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Enzymes fast and flexible

Enzymes are protein molecules — 'biological catalysts'. This article explains their structure and how they work so efficiently at relatively low temperatures.

Key words
Protein
Conformation
Active site
Activation energy

Enzymes are amazing molecules. They are very specific and can find a molecular 'needle' in a 'haystack' of millions of other molecules. They have such high catalytic efficiency that they make most chemical catalysts used in industry look positively sluggish — they can speed up chemical reactions by as much as a million (10^6) to a trillion (10^{12}) times. Some can do this at very low temperatures, in contrast to the catalysts used in chemical industries, most of which require high temperatures. This is the biological equivalent to speeding up your life from cradle to grave so that it is completed in a single heartbeat! How do they do it? The answer lies in their structure.

'Proteins that make haste'

This is what enzymes have been called. They are proteins that catalyse chemical reactions in living organisms. To understand how enzymes work, we need to understand the structure of proteins. A protein is a long, ribbon-like molecule made by joining together many subunits — amino acids (see Figure 1). Each amino acid has a central carbon atom, attached to which is an amino group ($-NH_2$), a carboxylic acid group (–COOH), and a side chain generally represented by R (which stands for radical). The amino and acidic groups are involved in joining the amino acids to one another to form the backbone of the protein chain. There are 20 different R groups and they stick out of this backbone. This means that there are 20 different amino acids which have different properties. They may be positively or negatively charged, hydrophobic or hydrophilic, bulky or tiny.

The protein molecule does not lie flat like a ribbon — far from it. In watery environments, such as inside living cells, a protein chain will fold into a unique three-dimensional shape so that its hydrophobic regions are (generally) towards the inside. Its hydrophilic regions are (generally) towards the outside. Weak bonds form between R groups and these help to stabilise the delicate shape, known as the **conformation**. The shape of the enzyme molecule has to be just right if the enzyme is to function. If the enzyme molecule is deformed, even slightly, it will not work (like a paper dart — the exact folding of the paper gives the dart its aerodynamic properties). Each different enzyme has its own highly specific shape with a pocket — the **active site**. It is here that the substrate will fit.

Only a few of the amino acids in the chain of an enzyme protein are involved in catalysis. These **catalytic amino acids** are not adjacent to each other in the chain. Instead they are quite spaced out along the length of the protein molecule (see Figure 1). When the molecule folds up into its three-dimensional conformation, however, the specific folding brings all the catalytic amino acids together in the active site region.

Collisions and catalysts

Once the substrate has fitted into the active site, the reaction is catalysed. Molecules only react when they collide with each other, but the collisions must occur in the right order and at the right speed. Consider a molecule of sucrose. It consists of a molecule of glucose joined

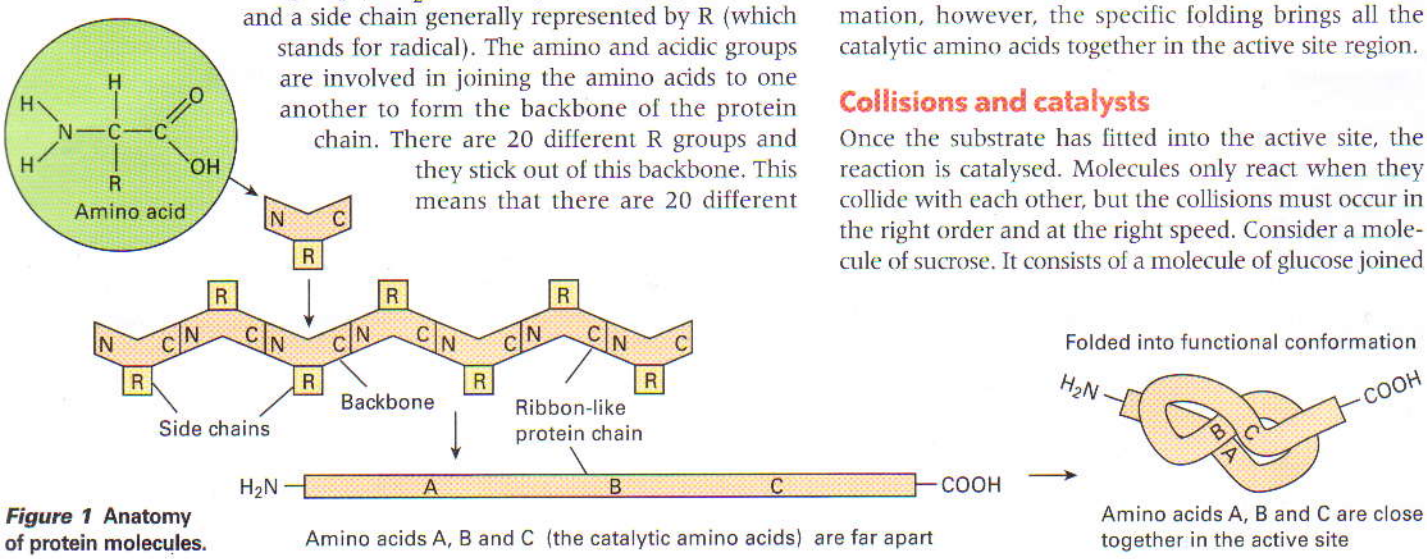

Figure 1 **Anatomy of protein molecules.**

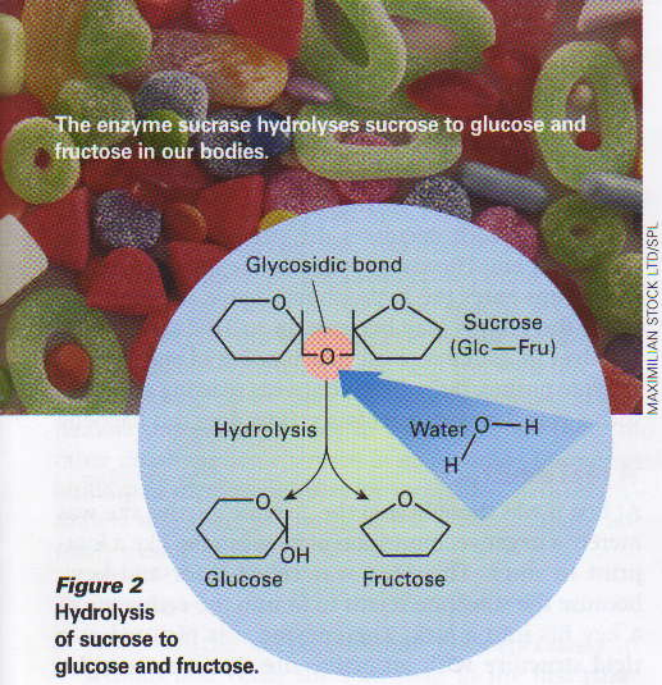

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Figure 2 **Hydrolysis of sucrose to glucose and fructose.**

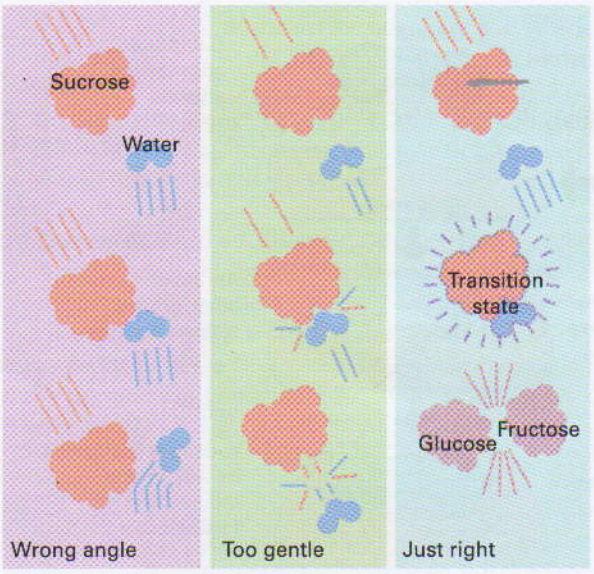

Figure 3 **Will sucrose and water ever get together? A sucrose molecule and a water molecule must collide at the correct angle and with sufficient energy if they are to react to form glucose and fructose.**

to a molecule of fructose by means of a glycosidic bond. Having eaten and enjoyed sucrose, we must digest it before it can be absorbed into the blood. We use the enzyme sucrase (found on the surface of the cells of the small intestine) to **hydrolyse** sucrose to glucose and fructose; water is used to break the oxygen bridge in the glycosidic bond (see Figure 2).

The reaction could be carried out in a test tube, without an enzyme — but it would not go very fast. Imagine a molecule of sucrose and a molecule of water crashing into each other. What happens? For a reaction to take place the correct bonds in the sugar have to be lined up with the correct bonds in the water (see Figure 3). If the alignment is not correct, the two molecules just bounce apart. If the alignment is correct but the impact is gentle, again, nothing happens since there is not enough energy to cause the rearrangement of electrons involved in making and breaking bonds. But if the two molecules are lined up *and* collide hard enough, the sucrose splits into glucose and fructose (see Figure 3).

This can be illustrated in an energy diagram (see Figure 4). The energy associated with each molecule is plotted against the progress of the reaction. At the start, sucrose and water have a certain amount of energy. They crash into one another and form an unstable high-energy intermediate, which quickly changes into glucose and fructose. In rearranging the bonds of sucrose and water, some energy is released, so the products have less energy than the starting molecules. The minimum amount of energy needed to get the reaction to go — to form the unstable intermediate known as the **transition state** — is called the activation energy. The transition state is an unstable arrangement of atoms which is a hybrid structure somewhere between reactants and products, having some of the features of each, with some bonds partly formed and some partly broken. In less than 10^{-13} seconds it collapses into either reactants or products.

Getting over the hump

Every chemical reaction has an energy barrier that has to be overcome before anything happens. A comparison that is often used is that of a boulder resting on top of a hill with a small mound of earth stopping it from rolling down. There are two ways to get the boulder to roll down the hill. You could push it over the mound until it reached the point where it would roll over by itself. This is equivalent to supplying heat to start a reaction. Alternatively you could dig away at the mound until the boulder started rolling of its own accord. This is equivalent to what a catalyst does. Instead of supplying energy, the enzyme reduces the height of the energy barrier so that many more molecules have enough energy to get over it. The reaction can go ahead without the need for any extra energy as the activation energy is halved for this particular reaction. This may not seem very impressive but it increases the rate of the reaction by a billion (10^9) times. How do enzymes do it?

Binding for activity

The answer is that enzymes work by binding their substrate molecules at the active site — the specially formed pocket open at the enzyme surface. Generally, the active site is lined with hydrophobic R groups and it contains groups that bind the substrate and other groups that catalyse the reaction. Substrates are usually much smaller than their enzymes. There are exceptions, such as the substrates of the protein-digesting and starch-digesting enzymes, but even here the enzyme

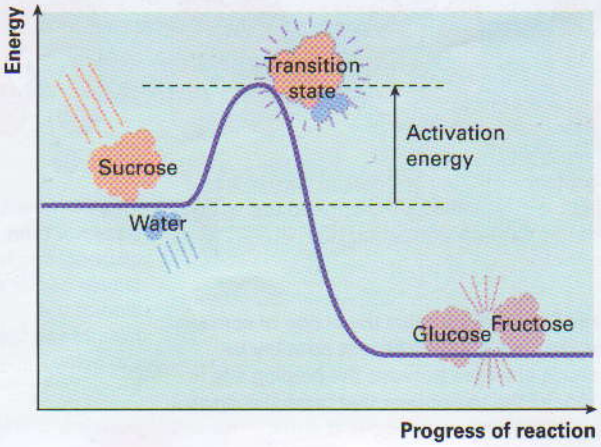

Figure 4 **Energy diagram. A sucrose molecule and a water molecule must collide with sufficient energy to form the unstable transition state structure if they are to react to form glucose and fructose.**

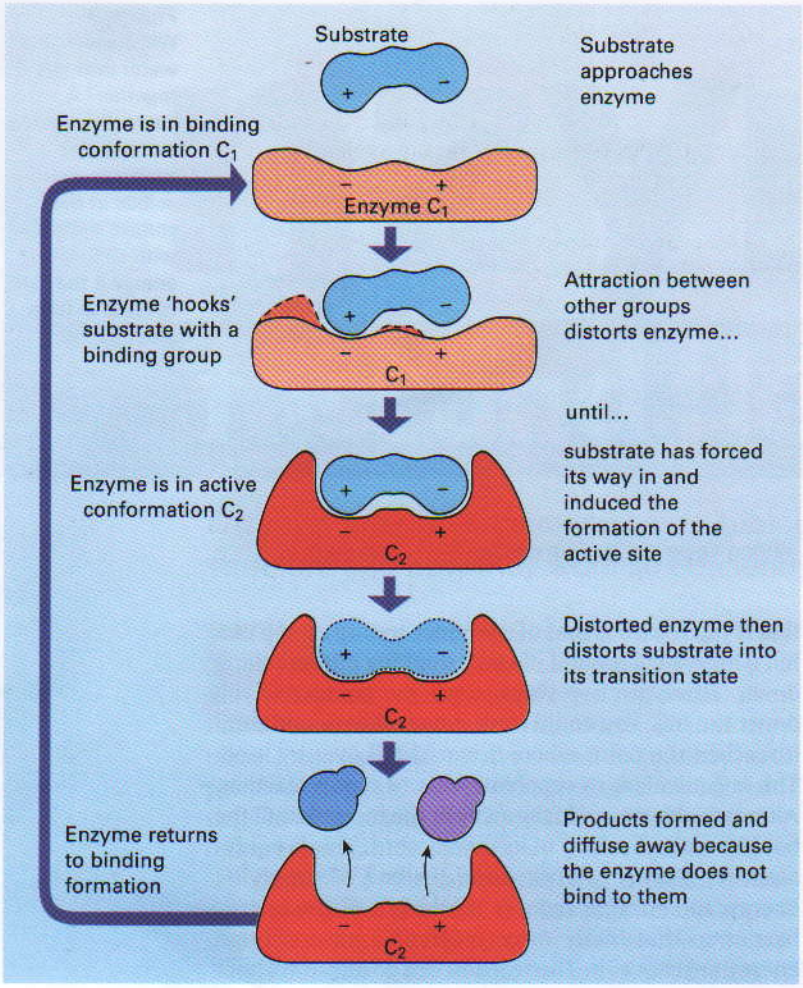

Figure 5 **The induced fit theory explains enzyme action.**

binds to only a small region of its massive substrate. When substrates bind to the enzyme, they slot neatly into the active site and find themselves close to the catalytic amino acids.

The binding is brought about by attractions between particular groups on the substrate and complementary ones in the active site. The substrate is held in precisely the correct position to react with other molecules, rather than having to collide with them by chance. Any charged groups in the substrate become more reactive when they are in the hydrophobic environment of the active site. In the watery cytoplasm, both positive and negative groups are surrounded by a shell of water molecules, which decreases the strength of attraction between them. These watery masks are discarded when the groups enter the hydrophobic active site, and as a result the groups may become up to 10^{70} times more reactive. In addition to all this, binding of substrate to enzyme pushes, pulls, bends or twists reacting groups in such a way that they react faster. How does this happen?

'A real embrace'

At first it was thought that the enzyme's active site was merely a negative impression of its substrate, like a footprint in mud. This idea was called 'lock-and-key', because the substrate seems to fit into the active site as a key fits into a lock. The enzyme was pictured as a rigid structure with an active site that was complementary to the substrate (just as a boot has a shape complementary to the foot that is to wear it). But this idea did not explain everything. In particular, it did not explain allosteric ('other site') effects — how small molecules (such as ATP) could alter an enzyme's activity by binding to the enzyme at some other site that was far from the active site. If the enzyme molecule were rigid, then a molecule binding to one part of it would not affect other parts of the protein. However, if the enzyme were flexible, such an allosteric effect could be explained.

A flexible enzyme molecule has two possible interchangeable conformations. One of these is the binding conformation (which is not catalytically active); the other is the catalytically active conformation (which does not bind). Binding groups in the enzyme attract and bind the substrate molecule. When the substrate binds, it disturbs the delicate balance of the flexible protein chain of the enzyme, causing it to alter its shape. This is why we refer to an **induced fit** of substrate to enzyme (see Figure 5). This rearrangement brings the catalytic amino acids into their correct positions in the active site so that catalysis can occur. Thus the shape of the enzyme molecule is affected by the substrate, just as the shape of a sock is affected by the foot that is wearing it. The process has been termed 'a real embrace' — the enzyme molecule wraps itself around the substrate. The distorted enzyme molecule in turn distorts the substrate molecule, straining or twisting the bonds. (This is how the activation energy is lowered.) Once catalysis occurs and products are formed, they no longer bind to the active site and diffuse away. The flexible enzyme then returns to its original shape, ready to bind the next molecule of substrate.

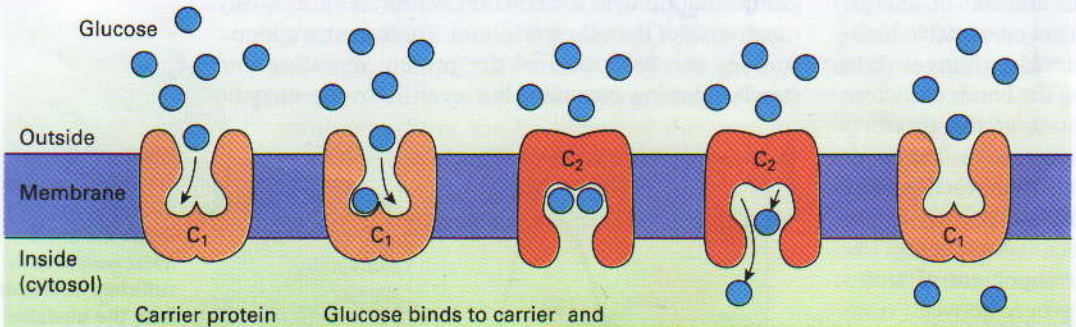

Figure 6 **The induced fit theory explains the action of some membrane-transport proteins. Glucose cannot possibly be transported in the wrong direction because the binding site is never found on the side of the membrane that faces the cytosol.**

This picture of how an enzyme works is more realistic than that given by the lock-and-key theory. Although induced fit was devised to explain the workings of enzymes, it can also explain how many other proteins bring about changes in cells. You may be familiar with the idea that carrier proteins within membranes actively transport substances, like glucose, against a concentration gradient. But have you ever wondered how this might be achieved? Figure 6 will help you with this. You might also like to consider the ideas of induced fit and flexible protein molecules in relation to a variety of other situations: ion channels in membranes, hormones binding to membrane receptor molecules, neurotransmitters and their receptors in the synapse...there are many examples.

Things to do

- Work out the average human life expectancy in seconds and verify the statement in the first paragraph about the degree to which enzymes speed up reactions.
- Molecules called competitive inhibitors interfere with particular enzymes because they are similar in shape to the normal substrate of the enzyme. Using the ideas of the binding and catalytically active conformation, explain why competitive inhibitors have their inhibitory effect.
- Enzymes are now widely used in biotechnology. Find the names of enzymes that are used by biotech companies and what they are used for.
- Type 'sucrase' into Google and then click on 'Images', and then on 'Image Search'. You will find a lovely image of sucrose in the active site of sucrase which makes it clear how the glycosidic bond is put under stress.
- Do the same thing for 'pepsin' and you will find different types of images of pepsin which will help you to understand its structure.

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KEY points

- Enzymes are protein molecules that are found in all living cells.
- The chain of amino acids in the enzyme molecule does not lie flat but spontaneously twists and folds to adopt a specific three-dimensional shape known as the conformation.
- Usually only a few of the amino acids are involved in catalysis. They are all found in a hydrophobic pocket or groove on the surface of the enzyme.
- Catalysis occurs when the substrate(s) is/are attracted into the active site and held in a particular position so that bonds can be either formed or broken.