Inhibition of enzyme activity (A2)

The inhibition of enzymes by naturally occuring small molecules can serve as a control mechanism in the cell. Many other small molecules or ions not generally found in cells can also inhibit enzyme activity. Inhibition can be reversible or non-reversible, depends on the type of interaction formed between the inhibitor and the enzyme molecule.

Competitive inhibition

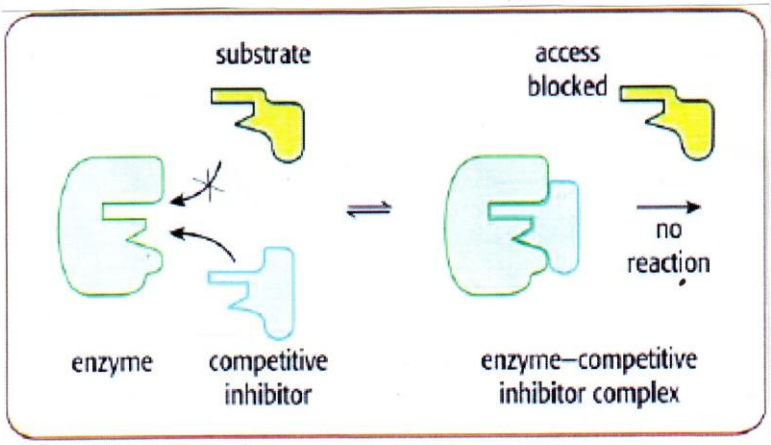
A competitive inhibitor imitates the substrate in the way it binds to the enzyme.

Competitive inhibitors of a particular enzyme are molecules that have a similar shape and charge distribution of the normal substrate.

These inhibitors can bind to the active site (by weak intermolecular attractive forces) but cannot take part in the enzyme-catalysed reaction.

When such inhibitor present in the active site, no reaction taking place and the correct substrate cannot attach to the enzyme.

When such an inhibitor is added to an enzyme/substrate mixture, a competition between the inhibitor and the substrate to occupy the active sites on the enzyme molecules.



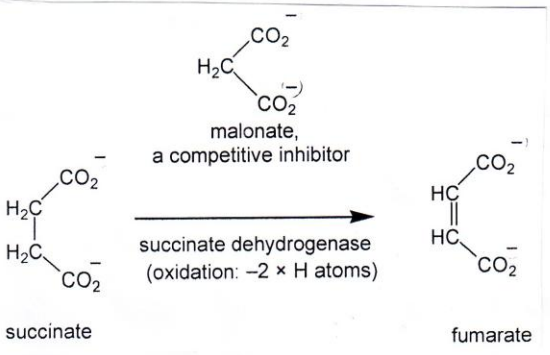
a model of action of a competitive inhibitor.

The result of this competition depends on the relative concentrations of the substrate and inhibitor.

The functionality of the enzyme molecule is not interfered with and the active sites are merely blocked.

This type of inhibition is reversible by an increase in the substrate concentration.

An example of competitive inhibition is the action of malonate on the enzyme succinic dehydrogenase.



A model of action of a competitive inhibitor

Malonate cannot be dehydrogenated to form a double bond since it does not have the $-CH_2-CH_2-$ group.

When the reaction is carried out in the presence of malonate, the enzyme activity decreases.

Increasing the concentration of malonate decreases the enzyme activity even further.

Increasing the concentration of succinate increases the enzyme activity.

Malonate is a successful competitive inhibitor because:

- it has two carboxylate groups, just like succinate.
- the length of the carbon chain is about the same to that of succinate.

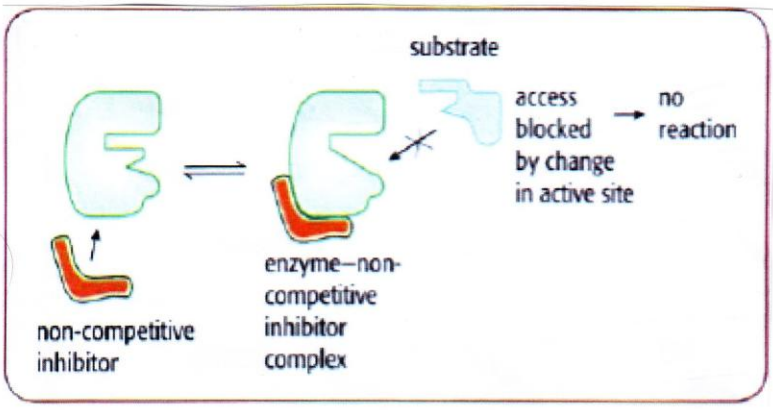
Non-competitive inhibition

A non-competitive inhibitor can bind on to regions of the enzyme other than the active site and affect enzyme activity.

The inhibitor binds to the enzyme, preventing the catalysed reaction from occuring.

The inhibitor does not bind to the active site.

The inhibitor binds to another position on the enzyme.



A scheme for non-competitive inhibition.

This binding causes one of the following:

- the active site to change shape so that the substrate cannot bind.
- the enzyme-substrate complex to change shape so that the reaction cannot take place.

The inhibitor is not shaped like the substrate and there is no competition between the substrate and the inhibitor.

The inhibition cannot be overcome by adding more substrate.

The effect is reduction of the number of active enzyme molecules available.

The binding between non-competitive inhibitor and enzyme involved weak intermolecular attractive forces.

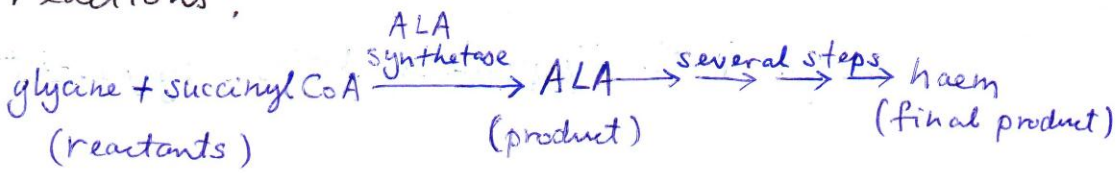
If the concentration of inhibitor fails, the enzyme-inhibitor complex falls apart and the functional shape of the enzyme is restored.

This type of inhibition is reversible and can provide an important mechanism for feedback control of a metabolic pathway in cells.

An <u>example</u> is the control of haem synthesis.

Haem is part of the haemoglobin molecule which is responsible for the transport of oxygen around the body.

Haem is made by a sequence of enzyme-catalysed reactions.



The enzyme which catalyses the first step in this sequence (ALA synthetase) is inhibited by haem non-competitively.

When the concentration of haem in the body rises:

- haem binds to the second site on the ALA synthetase.
- this changes the shape of the active site so that no more product is formed.
- the concentration of haem in the body falls.

Exercise 1

Use the example of the control of haem synthesis to answer the following questions.

a) Explain what happens when the concentration of haem in the cell is low. Your answer should explain the steps leading first of all to a higher haem concentration, and finally to the haem concentration becoming low again.

b) The synthesis of ALA is the rate-determining step.

(i) What is the meaning of rate determining step?

(ii) What advantage is there to the cell that the first step in this reaction is the rate determining step?

Workings.

a)

1. When the concentration of haem is low, there is less haem to bind to enzyme molecules.
2. This causes fewer enzyme molecules are inhibited and more enzyme molecules are active.
3. The active enzyme molecules catalyse the ALA synthesis, more haem is synthesised.

4. Haem concentrations become high, there is more haem to bind to enzyme molecules.

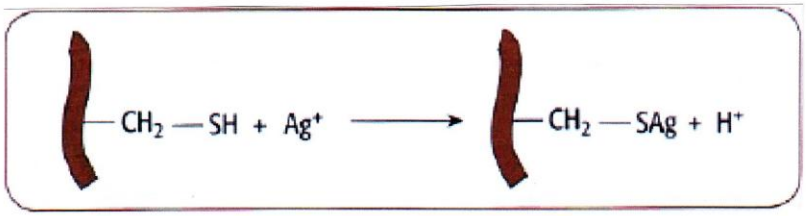
5. As a result, more enzyme molecules are inhibited, and fewer enzyme molecules are active.

6. There are few active enzyme molecules to catalyse the ALA synthesis, less haem is synthesised.

7. The concentration of haem becomes low again, and the whole process restart.

Heavy metals as inhibitors to enzymes

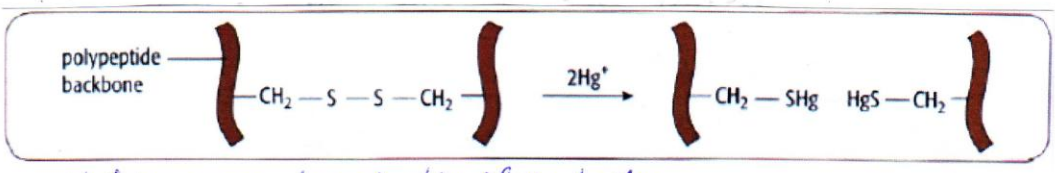
Heavy metals, such as Ag^+ ions and Hg^+ ions, react with one or more $-SH$ (sulfhydryl) groups, replacing the hydrogen atom with a metal ion.



The reaction between silver ion and a sulfhydryl group

The resulting modification does not allow disulfide bridge bridges to form, and so changes the shape of the enzyme sufficiently enough to prevent the catalysed reaction taking place.
The enzyme is temporarily denatured.

Hg ions can inhibit enzyme activity by breaking disulfide bridges.



Hg^+ ions can break disulfide bridge.

The tertiary structure of the protein is altered because the disulfide bridges no longer play a part in holding the polypeptide chains in the correct position.
These reactions can often be reversed by atmospheric oxidation.

Exercise 2

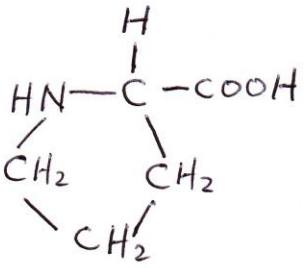
Describe three differences between a compatitive inhibitor and a non-competitive inhibitor.

Workings

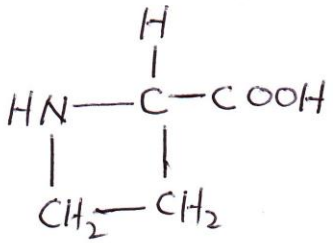
competitive inhibitor	non-competitive inhibitor
the inhibition can be overcome by increasing the normal substrate concentration	increasing the normal substrate concentration has no effect on the inhibition
acts at the active site of the enzyme.	usually acts at a second site on the enzyme.
has a similar shape and similar charge distribution characteristics to the normal substrate.	does not have the similar shape and charge distribution characteristics to the normal substrate

Exercise 3

The structures of proline and azetidine-2-carboxylic acid are as follow:



proline



azetidine-2-carboxylic acid.

Azetidine-2-carboxylic acid (A2C) can act as a competitive inhibitor of proline.
What features of A2C make it a good competitive inhibitor of proline?

Workings

Both A2C and proline have similar structure.

1. Both are secondary amine
2. Both have one lone pair on the nitrogen
3. Both have one -COOH group at similar position
4. The size of the ring in A2C is about similar to that of proline.

Exercise 4

An enzyme uses proline as a substrate.
The data in the table shows the effect of different mixtures of proline and azetidine-2-carboxylic acid (A2C) on the rate of reaction.

Experiment	1	2	3	4	5
[proline] / mol dm^{-3}	3×10^{-4}	3×10^{-4}	3×10^{-4}	6×10^{-4}	9×10^{-4}
[A2C] / mol dm^{-3}	0	3×10^{-4}	6×10^{-4}	3×10^{-4}	3×10^{-4}
Relative rate of rxn	5.2	4.8	4.4	5.0	5.1

How does this data show that A2C is a competitive inhibitor of proline?

Workings

From experiments 1, 2 and 3, the increase in the concentration of A2C decreases the rate of reaction.
Thus A2C is an inhibitor.
From experiments 2, 4 and 5, the inhibitory effect of A2C is lessened by increasing the concentration of substrate present, the less the inhibitory effect.
Therefore A2C is a competitive inhibitor.

Exercise 5

Thallium is a heavy metal.

i) Write an equation to show the reaction of thallium(I) ions, Tl^+, with the sulfhydryl group in a cysteine residue.

ii) What type of inhibitors are thallium(I) ions? Explain your answer.

Workings

i) $RCH_2SH + Tl^+ \longrightarrow RCH_2STl + H^+$

ii) Tl^+ is a non-competitive inhibitor because it forms a covalent bond with the sulfur atom. This can only be reversed by reaction with a compound containing $-SH$ groups.
(competitive inhibitors only form weak intermolecular forces at the active site)