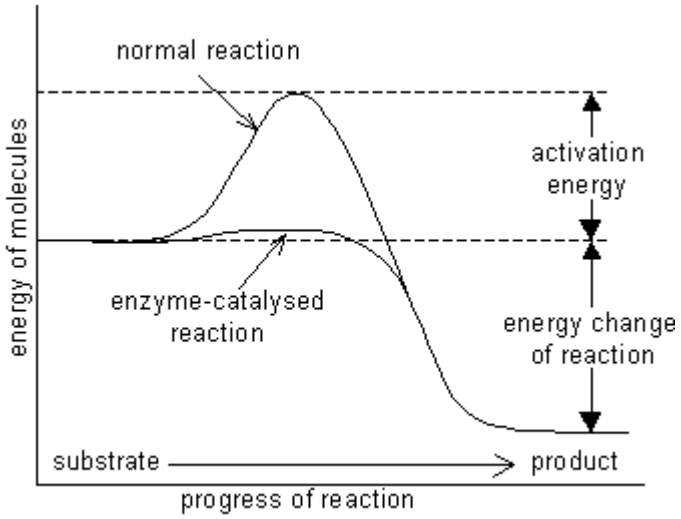
Enzymes

ENERGY CHANGES

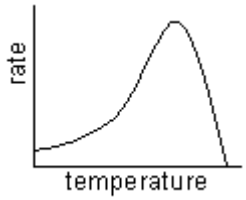
The way enzymes work can also be shown by considering the energy changes that take place during a chemical reaction. We shall consider a reaction where the product has a lower energy than the substrate, so the substrate naturally turns into product (in other words the equilibrium lies in the direction of the product). Before it can change into product, the substrate must overcome an "energy barrier" called the activation energy (E_A). The larger the activation energy, the slower the reaction will be because only a few substrate molecules will by chance 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 physiological reactions have large activation energies, so they simply don't happen on a useful time scale. Enzymes dramatically reduce the activation energy of a reaction, so that most molecules can easily get over the activation energy barrier and quickly turn into product.



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 of iron filings, and just 1 kJ mol^{-1} in the presence of the enzyme catalase. The activation energy is actually the energy required to form the transition state, so enzymes lower the activation energy by stabilising the transition state, and they do this by changing the conditions within the active site of the enzyme. So the three ideas above are really three ways of describing the same process.

FACTORS THAT AFFECT ENZYME ACTIVITY

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.

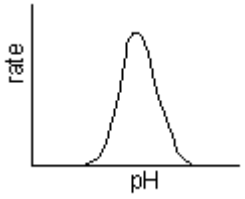
Up to the optimum temperature the rate increases geometrically with temperature (i.e. it's a curve, not a straight line). The rate increases because the enzyme and substrate molecules both have more kinetic energy so collide more often, and also because more molecules have sufficient energy to overcome the (greatly reduced) activation energy. The increase in rate with temperature can be quantified as a Q_{10}, which is the relative increase for a 10°C rise in temperature. Q_{10} is usually 2-3 for enzyme-catalysed reactions (i.e. the rate doubles every 10°C) and usually less than 2 for non-enzyme reactions.

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

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.

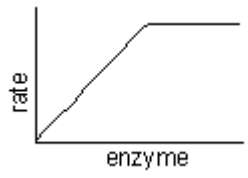
Above the optimum temperature the rate decreases as more and more of the enzyme molecules denature. The thermal energy breaks the hydrogen bonds holding the secondary and tertiary structure of the enzyme together, so the enzyme (and especially the active site) loses its shape to become a random coil. The substrate can no longer bind, and the reaction is no longer catalysed. At very high temperatures this is irreversible. Remember that only the weak hydrogen bonds are broken at these mild temperatures; to break strong covalent bonds you need to boil in concentrated acid for many hours.

2. pH



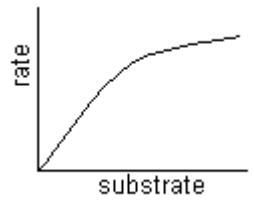
Enzymes have an optimum pH at which they work fastest. For most enzymes this is about pH 7-8 (physiological pH of most cells), but a few enzymes can work at extreme pH, such as protease enzymes in animal stomachs, which have an optimum of pH 1. The pH affects the charge of the amino acids at the active 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 increases linearly, because there are more enzyme molecules available to catalyse the reaction. At very high enzyme concentration the substrate concentration may become rate-limiting, so the rate stops increasing. Normally enzymes are present in cells in rather low concentrations.

4. Substrate concentration



The rate of an enzyme-catalysed reaction shows a curved dependence on substrate concentration. As the substrate concentration increases, the rate increases because more substrate molecules can collide with enzyme molecules, so more reactions will take place. At higher concentrations the enzyme molecules become saturated with substrate, so there are few free enzyme molecules, so adding more substrate doesn't make much difference (though it will increase the rate of E-S collisions).

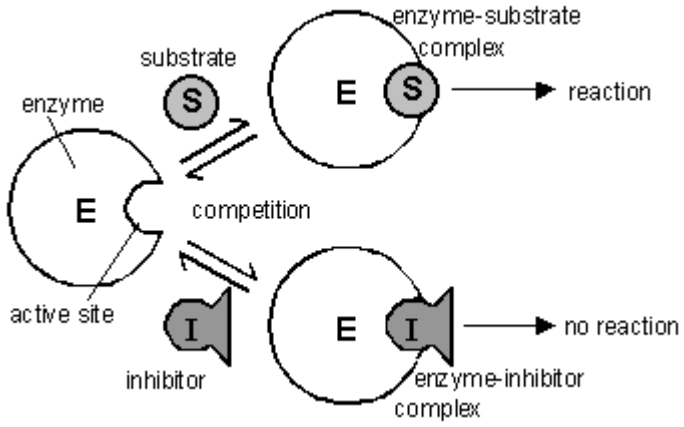
The maximum rate at infinite substrate concentration is called v_{max}, and the substrate concentration that give a rate of half v_{max} is called K_M. These quantities are useful for characterising an enzyme. A good enzyme has a high v_{max} and a low K_M.

5. Covalent modification

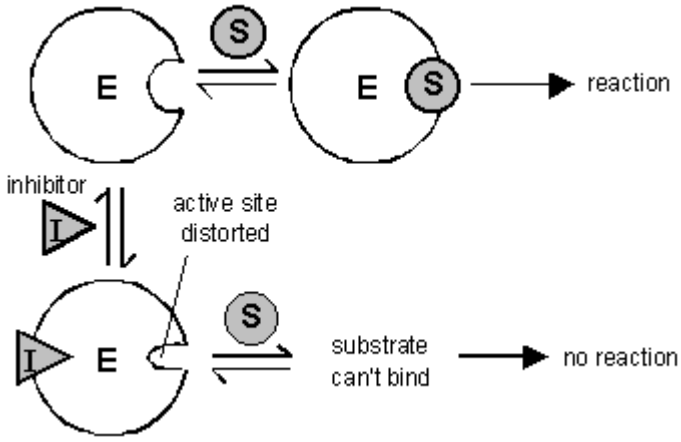
The activity of some enzymes is controlled by other enzymes, which modify the protein chain by cutting it, or adding a phosphate or methyl group. This modification can turn an inactive enzyme into an active enzyme (or vice versa), and this is used to control many metabolic enzymes and to switch on enzymes in the gut (see later) e.g. hydrochloric acid 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 normal substrate molecule, and 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. 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. The sulphonamide anti-bacterial drugs are competitive inhibitors.

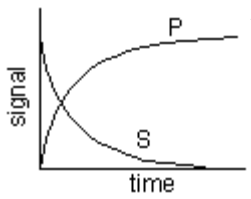


(b) A non-competitive inhibitor molecule is quite different in structure from the substrate molecule and does not fit into the active site. It binds to another part of the enzyme molecule, changing the shape of the whole enzyme, including the active site, so that it can no longer bind substrate molecules. Non-competitive inhibitors therefore simply reduce the amount of active enzyme (just like decreasing the enzyme concentration), so they decrease v_{max}, but have no effect on K_M. Inhibitors that bind fairly weakly and can be washed out are sometimes called reversible inhibitors, while those that bind tightly and cannot be washed out are called irreversible inhibitors. Poisons like cyanide, heavy metal ions and some insecticides are all non-competitive inhibitors.

ENZYME KINETICS

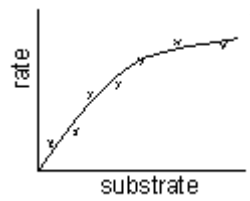
This means measuring the rate of enzyme reactions.

1. Firstly you need a signal to measure that shows the progress of the reaction. The signal should change with either substrate or product concentration, and it should preferably be something that can be measured continuously. Typical signals include colour changes, pH changes, mass changes, gas production, volume changes or turbidity changes. If the reaction has none of these properties, it can sometimes be linked to a second reaction, which does generate one of these changes.



2. If you mix your substrate with enzyme and measure your signal, you will obtain a time-course. If the signal is proportional to substrate concentration it will start high and decrease, while if the signal is proportional to product it will start low and increase. In both cases the time-course will be curved (actually an exponential curve).

3. How do you obtain a rate from this time-course? One thing that is not a good idea is to measure the time taken for the reaction, for as the time-course shows it is very difficult to say when the reaction ends: it just gradually approaches the end-point. A better method is to measure the initial rate - that is the initial slope of the time-course. This also means you don't need to record the whole time-course, but simply take one measurement a short time after mixing.



4. Repeat this initial rate measurement under different conditions (such as different substrate concentrations) and then plot a graph of rate *vs.* the factor. Each point on this second graph is taken from a separate initial rate measurement (or better still is an average of several initial rate measurements under the same conditions). Draw a smooth curve through the points.

Be careful not to confuse the two kinds of graph (the time-course and rate graphs) when interpreting your data.

One useful trick is to dissolve the substrate in agar in an agar plate. If a source of enzyme is placed in the agar plate, the enzyme will diffuse out through the agar, turning the substrate into product as it goes. There must be a way to distinguish the substrate from the product, and the reaction will then show up as a ring around the enzyme source. The higher the concentration of enzyme, the higher the diffusion gradient, so the faster the enzyme diffuses through the agar, so the larger the ring in a given time. The diameter of the ring is therefore proportional to the enzyme concentration. This can be done for many enzymes, e.g. a protein agar plate can be used for a protease enzyme, or a <u>starch agar plate</u> can be used for the enzyme amylase.

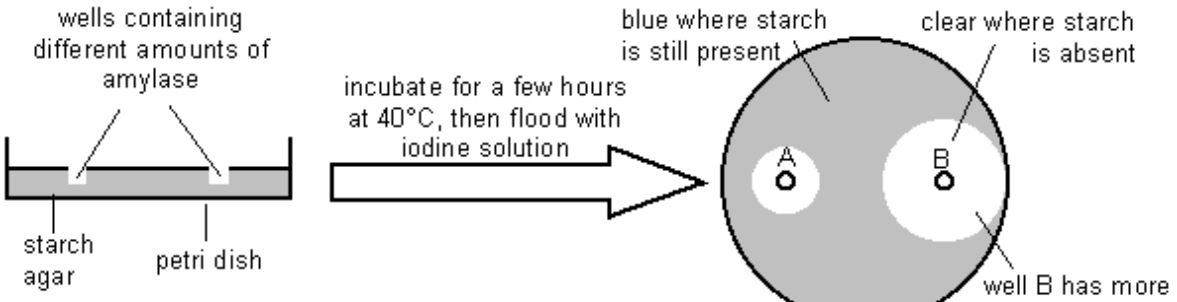


Figure 1. starch agar plate

<u>QUESTIONS:</u>

1) a) Why does a 'good' enzyme have a high V_{max} and low K_m?

   b) Compare the impact of a competitive inhibitor and non-competitive inhibitor on the above values.

2) Identify six possible signals used to measure the progress a reaction.

3) Explain how the rate of a reaction can be determined by using a time-course.

4) Explain what the Figure 1. Diagram (starch agar plate) is illustrating.