponential term $e^{-E_a/RT}$ is simply the fraction of collisions that have sufficient energy to react.

This fraction goes up when T is increased because of the negative sign in the exponential term.

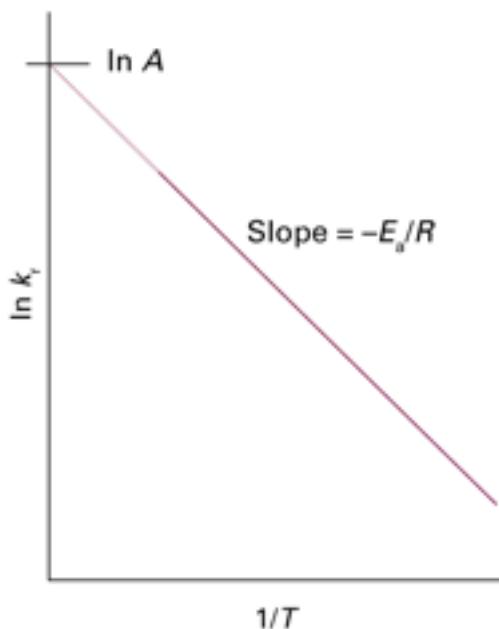
However, most of the collisions calculated by $e^{-E_a/RT}$ do not lead to products, and so

- A** = the frequency factor or pre-exponential factor (same units as k), is the fraction of sufficiently energetic collisions that actually lead to reaction.
- T** = Kelvin temperature
- R** = ideal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$)
- k** is the rate constant

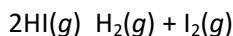
Logarithmic form of the Arrhenius equation:

$$\ln k = \ln A - \frac{E_a}{RT}$$

A plot of $\ln k$ versus $1/T$ gives **slope= $-E_a/R$** and **intercept= $\ln A$**



Consider the reaction:

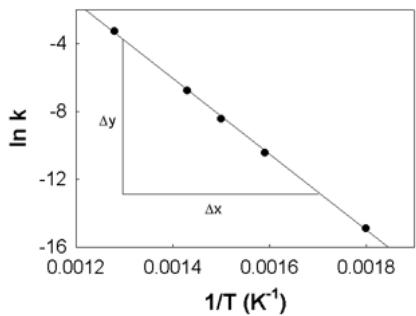


The following data was measured:

$k(\text{M}^{-1}\text{s}^{-1})$	$T(\text{°C})$
3.52×10^{-7}	283
3.02×10^{-5}	356
2.19×10^{-4}	393
1.16×10^{-3}	427
3.95×10^{-2}	508

To answer the question, we need to find $\ln k$, T in K, $1/T$, and then plot $\ln k$ vs. $1/T$. The slope of the line will lead us to the activation energy.

k	$\ln k$	$T (\text{°C})$	$T (\text{K})$	$1/T$
3.52×10^{-7}	-14.860	283	556	1.80×10^{-3}
3.02×10^{-5}	-10.408	356	629	1.59×10^{-3}
2.19×10^{-4}	-8.426	393	666	1.50×10^{-3}
1.16×10^{-3}	-6.759	427	700	1.43×10^{-3}
3.95×10^{-2}	-3.231	508	781	1.28×10^{-3}

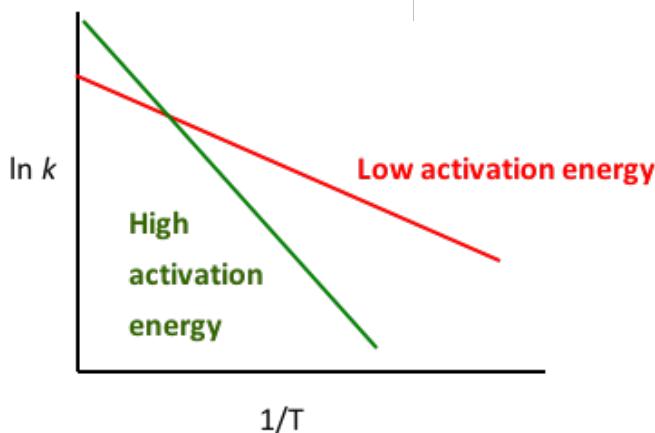


$$\text{slope} = \frac{\Delta y}{\Delta x} = \frac{[(-3.8) - (-12.8)]}{[0.00130 - 0.00170]} = -23000$$

$$\text{slope} = -\frac{E_a}{R}$$

so

$$E_a = -\text{slope} \times R = -(-23000) \times (8.314) = 190000 \text{ J/mol} = 190 \text{ kJ/mol}$$



High activation energy corresponds to a reaction rate that is very sensitive to temperature (the Arrhenius plot has a steep slope). Converse also applies.

- Manipulation of Arrhenius equation:

Once the activation energy of a reaction is known, it is a simple matter to predict the value of a rate constant k' at a temperature,

T' from another value of k at another temperature, T .

$$\begin{aligned} \ln k' &= \ln A - E_a/R T' \\ \ln k &= \ln A - E_a/R T \end{aligned} \quad \begin{matrix} \nearrow \\ \text{Subtract these equations} \end{matrix}$$

$$\ln k' - \ln k = \ln A - \ln A - E_a/R T' - (-E_a/R T)$$

$$\ln\left(\frac{k'}{k}\right) = \frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T'} \right)$$

Can also find E_a if k' , k , T' and T are known.

How to experimentally determine an activation energy, E_a :

For the reaction $\text{C}_2\text{H}_5\text{I} \rightarrow \text{C}_2\text{H}_4 + \text{HI}$

At 600K, $k = 1.6 \times 10^{-5} \text{ s}^{-1}$. At 700K, $k = 6.36 \times 10^{-3} \text{ s}^{-1}$ What is E_a ?

$E_a = 2.09 \times 10^{-5} \text{ J/mol}$ or 209 kJ/mol .

How to find how fast does a reaction rate changes when we increase the temperature:

The rate constant for a first order reaction is $9.16 \times 10^{-3} \text{ s}^{-1}$ at 0C. E_a is 88 kJ/mol . If the temperature is raised 2C, what is the new rate constant?



First we tabulate the values, remembering to convert temperature to Kelvin scale.

$E_a = 88.000 \text{ J/mol}$ $R = 8.314 \text{ J/mol K}$

$k_1 = 9.16 \times 10^{-3} \text{ s}^{-1}$ at $T_1 = 273 \text{ K}$

$k_2 = ?$ at $T_2 = 275 \text{ K}$

We use these values in the Arrhenius equation. Taking inverse

(natural) logarithms of both sides.

$$k_2 = 1.32 (9.16 \times 10^{-3} \text{ s}^{-1}) = 1.21 \times 10^{-2} \text{ s}^{-1}$$

We see that a very small temperature difference, only 2C causes an increase to the rate constant (and hence in the reaction rate for the same concentrations) of about 32%.

Enzyme Kinetics Michaelis-Menten equation



Velocity of the reaction: $v_o = \frac{d[P]}{dt} = k_2[ES]$

Looking at [ES]:

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

- We assume that $k_{-1} \gg k_2$
- Therefore: $\frac{d[ES]}{dt} = 0$

First step of reaction achieves equilibrium

$$K_S = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

K_S is the dissociation constant

This assumption is not often correct

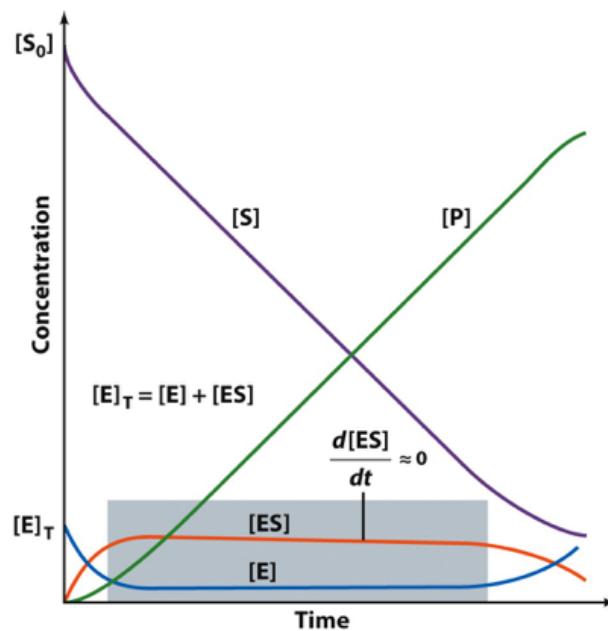


Figure 14-7
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$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

If $\frac{d[ES]}{dt} = 0$ then:

$$0 = k_1[E][S] - k_{-1}[ES] - k_2[ES] \text{ then:}$$

$$k_1[E][S] = k_{-1}[ES] + k_2[ES]$$

$$[ES] = \frac{[E][S]}{K_M}$$

[E] can be substituted by:

$$[E_T] = [E] + [ES]$$

$$[E] = [E_T] - [ES]$$

So:

$$[ES] = \frac{([E_T] - [ES])[S]}{K_M}$$

$$[ES] = \frac{([E_T] - [ES])[S]}{K_M}$$

Rearranging the equation we get:

$$[ES] = \frac{[E_T][S]}{K_M + [S]}$$

Remember: $v_o = k_2[ES]$

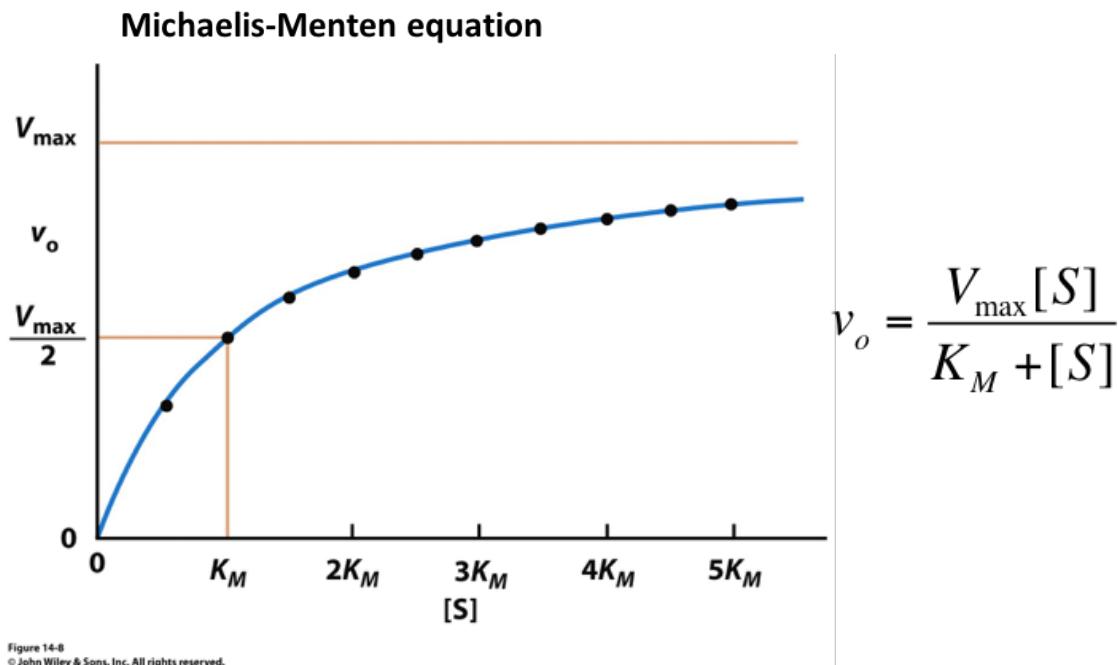
So: $v_o = \frac{k_2[E_T][S]}{K_M + [S]}$

We need to calculate max velocity. How? Adding more substrate most of the enzyme is saturated and it is in [ES] form

$$\text{So: } V_{\max} = k_2 [E_T]$$

Substituting in:

$$v_o = \frac{k_2 [E_T][S]}{K_M + [S]} = \boxed{\frac{V_{\max}[S]}{K_M + [S]}}$$



K_m is also the affinity of the substrate (how much substrate you need, to achieve V_{\max})

- The Michaelis constant, has a simple operational definition: When $[S] = K_m$: the Michaelis-Menten equation yields $v_o = V_{\max}/2$
Therefore, the **K_m is the substrate concentration at which the reaction rate is half the maximal rate**

- When $[S]$ is low, i.e. $[S] \ll K_m$:
the Michaelis equation approximates to $v_o = (V_{\max}[S]) / K_m$
The initial rate is proportional to $[S]$

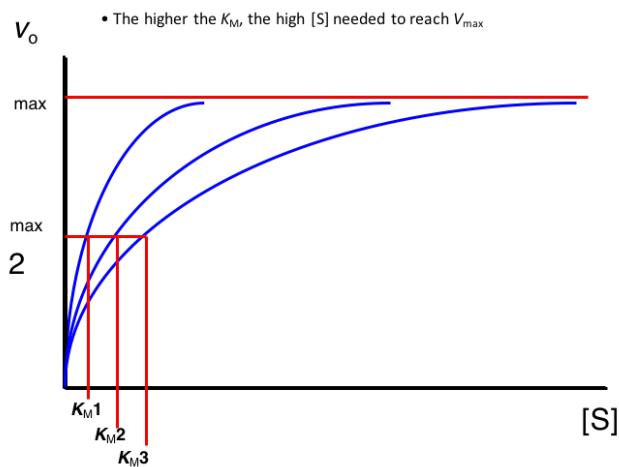
- When $[S]$ is high, i.e. $[S] \gg K_m$:
the Michaelis eqn approximates to $v_o = V_{\max}$
The initial rate is independent of $[S]$

The K_M of an enzyme is the substrate concentration at which the reaction occurs at half of the maximum rate

- If an enzyme has a low K_M , it achieves maximal catalytic efficiency at low [S].
- K_M is different between enzymes and for different substrates.
- K_M alters with temperature and pH.
- K_M can also be expressed as:

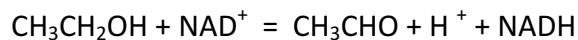
$$K_M = \frac{k_{-1} + k_2}{k_1} = \frac{k_{-1}}{k_1} + \frac{k_2}{k_1} = K_S + \frac{k_2}{k_1}$$

- Since K_S is the dissociation equilibrium constant for the Michaelis-Menten complex, as K_S decreases, the affinity of the enzyme for the substrate increases, therefore K_M is a measure of substrate affinity, provided that k_2/k_1 is small compared to K_S , i.e., $k_2 < k_{-1}$



THE CONSEQUENCE OF K_M

Individuals sensitive to alcohol...facial flushing & tachycardia even after ingesting a small amount



- Acetaldehyde is normally processed by acetaldehyde dehydrogenase



- Most people have two forms of acetaldehyde dehydrogenase a low K_M **mitochondrial** enzyme and a high K_M **cytosolic** form

- In susceptible individuals, the mitochondrial enzyme is less active due to a single amino acid mutation and processing is via the cytosolic one

- Less is converted (high K_M) thus more in the blood causing ‘effects’

Some K_M values

Enzymes	[S]	K_M (μM)
Chymotrypsin	Acetyl-L-trp	5000
Pyruvate Carboxylase	Pyruvate	400
Penicillinase	Benzylpenecillin	50
Lysozyme	Hexa-N-acetylgluc.	6
Arg-tRNA synthetase	tRNA	0.4

CATALYTIC EFFICIENCY OF ENZYMES



$$V_{\max} = k_2 [E_T] = k_{cat} [E_T]$$

Kinetic parameters provide a measure of its catalytic efficiency

k_2 is the turnover number k_{cat} :

Number of reaction processes that each active site catalyses per unit time

Some k_{cat} (turnover) values

Enzymes	[S]	k_{cat} (s^{-1})
Carbonic anhydrase	CO_2	1 000 000
Acetylcholinesterase	acetylcholine	14 000
Penicillinase	Benzylpenecillin	2000
Chymotrypsin	Hexa-N-acetylgluc.	100
DNA pol I	nucleotides	15
Lysozyme	Hexa-N-acetylgluc	0.5

- When $[S] \ll K_M$ very little ES is formed
- Therefore: $[E]=[E_T]$

$$v_o \approx \frac{k_2[E_T][S]}{K_M} \approx \frac{k_{cat}}{K_M}[E][S]$$

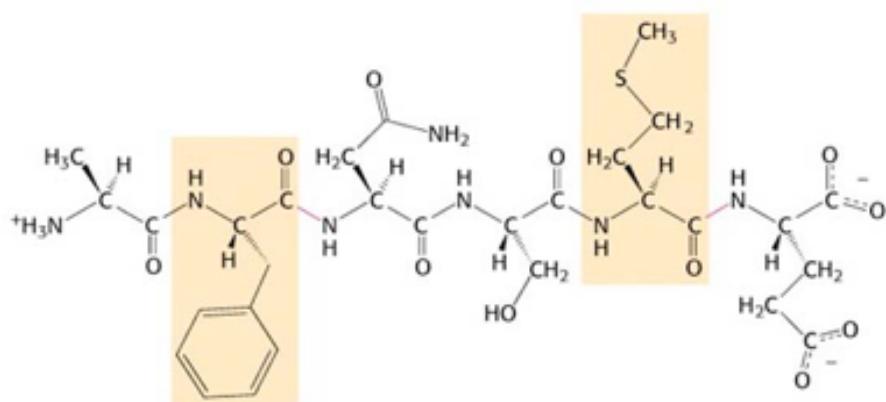
$\frac{k_{cat}}{K_M}$ is a measure of an enzyme's catalytic efficiency

- K_M is a measure of substrate binding affinity and k_{cat} is a measure of turnover efficiency, k_{cat}/K_M is a measure of **catalytic efficiency**. The higher, the better the enzyme (if comparing similar enzyme reactions).
- In industrial situations, it is desirable to have the fastest reaction rates under the allowable conditions (which may not always be optimal). Comparing the catalytic efficiencies of enzymes allows one to select appropriate enzymes, i.e. an enzyme which has a catalytic efficiency 10-times that of another, can be used 10-fold less to get the same v_o .
- Comparing the catalytic efficiencies of similar enzymes from different species (having varying K_M and k_{cat} , show that the catalytic efficiencies are approximately the same,

e.g. The β -lactamases from various bacteria

USES OF K_{cat}/K_m

1. enzyme's substrate preference
 2. enzyme's catalytic efficiency
1. enzyme's preference for different substrates (substrate specificity)
 - The higher the k_{cat}/K_m , the better the enzyme works on that substrate.
 - e.g., chymotrypsin: protease that clearly "prefers" to cleave after bulky hydrophobic and aromatic side chains.



- chymotrypsin specificity: active site best accommodates substrates with a bulky hydrophobic or aromatic residue contributing carbonyl group to peptide bond to be hydrolysed.
- Chymotrypsin also catalyses hydrolysis of ESTER bonds whose carboxylic acid component has a bulky, hydrophobic and/or aromatic R-group.

CATALYTIC PERFECTION

$$\frac{k_{cat}}{K_M} = \frac{k_2}{K_M} = \frac{k_1 k_2}{k_{-1} + k_2}$$

- The ratio is maximal when $k_2 \gg k_{-1}$, that is when the formation of the [P] from the [ES] complex is fast compared to the breakdown back to the substrate and enzyme.
- Then, $k_{cat}/K_M = k_1$ the term, k_1 can not be faster than rate of collision of reactants. This is the diffusion-controlled limit and is about $10^8\text{-}10^9 \text{ M}^{-1}\text{s}^{-1}$, so enzymes with a $k_{cat}/K_M = 10^8\text{-}10^9 \text{ M}^{-1}\text{s}^{-1}$ must catalyse a reaction almost every time they encounter substrate.

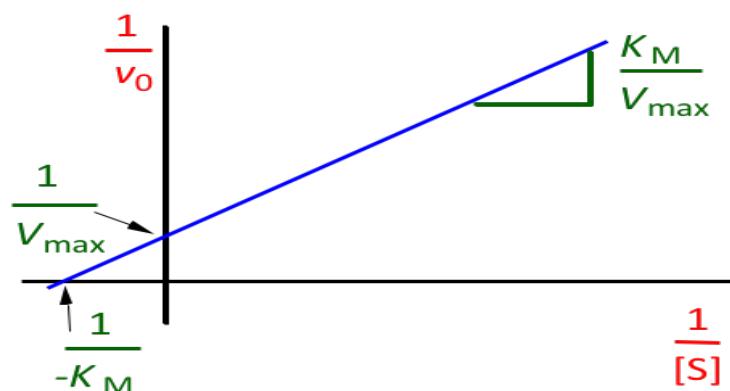
Amino acid are guiding the substrates to the active site--> reach catalytic perfection

LINEWEAVER-BURK PLOT

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

Linearising it in double-reciprocal form:

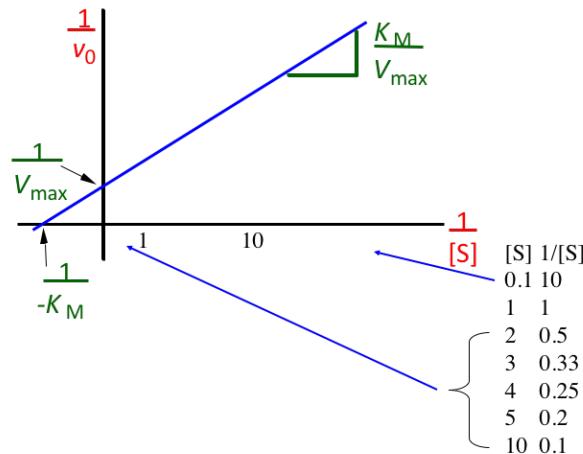
$$\frac{1}{v_o} = \frac{K_M}{V_{max}} \frac{1}{S} + \frac{1}{V_{max}}$$



A plot of $1/v_o$ vs $1/[S]$ is linear

- slope $= K_M / V_{max}$ and
- y-axis intercept of $1/V_{max}$
- x-axis intercept of $-1/K_{cat}$

- The disadvantage of this plot is that most measurements of $[S]$ are at high values, and the $1/[S]$ values all get crowded on the left side of the graph, making drawing a straight line difficult and inaccurate.
- Secondly, for small $[S]$, small errors in v_0 lead to large errors in $1/v_0$ and hence large errors in K_M and V_{\max}
- $1/v$ approaches infinity as $[S]$ decreases
 - gives undue weight to inaccurate measurement made at low concentration

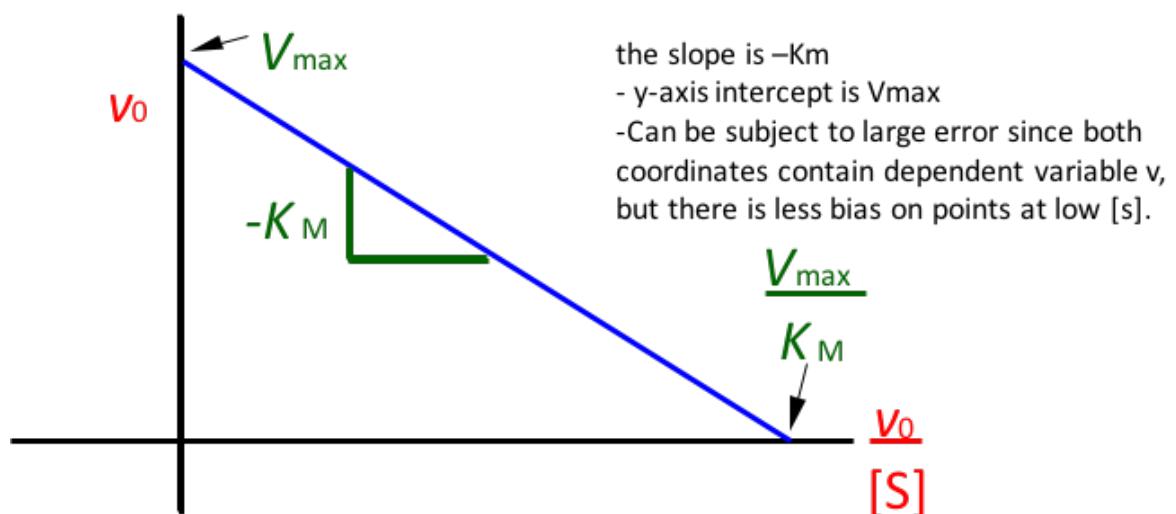


- give insufficient weight to more accurate measurements at high concentration.

There are other plots which have their advantages and disadvantages

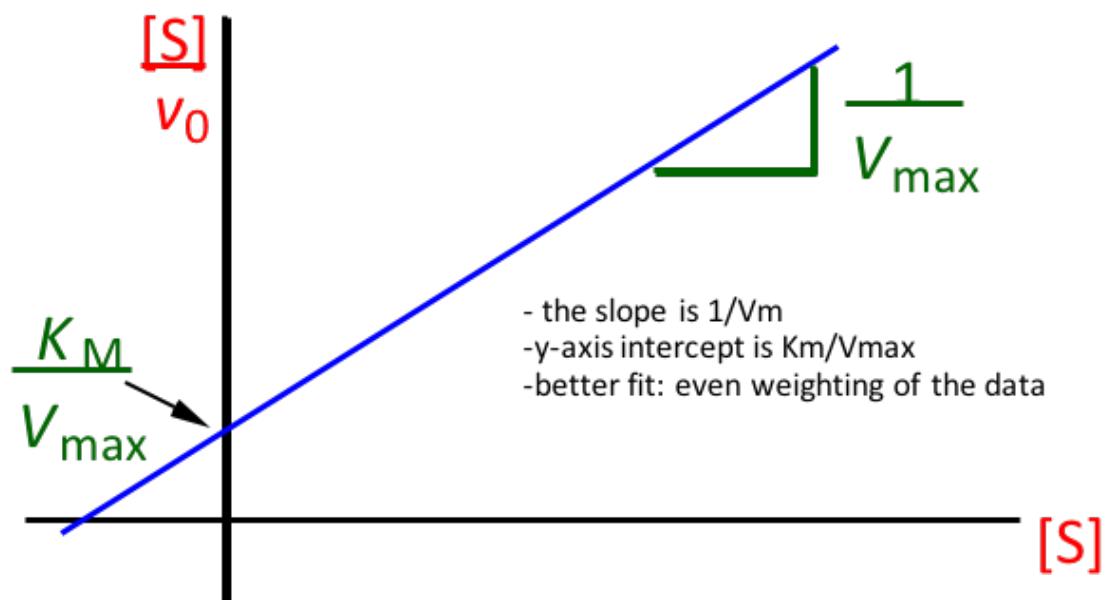
HOFSTEE-EADIE PLOT

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



HANES-WOOLF PLOT

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

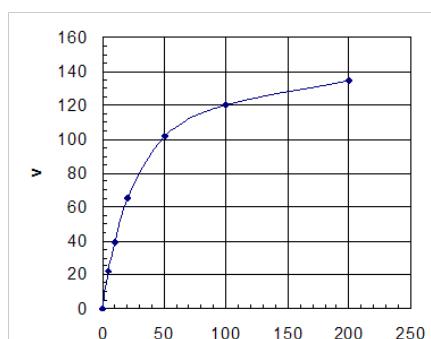


EXERCISE

Estimate V_{max} and K_m for the following data

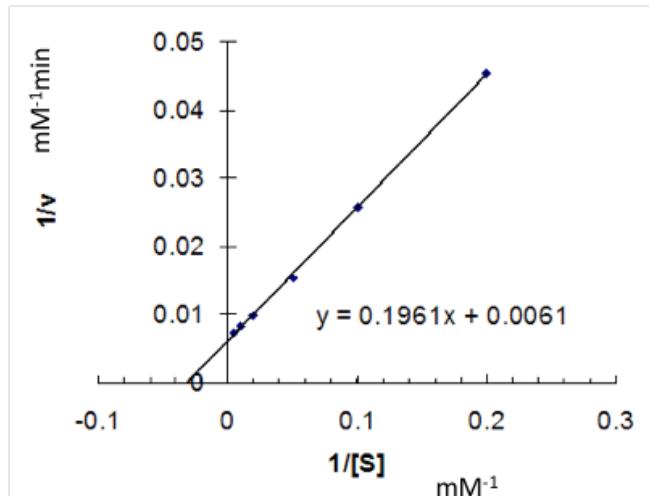
[S] mM	v (mM-min ⁻¹)
5	22
10	39
20	65
50	102
100	120
200	135

- $V_{max} = 150 \text{ mM/min}$, in which case K_m is about 25 mM.



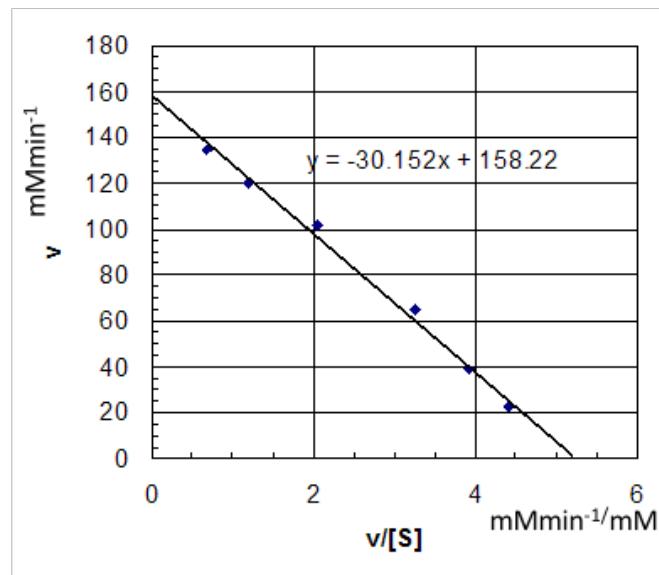
Lineweaver-Burke

- $V_{max} = 1/0.0061 = 164\text{mM/min}$; $K_m/V_{max} = 0.1961$, so $K_m = 164 * 0.1961 = 32\text{mM}$ or $-1/K_m = -(1/-0.031) = 32\text{mM}$



Eadie-Hofstee

- $K_m = 30\text{mM}$, $V_{max} = 158\text{mM/min}$



- How about kcat and kcat/K_M?
- Let's assume we have total enzyme concentration of 1 nM.

$$V_{\max} = k_{cat}[E_T]$$

$$k_{cat} = \frac{V_{\max}}{[E_T]} = \frac{164 \text{ mM min}^{-1}}{1 \times 10^{-3} \text{ mM}} = 164 \times 10^3 \text{ min}^{-1} = 1.64 \times 10^5 \text{ min}^{-1}$$

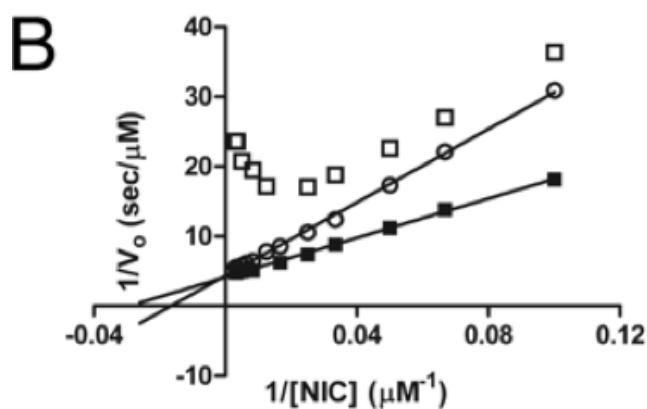
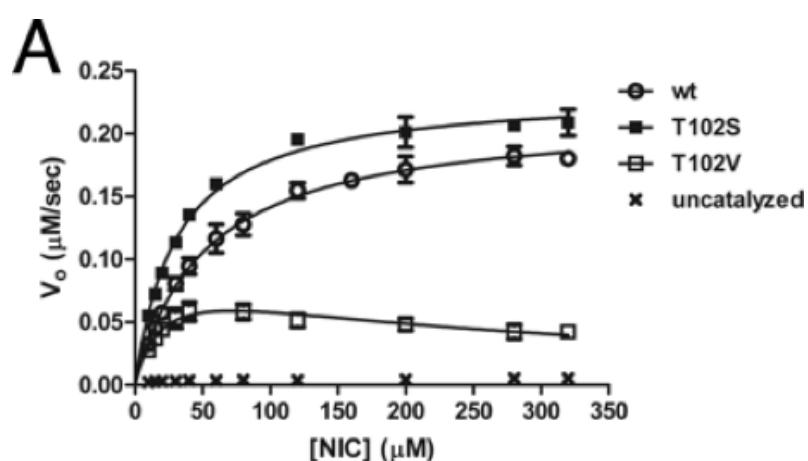
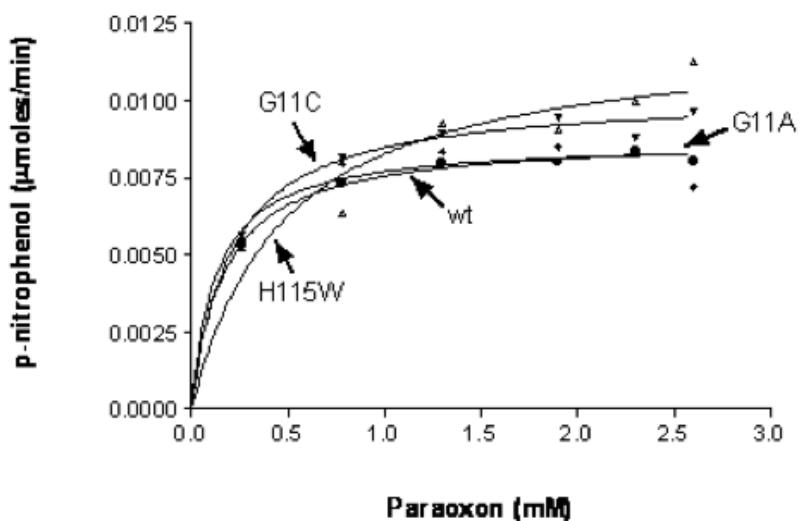
$$k_{cat} = 1.64 \times 10^5 \text{ min}^{-1} = (1.64 \times 10^5 \text{ min}^{-1}) \left(\frac{1 \text{ min}}{60 \text{ s}} \right) = 2,733 \text{ s}^{-1}$$

- We can now calculate kcat/K_M

$$\frac{k_{cat}}{K_M} = \frac{2,733 \text{ s}^{-1}}{32 \text{ mM}} = 8.54 \times 10^7 \text{ s}^{-1} M^{-1}$$

We can produce mutations in the enzymes and see the change in activity studying kinetics.

Paraoxonase Activity



REVERSIBLE REACTION

- The Michaelis-Menten model implicitly assumes that the reverse reaction may be neglected.
- Many reactions are reversible (small ΔG) which have products which “back-react” to form substrates at a significant rate.



- Michaelis-Menten equation can be modified to consider the back reaction from product to substrate
- This treats [ES] as a reaction intermediate and may be considered a one intermediate Michaelis-Menten equation
- Much more complicated but still contains terms that are measurable and solvable

$$v = \frac{\frac{V_{\max}^f[S]}{K_M^S} - \frac{V_{\max}^r[P]}{K_M^P}}{1 + \frac{[S]}{K_M^S} + \frac{[P]}{K_M^P}}$$

THE HALDANE RELATIONSHIP

At equilibrium $v=0$

$$K_{eq} = \frac{[P]_{eq}}{[S]_{eq}} = \frac{V_{\max}^f K_M^P}{V_{\max}^r K_M^S}$$

The kinetic parameters are not independent of each other. They are related by the equilibrium constant for the overall reaction

EFFECTS OF pH

Enzymes are pH sensitive and are usually only active in a narrow pH range (usually 5-9) due to:

- (1) pH sensitivity of substrate binding
- (2) reduced catalytic efficiency of the enzyme
- (3) ionization of substrate
- (4) protein structural changes (usually at pH extremes)

Kinetic analysis as a function of pH provides information about the nature of functionally (catalytically) important residues

General chemical equation for enzyme with two ionizable groups assumes only EH and ESH are active

EVALUATION OF IONIZATION CONSTANTS

- The ionization constants in our pH dependent general chemical reaction can be determined by plotting $\log V'_{\max}$ vs pH and $\log(V'_{\max} / K'M)$ vs pH
- $\log(V'_{\max} / K'M)$ vs pH provides information about KE1 and KE2 or the first step of the reaction
- Knowing the ionization constant of a catalytic residue gives a clue to its identity
- A pK_E of 4 would suggest a carboxylate group such as an Asp or Glu; the pK_a of equivalent free amino acids due to the local environment

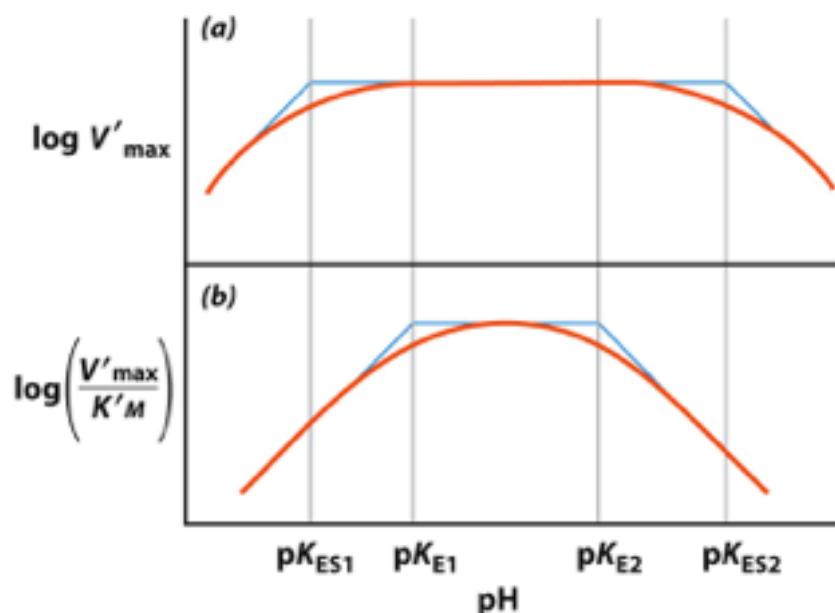
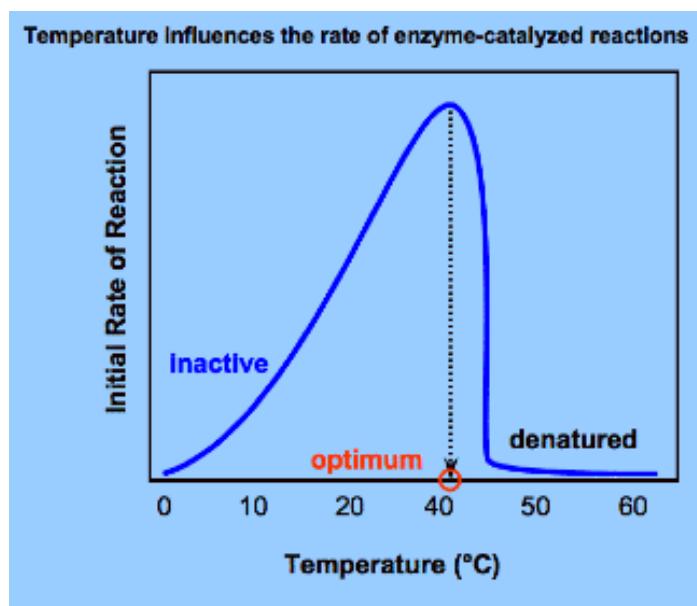


Figure 10-18
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EFFECT OF TEMPERATURE

- The effects of temperature on rate due to the fact that as temp. increases more molecules will have the energy required to achieve activation energy, E_a . (Arrhenius equation and the Maxwell-Boltzmann Distribution)
- The effects of temperature on protein stability: at high temperatures the protein denatures.



BISUBSTRATE REACTIONS

- ~60 % of all enzymatic reaction are bisubstrate reactions that require two substrates and yield two products
- Almost all bisubstrate reactions fall into two groups:
 - *transferase reactions* – one functional group is transferred between the substrates
 - *redox reactions* – reducing equivalents are transferred between the substrates
- While there are many possible bisubstrate reaction mechanism, only a few types commonly occur

CLELAND NOMENCLATURE

- Substrates are A, B, C, D *in the order they add to the enzyme* Products are P, Q, R, S, T *in the order they leave the enzyme*
- Stable enzyme forms are E, F, G; stable enzyme forms are defined as those incapable of converting to another stable form by itself

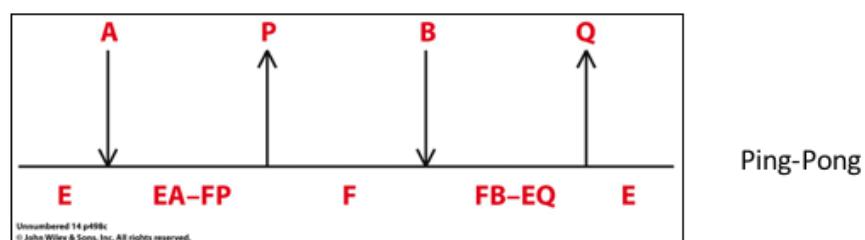
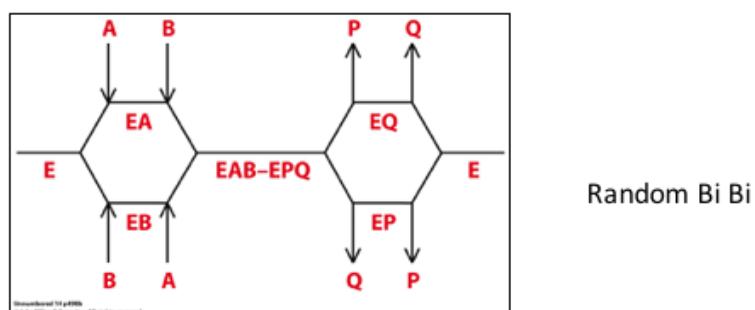
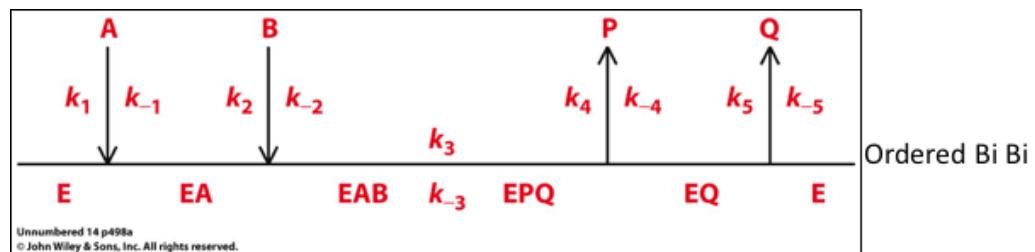
- Number of reactants and products in a given reaction are specified by the terms *uni*, *bi*, *ter* and *quad*
- e.g. Reaction with 1 substrate and two products - Uni Bi reaction Bisubstrate reactions with two products - Bi Bi reaction

Two major mechanism for bisubstrate reactions

- Sequential Reactions – all substrates must combine with the enzyme before the reaction can occur and products can be released. Single step conversion of substrate to product gives rise to alternate name, single displacement reaction

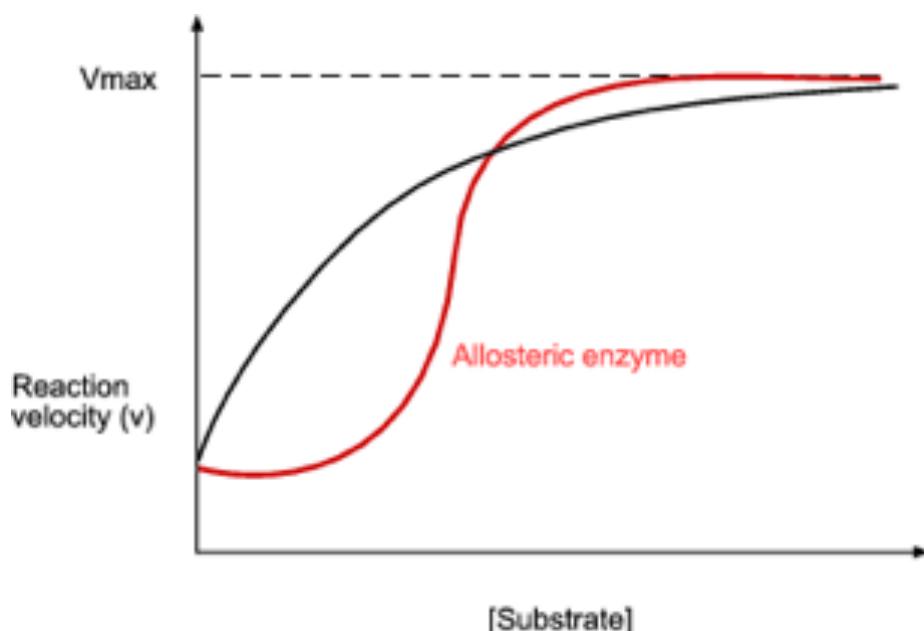
Subdivided into Ordered and Random

- Ordered Sequential mechanism require each substrate to bind and each product to be released in particular order
- Random Sequential mechanism allow substrate to bind and product to be released in any order
- Ping-Pong Reactions – one or more products are released before all substrates have combined with the enzyme also referred to as double displacement reaction



REACTIONS NOT OBEYING MICHAELIS-MENTEN

- Allosteric enzymes do not follow the Michaelis-Menten Kinetics. This is because allosteric enzymes have multiple active sites. These multiple active sites exhibit the property of cooperativity, where the binding of one active site affects the affinity of other active sites on the enzyme.

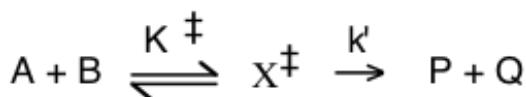


TRANSITION STATE THEORY

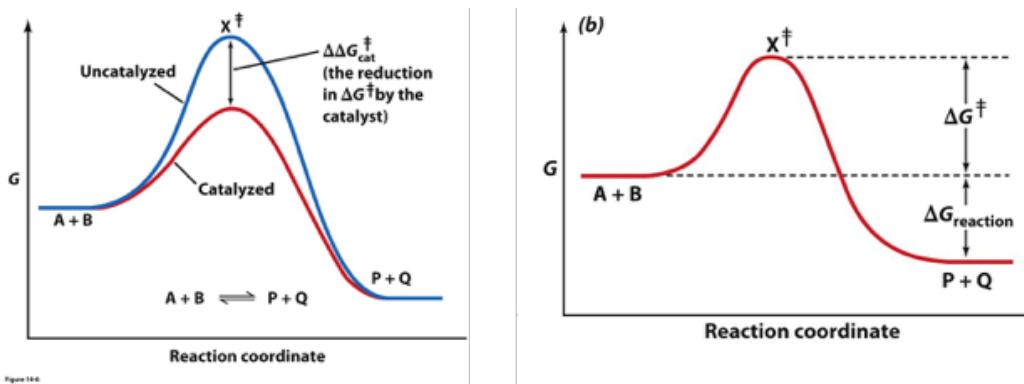
Active site is small relative to the total volume of the enzyme:

1. Usually occur in clefts and crevices in the protein. Excluding solvents which would otherwise reduce the catalytic activity of the enzyme.
 2. Amino acids and cofactors are held in precise arrangement with respect to structure of the substrate
 3. Complements the structure of the substrate molecule
- Enzymes enhance the rate of reaction by stabilizing the **transition state** of the reaction
 Enzyme catalysis do not alter the equilibrium of a reversible reaction

Consider the reaction $A + B \rightarrow P + Q$



K^\ddagger is the equilibrium constant between $A + B$ and X^\ddagger and k' is the rate constant for conversion of X^\ddagger to $P + Q$.



The transition state, X^\ddagger , is metastable. The transition state can be thought of as sharing some features of the reactants and some features of the products. That is, some bonds in the substrate are on their way to being broken and some bonds in the product are partially formed.

The transition state, X^\ddagger , is in rapid equilibrium with reactants

with equilibrium constant K^\ddagger .

$$K^\ddagger = \frac{X^\ddagger}{[A][B]}$$

ΔG^\ddagger , the activation energy, is the difference in Gibbs free energy between the transition state, X^\ddagger , and the reactants. Since K^\ddagger is an equilibrium constant, the now familiar equation applies:

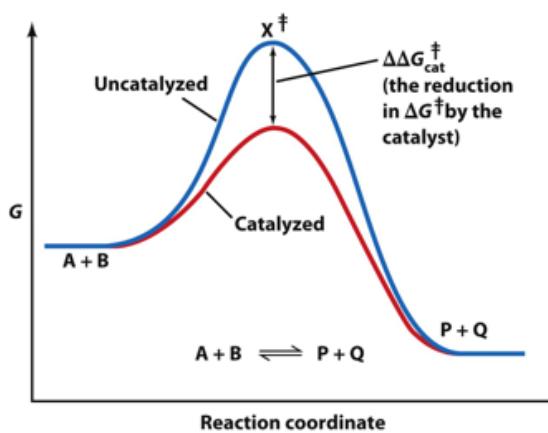
$$-RT \ln K^\ddagger = \Delta G^\ddagger$$

The frequency with which reactants achieve the transition state is inversely proportional to the activation energy barrier between the two.

The observed rate of the reaction, k_{obs} , will be a function of the concentration of the reactants, the rate of conversion of $X \rightleftharpoons[]{} P + Q$, k' , and will decrease exponentially with an increase in ΔG^\ddagger .

$$k_{\text{obs}} = k' e^{-\Delta G^\ddagger / RT} [A][B]$$

Enzymatic rate accelerations are achieved by lowering the activation barrier between reactants and the transition state, thereby increasing the fraction of reactants able to achieve the transition state.



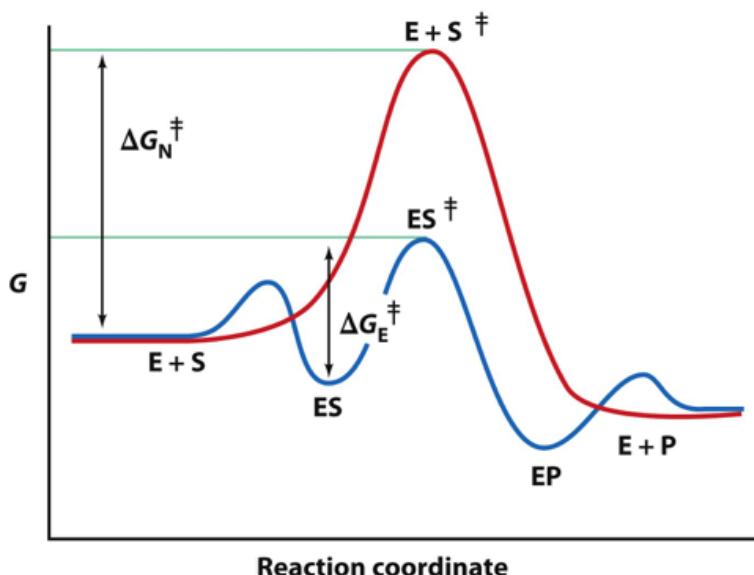
Enzymes accelerate reactions by lowering the energy barrier between reactants and products.

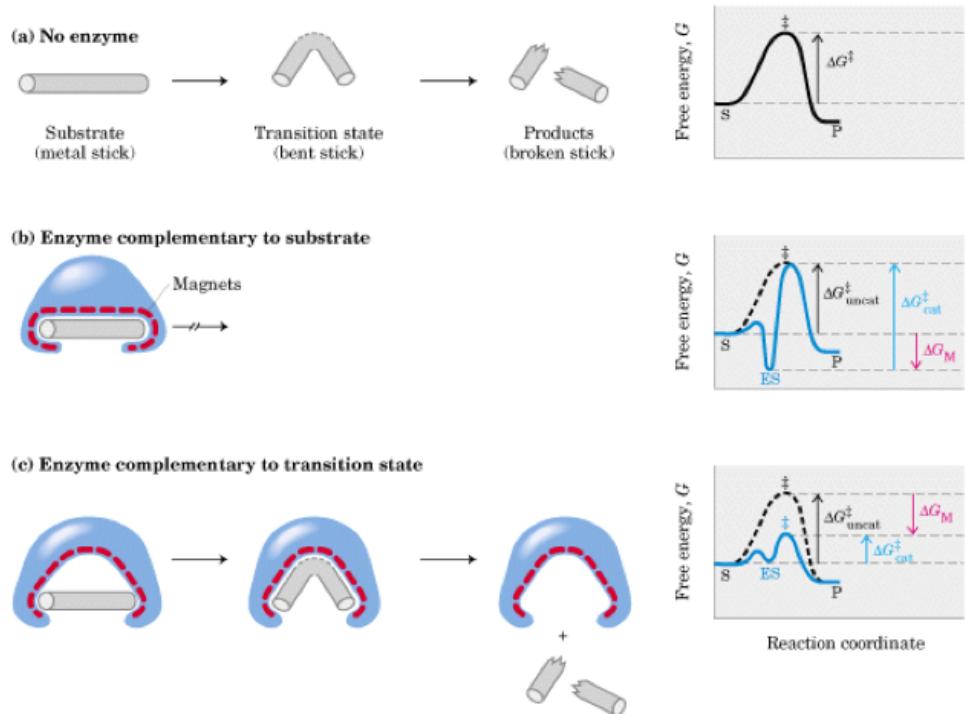
$$\Delta \Delta G^\ddagger = \Delta G^\ddagger_{\text{uncatalyzed}} - \Delta G^\ddagger_{\text{catalyzed}}$$

Although less energy is required to form the transition state in the catalyzed reaction, the ground states of the free substrates and products remain the same. The kinetic barrier is lowered by the same extent for the forward and reverse reactions. Consequently, a catalyst accelerates the reaction without affecting its equilibrium.

If a catalyst lowers the activation barrier by $\Delta \Delta G^\ddagger$, the rate of the reaction is enhanced by the factor $e^{\Delta \Delta G^\ddagger / RT}$. Consequently, a ten-fold rate enhancement requires that $\Delta \Delta G^\ddagger = 1.36 \text{ kcal/mole}$, less than the energy of a single hydrogen bond.

$$(\Delta \Delta G^\ddagger = RT/n10 = 1.98 \times 10^{-3} \text{ kcal/mol} \cdot \text{K} \times 298 \text{ K} / n(10) = 1.36 \text{ kcal/mol})$$

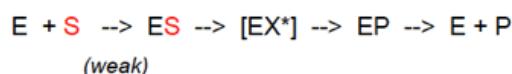
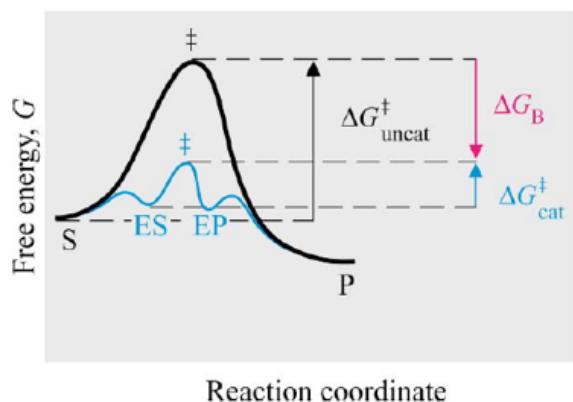




In most case, initial interaction is noncovalent (ES) making use of hydrogen bonding, electrostatic, hydrophobic and van der Waals

force to effect binding

ES: Catalytic groups are now an integral part of the same molecule, the reaction of enzyme bound substrates will follow first order rather than second order kinetics.



- Change in free energy ΔG_B

Favorable interaction between the enzyme and substrate result in a favorable intrinsic binding energy.

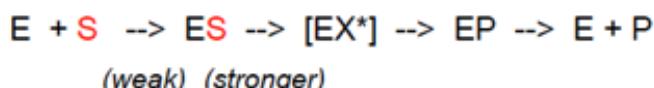
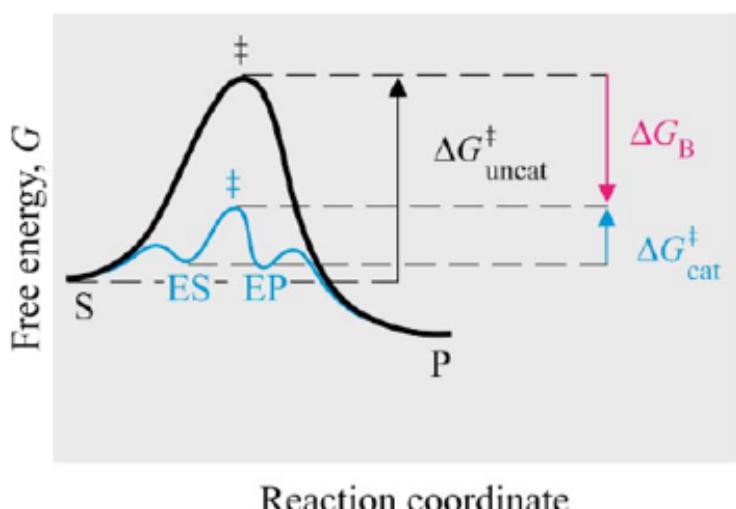
Entropy is lost when substrate binds to the enzyme

- (a) Two entities become one

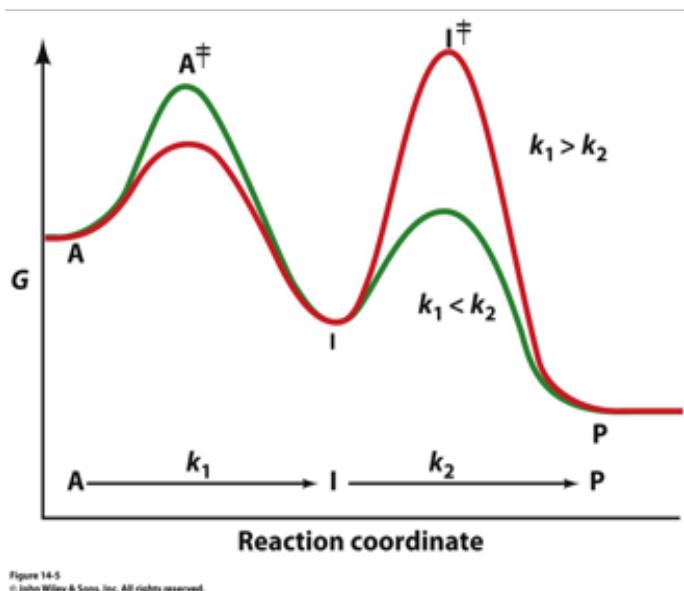
(b) Substrate is less able to rotate

(c) Substrate become more ordered

Weak interactions between the enzyme and substrate optimise and stabilise the transition state.



For a reaction that involves several steps, each step will have a corresponding transition state.

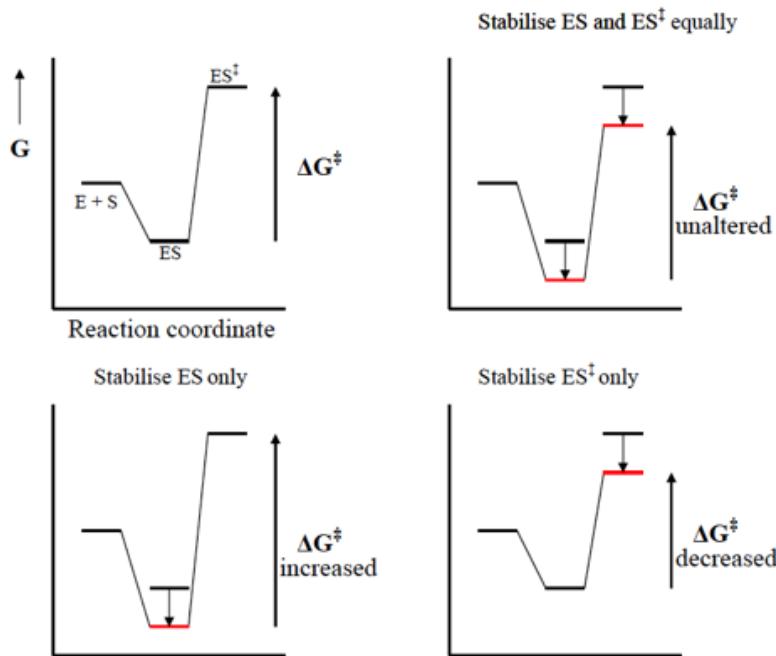


If the formation of I, an intermediate, from A is slower than the formation of P from I ($k_1 < k_2$) the activation barrier for the first step must be higher than the activation barrier for the second step (thick line). If k_1 is much slower than k_2 , conversion of A to I is the rate-determining step for the reaction. That is, the overall reaction proceeds at a rate that can be no faster than k_1 . Conversely, if formation of P from I is much slower than formation of I from A ($k_2 < k_1$), the activation barrier for the second step is higher (thin line) and formation of P from I is rate-determining.

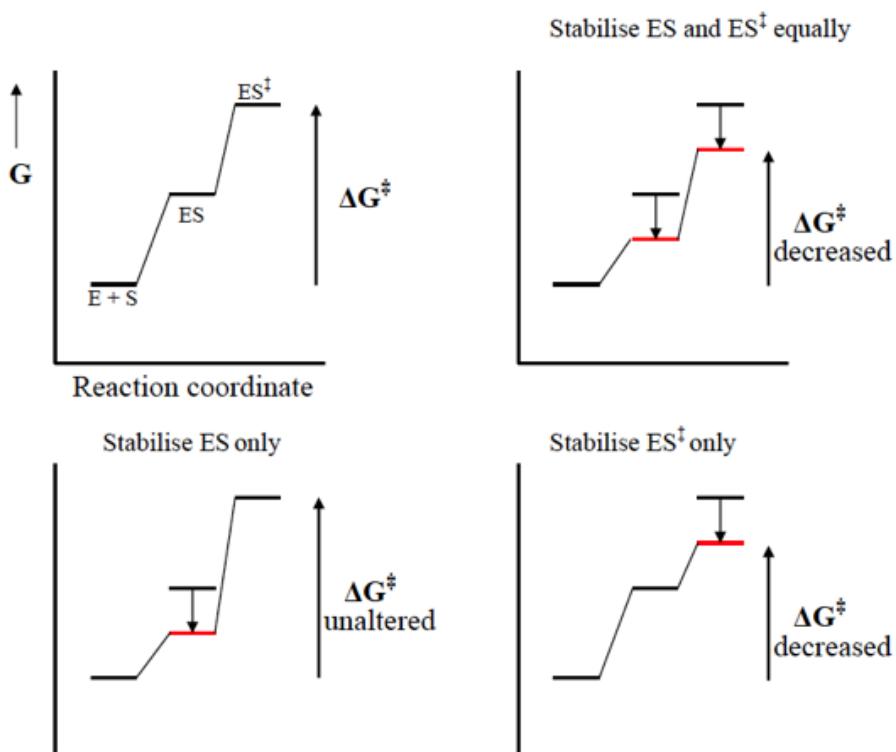
Figure 14-5
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Which states should an enzyme stabilise to increase catalysis?

Case 1: $[S] > K_m$ so that ES favoured over E+S (ΔG^\ddagger determines k_{cat})



Case 2: $[S] < K_m$ so that E + S favoured over ES (ΔG^\ddagger determines k_{cat}/K_m)



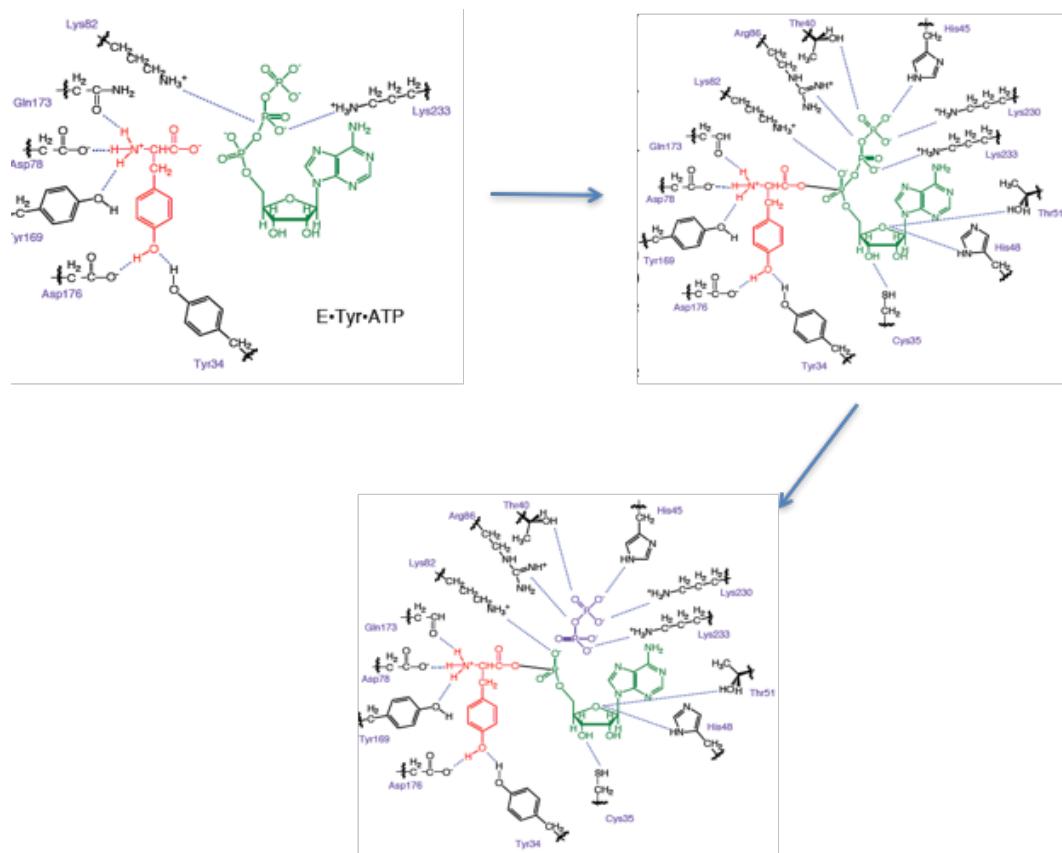
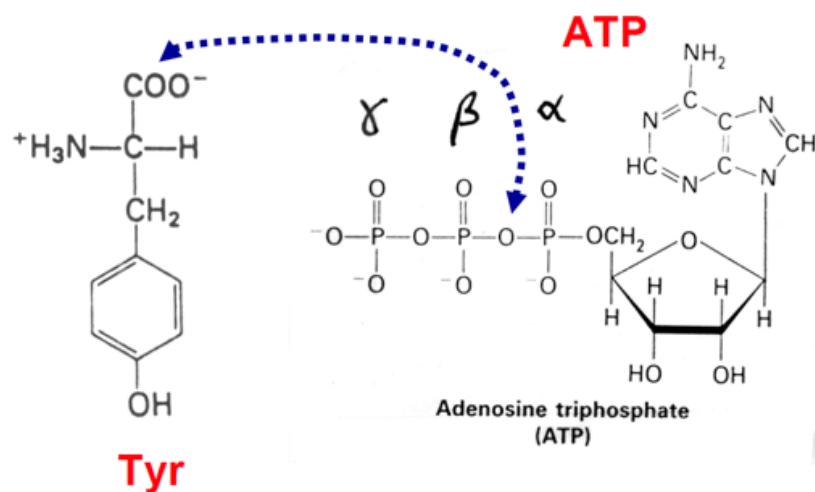
- TYROSYL tRNA SYNTHETASE

Tyrosyl tRNA synthetase is a dimeric protein with each monomer being predominantly α -helical with a central 6-stranded β -sheet core

The C-terminal portion tends to be disordered in the absence of tRNA.

The error rate for most aminoacyl tRNA synthetases is very low. (in the case of Tyr tRNA synthetase erroneous incorporation of Phe is on the order of 1 in 5×10^4)

Tyrosyl tRNA synthetase binds Tyr with a K_D of $\sim 2 \times 10^{-6}$ M (approx. 5 orders of magnitude greater than for Phe).



Enzyme	k_{cat} (s ⁻¹)	K _m (mM)	k_{cat}/K_m (s ⁻¹ M ⁻¹)
Wild-type	7.6	0.9	8.4×10^3
His48Gly (loss of H-bond to ribose)	1.6	1.4	1.14×10^3
Cys35Gly (loss of H-bond to ribose)	2.8	2.6	1.12×10^3
His45Asn (loss of H-bond to γ -phosphoryl group during formation of transition state)	0.003	1.0	3

- Loss of binding energy in formation of TS but not binding of ATP lowers k_{cat}

- **CATALYTIC ANTIBODIES**

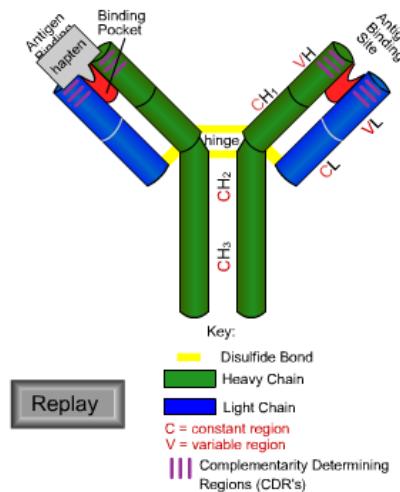
Antibodies tightly bind the antigen, but do not specifically alter its chemical nature.

Enzymes bind and catalyse substrates to new products.

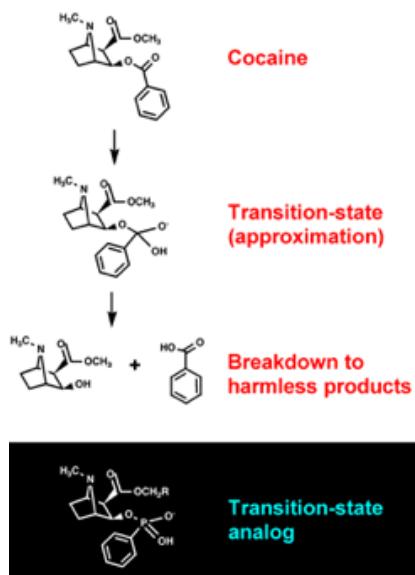
Enzymes catalyse a reaction by stabilising the transition state.

Theoretically, if an antibody binds to a transition-state molecule, it may be expected to catalyse a corresponding chemical reaction by forcing substrates into transition-state geometry.

Synthesis of transition-state analogs. These molecules are more stable than the transition state itself, but mimic its three-dimensional structure. If injected into the bloodstream of an animal, transition state analogs act as haptens, and elicit antibody production. Antibodies are isolated from the serum of the animal, and then screened by experimental assays to determine which catalyse the selected reaction.



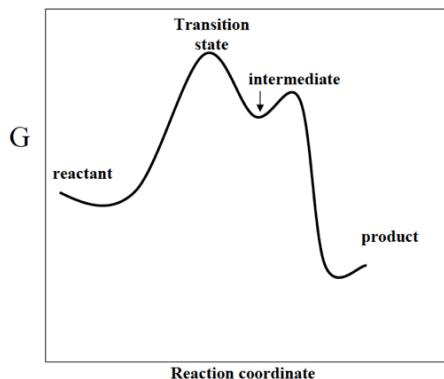
- Abzymes have been implicated for use in the detoxification of cocaine. Catalytic antibodies have been generated that cleave the cocaine molecule at specific bonds, thereby eliminating the toxic effect of the drug.



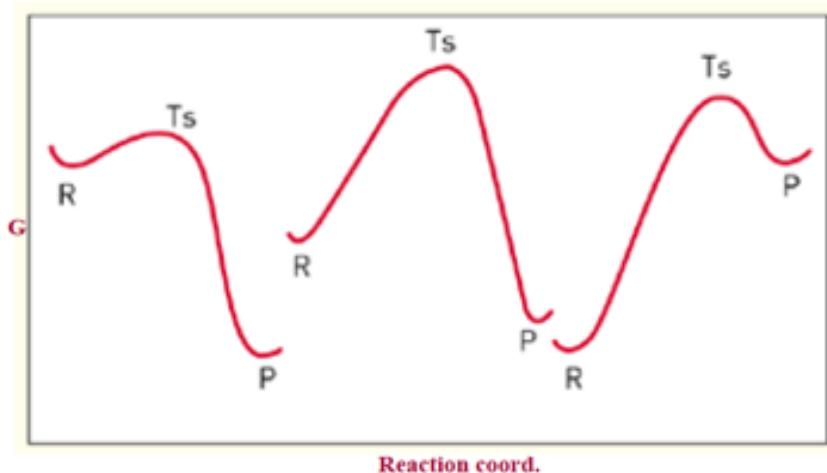
- **HAMMOND POSTULATE**

"If two states, as for example, a transition state and an unstable intermediate, occur consecutively during a reaction process and have nearly the same energy content, their interconversion will involve only a small reorganization of the molecular structures."

George S. Hammond, 1955



Hammond postulated that in highly exothermic reactions (left) the transition state (T_s) is structurally similar to the reactant (R), but that in highly endothermic reactions (right) the product (P) is a better model of the transition state



The Hammond postulate suggests that species that are sequential on the reaction coordinate and similar in energy are therefore similar in

Structure

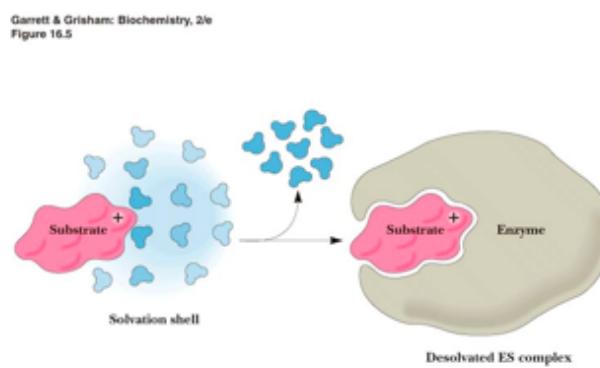
- Therefore, an unstable intermediate on the reaction pathway (whose structure may be obtained by experimental means) is predicted to resemble the structure of the transition state
- Conversely, changes in structure that stabilise or destabilise reactive intermediates will stabilise or destabilise transition states leading to them
- Elucidating the structure of the transition state can lead to the design of transition state analogs whose ground state geometry is similar to the transition state
- These analogues can be used as enzyme inhibitors (Drug therapy)
- These analogues can be used to elucidate the mechanism of the enzyme mediated reaction (Mechanistic details)
- **DESOLVATION**

When substrate binds to the enzyme surrounding water in solution is replaced by the enzyme. This makes the substrate more reactive by destabilizing the charge on the substrate

Expose a water charged group on the substrate for interaction with the enzyme

Lowers the entropy of the substrate

When substrate bind to the enzyme, it may induces a conformational change in the active site to fit to a transition state



Saunders College Publishing

Frequently, in the transition state, the substrate and the enzyme have slightly different structure (strain or distortion) and increase the reactivity of the substrate.

- **PROXIMITY**
- Rate increase due to two reactants being brought out of a dilute environment and placed closer together
- Enzymes do this by providing a docking site and a micro environment allowing proper substrate orientation for reaction
- Contributes to the loss of the substrates freedom of movement (loss of entropy). Increases chances of reaction --molecules are closer together on enzyme surface – increase in effective concentration

INHIBITION

- Enzyme inhibitors are molecules that bind to enzymes and decrease their activity. Blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors.
- Reversible or irreversible
- Irreversible inhibitors usually react with the enzyme and change it chemically
- Reversible inhibitors bind non-covalently and different types of inhibition are produced
- Inhibitor competes directly with substrate for the enzymes active site
 - Resembles the substrate in structure
 - Binds to active site (not always) but it is unreactive
- Sulfanilamide is an antibiotic useful in the treatment of some kidney infection; structural analog of p-aminobenzoic acid (PABA); PABA is a structural part of folic acid
- Some bacteria require folic acid for their growth and division. Sulfanilamide is a competitive inhibitor for bacterial **dihydrofolate synthetase**. Thus bacteria are starved of the required folate and cannot grow and divide. Sulfanilamide is highly toxic to bacteria that must synthesise their own folic acid. Since humans require folate from dietary source, the sulfanilamide is not harmful at the doses that kill bacteria

The initial velocity for uninhibited reaction is given by:

$$v_o = k_2[ES]$$

Assume that the inhibitor binds reversibly and in rapid equilibrium

$$K_I = \frac{[E][I]}{[EI]}$$

EI is catalytically inactive. Lowers the concentration of free enzyme available for substrate to bind

Therefore:

$$[E_T] = [E] + [ES] + [EI]$$

Express enzyme concentration in terms of [ES]

$$[E] = \frac{K_M[ES]}{[S]}$$

For the enzyme-inhibitor complex:

$$[EI] = \frac{[E][I]}{K_I} = \frac{K_M[ES][I]}{[S]K_I}$$

So:

$$[E_T] = [ES] \left\{ \frac{K_M}{[S]} \left(1 + \frac{I}{K_I} \right) + 1 \right\}$$

Solving the equation for [ES]:

$$[ES] = \frac{[E_T][S]}{K_M \left(1 + \frac{I}{K_I} \right) + [S]}$$

- The initial velocity can be expressed:

$$v_o = k_2 [ES] = \frac{k_2 [E_T][S]}{K_M \left(1 + \frac{I}{K_I} \right) + [S]}$$

$\left(1 + \frac{I}{K_I} \right)$ can be defined as α (alpha), a function of the inhibitor concentration

- We know that $V_{max} = k_2 [E_T]$

So:

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

To obtain $v_o = V_{max}/2$ the value of $[S]$ must be αK_M

The K_M of the inhibited reaction is referred as $K_{M,app}$ and it is equal to αK_M

$V_{max,app}$ refers to the V_{max} of the inhibited reaction

The larger the value of α the higher the $[S]$ to approach V_{max}

Ie the V_{max} stays the same but the K_M changes (increases) since we need higher $[S]$ to achieve V_{max}

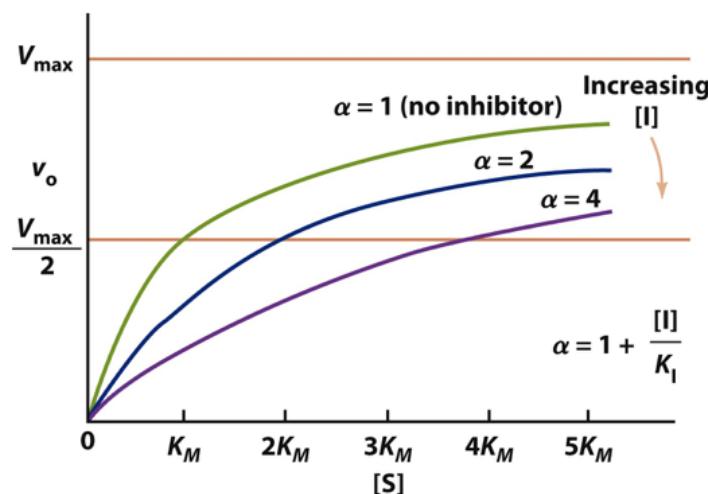
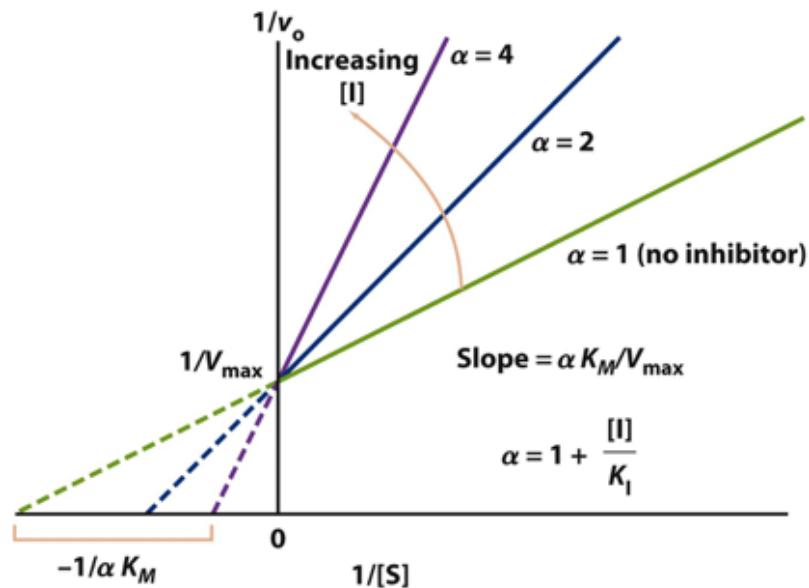


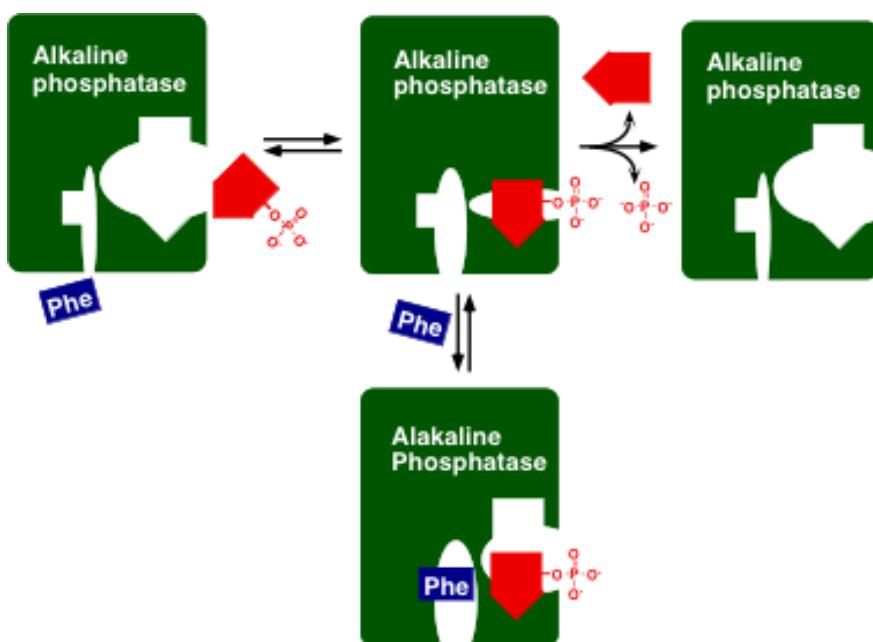
Figure 14-11
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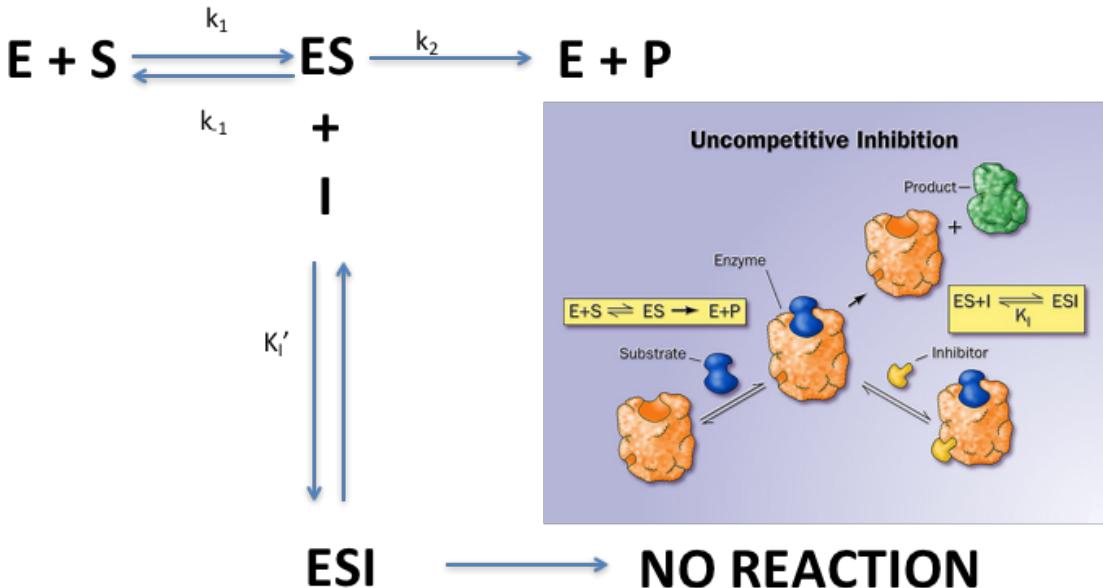
$$\frac{1}{v_o} = \left(\frac{\alpha K_M}{V_{\max}} \right) \frac{1}{[S]} + \frac{1}{V_{\max}}$$



UNCOMPETITIVE INHIBITION

- The inhibitor binds directly to the enzyme-substrate complex but not to the free enzyme
- It does not bind to the active site
- It can cause distortion of the active site
- Alkaline phosphatase catalyses the release of inorganic phosphate from phosphate esters. It is found in a number of tissues, including liver, bile ducts, intestine, bone, kidney, placenta, and leukocytes. Serum alkaline phosphatase levels are important diagnostic markers for bone and liver disease.





- The inhibitor binding step has a dissociation constant of:

$$K'_I = \frac{[ES][I]}{[ESI]}$$

- The amount of $[E_T] = [E] + [ES] + [ESI]$

$$[E_T] = [ES] \left(\frac{K_M}{[S]} + 1 + \frac{[I]}{K'_I} \right)$$

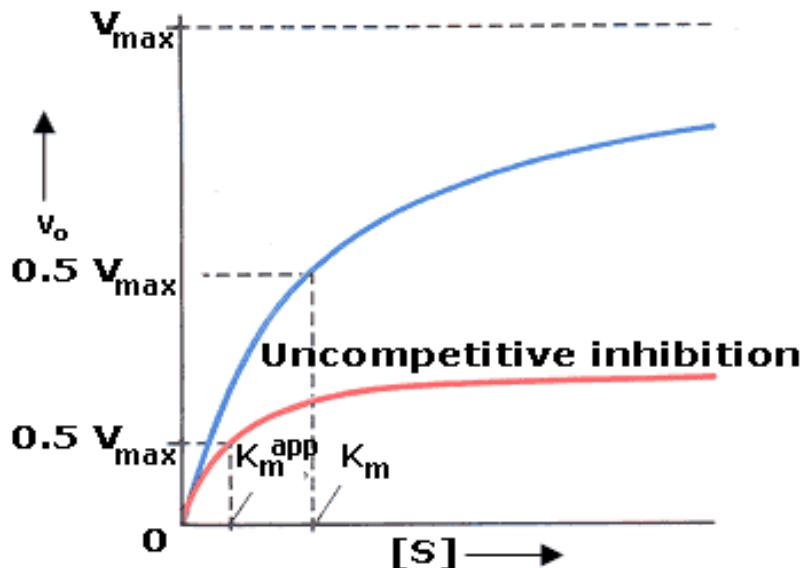
- We can now define factor α' :

$$\alpha' = \left(1 + \frac{[I]}{K'_I} \right)$$

- The rate for uncompetitive inhibitor can be given by:

$$v_o = \frac{V_{\max}[S]}{K_M + \alpha'[S]}$$

- At high $[S]$, v_o approaches V_{\max}/α'
- The V_{\max} is affected. Increasing the amount of $[S]$ cannot achieve V_{\max}
- Lowering the $[S]$ so $[S] \ll K_M$ the effect of the inhibitor becomes negligible



$$\frac{1}{v_o} = \left(\frac{K_M}{V_{max}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

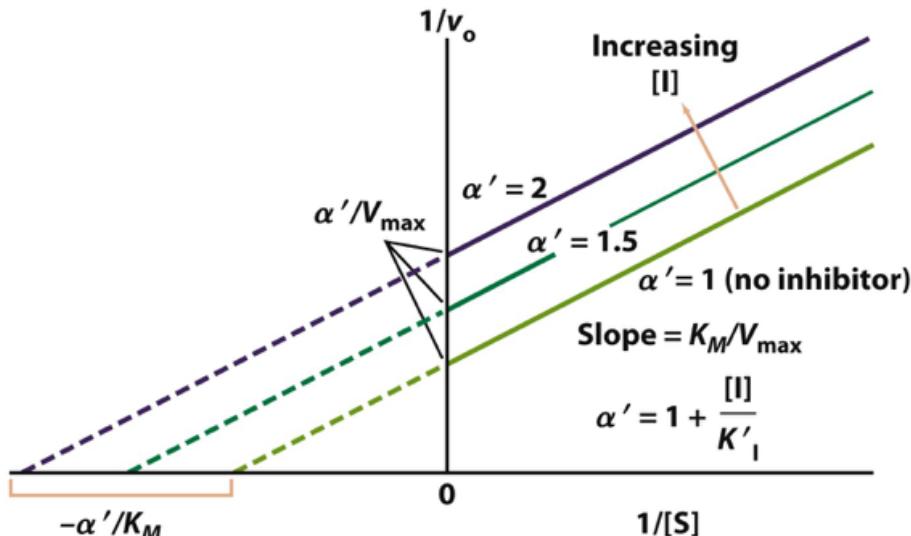
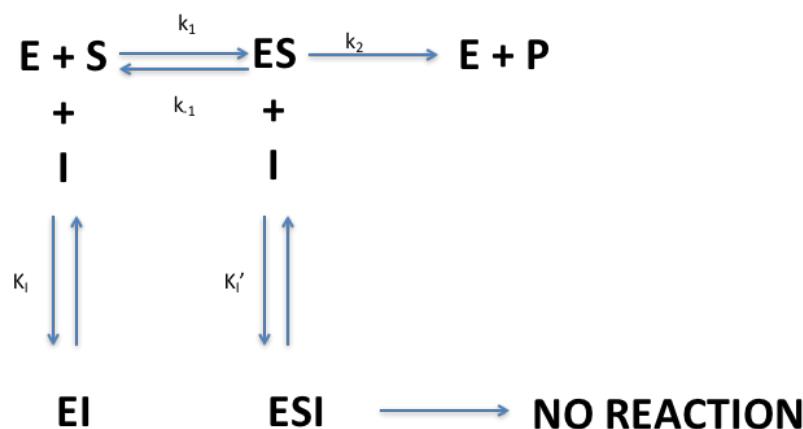
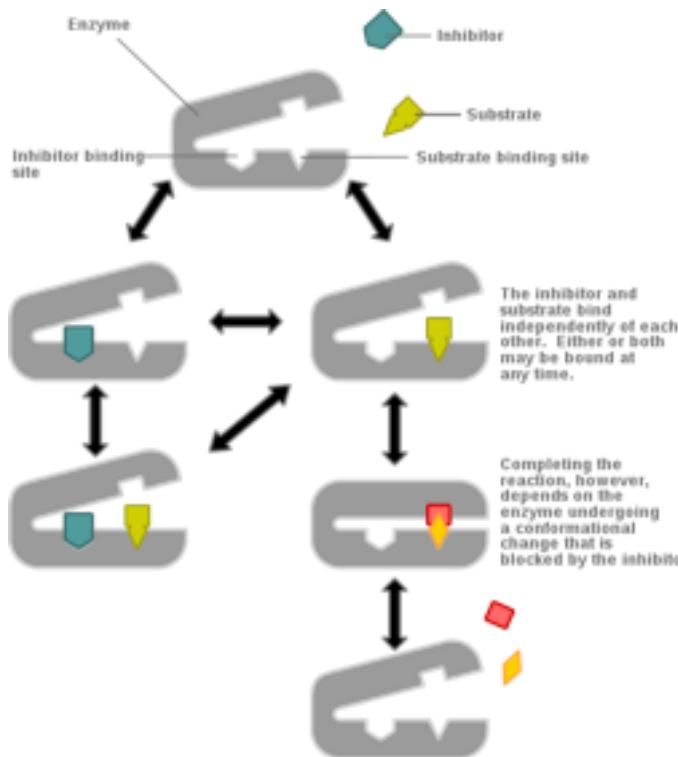


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The y intercept will give us the v_{max} (alpha factor over v_{max}), x intercept gives the K_m ; the slope is not affected but is K_m/V_{max} .

MIXED INHIBITION

- Mixed inhibitors do not bind directly in the active site, and therefore do not block substrate binding, but instead bind at sites that can be proximal or distal from the active site
- The inhibitor-distorted active site has trouble converting the substrate to product before it dissociates, resulting in a lowered apparent substrate binding affinity.
- Mixed inhibitors distort the active site. Therefore the enzymatic turnover rate is slowed.
- Mixed inhibitors can also bind the enzyme-substrate complex



The inhibitor has different affinities for the enzyme substrate complex. Have different dissociation constants (for free enzyme, enzyme complexes...).

The inhibitor binding steps are assumed to be at equilibrium but with different dissociation constants:

$$K_I = \frac{[E][I]}{[EI]} \qquad K'_I = \frac{[ES][I]}{[ESI]}$$

- The Michaelis-Menten equation becomes:

$$v_o = \frac{V_{max}[S]}{\alpha K_M + \alpha'[S]}$$

Mixed because α multiplies K_M (competitive) and α' the $[S]$ (uncompetitive)

Effective at both high and low $[S]$

$$\frac{1}{v_o} = \left(\frac{\alpha K_M}{V_{\max}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{\max}}$$

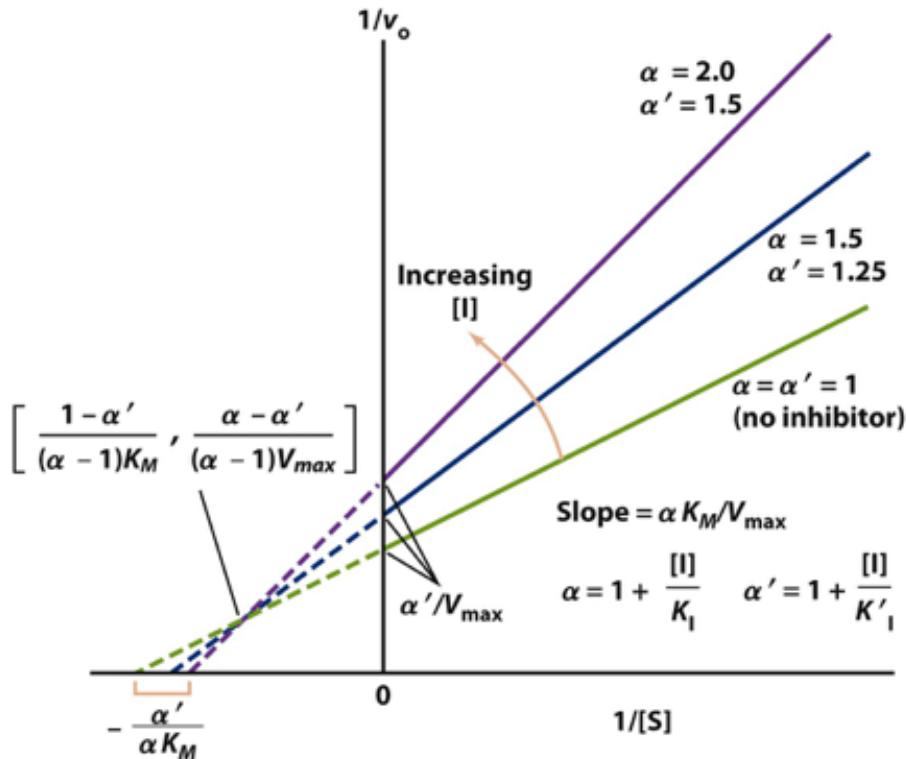


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NON COMPETITIVE INHIBITOR

- Do not bind to the active site; they bind to other parts of the enzyme, which can be remote from the active site. The extent of inhibition depends entirely on the inhibitor concentration and will not be affected by the substrate concentration.
- Noncompetitive inhibition is a special case of mixed inhibition where the affinity of inhibitor for E and ES is the same
- Special case of mixed inhibition where
 - $\alpha = \alpha'$
 - $K_I = K'_I$
- V_{\max} lowers as increase $[I]$
- Lines intersect on x-axis
 - K_M does not change

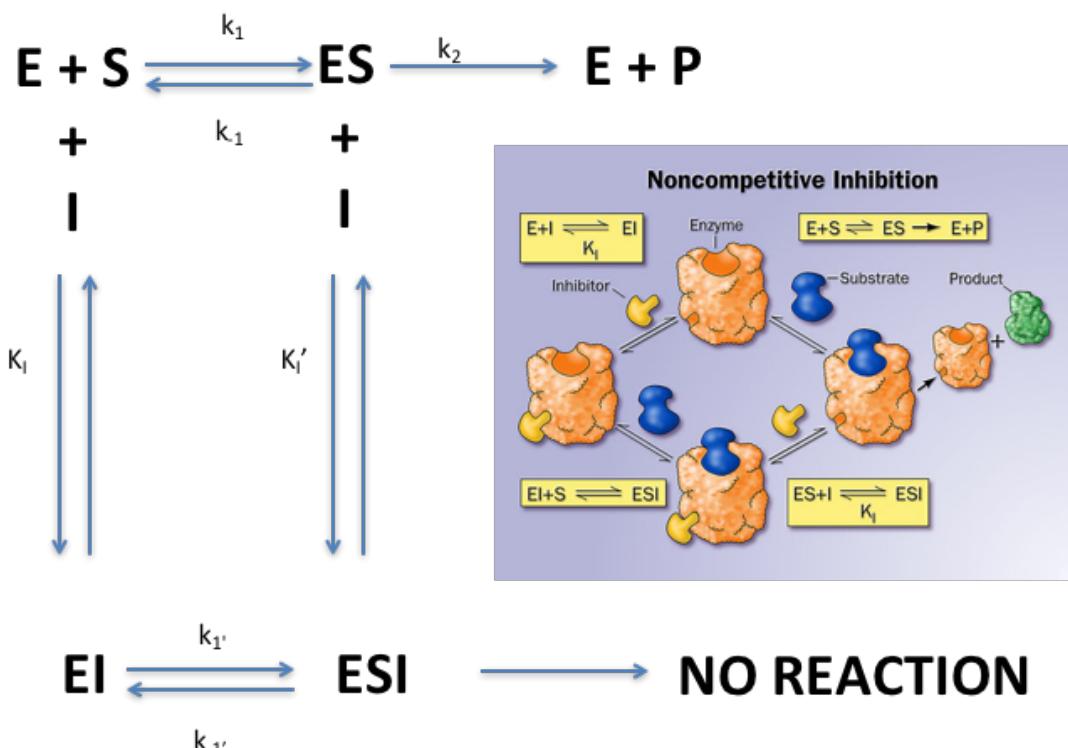


Table 14-2 Effects of Inhibitors on the Parameters of the Michaelis–Menten Equation^a

Type of Inhibition	$V_{\text{max}}^{\text{app}}$	K_M^{app}
None	V_{max}	K_M
Competitive	V_{max}	αK_M
Uncompetitive	V_{max}/α'	K_M/α'
Mixed	V_{max}/α'	$\alpha K_M/\alpha'$

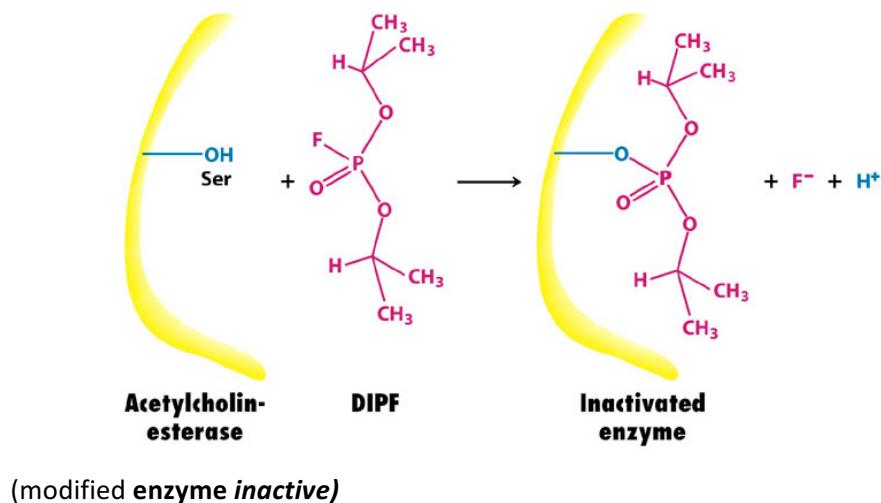
$$^a \alpha = 1 + \frac{[I]}{K_I} \text{ and } \alpha' = 1 + \frac{[I]}{K'_I}.$$

Table 14-2
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IRREVERSIBLE-SUICIDE INHIBITION

- 1) group-specific covalent modifying agents
 - 2) suicide inhibitors (mechanism-based inhibitors)
 - 3) affinity labels
 - 4) transition state analogs
- **Group-specific covalent modifying agents**
 - react with specific type of enzyme functional group (e.g., with Ser-OH, or with Cys-SH, or with His imidazole)
 - Diiisopropylphosphofluoridate (DIPF), a potent nerve gas (poison) reacts with specific, reactive Ser-OH on enzymes

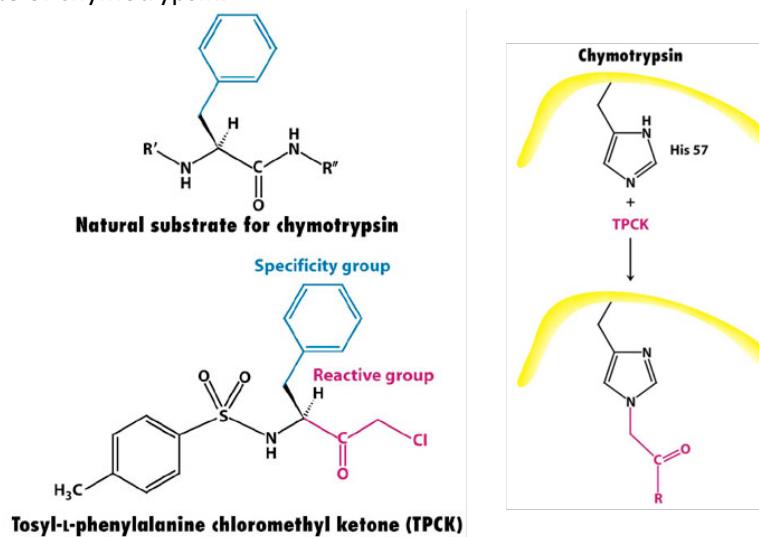
example: reaction with the **reactive, catalytic OH group** of the enzyme **acetylcholinesterase** at synaptic junctions



- **Affinity labels**

structural similarity to substrate "guides" reagent to active site reaction at active site covalently inactivates enzyme

Tosyl phenylalanyl chloromethylketone (TPCK) has a *phenyl group* that binds in substrate specificity site of chymotrypsin.



- **Suicide substrates (mechanism-based inhibitors)**

Structural similarity to substrate "guides" reagent to active site, and enzyme TREATS IT AS A SUBSTRATE, starting chemical catalytic process with the inhibitor.

However, chemical mechanism itself leads enzyme to react covalently with inhibitor, thus "committing suicide"

Mechanism-based inhibition depends on chemical mechanism of catalysis.

Example: penicillin (inhibits an enzyme, a transpeptidase, required for bacterial cell wall synthesis)

PENICILLIN

Discovered in 1928 by Alexander Fleming

Penicillium notatum

Inhibits **glycopeptide transpeptidase**, a bacterial enzyme that catalyses the cross-linking of peptidoglycan chains during the synthesis of cell walls

Does not kill existing bacteria but daughter cells are susceptible to osmotic lysis and this halts the growth of the population.

- **TRANSITION STATE ANALOGUES**

Structurally similar to transition state, which binds even more tightly to enzyme than substrate binds, so very high affinity for active site transition state analogs useful for:

understanding catalytic mechanisms (clues about structure of transition state)

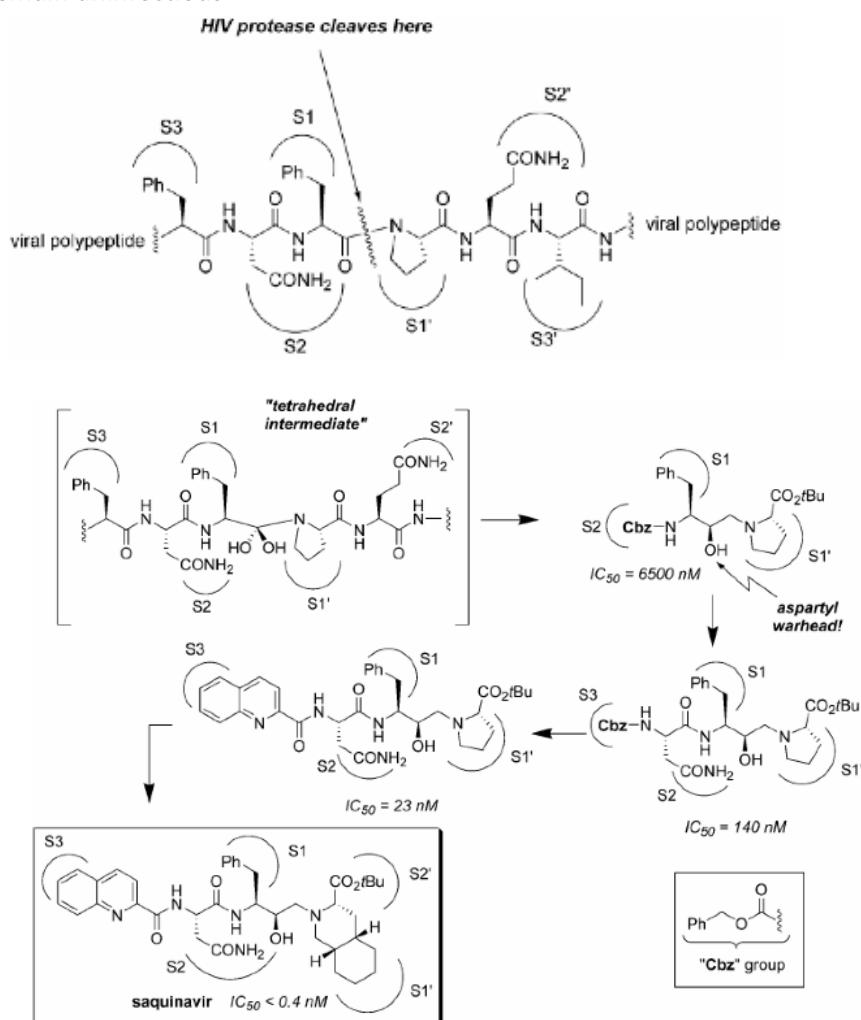
very specific inhibitors of enzymes (pharmaceutical applications)

antigens for immunizing lab animals to generate antibodies with binding sites complementary to the transition state

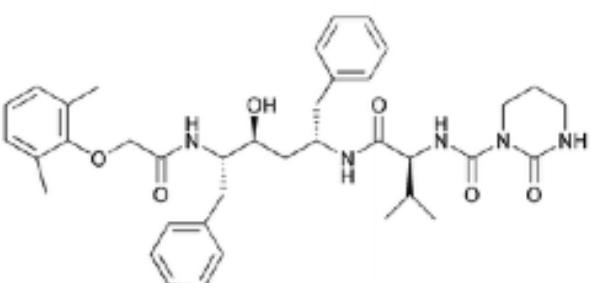
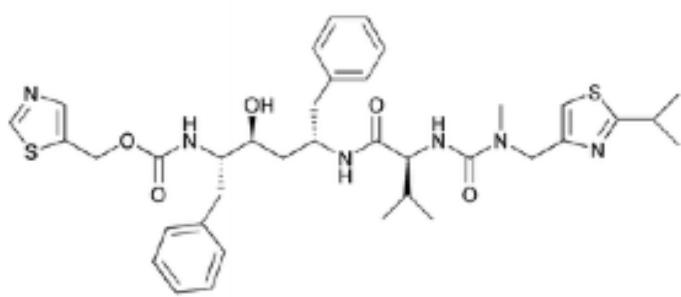
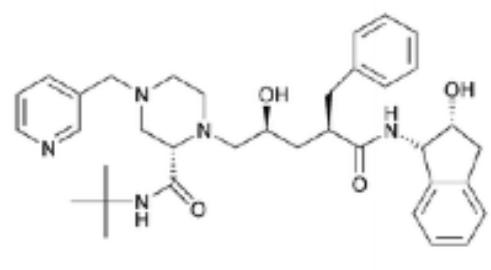
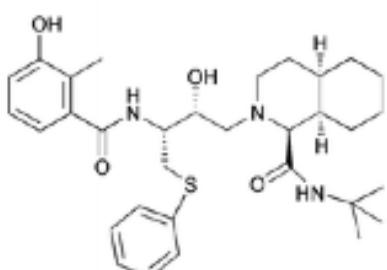
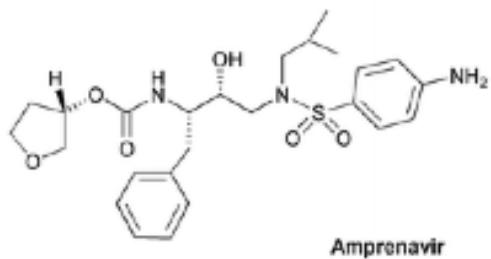
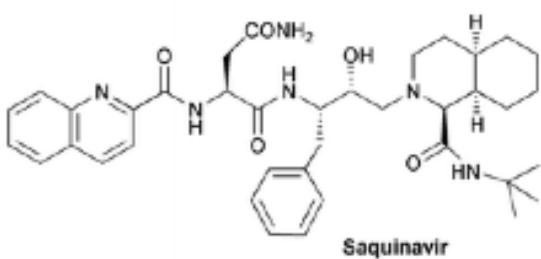
HIV PROTEASE

HIV-1 protease is an aspartic protease that is essential for the life-cycle of HIV

HIV protease cleaves newly synthesised polyproteins at the appropriate places to create the mature protein components of an infectious HIV virion. Without effective HIV protease, HIV virions remain uninfected.



FDA approved HIV-1 protease inhibitors



ENZYME CATALYSIS

Mode of Catalysis:

- Acid Base catalysis
- Covalent catalysis
- Metal ion catalysis
- Electrostatic catalysis
- Proximity and orientation effects
- Transition state binding catalysis

Acid Base Catalysis:

- Specific functional groups in enzyme structure positioned to
 - donate a proton (act as a general acid), or
 - accept a proton (act as a general base)
 - helps enzyme avoid unstable charged intermediates in reaction
 - Group that donates a proton (acts as a general acid) in catalysis has to then accept a proton (act as a general base) later in catalytic mechanism for catalyst to be regenerated in its original conjugate acid form
 - Likewise, general base that accepts a proton must give it up later.
- **General acid catalysis:**
Partial proton transfer from Bronsted acid
- **General base catalysis:**
Its rate is increased by partial proton abstraction by a Bronsted base
 - **Bronsted acid:** a species that can donate protons:
$$\text{HA} \rightleftharpoons \text{H}^+ + \text{A}^-$$
$$\text{HA} + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{A}^-$$

HA is bronsted acid, H₂O is bronsted base, A- is CONJUGATE BASE
 - **Bronsted base:** a species that can accept protons:
$$\text{BH}^+ \rightleftharpoons \text{H}^+ + \text{B}$$

B is base, BH+ is CONJUGATE ACID
(revise about Lewis acid/base)
- **General Acid-Base catalysis** is a process in which partial proton transfer (from acid) or partial proton abstraction (from base) lowers the free energy of the transition state
- **Specific Acid-Base** catalysis refers to reactivity of protons or hydroxide ions respectively

- Concerted General Acid-Base catalysed reaction is one where both processes occur simultaneously

KETO-ENOL TAUTOMERIZATION

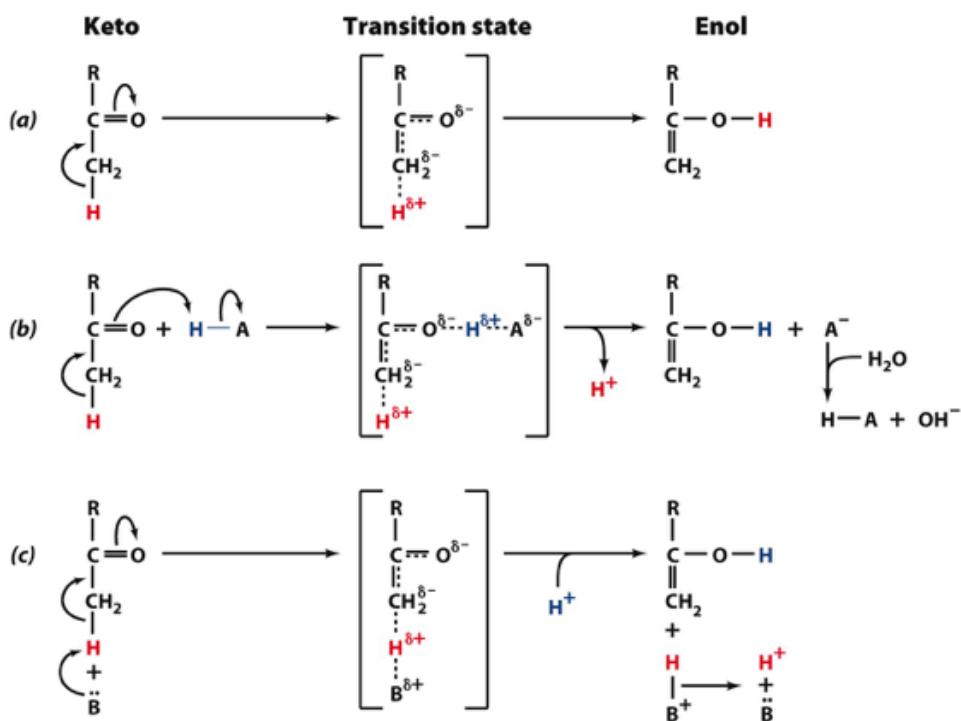
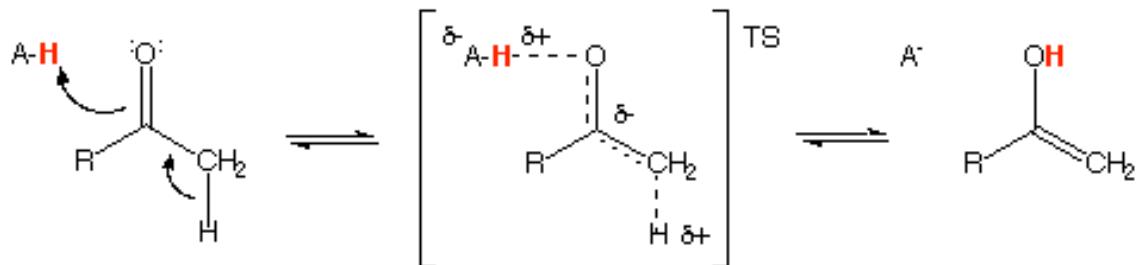
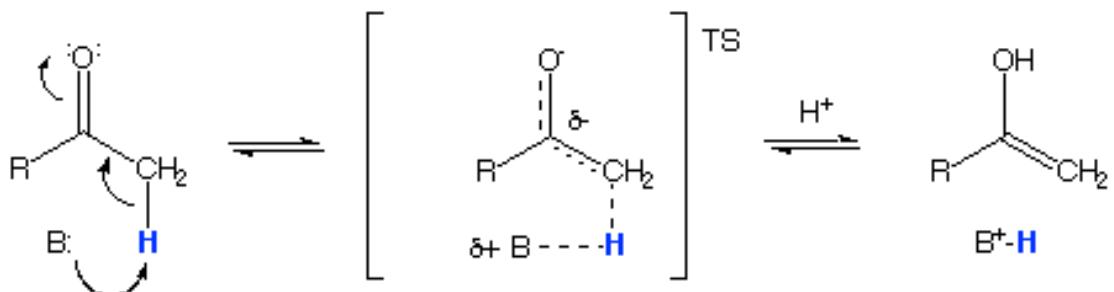


Figure 15-1
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In the enzyme active site, a general acid donates a proton to aid in the stabilisation of the negative oxygen that forms during the reaction:

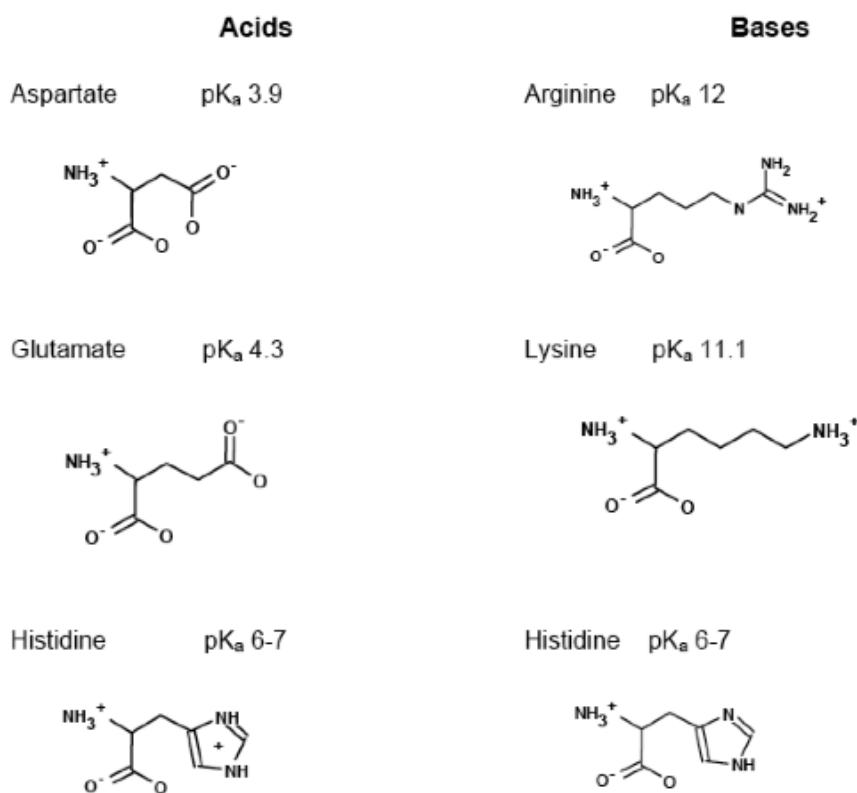


A general base pulls the hydrogen off, thus neutralise the positive charge that the proton generates:



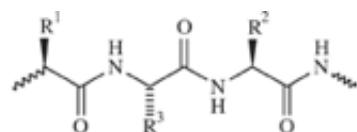
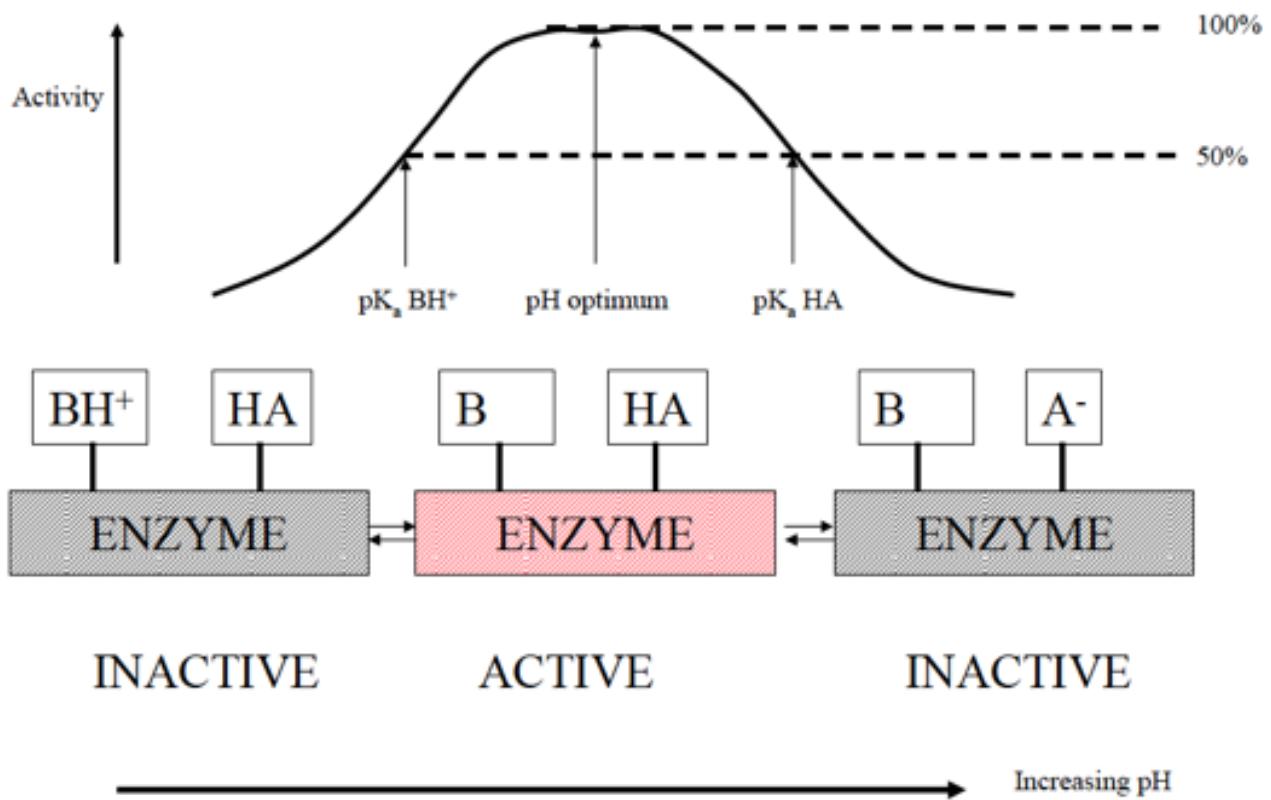
- The acid is often the proton and the base is often a hydroxyl ion
- It involves the acidic and basic side chains of amino acids: Asp, Glu, His, Lys, Cys, Tyr
- Acid - base reactions are governed by sidechain pKa's
- Catalysis often sensitive to pH changes (pKa-e.g.)
- pH - rate profiles can distinguish between acid-base catalysis and lead to the identification of participating catalytic residues (mutagenesis)

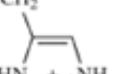
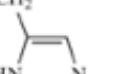
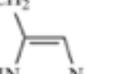
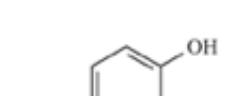
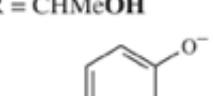
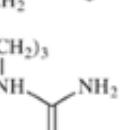
Amino acid side chains used in acid-base catalysis



- The effectiveness of general acid-base catalysts is dependent on pH since it will affect their ionisation state
- For an acid to act as an acid-catalyst it must be in its protonated form
- For a base to act as a basic catalyst it must be deprotonated
- Although an acid of pK_a 5 is a much better general acid catalyst than an acid of pK_a 7, the efficiency will depend on the pH of the reaction:
 - At pH 7, 99% of the pK_a 5 acid is in the inactive form and only 1% is protonated
 - At pH 7, a pK_a 7 acid will be 50% protonated thus making it more effective catalyst

The pH value where the titratable group is half-protonated is equal to the pK_a



Amino acid	Acid groups	pK_a	Basic groups	Nucleophilic groups
Aspartic acid	$R = \text{CH}_2\text{CO}_2\text{H}$	~4	$R = \text{CH}_2\text{CO}_2^-$	$R = \text{CH}_2\text{CO}_2^-$
Glutamic acid	$R = \text{CH}_2\text{CH}_2\text{CO}_2\text{H}$	~4	$R = \text{CH}_2\text{CH}_2\text{CO}_2^-$	$R = \text{CH}_2\text{CH}_2\text{CO}_2^-$
Histidine	$R = \text{CH}_2$ 	~7	$R = \text{CH}_2$ 	$R = \text{CH}_2$ 
Cysteine		8–9		$R = \text{CH}_2\text{SH}$ (or anion)
Lysine	$R = (\text{CH}_2)_4\text{NH}_3^+$	~9	$R = (\text{CH}_2)_4\text{NH}_2$	$R = (\text{CH}_2)_4\text{NH}_2$
Serine		High		$R = \text{CH}_2\text{OH}$ (needs gb)
Threonine		High		$R = \text{CHMeOH}$
Tyrosine	$R = \text{CH}_2$ 	~10		$R = \text{CH}_2$ 
Arginine	$R = (\text{CH}_2)_3$ 	12–13		

ACID BASE CATALYSIS RNA

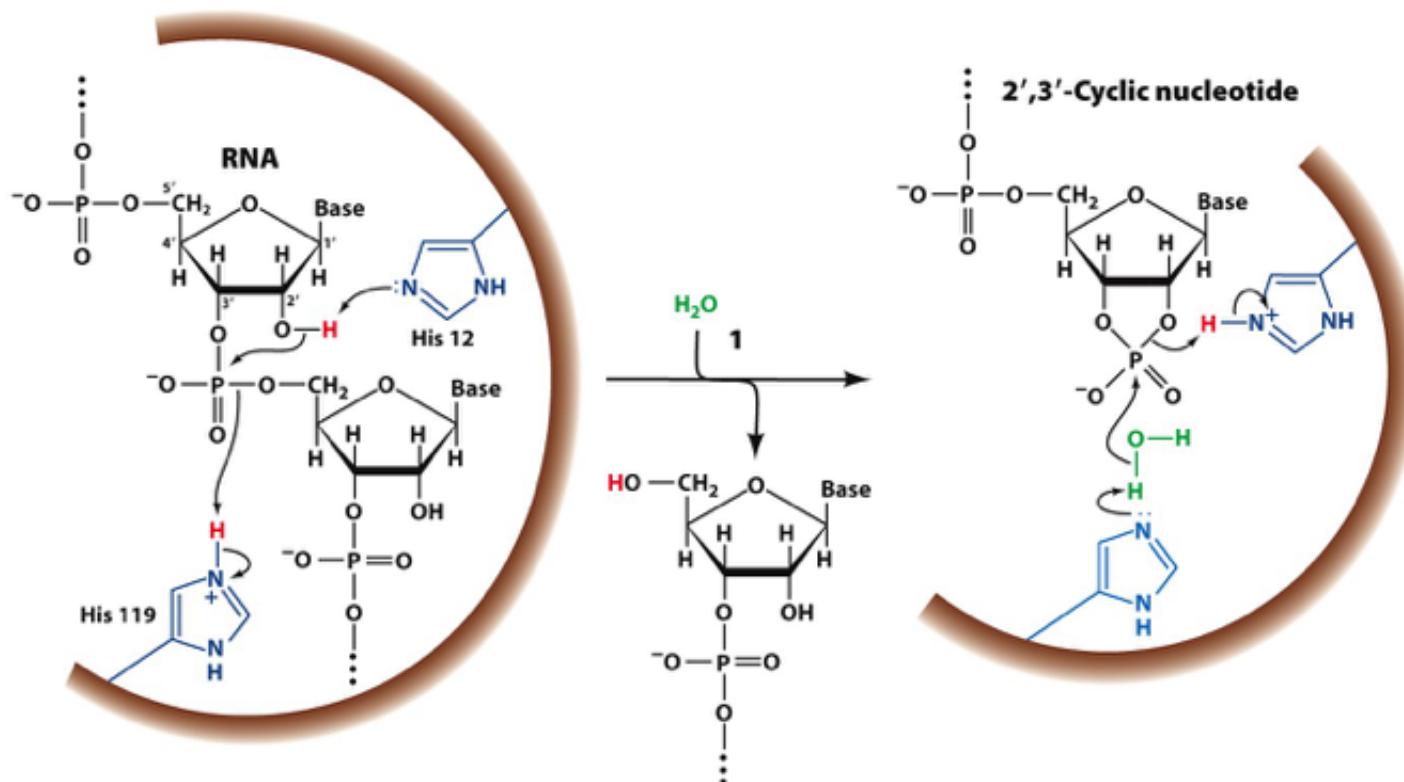


Figure 15-3 part 1
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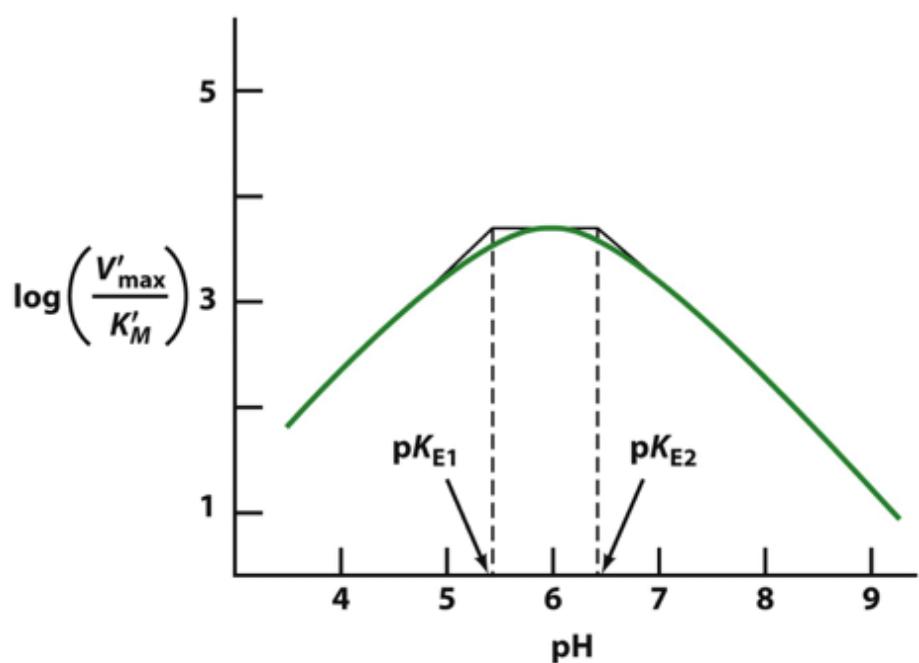
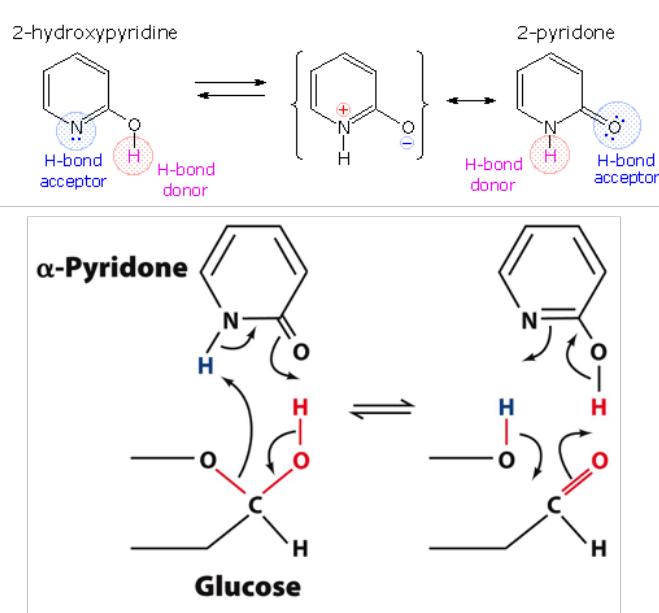
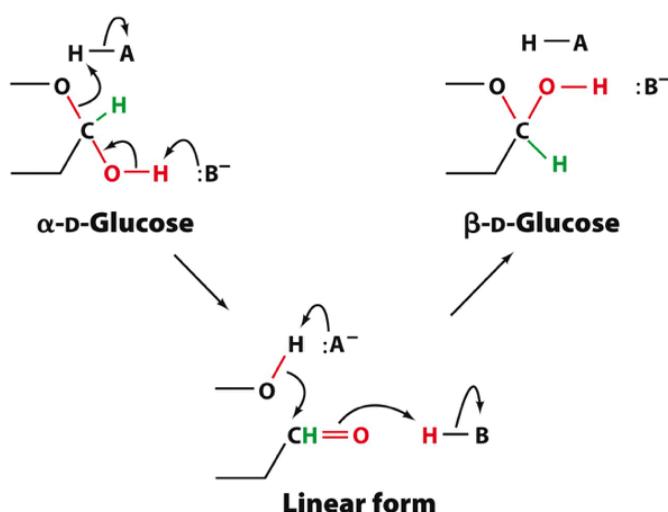
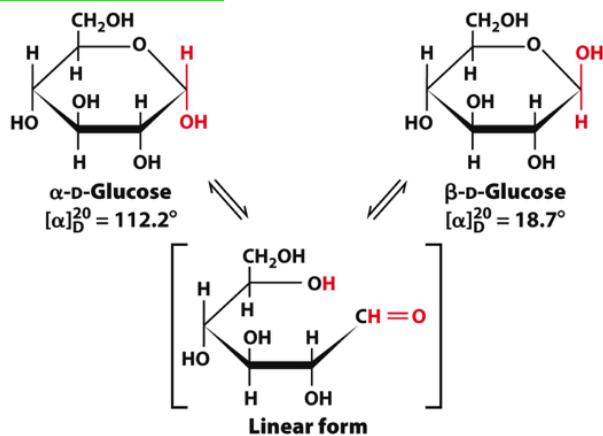


Figure 15-2
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ACID BASE: MUTAROTATION



Pyridone mimics the enzyme active site

COVALENT CATALYSIS

Transient formation of a catalyst-substrate covalent bond

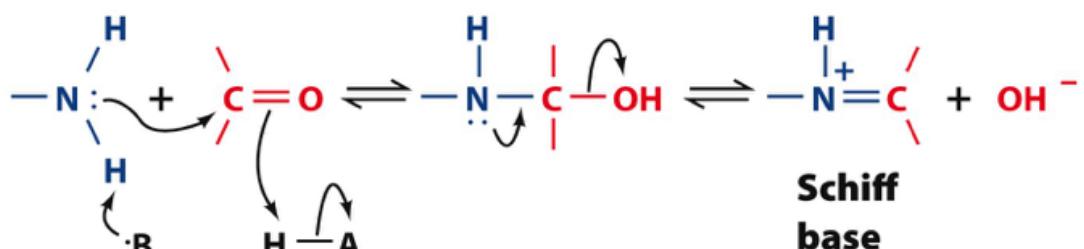
- The nucleophilic reaction between the catalyst and the substrate to form a covalent bond
- The withdrawal of electrons from the reaction center by the now electrophilic catalyst
- The elimination of the catalyst (reverse of 1.)

Nucleophilic catalysis - covalent bond formation is limiting

Electrophilic catalysis-withdrawal of electrons is rate limiting

Schiff Base Formation

- A Schiff base may form from the condensation of an amine with a carbonyl compound
- The Schiff base (protonated at neutral pH) acts as an electron sink that greatly stabilises negative charge that develops on the adjacent carbon



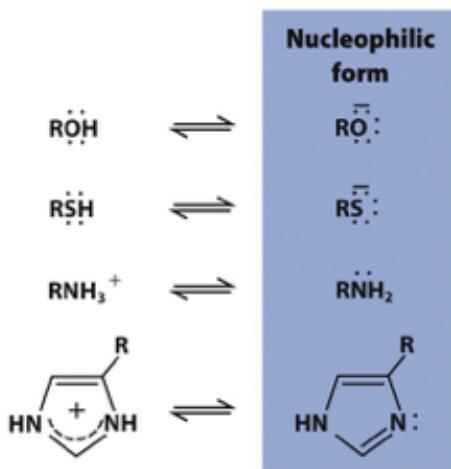
Unnumbered 15 p510
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- **Nucleophilicity is related to basicity.** Instead of abstracting a proton, nucleophilically attacks to make covalent bond
- Good covalent catalysts must have high nucleophilicity and ability to form a good leaving group.
- Polarized groups (highly mobile e-) are good covalent catalysts: imidazole, thiols
- Coenzymes: thiamine pyrophosphate, pyridoxal phosphate.

What kind of groups in proteins are good nucleophiles:

• Aspartate	caboxylates	$\text{R} = -\text{CH}_2-\text{C}(=\text{O})\text{O}^-$
Glutamates	caboxylates	$\text{R} = -\text{CH}_2-\text{CH}_2-\text{C}(=\text{O})\text{O}^-$
• Cystine	thiol-	$\text{R} = -\text{CH}_2\text{SH}$
Serine	hydroxyl-	$\text{R} = -\text{CH}_2\text{OH}$
Tyrosine	hydroxyl-	$\text{R} = -\text{CH}_2-\text{C}_6\text{H}_4-\text{OH}$
• Lysine	amino-	$\text{R} = -\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_3^+$
Histadine	imidazolyl-	$\text{R} = -\text{CH}_2-\text{C}_5\text{H}_4\text{N}(\text{H}^+)_2$

(a) Nucleophiles



(b) Electrophiles

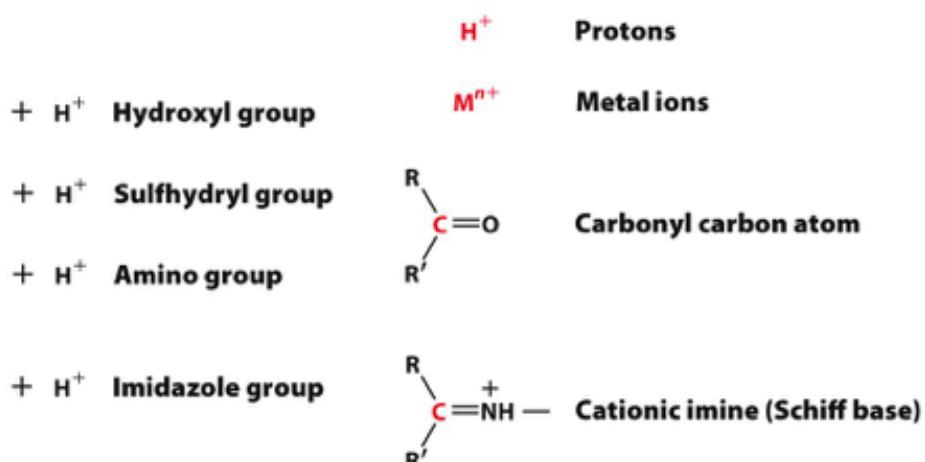


Figure 16-5

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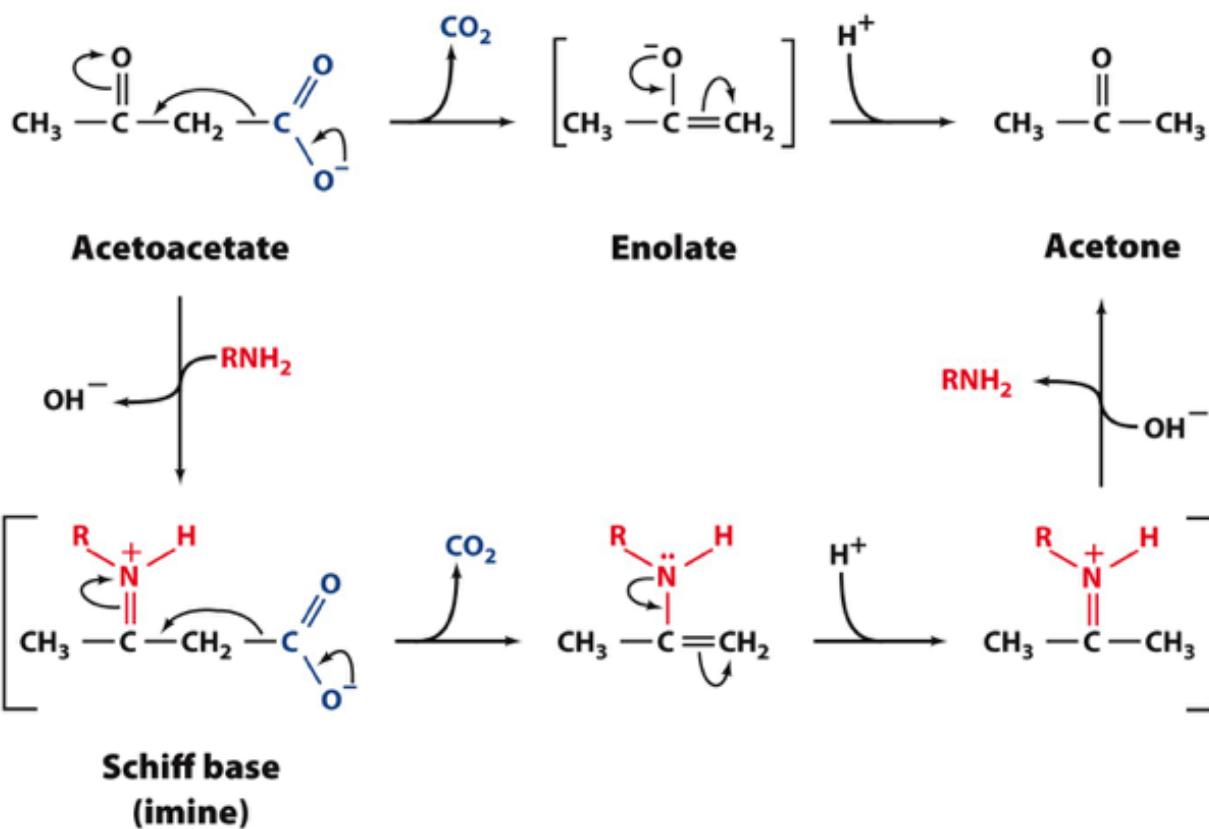


Figure 15-4
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PENICILLIN

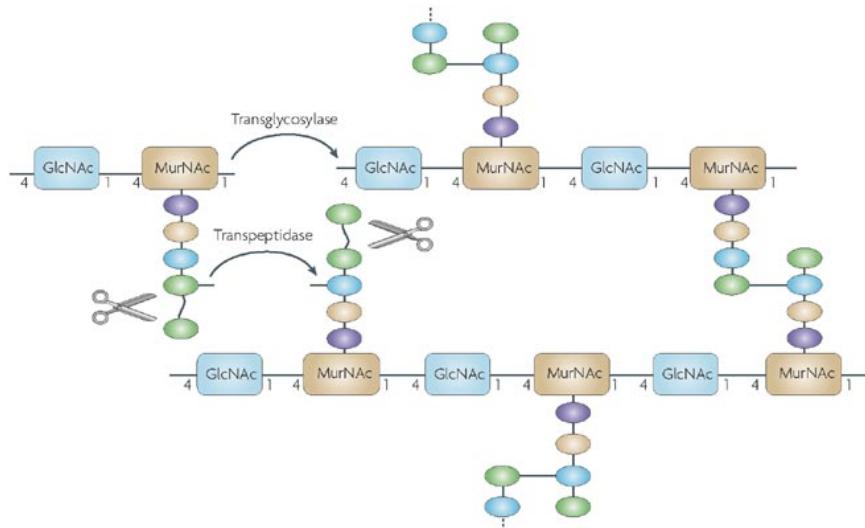
Discovered in 1928 by Alexander Fleming

Penicillium notatum

Inhibits **glycopeptide transpeptidase**, a bacterial enzyme that catalyses the cross-linking of peptidoglycan chains during the synthesis of cell walls

Does not kill existing bacteria but daughter cells are susceptible to osmotic lysis and this halts the growth of the population.

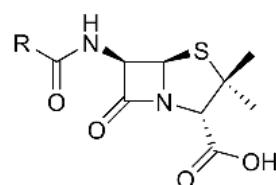
FORMATION OF CELL WALL: (Cuts d-ala and d-ala, cross linking)



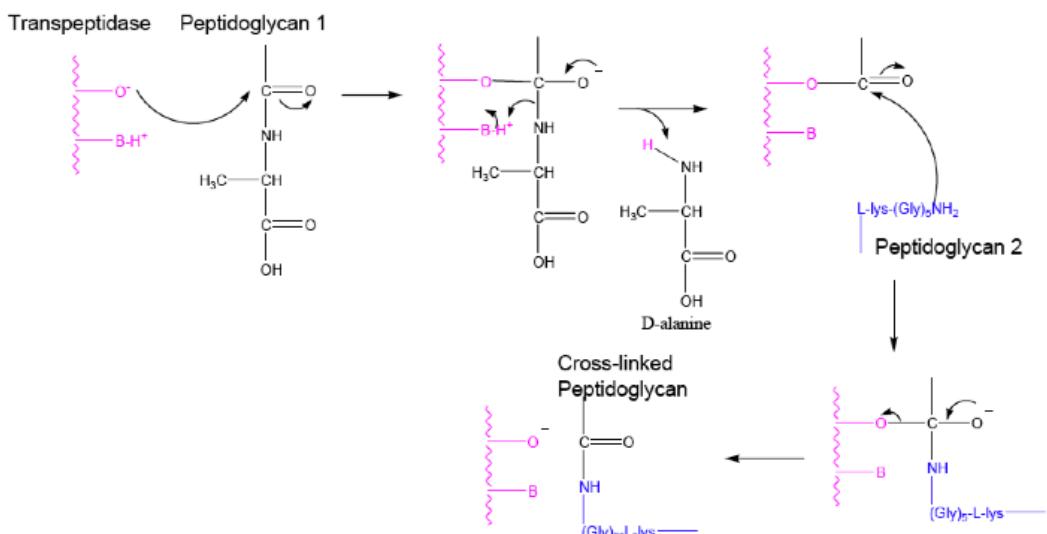
Nature Reviews | Microbiology

Most important part of all penicillins is the β -lactam- ring

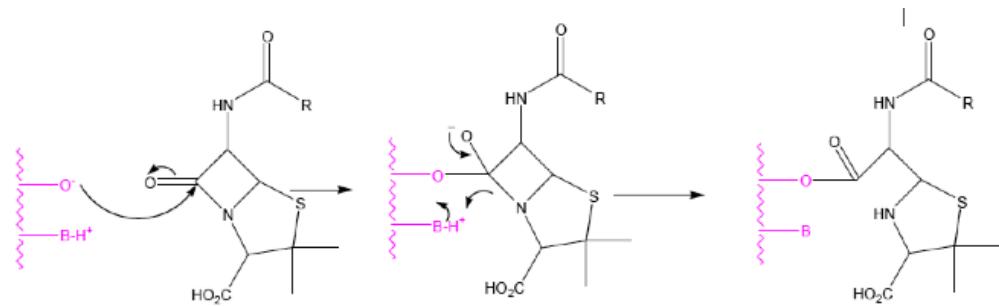
The derivatives have different substituents (-R) with specific functions



TRANSPEPTIDASE MECHANISM

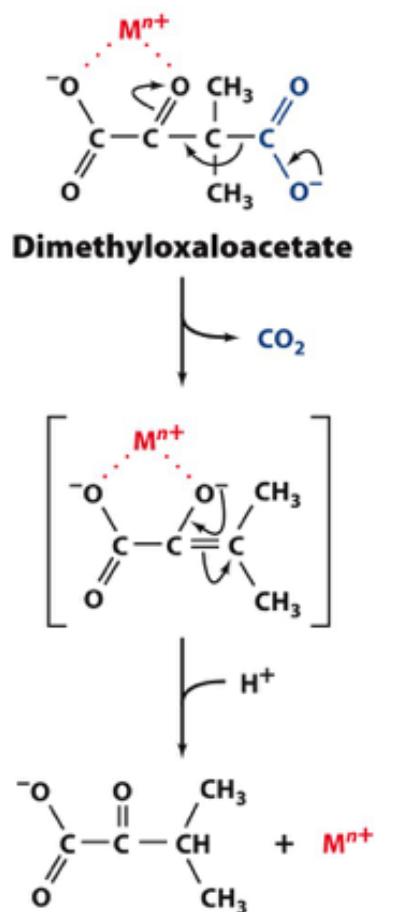


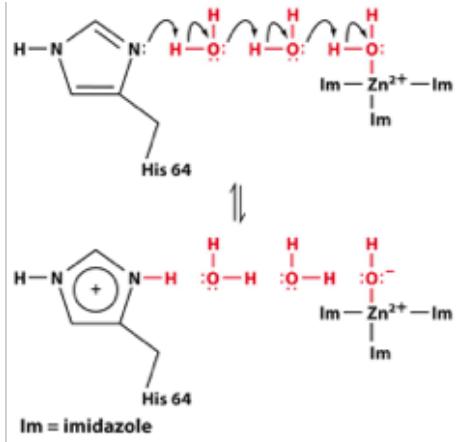
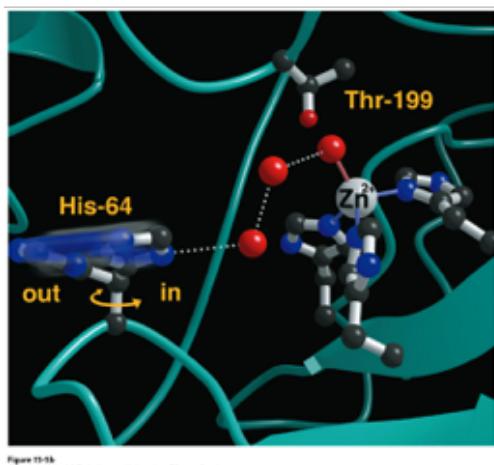
Penicillin inhibits transpeptidation : Serine residue, Tetrahedral intermediate, collapses, reduction of N



METAL ION CATALYSIS

- **Metalloenzymes:**
 - Tightly bound metal ions eg. Fe^{2+} , Fe^{3+} , Cu^{2+} , Mn^{2+} , Zn^{2+}
- **Metal-activated enzymes:**
 - Loosely bind metals from solution eg. Na^+ , Mg^{2+} , Ca^{2+}
 - Binding to substrates and orient them properly
 - Mediate oxidation/reduction reactions through changes in the metals oxidation state
 - Shielding negative charges (electrostatic stabilisation)



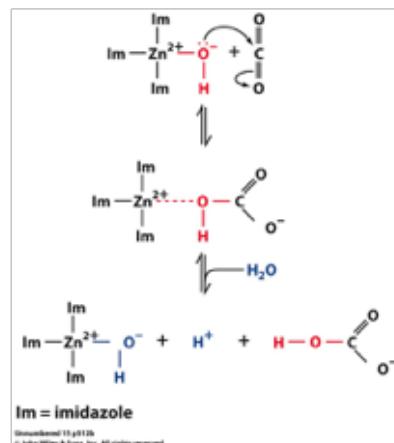


Zn assists in the formation of a nucleophile (OH^-)

CO_2 binds at the active site and positioned to react with the hydroxide

OH^- attacks the CO_2 converting it to bicarbonate

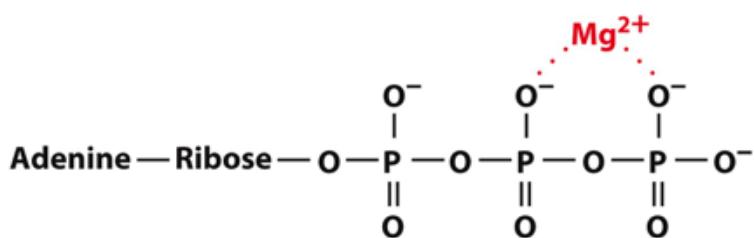
Water binds to the active site and releasing product



- Metals can act as 'superacids':
metal ions can be present at high concentrations at neutral pH and have charges greater than +1
- Acts as a Lewis acid
- Nucleophilic catalysis via water ionisation
- Metal-bound hydroxyl groups are potent nucleophiles at neutral pH
 - The pKa for free water is ~15; difficult to get hold of a hydroxide ion at neutral pH
 - A metal-bound water molecule of pKa 7 is most effective for nucleophilic attack

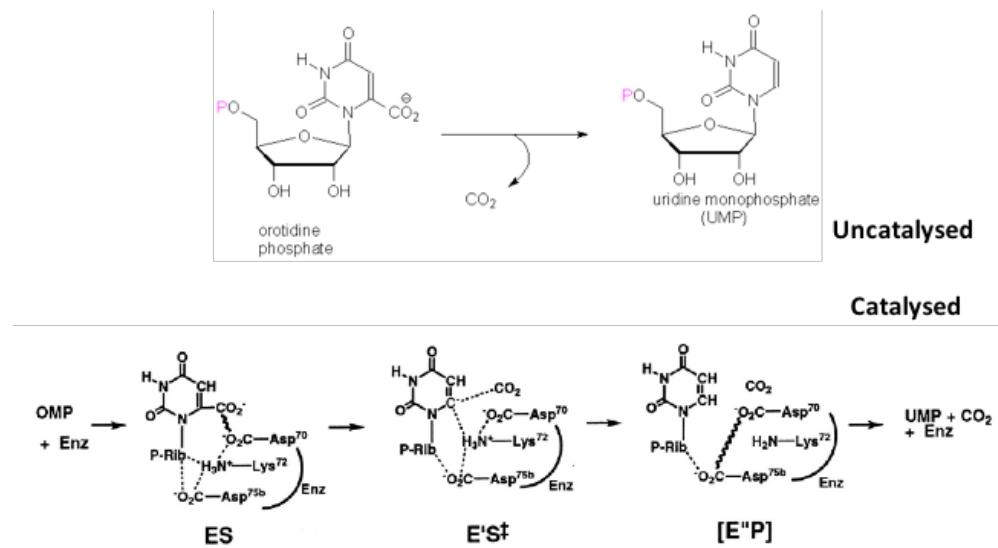
CHARGE SHIELDING

- Electrostatically shielding of negative charges
eg. Negative charges in the phosphate groups of ATP are shielded by Mg^{2+}



ELECTROSTATIC CATALYSIS

- Enzymes arrange active site charge distributions to stabilise the transition states of catalysed reactions
- Substrate binding generally excludes water from an enzyme active site generating a low dielectric constant within the active site
- Electrostatic interactions are stronger: pKa can vary by several pH units due to proximity of charged groups
- Alternative form of electrostatic catalysis: Several enzymes (eg. superoxide dismutase) apparently use charge distributions to guide polar substrates to their active sites (substrate channeling)

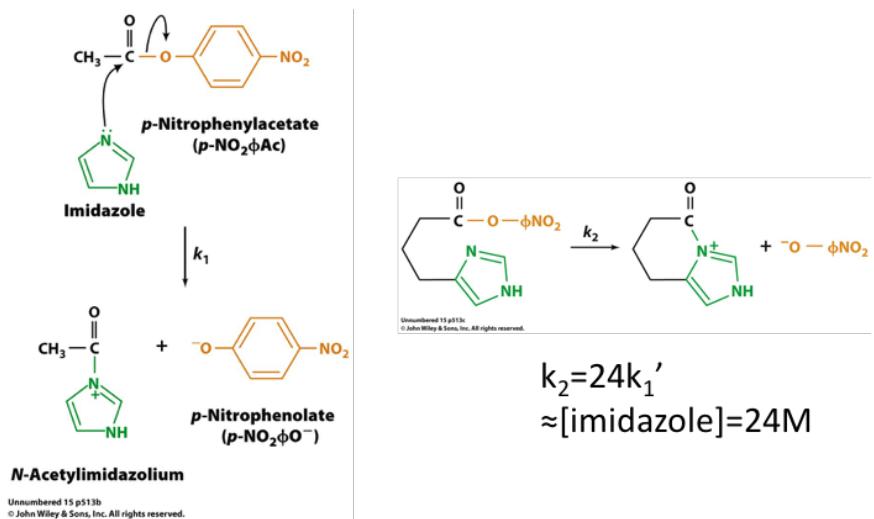


OROTIDINE 5'-PHOSPHATE DECARBOXYLASE

The enzyme can catalyse the substrate in 18 milliseconds, in a reaction that would take 78 million years to complete without the enzyme

PROXIMITY AND ORIENTATION EFFECTS

- Substrate binding has additional effects that enhance reaction rates: proximity & orientation
- Reactants must come together with the proper spatial relationship for a reaction to occur
- Proximity effects (minor) are most readily observed by comparing equivalent inter- and intramolecular reactions
- Intramolecular reactions are typically 10-100 fold more rapid
- Orientation effects are more significant though difficult to quantify
- Theory suggest molecules are maximally reactive when their orbitals are aligned so the electronic energy of the transition state is minimised (stereoelectronic assistance)



$$k_1' = 0.043 \text{ s}^{-1}$$

$$[\text{imidazole}] = 1\text{ M}$$

STEREOELECTRONIC ASSISTANCE

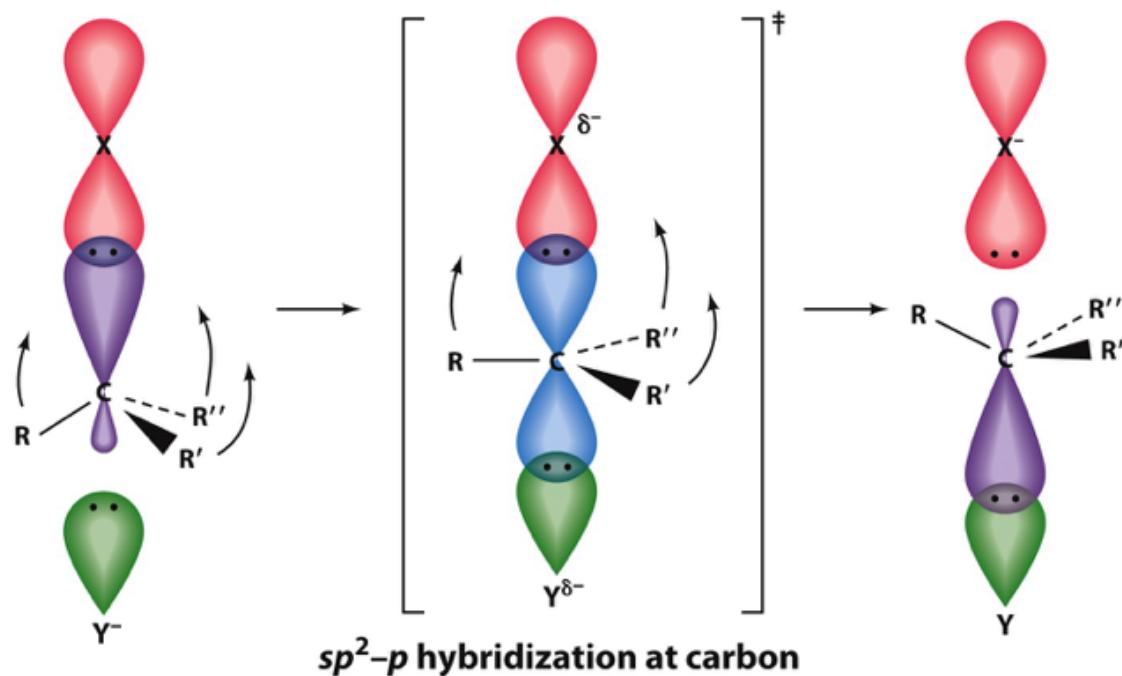


Figure 15-6
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TRANSITION STATE BINDING

- Enzymes bind the transition state with higher affinity than the substrate or product
- Transition state analogues are excellent competitive inhibitors
- Together with proximity and orientation effects, accounts for rate enhancement in many enzymes
- Enzyme mechanically strain substrates towards transition states

- Rate enhancement (ie. $\text{DDG}\ddagger$) can be expressed in terms of enzyme affinity for transition state compared relative to substrate
- Explains why good and bad substrates typically have similar K_M value but different k_{cat} values
- A good substrate does not need to bind tightly to the enzyme but must bind tightly when activated to the transition state

TRANSITION STATE ANALOGUES

Proline racemase

