Medicinal Chemistry

Chapter 4

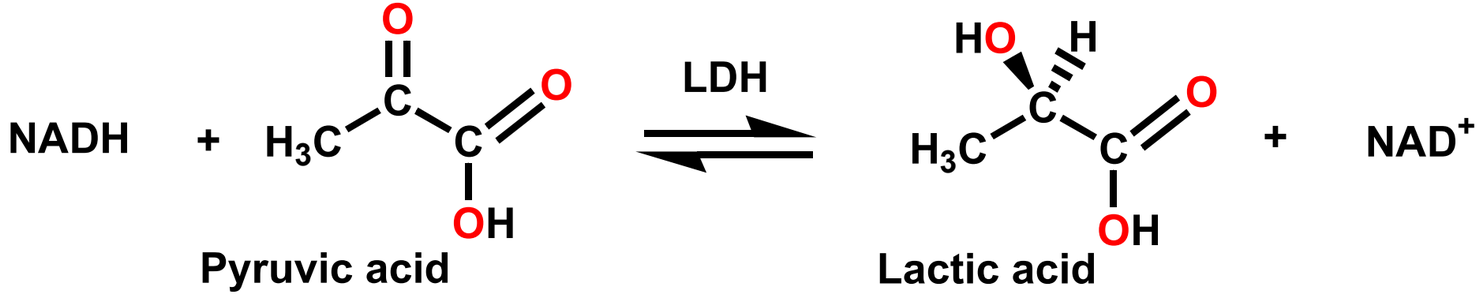
DRUG TARGETS: ENZYMES

Contents

1. Structure and function of enzymes

Enzymes are proteins which act as the body's catalysts agents that speed up a chemical reaction without being consumed themselves. Without them, the cell's chemical reactions would either be too slow or not take place at all.

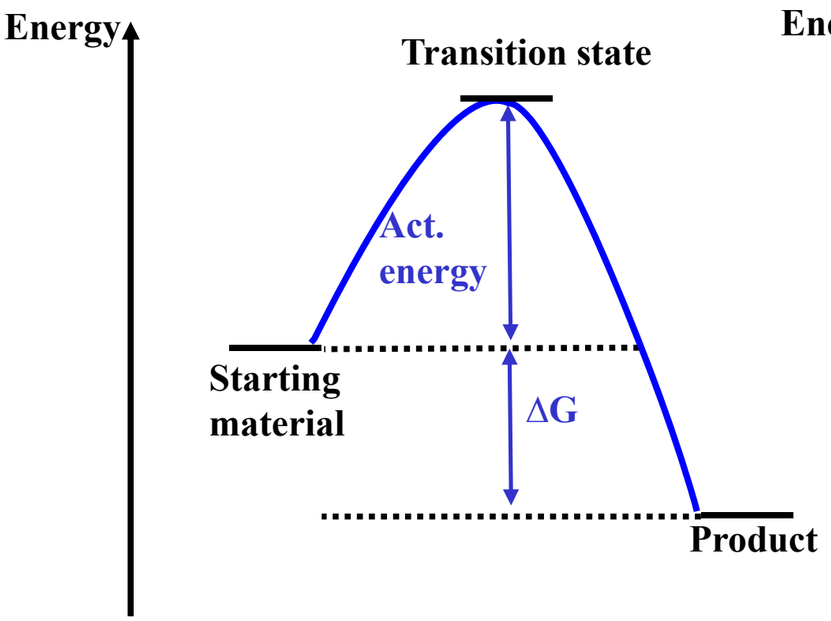
Example:



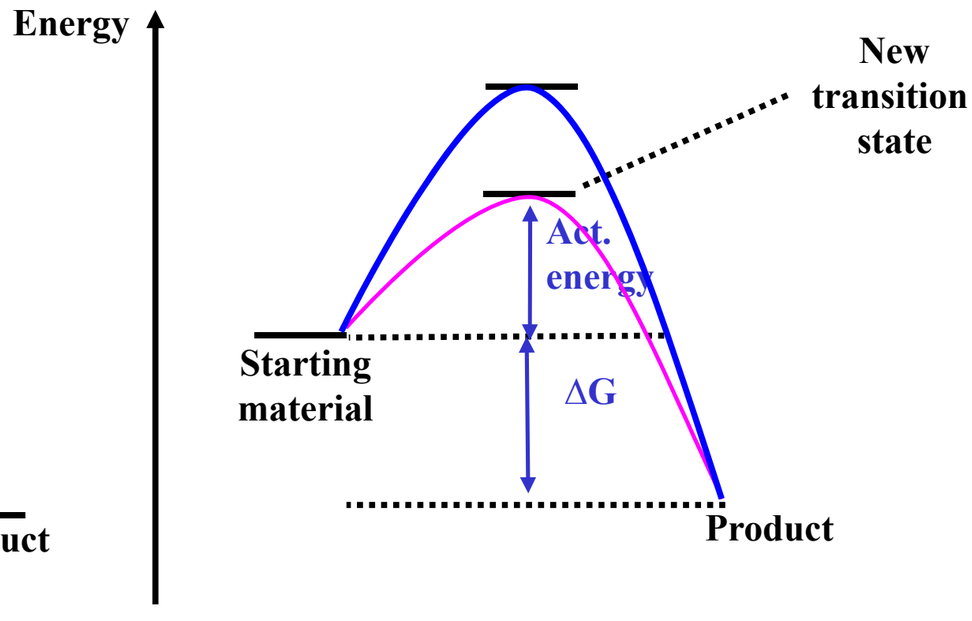
LDH = Lactate dehydrogenase (enzyme)
NADH = Nicotinamide adenosine dinucleotide (reducing agent & cofactor)
Pyruvic acid = Substrate

1. Structure and function of enzymes

An enzyme acts to lower the activation energy by helping to stabilize the transition state.



WITHOUT ENZYME



WITH ENZYME

The energy of the substrate and products are unaffected.

The equilibrium ratio of substrate to product is unaffected.

$\Delta G = -RT \ln K$
where K is the equilibrium constant (= [products]/[reactants]), R is the gas constant (= 8.314 J mol $^{-1}$ K $^{-1}$), and T is the temp.

1. Structure and function of enzymes

Methods of enzyme catalysis

- **Provides a reaction surface (the active site)**
- **Provides a suitable environment (hydrophobic)**
- **Brings reactants together**
- **Positions reactants correctly for reaction**
- **Weakens bonds in the reactants**
- **Provides acid / base catalysis**
- **Provides nucleophilic groups**
- **Stabilises the transition state with intermolecular bonds**

2. The Active Site

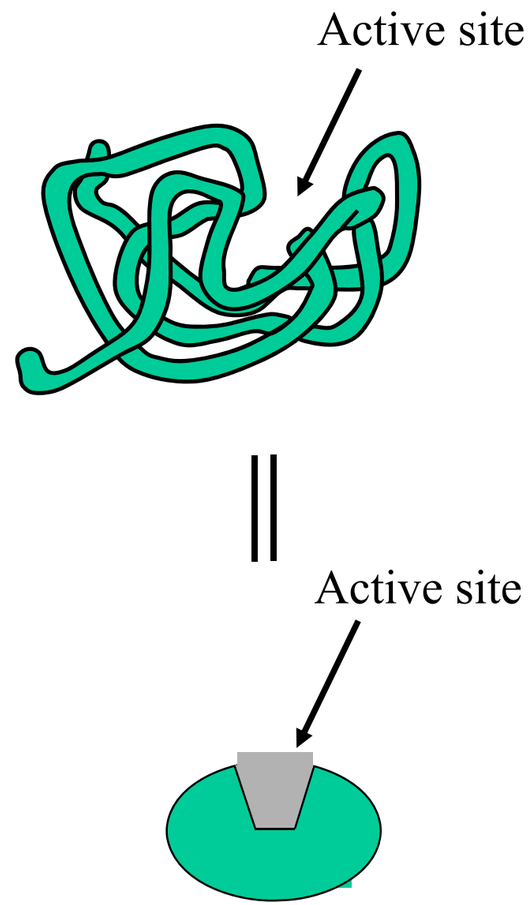
The site could be a groove, hollow, or gully allowing the substrate to sink into the enzyme.

Normally, the active site is more hydrophobic in character than the surface of the enzyme, providing a suitable environment for many reactions that would be difficult or impossible to carry out in an aqueous environment.

Amino acids present in the active site can have one of two roles:

- binding—the amino acid residue is involved in binding the substrate or a cofactor to the active site;

- catalytic—participate in the enzyme-catalysed reaction

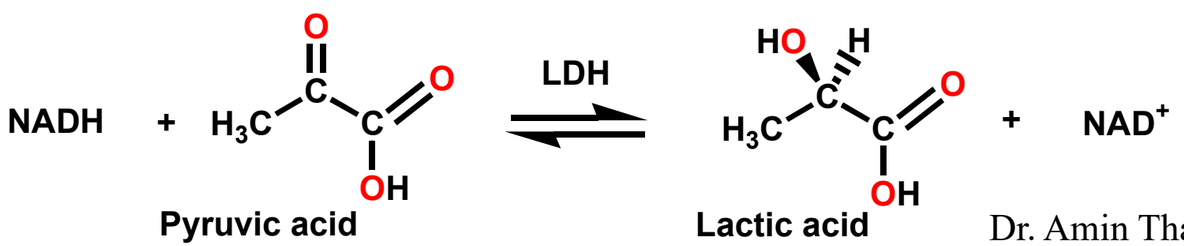


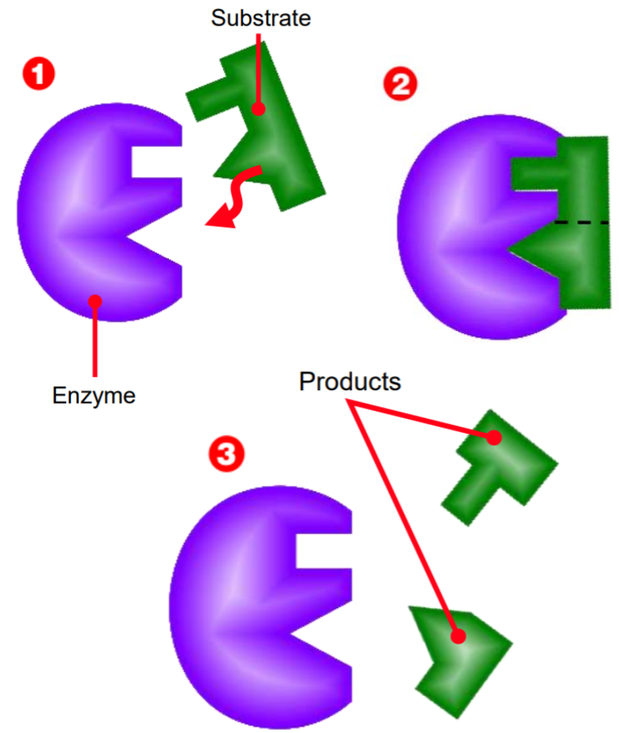
3. Substrate Binding

Key & Lock

In the past, it was thought that a substrate fitted its active site in a similar way to a key fitting a lock (**Fischer's lock and key hypothesis**).

- However, this does not explain how some enzymes can catalyse a reaction on a range of different substrates.
- This theory implies that only enzyme has an optimum substrate that fits it perfectly and can be catalysed whereas all other substrates are catalysed less efficiently.
- This is not the case, so the lock and key analogy is invalid.





1. A substrate is drawn into the active sites of the enzyme.
2. The substrate shape must be compatible with the enzymes active site in order to fit and be reacted upon.
3. The enzyme modifies the substrate. In this instance the substrate is broken down, releasing two products

3. Substrate Binding

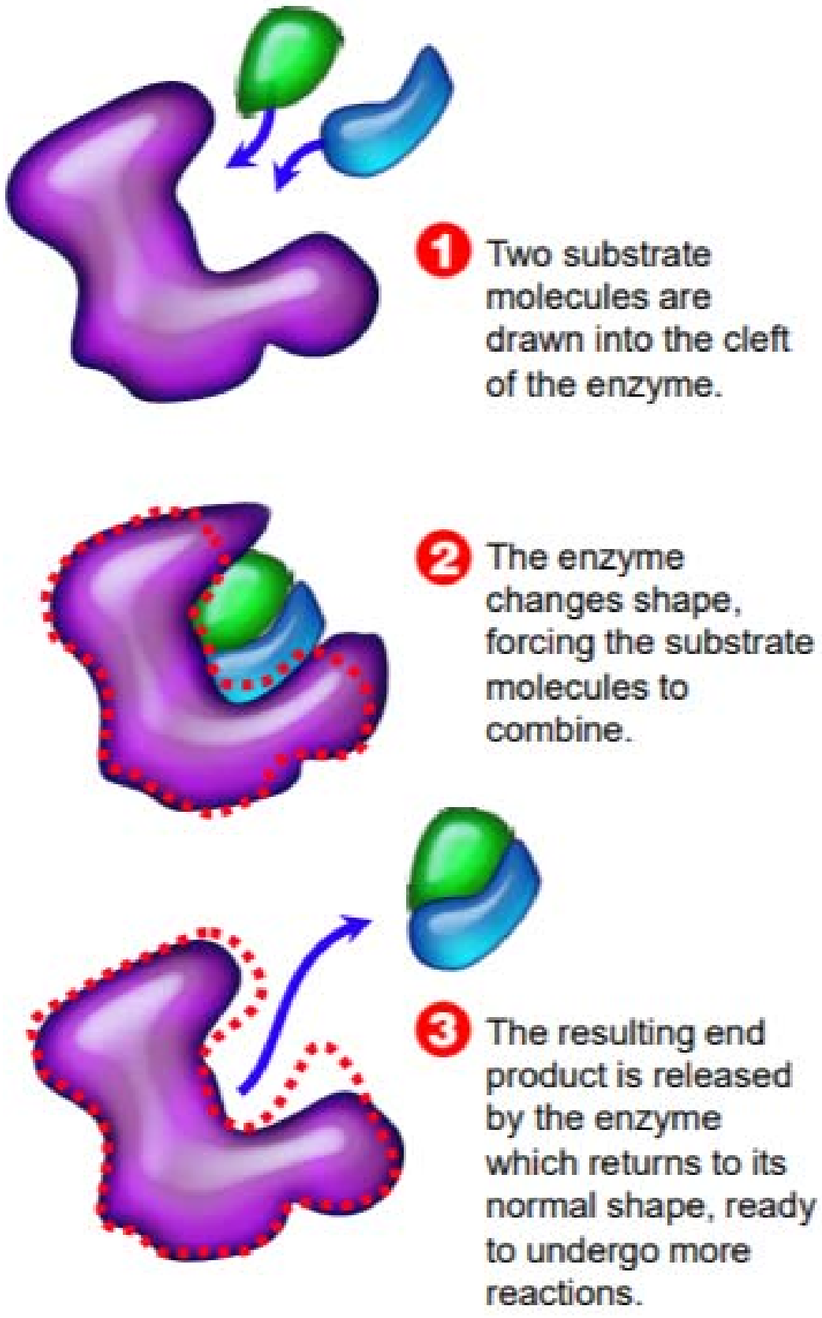
Induced fit

More recent studies have revealed that the process is much more likely to involve an induced fit.

The enzyme or the reactants (substrate) change their shape slightly.

The reactants become bound to enzymes by weak chemical bonds.

This binding can weaken bonds within the reactants themselves, allowing the reaction to proceed more readily

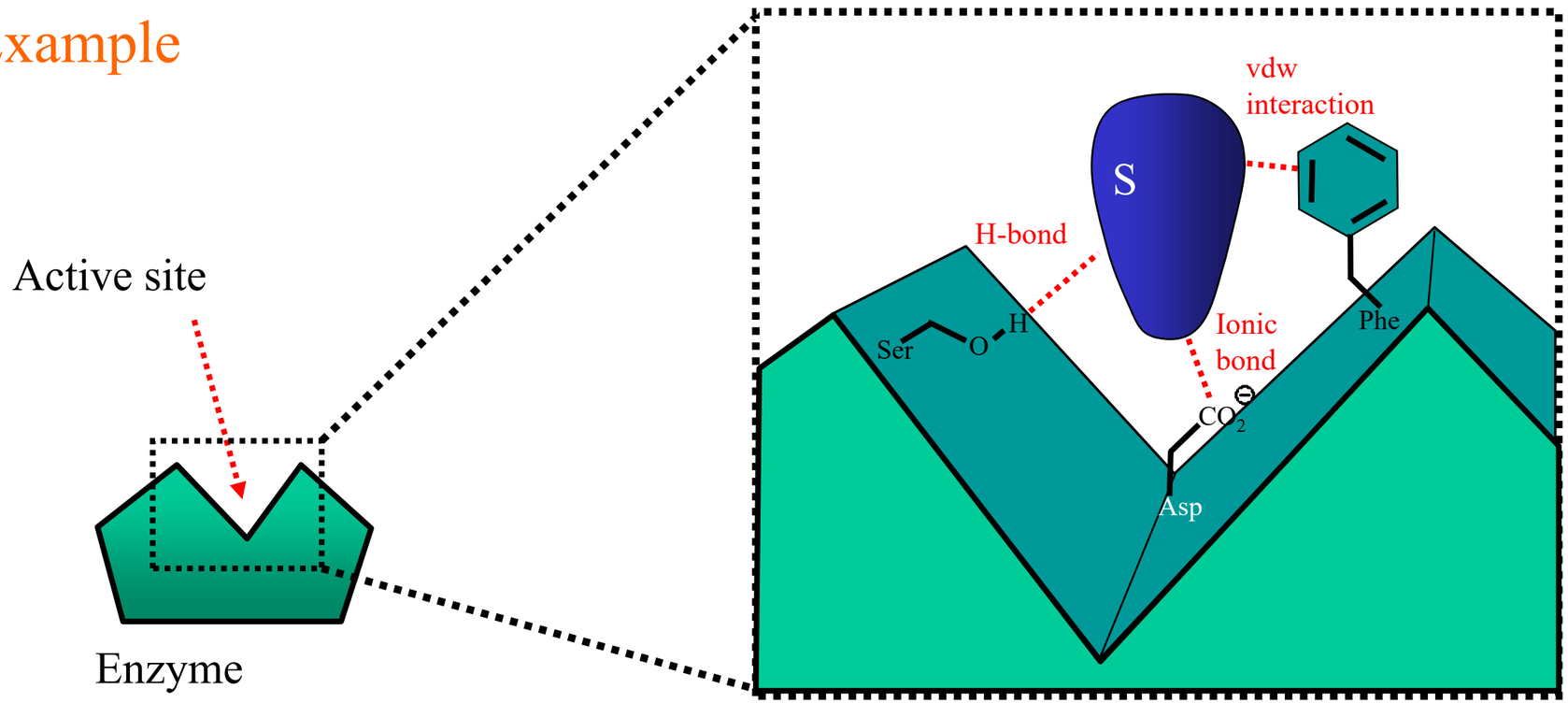


3. Substrate Binding

Bonding forces

- Ionic
- H-bonding
- van der Waals

Example

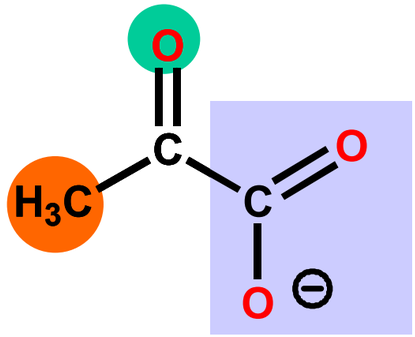


3. Substrate Binding

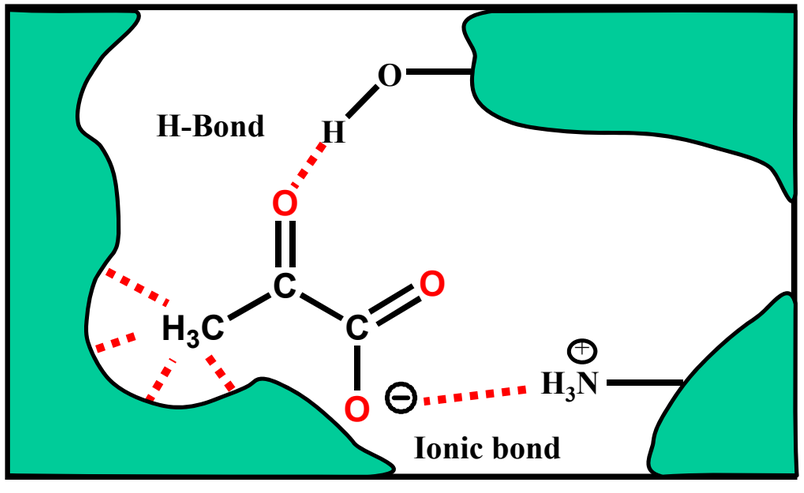
Bonding forces

A pyruvic acid as a substrate, three possible interactions by which it might bind to its active site

- ionic interaction involving the ionized carboxylate group
- hydrogen bond involving the ketonic oxygen
- van der Waals interaction involving the methyl group



Possible interactions

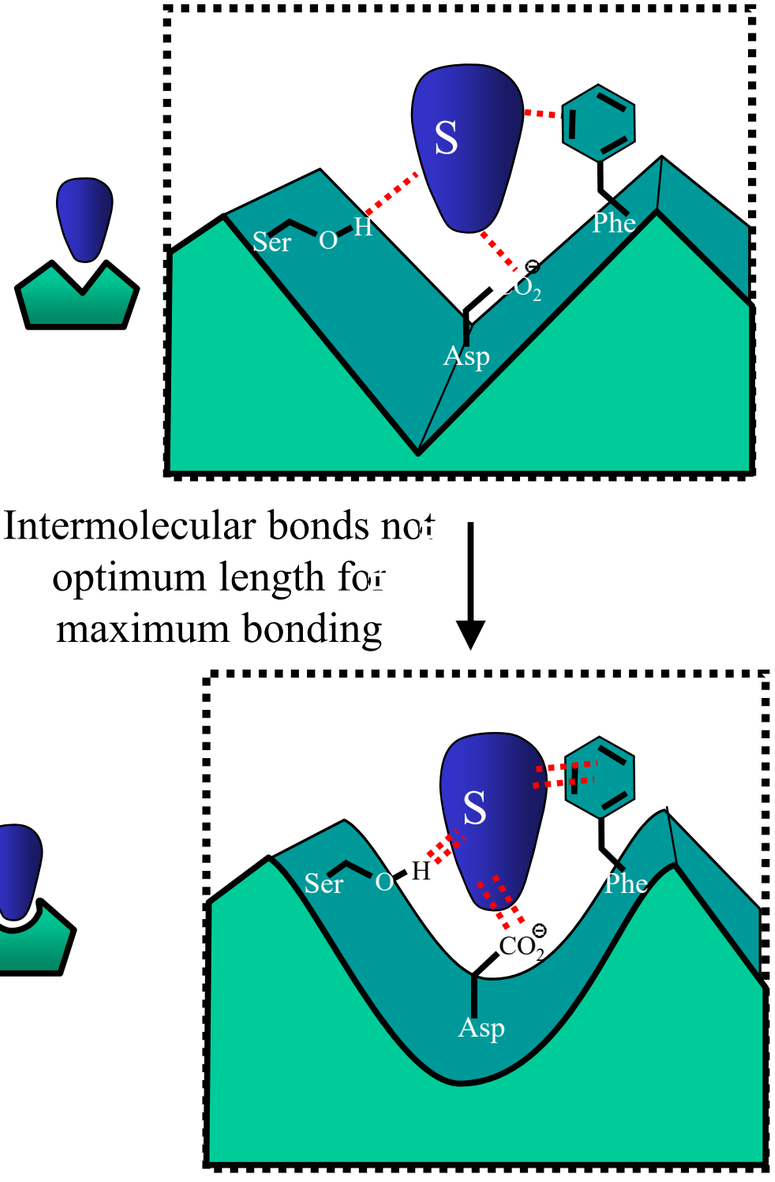


3. Substrate Binding

Bonding forces

The binding groups may be slightly too far away from the corresponding binding regions in the active site. In order to maximize the strength of these bonds, the enzyme changes shape such that the amino acid residues involved in the binding move closer to the substrate.

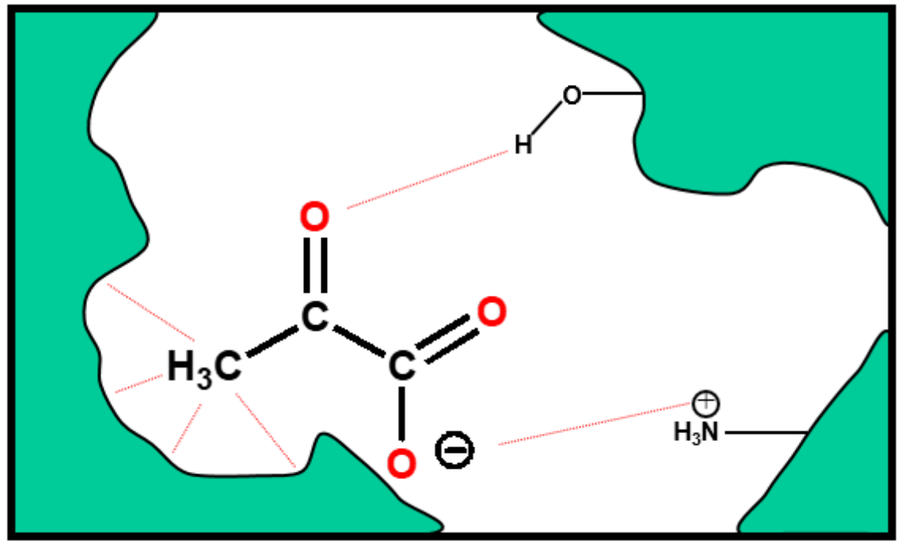
As the enzyme changes shape to maximize bonding interactions, the same thing can happen to the substrate. It too may alter shape. Bond rotation may occur to fix the substrate in a particular conformation—and not necessarily the most stable one. Bonds may even be stretched and weakened.



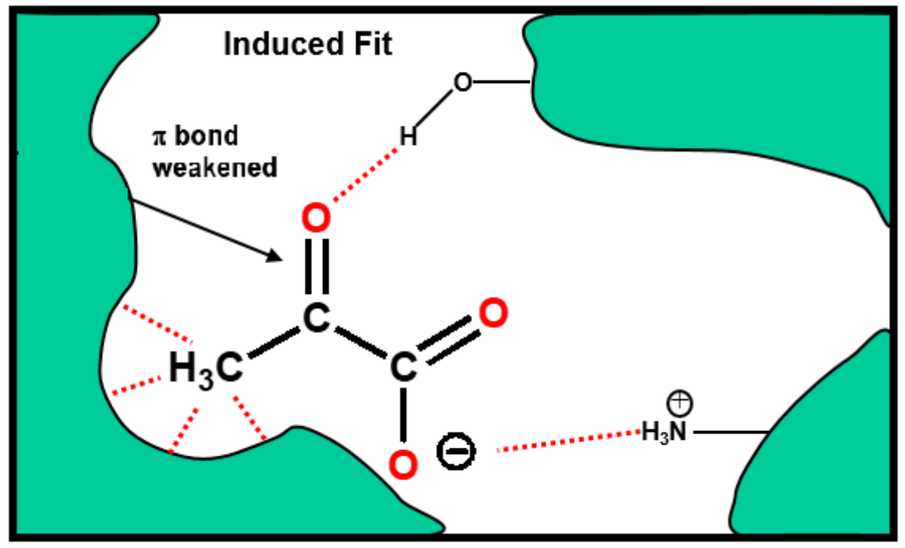
3. Substrate Binding

Binding of pyruvic acid in LDH

The fit is not perfect, the three bonding interactions are not ideal either. Each substrate induces the active site into a shape that is ideal for it and, as long as the moulding process does not distort the active site so much that the reaction mechanism proves impossible, the reaction can proceed.



This moulding process designed to **maximize binding interactions** may force the substrate into the **ideal conformation** for the reaction **to follow and may also weaken the very bonds that have to be broken.** Once bound to an active site, the substrate is now held ready for the subsequent reaction. **Binding has fixed the 'victim' (substrate**) so that it cannot evade attack, and this same binding has weakened its defenses (bonds) so that reaction is easier (a lower activation energy).



4. Catalysis mechanisms

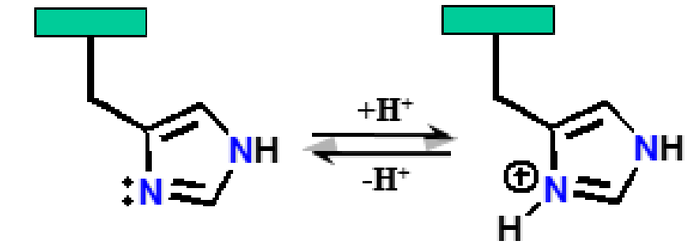
4.1 Acid/base catalysis

Acid/base catalysis is often provided by the amino acid **histidine** , which contains an imidazole ring as part of its side chain.

The imidazole ring acts as a weak base, which means that it exists in equilibrium between its protonated and free base forms allowing it to accept or donate protons during a reaction mechanism.

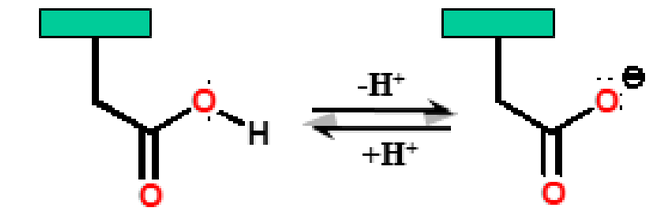
This is important, as there are often very few water molecules present in an active site to carry out this role.

Aspartic acid and aspartate residues act as proton donors and proton acceptors, respectively, in other enzyme-catalysed reactions



Non-ionised
Acts as a basic catalyst
(proton 'sink')

Ionised
Acts as an acid catalyst
(proton source)



Non-ionised
Acts as an acid catalyst
(proton source)

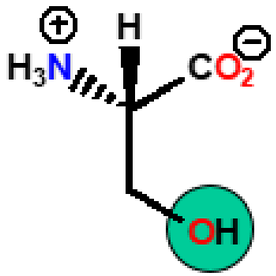
Ionised
Acts as a basic catalyst
(proton 'sink')

4. Catalysis mechanisms

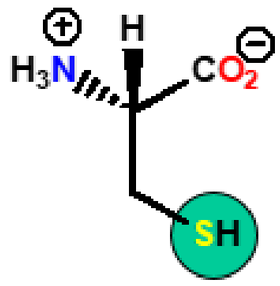
4.2 Nucleophilic residues

The amino acids serine and cysteine are present in the active sites of some enzymes. These amino acids have nucleophilic residues which are able to participate in the reaction mechanism.

They do this by reacting with the substrate to form intermediates that would not be formed in the uncatalysed reaction. These intermediates offer an alternative reaction pathway that may avoid a high-energy transition state and hence increase the rate of the reaction.



L-Serine

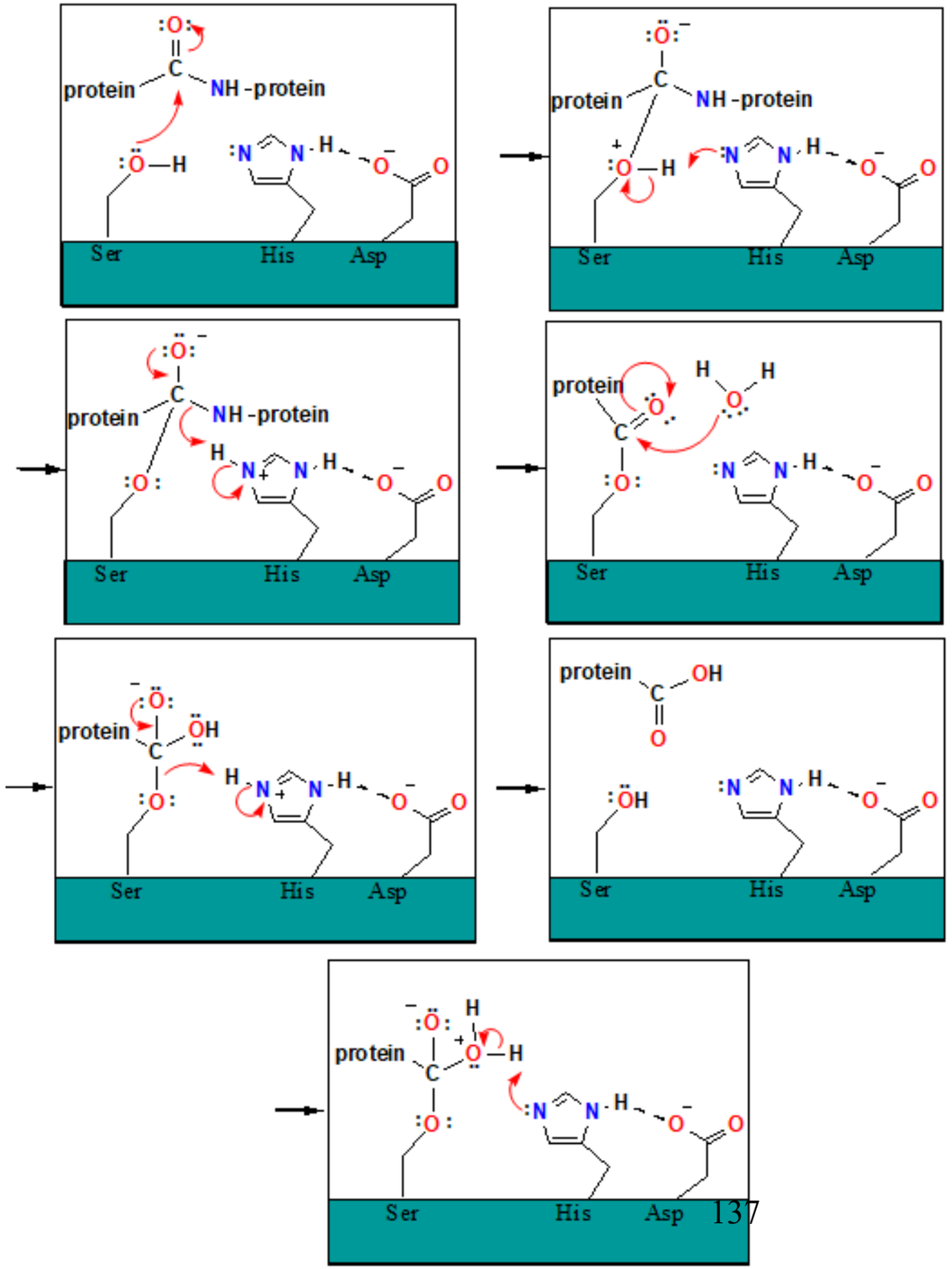


L-Cysteine

4. Catalysis mechanisms

Mechanism for chymotrypsin

Chymotrypsin hydrolyses peptide bonds involves a **catalytic triad (three strength)** of amino acids—serine, histidine, and aspartic acid. Serine and histidine participate in the mechanism as a nucleophile and acid/base catalyst respectively. The aspartate group interacts with the histidine ring and serves to activate and orient it correctly for the mechanism.



5. Naming and classification of enzymes

Systematic Name

- According to the International union Of Biochemistry an enzyme name has twoparts:

- First part is the name of the substrates for the enzyme.
- Second part is the type of reaction catalyzed by the enzyme.This part ends with the suffix “ase”.

Example: Lactate dehydrogenase

IUB Classification of Enzymes

- Enzymes are classified according to the reaction they catalyze.

Class	Reactions catalyzed
• Oxidoreductases	**Oxidation-reduction**
• Transferases	**Transfer groups of atoms**
• Hydrolases	**Hydrolysis**
• Lyases	**Add atoms/remove atoms to/from a double bond**
• Isomerases	**Rearrange atoms**
• Ligases	**Use ATP to combine molecules**

EC 1. Oxidoreductases

- Catalyze the transfer of hydrogen or oxygen atoms or electrons from one substrate to another.

$$A\,H_2 + B \rightarrow A + BH_2$$

EC 2. Transferases

- Catalyze group transfer reactions, excluding oxidoreductases (which transfer hydrogen or oxygen and are EC 1). These are of the general form:

$$A\text{-}X + B \leftrightarrow BX + A$$

EC 3. Hydrolases

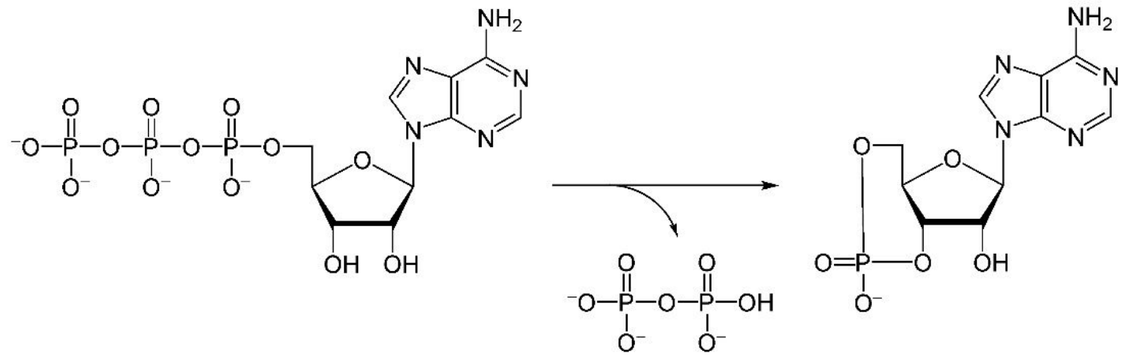
- Catalyze hydrolytic reactions. Includes.

$$A\text{-}X + H_2O \leftrightarrow X\text{-}OH + A\text{-}H$$

EC 4. Lyases

- Catalyze non-hydrolytic (covered in EC 3) removal of functional groups from substrates, often creating a double bond in the product; *or the reverse reaction, ie, addition of function groups across a double bond.*

$$A\text{-}X + B\text{-}Y \rightarrow A{=}B + X\text{-}Y$$

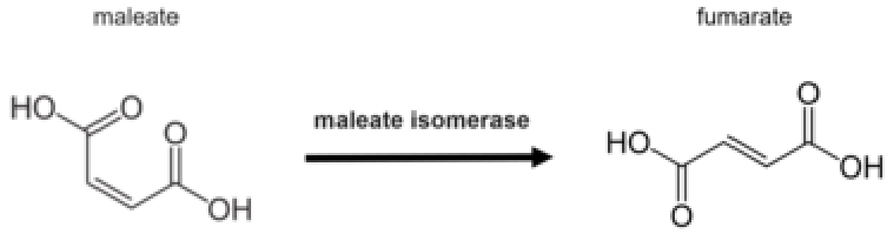


EC 5. Isomerases

- Catalyzes isomerization reactions, including epimerizations and cis-trans
- isomerizations.

$$A \rightarrow A'$$

Ex: Isomerases (Cis-Trans), Epimerases (D—L)

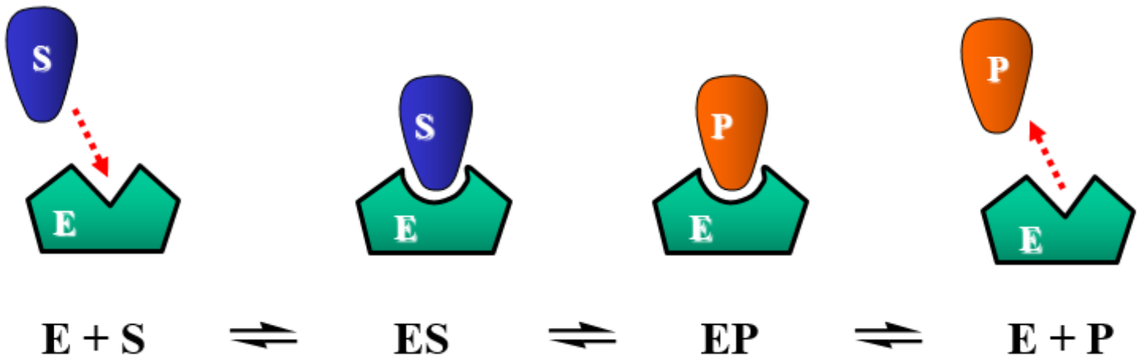


EC 6. Ligases

- Catalyzes the synthesis of various (mostly C-X) bonds, coupled with the breakdown of energy- containing substrates, *usually ATP*.

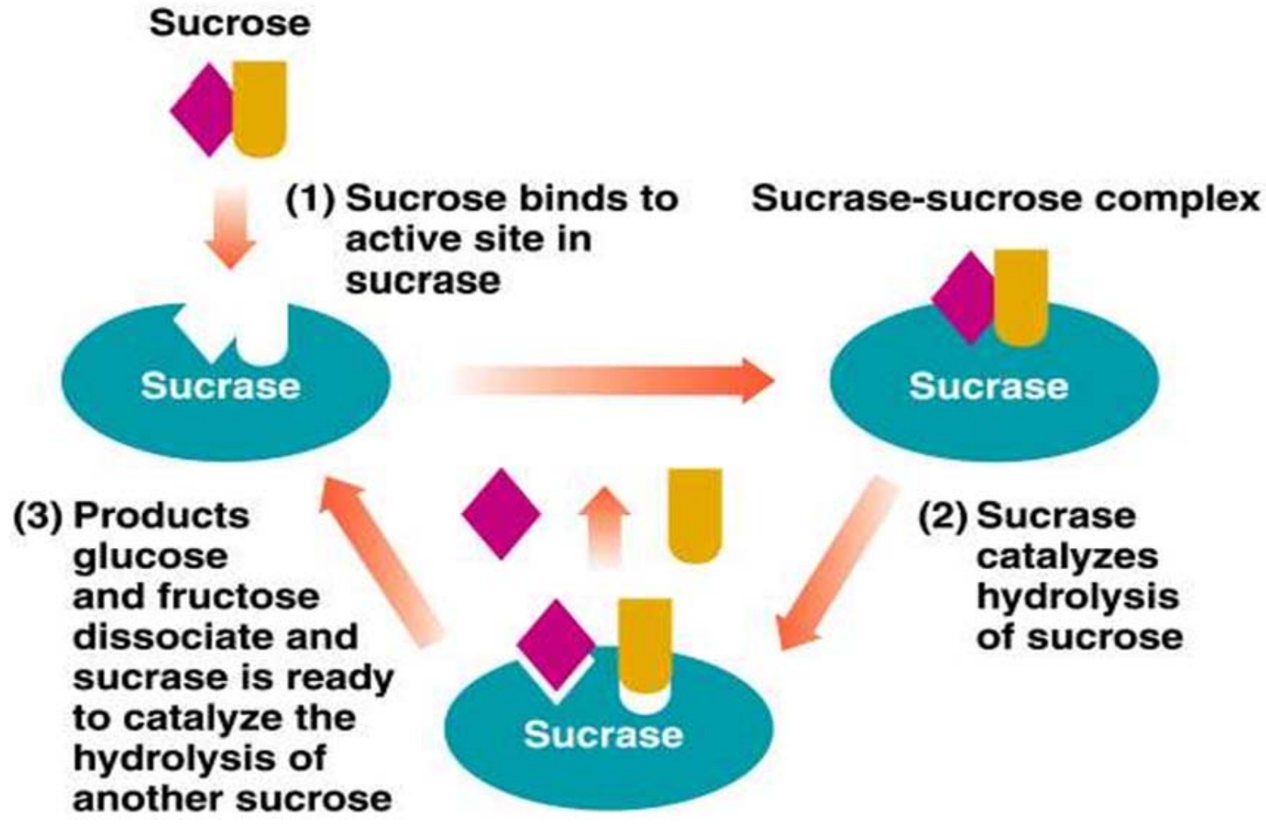
$$A + B \rightarrow A\text{-}B$$

6. Overall Process of Enzyme Catalysis



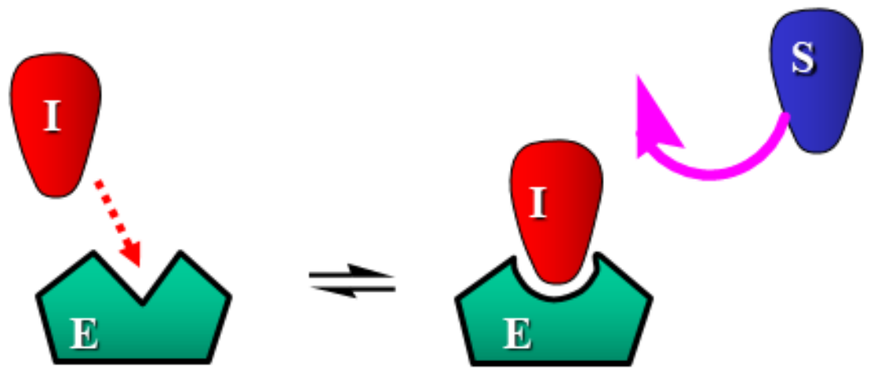
Notes:

- Binding interactions must be strong enough to hold the substrate sufficiently long for the reaction to occur
- Interactions must be weak enough to allow the product to depart
- Interactions stabilise the transition state



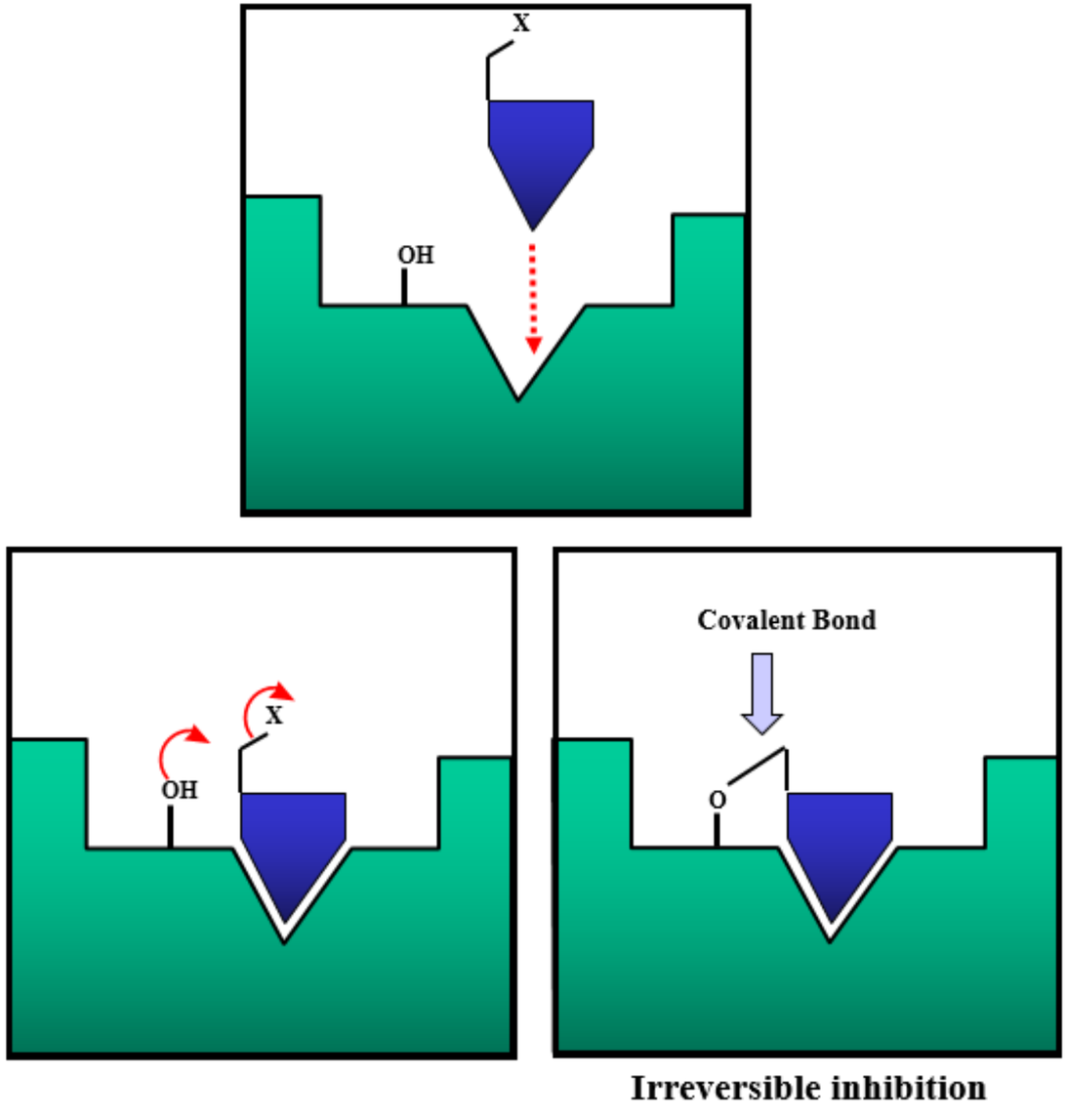
7. Competitive (reversible) inhibitors

- Inhibitor binds reversibly to the active site
- Intermolecular bonds are involved in binding
- No reaction takes place on the inhibitor
- Inhibition depends on the strength of inhibitor binding and inhibitor concentration
- Substrate is blocked from the active site
- Increasing substrate concentration reverses inhibition
- Inhibitor likely to be similar in structure to the substrate



8. Non competitive (irreversible) inhibitors

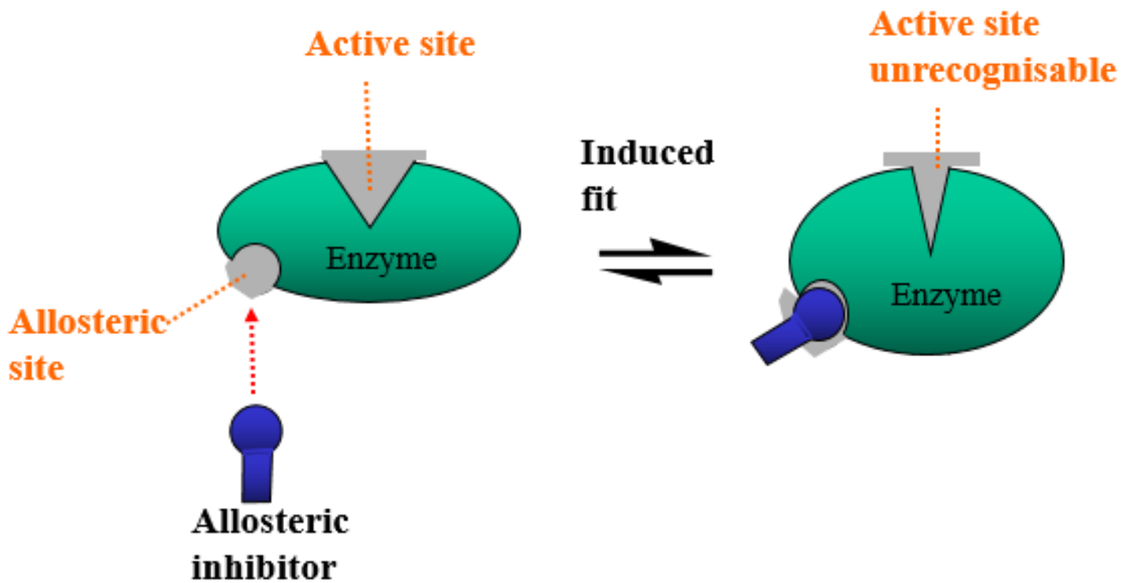
- Inhibitor binds irreversibly to the active site
- Covalent bond formed between the drug and the enzyme
- Substrate is blocked from the active site
- Increasing substrate concentration does not reverse inhibition
- Inhibitor likely to be similar in structure to the substrate



Irreversible inhibition

9. Allosteric inhibitors

- Inhibitor binds reversibly to the allosteric site
- Intermolecular bonds are formed
- Induced fit alters the shape of the enzyme
- Active site is distorted and is not recognised by the substrate
- Increasing substrate concentration does not reverse inhibition
- Inhibitor is not similar in structure to the substrate

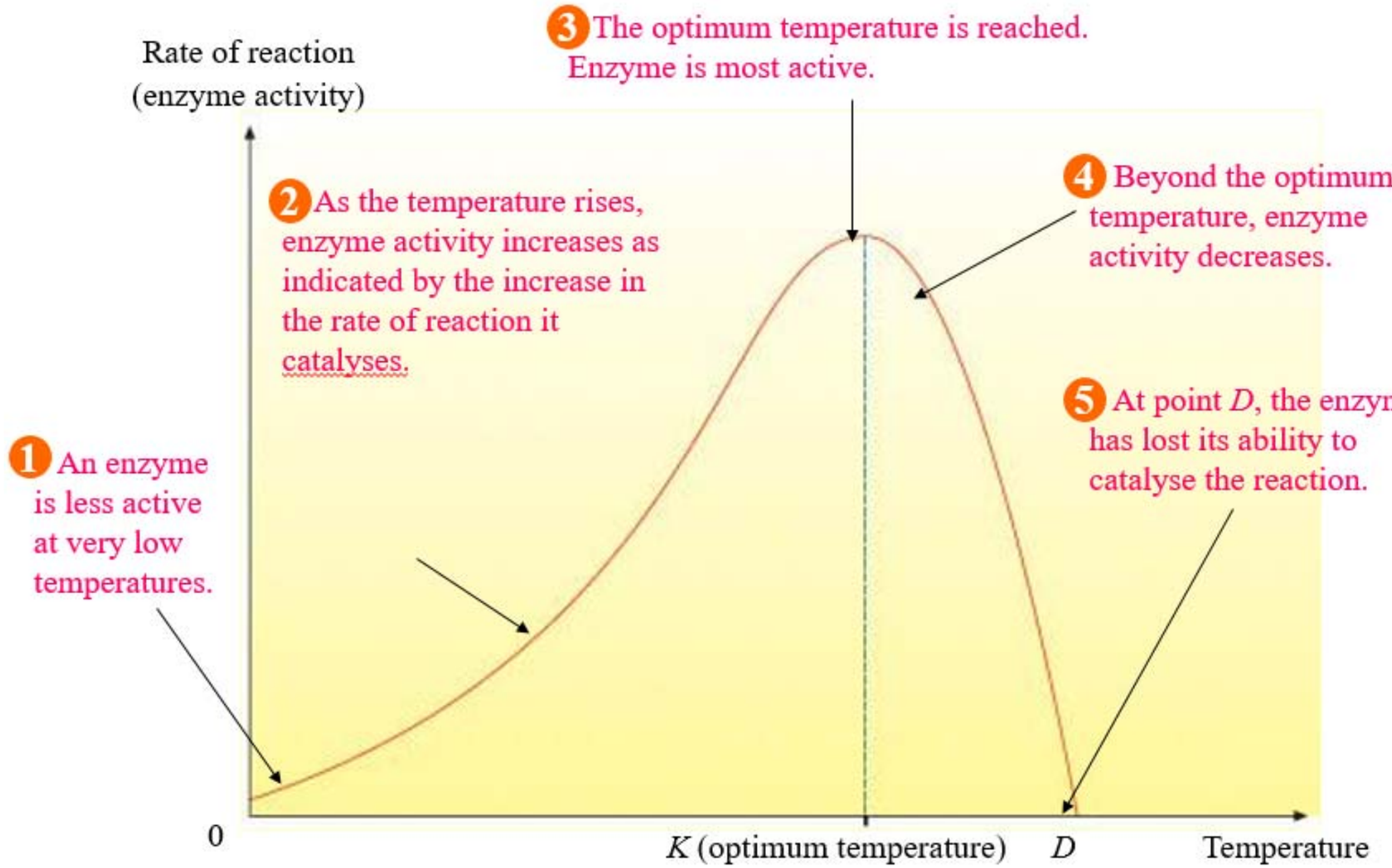


10. Factors affect the activity

Temperature and Enzyme Action

Enzymes:

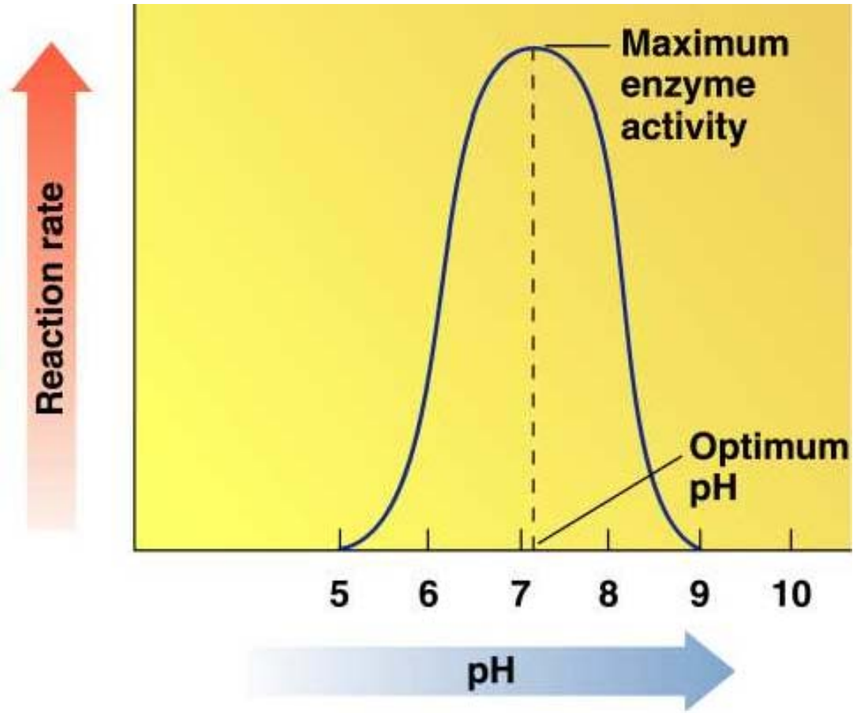
- Are most active at an optimum temperature (usually 37° C in humans).
- Show little activity at low temperatures.
- Lose activity at high temperatures as denaturation occurs.



pH and Enzyme Action

Enzymes:

- Are most active at optimum pH.
- Contain R groups of amino acids with proper charges at optimum pH.
- Lose activity in low or high pH as tertiary structure is disrupted.



Optimum pH Values

- Most enzymes of the body have an optimumpH of about 7.4.
- In certain organs, enzymes operate at lower and higher optimum pH values.

Table 21.5 Optimum pH for Selected Enzymes

Enzyme	Location	Substrate	Optimum pH
Pepsin	Stomach	Peptide bonds	2
Urease	Liver	Urea	5
Sucrase	Small intestine	Sucrose	6.2
Pancreatic amylase	Pancreas	Amylose	7
Trypsin	Small intestine	Peptide bonds	8
Arginase	Liver	Arginine	9.7

Enzymes

Biological catalysts, which are mainly made of proteins. They speed up the rate of chemical reactions without themselves being chemically changed at the end of the reactions.

Functions

- Building up or synthesising complex substances
- Breaking down food substances in cells to release energy (cellular respiration)
- Breaking down poisonous substances in cells

Characteristics

- Speed up chemical reactions
- Required in small amounts
- Highly specific
- Work best at an optimum temperature and pH
- May need coenzymes for activity
- Some catalayse reversible reactions

Mode of Action

- Lower the activation energy of a reaction
- Interact with the substrate according to lock and key hypothesis to form an enzyme-substrate complex

Classes

based on the type of reaction catalysed e.g.

- Hydrolases
- Oxidation-reduction enzymes

Limiting factors

Temperature / pH

- Increase in temperature increases the rate of enzyme reaction until optimum temperature is reached
- Increase in pH increases the rate of enzyme reaction until optimum pH is reached

Please find the book chapter and All chapter 4 attachment through the link:

http://u.pc.cd/AYf

Homework assignment: please answer questions 2 and 3 at the end of the chapter