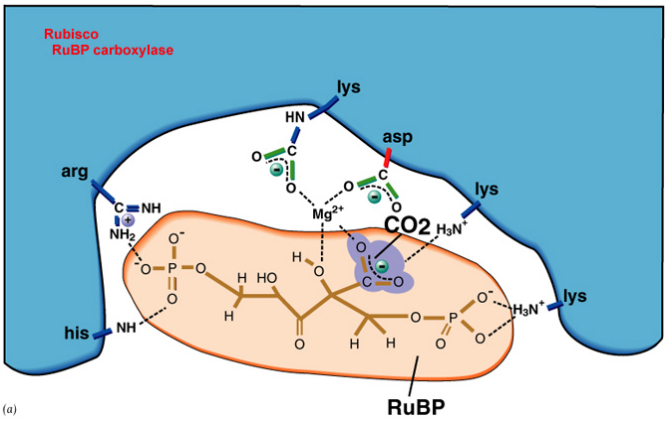
AS Biology - Module 1

CHAPTER 4:

ENZYMES

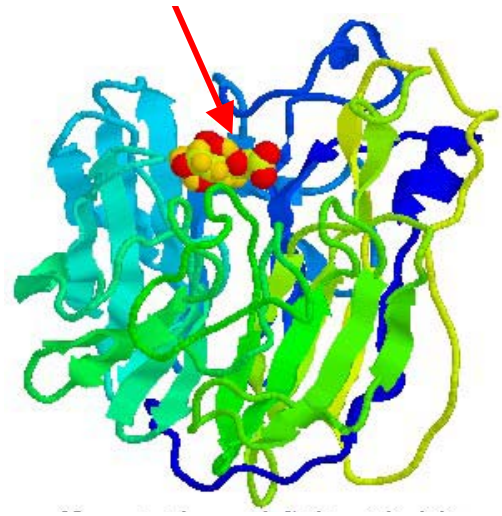
Enzymes are biological catalysts. There are about 40,000 different enzymes in human cells, each controlling a different chemical reaction. They increase the rate of reactions by a factor of between 10^6 to 10^{12} times, allowing the chemical reactions that make life possible to take place at normal temperatures. They were discovered in fermenting yeast in 1900 by Buchner, and the name enzyme means "in yeast". As well as catalysing all the metabolic reactions of cells (such as respiration, photosynthesis and digestion), they may also act as motors, membrane pumps and receptors.



The active site of RUBISCO, the key enzyme in photosynthesis, contains just 6 amino-acids.

Enzyme Structure

Substrate in active site



Enzymes are proteins, and their function is determined by their complex structure. The reaction takes place in a small part of the enzyme called the **active site**, while the rest of the protein acts as "scaffolding". This is shown in this diagram of a molecule of the enzyme **trypsin**, with a short length of protein being digested in its active site. The amino acids around the active site attach to the substrate molecule and hold it in position while the reaction takes place. This makes the enzyme **specific** for one reaction only, as other molecules won't fit into the active site – their shape is wrong.

Many enzymes need **cofactors** (or **coenzymes**) to work properly. These can be metal ions (such as Fe^{2+}, Mg^{2+}, Cu^{2+}) or organic molecules (such as haem, biotin, FAD, NAD or coenzyme A). Many of these are derived from dietary vitamins, which is why they are so important. The complete active enzyme with its cofactor is called a **holoenzyme**, while just the protein part without its cofactor is called the **apoenzyme**.

How do enzymes work?

There are three parts to our thinking about enzyme catalysis. They each describe different aspects of the same process, and you should know about each of them.

1. Reaction Mechanism

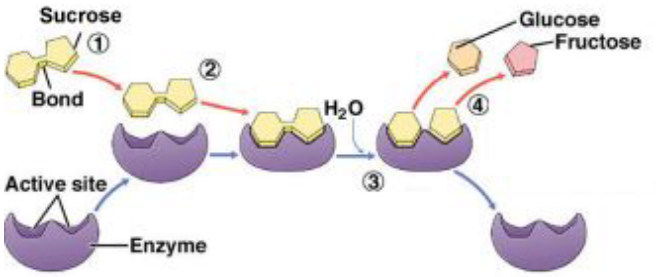
In any chemical reaction, a **substrate** (S) is converted into a **product** (P):

S □ P

(There may be more than one substrate and more than one product, but that doesn't matter here.) In an enzyme-catalysed reaction, the substrate first binds to the active site of the enzyme to form an **enzyme-substrate (ES) complex**, then the substrate is converted into product *whilst attached to the enzyme*, and finally the product is released, thus allowing the enzyme to start all over again (*see right*)

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

Where E = Enzyme
S = Substrate
P = Products



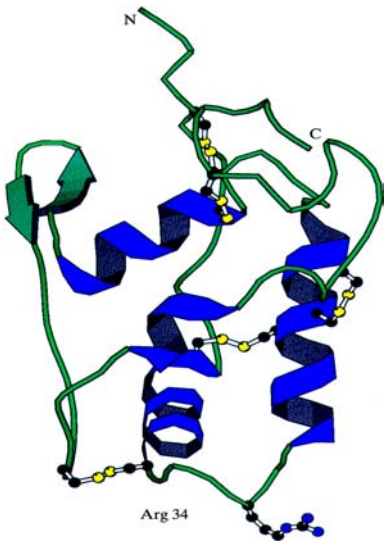
An example is the action of the enzyme **sucrase** hydrolysing sucrose into glucose and fructose (*see left*)

2. Molecular Geometry

The substrate molecule is **complementary** in shape to that of the active site. It **was** thought that the substrate **exactly** fitted into the active site of the enzyme molecule like a key fitting into a lock (the now discredited **'lock and key'** theory). This explained why an enzyme would only work on one substrate (specificity), but failed to explain **why** the reaction happened.

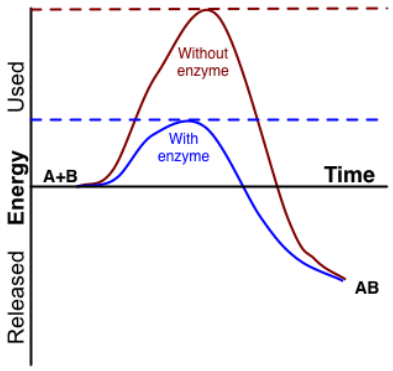
It is now known that the substrate and the active site **both change shape** when the enzyme-substrate complex is formed, bending (and thus weakening) the target bonds. For example, if a substrate is to be split, a bond might be stretched by the enzyme, making it more likely to break. Alternatively the enzyme can make the local conditions inside the active site quite different from those outside (such as pH, water concentration, charge), so that the reaction is more likely to happen.

Although enzymes can change the speed of a chemical reaction, they cannot change its direction, otherwise they could make "impossible" reactions happen and break the laws of thermodynamics. So an enzyme can just as easily turn a product into a substrate as turn a substrate into a product, depending on the local concentrations. The **transition state** is the name given to the distorted shape of the active site and substrate.



This diagram shows another enzyme with its 5 disuphide bridges in yellow and regions of α –helix in blue. The active site is near the region of the arrows.

3. Energy Changes

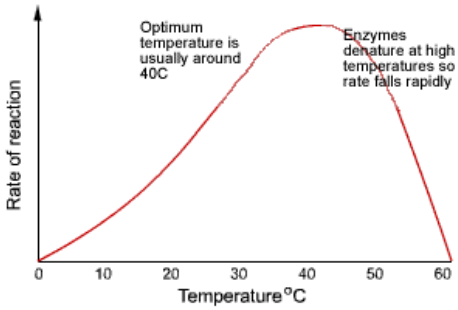


The way enzymes work can also be shown by looking at the energy changes during a chemical reaction. In a reaction where the product has a lower energy than the substrate, the substrate naturally turns into product (i.e. the equilibrium lies in the direction of the product). Before it can change into product, the substrate must overcome an "energy barrier" called the **activation energy**. The **larger** the activation energy is, the **slower** the reaction will be. This is because only a few substrate molecules will have sufficient energy to overcome the activation energy barrier. Imagine pushing boulders over a hump before they can roll down hill, and you have the idea. Most biological reactions have **large** activation energies, so they without enzymes they happen far too slowly to be useful. **Enzymes reduce the activation energy** of a reaction so that the **kinetic energy of most molecules exceeds the activation energy required** and so they can react.

For example, for the **catalase** reaction ($2H_2O_2 \rightarrow 2H_2O + O_2$) the activation energy is 86 kJ mol^{-1} with **no** catalyst, 62 kJ mol^{-1} with an **inorganic** catalyst, and just 1 kJ mol^{-1} with the enzyme catalase.

Factors that Affect the Rate of Enzyme Reactions

1. Temperature



Enzymes have an **optimum temperature** at which they work fastest. For mammalian enzymes this is about 40°C, but there are enzymes that work best at very different temperatures, e.g. enzymes from the arctic snow flea work at -10°C, and enzymes from thermophilic bacteria work at 90°C.

Up to the optimum temperature the rate increases geometrically with temperature (i.e. it's a curve, not a straight line). The rate increases because the **enzyme and substrate molecules both have more kinetic energy** and so **collide more often**, and also because **more molecules have sufficient energy to overcome the activation energy**.

Above the optimum temperature the rate decreases as more of the enzyme molecules **denature**. The thermal energy **breaks the hydrogen bonds** holding the secondary and **tertiary structure** of the enzyme together, so the **enzyme loses its shape** and becomes a random coil - and the **substrate can no longer fit into the active site**. This is irreversible. Remember that **only the hydrogen bonds** are broken at normal temperatures; to break the primary structure (the peptide bonds) you need to boil in strong acid for several hours – or use a **protease** enzyme!

The increase in rate with temperature can be quantified as the Q_{10}, which is the relative increase for a 10°C rise in temperature. Q_{10} is usually around 2 for enzyme-catalysed reactions *(i.e. the rate doubles every 10°C)* and usually less than 2 for non-enzyme reactions.

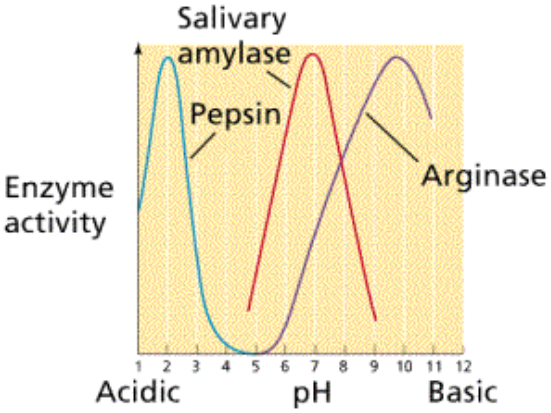
$$Q_{10} = \frac{\text{rate at temp } (t+10)°C}{\text{rate at temp } t°C}$$

The rate is not zero at 0°C, so enzymes still work in the fridge (and food still goes off), but they work slowly. Enzymes can even work in ice, though the rate is extremely slow due to the very slow diffusion of enzyme and substrate molecules through the ice lattice.

2. pH

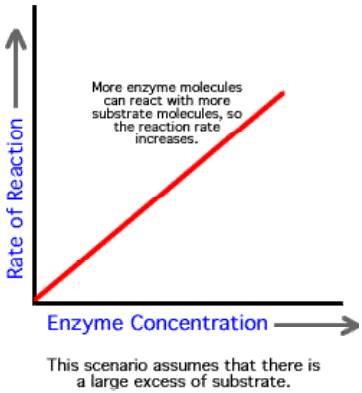
Enzymes have an **optimum pH** at which they work fastest. For most enzymes this is about pH 7-8 (normal body pH), but a few enzymes can work at extreme pH, such as **gastric protease (pepsin)** in our stomach, which has an optimum of pH 1.

The pH affects the charge of the amino acids at the active site, so the properties of the active site change and the substrate can no longer bind. For example a carboxyl acid R groups will be uncharged a low pH (COOH), but charged at high pH (COO^-).

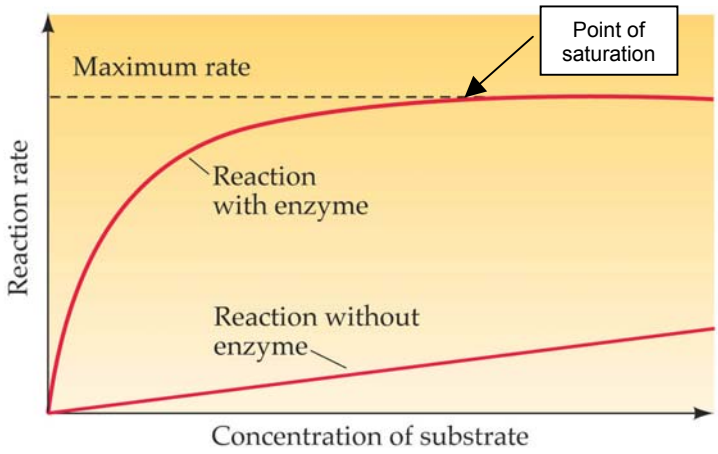


3. Enzyme concentration

As the enzyme concentration increases the rate of the reaction also increases, because there are more enzyme molecules (and so more active sites), available to catalyse the reaction therefore **more enzyme-substrate complexes form**. In cells, the substrate is always in excess, so the graph **does not level** out. In the lab, these conditions need not apply and a plateau can be reached.



4. Substrate concentration



The rate of an enzyme-catalysed reaction is also affected by substrate concentration. As the substrate concentration increases, the rate increases because **more substrate molecules can collide with active sites**, so more **enzyme-substrate complexes form.**

At higher concentrations the enzyme molecules become **saturated** with substrate, and there are few free active sites, so adding more substrate doesn't make much difference (though it **will** increase the rate of E-S collisions).

The maximum rate at infinite substrate concentration is called v_{max}, and the substrate concentration that gives a rate of half v_{max} is called K_M. These quantities are useful for characterising an enzyme. A good enzyme has a high v_{max} and a low K_M.

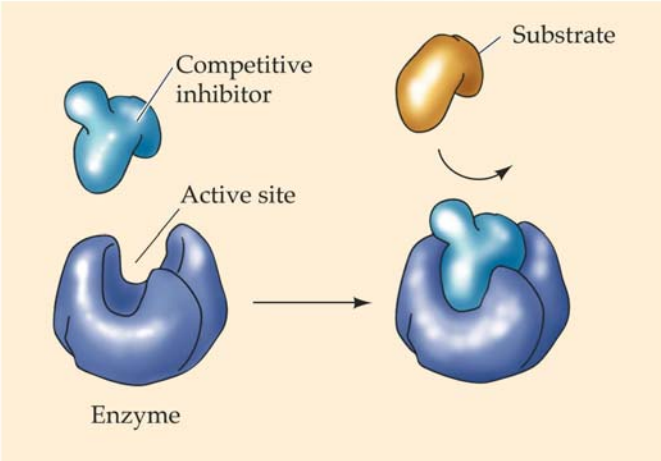
5. Covalent modification

The activity of some enzymes is controlled by other enzymes, which modify the protein chain by cutting it, or adding a phosphate or methyl group. This modification can turn an inactive enzyme into an active enzyme (or vice versa), and this is used to control many metabolic enzymes and to switch on enzymes in the gut e.g. HCl in stomach → activates pepsin → activates rennin.

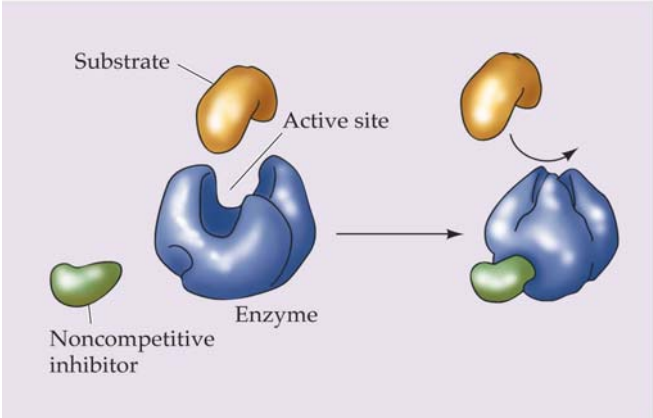
6. Inhibitors

Inhibitors inhibit the activity of enzymes, reducing the rate of their reactions. They are found naturally, but are also used artificially as drugs, pesticides and research tools. There are two kinds of inhibitors.

(a) A competitive inhibitor molecule has a **similar structure to the substrate molecule**, and so it can fit into the active site of the enzyme. It therefore **competes** with the substrate for the active site, so the reaction is slower. Increasing the concentration of substrate restores the reaction rate and the inhibition is usually temporary and reversible. Competitive inhibitors increase K_M for the enzyme, but have no effect on v_{max}, so the rate can approach a normal rate if the substrate concentration is increased high enough.



(b) A non-competitive inhibitor molecule is quite different in structure from the substrate and does not fit into the active site. It binds to another part of the enzyme molecule, changing the shape of the whole enzyme, including the active site, so that it can no longer bind substrate molecules. Non-competitive inhibitors therefore simply reduce the amount of active enzyme. This is the same as decreasing the enzyme concentration, so they decrease v_{max}, but have no effect on K_M. This kind of inhibitor **tends** to bind tightly and irreversibly – such as the poisons cyanide and heavy metal ions. Many nerve poisons (insecticides) work in this way too.



7. Feedback Inhibition (Allosteric Effectors)

The activity of some enzymes is controlled by certain molecules binding to a specific regulatory (or **allosteric**) site on the enzyme, distinct from the active site. Different molecules can either inhibit or activate the enzyme, allowing sophisticated control of the rate. Only a few enzymes can do this, and they are often at the start of a long biochemical pathway. They are generally **activated by the substrate** of the pathway and **inhibited by the product** of the pathway, thus only turning the pathway on when it is needed. This process is known as **feedback inhibition.**