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Investigating the effect of copper sulfate concentration on catalase activity using paper discs Transcript

Enzymes are proteins that act as catalysts. They increase the rate of a reaction but are not changed in this process.

Enzymes achieve this by providing a complementary active site that binds to the substrate to form an enzyme-substrate complex. This decreases the activation energy required to make the products of the reaction.

An example of an enzyme is the protein catalase. It has been estimated that one catalase molecule can catalyse the decomposition of 44 million molecules of hydrogen peroxide, its substrate, in one second.

Hydrogen peroxide is a toxic by-product of metabolic processes in the cell and is found in plant and animal tissues.

When hydrogen peroxide breaks down, water and oxygen gas are the products. This produces bubbles and is a product that can be easily measured.

There are several factors that affect the ability of enzymes to catalyse specific biochemical reactions. These include temperature, pH, the concentration of substrate and the concentration of the enzyme in the reaction mixture.

Another is the presence and concentration of inhibitors, substances that reduce their affinity for their substrate.

Two groups of inhibitors can affect enzyme activity.

In competitive inhibition, the inhibitor has a similar structure to the substrate molecule and can fit into the active site of the enzyme. The inhibitor competes with the substrate for the active site, so the reaction is slower.

In non-competitive inhibition, the inhibitor has a different in structure from the substrate molecule and do not fit into the active site. They bind to another part of the enzyme molecule, sometimes called the allosteric site. This changes the tertiary structure of the enzyme, including the active site, so that it can no longer bind substrate molecules.

In this investigation, celery extract is used as the source of catalase. Breaking up celery cells allows the catalase inside them to dissolve in water to form a solution.

The concentration of a known inhibitor of catalase, copper sulfate, will be changed to investigate the effect of this factor on the rate of the enzyme-catalysed reaction.

In this investigation, the rate of reaction can be estimated by determining the rate of formation of a product – oxygen gas.

One approach that can be used to do this is to soak a small paper disc in celery extract, containing the enzyme, and then placing this into a solution of the substrate in a test-tube – hydrogen peroxide.

The inhibitor, copper sulfate, can be added to the celery extract containing catalase.

Recording the time taken for this disc to begin rising can provide an indication of the rate of the reaction. The faster the gas is released from the surface of the disc, the shorter the time taken for the disc to start to rise.

To prepare the first reaction mixture, 9 cm3 celery extract is put into a test tube using a syringe.

Next, 1 cm^3 of the first copper sulfate stock solution is transferred from the stock beaker into the same test tube using a smaller syringe.

This solution, which is a concentration of 0.1 $moldm^{-3}$ copper sulfate mixed with celery extract containing catalase, is inverted to mix.

Note that the concentration of the copper sulfate has been reduced by a tenth compared with the stock solution.

To prepare the second reaction mixture, 9 cm3 celery extract is put into a test tube using the same 10 cm^3 syringe.

Next, 1 cm^3 of the second, more dilute, copper sulfate stock solution is transferred from the stock beaker into the same test tube using a different 1 cm^3 syringe to the one that was used before. This is to prevent contamination of one inhibitor solution with the other.

This solution, which now contains a concentration of 0.01 $moldm^{-3}$ copper sulfate, is inverted to mix.

Note that again the concentration of the copper sulfate has been reduced by a tenth compared with the stock solution.

A final solution is prepared in a third test-tube by adding 9 cm^3 celery extract using the same 10 cm^3 syringe.

Next, 1 cm^3 of distilled water is transferred from the stock beaker into the same test tube using a different 1 cm^3 syringe to the one that was used before. This is to prevent contamination of this syringe with inhibitor.

Adding an equal volume of distilled water means that this test-tube will act as a control experiment in this investigation.

This solution, which contains no inhibitor, is inverted to mix.

These steps have prepared three solutions that will kept stored at room temperature for use later in the investigation.

It is now time to start the first experiment.

Before this can happen, a holepunch must be used to prepare some small discs of filter paper of an identical size.

One by one, these filter paper discs can be used to determine the rate of the enzyme-catalysed reaction in the three test-tubes. In this first experiment, the test tube containing no inhibitor will be used.

Note that after using them it is important to wash the forceps, so that they do not contaminate the next experiment.

It is important to start the timer as soon as the substrate, hydrogen peroxide, is added to the test-tube. This is done using a new syringe that has not been used to transfer any other solution.

Very soon, bubbles begin to form on the surface of the disc. Not long afterwards, the disc begins to rise. It is at this moment that the timer is stopped.

This is repeated another two times to obtain three readings for the time, which can be used to calculate a mean value.

This procedure is then repeated three times for the next concentration of inhibitor, and then again three times for the final concentration of inhibitor.

In this investigation, five concentrations of substrate, hydrogen peroxide, are used. The procedure described is carried out for each of the concentrations of hydrogen peroxide.

The value recorded for time for each experiment can be converted into a value representing the initial rate of reaction.

This is more appropriate for this investigation as the effect of the inhibitor on enzyme activity can be more easily determined and presented on a graph later.

To do this, the formula 1000/ t is used where t = the time taken for the paper disc to start rising. The initial rate of reaction has units that are $seconds^{-1}$.

This is a table that can be drawn to record the data from this investigation.

For each inhibitor concentration, three values of time are measured, and the mean values are shown here.

It is possible to present this data in the form of a line graph.

At low concentrations of hydrogen peroxide, the rate of the reaction increases almost linearly as the concentration increases.

However, at higher concentrations of hydrogen peroxide, the rate of the enzyme-catalyzed reaction plateaus and reaches a maximum value. This is called the V_{max}.

The results show that the maximum rate of reaction, called V_{max}, is affected by the inhibitor. However, the value of the Michaelis-Menten constant, Km, is constant.

This confirms that copper sulfate is a non-competitive inhibitor of catalase. It likely binds to the allosteric site of catalase and reduces the rate of formation of enzyme-substrate complexes and oxygen release.

This experiment showed the effect of different copper sulfate concentrations on catalase activity.

Finding out how more about the effect of copper ions on enzymes will help determine the changes in growth of plants and the health of animals living near sites of contamination, such as copper mines, and those exposed to copper ions used in some types of pesticide.