Amylase

Introduction

The digestion of starch by amylase is likely to be one of the first enzyme reactions encountered by students, probably using iodine solution to show the disappearance of starch when it is mixed with amylase.

Background

Amylase is a general term for several different enzymes that hydrolyse starch.
There are many natural sources of amylase, the most commonly used being those from mammalian saliva and pancreas; plant amylase from sweet potatoes or from grains such as barley; fungal amylase and bacterial amylase.
Starch is a mixture of **amylose**, a straight chain of glucose molecules and **amylopectin**, a branching chain. Most starches are about 20% amylase and 80% amylopectin.
α-amylases digest starch by randomly breaking the glycosidic bonds between glucose molecules. The product is therefore a mixture of small molecules containing two, three or more glucose molecules, (i.e. a mixture of maltose and dextrins).
β-amylases digest starch by cleaving every second bond starting from one end, producing maltose.
Salivary amylase, (ptyalin), is an α-amylase, as are fungal and bacterial amylases.
β-amylases are obtained from plant sources, such as barley or sweet potato.

Suggestions for investigations

- compare amylase from different sources
- measure optimum temperature and pH of amylases
- investigate the products of the reaction

Investigations with α-amylase

α-amylase is present in saliva. Salivary amylase, (*ptyalin*), is readily available and is considered safe to use when simple precautions are followed.
There are a number of different bacterial and fungal α-anylases available in liquid or powdered form.

SAFETY

Each student should collect and use only THEIR OWN saliva.
After use test-tubes and other laboratory equipment that has been in contact with saliva should be soaked in a sterilising solution such as dilute bleach before being washed up.

If powdered enzymes from bacteria or fungi are used then precautions must be taken when preparing solutions as the enzyme powders may provoke allergic reactions. Solutions should be prepared in a fume cupboard, or with care while wearing a dust mask. Skin contact with powdered enzymes and prepared solutions should be avoided.

The reaction between soluble starch and α-amylase can be followed by monitoring the disappearance of the blue/black starch-iodine complex or the appearance of reducing sugars using DNSA reagent or Benedict's reagent.
Salivary amylase is good for showing the effects of temperature and pH on enzyme activity as it is relatively sensitive to both conditions, though the optimum temperature is considerably higher than is usually given in school textbooks.

To collect salivary amylase

- Rinse the mouth out first with water.
- Take a little water into the mouth, swirl it around and spit out into a container labelled to identify the person whose saliva it contains.

Reaction mixture

A suitable reaction protocol for α-amylase is as follows;

- 0.5cm^3 of a 5% solution of soluble starch
- 4.4cm^3 of pH7 buffer, (the reaction will work with water instead of buffer).
- 0.1cm^3 of amylase, (salivary amylase or a 0.1% solution of bacterial or fungal amylase).

Before adding it to the enzyme the substrate/buffer mixture should be equilibrated to the reaction temperature; 35°C works well, but lower temperatures, though slower, may be easier to maintain.
At 30 second intervals remove 0.1cm^3 of the reaction mixture and add it to 3cm^3 of iodine solution, (2% iodine stock solution in 0.1M HCl)
Read absorbance using red light.

More details, suggestions for investigations and sample results can be viewed on the *Mystrica* website, www.mystrica.com

Investigations with β –amylase

Sweet potato is an excellent source of β-amylase.

Enzyme extraction
Sweet potato (*Ipomoea batatas*) is crushed in a pestle and mortar, or blended, with water using 1cm^3 of water for each gram of sweet potato. 30g of sweet potato gives a useful amount of extract and we have found that large sweet potatoes give a better yield of enzyme than small, young potatoes, though small ones still give good yields.
The extract is filtered through several layers of butter muslin.
If a centrifuge is available it can be used to remove cell debris but the extract will give good results without this stage.
The extract will keep for several weeks refrigerated without much loss of activity.

Enzyme activity can be shown using soluble starch as the substrate and DNSA reagent or Benedict's reagent, to measure the maltose produced. Soluble starch will not be completely broken down by β –amylase as the enzyme can only digest straight chains of glucose an is stopped by branches.
An alternative method is to use **amylose** prepared from potato starch as the substrate as this is completely broken down to maltose, (since there are no branches in the chains to stop the progress of the enzyme), and the blue colour obtained with iodine solution will disappear completely. The method for preparing amylose is given on the Mystrica website, www.mystrica.com

Reaction mixture

Suitable conditions for this reaction are a temperature of 35°C in pH6 buffer.
The buffer/substrate mixture should be allowed to equilibrate to the reaction temperature before being added to the enzyme.

Using soluble starch as the substrate and measuring the production of maltose.
Mix 1.5cm^3 of a 5% solution of soluble starch with 1.5cm^3 of buffer.
Add this to 0.1cm^3 of the enzyme extract to start the reaction.
At intervals of 1 minute remove 0.3cm^3 and add to 0.3cm^3 DNSA reagent in a test tube.
Stand the tube in boiling water for 5 minutes then add 3cm^3 of cold water and read the absorbance using green light.

Using amylose as the substrate and measuring the colour of the blue complex formed between amylose and iodine.
Mix 2cm^3 of amylose from potato starch with 2cm^3 of buffer.
Add this to 0.1cm^3 of the enzyme extract to start the reaction.
At intervals of 30 seconds remove 0.1cm^3 into 3cm^3 of iodine solution, (2% iodine stock solution in 0.1M HCl)
Read absorbance using red light

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