Phosphorylase

Introduction

Potato phosphorylase can be used to demonstrate the synthesis of starch. It can also be used to investigate the equilibrium in a reversible reaction.

Background

Phosphorylase, is usually associated with the breakdown of starch or glycogen by the release of terminal glucose molecules as glucose-1-phosphate. However, if provided with glucose-1-phosphate and in the absence of inorganic phosphate, the reaction proceeds in the opposite direction and starch is synthesised by the addition of successive glucose molecules to pre-existing polysaccharide chains.

Suggestions for investigations

Starch phosphorylase extracted from potato can be used to demonstrate the synthesis reaction in the presence of glucose-1-phosphate. The reaction requires priming with small fragments of partially hydrolysed starch which can grow with the addition of further glucose molecules. It also requires the absence of inorganic phosphate since this will cause the reaction to proceed in the opposite direction.
This system can also be used as a good demonstration of reversibility and equilibrium in an enzyme reaction. The direction of the reaction depends on the ratio of glucose-1-phosphate : inorganic phosphate. By adding phosphate during the course of the reaction the direction can be reversed and synthesis of starch becomes degradation of starch.
In both of these investigations the reaction can be followed using a colorimeter to measure the starch-iodine complex.

Extraction of potato phosphorylase

Try to keep everything as cold as possible during this extraction.
Use a blender, or a pestle and mortar to liquidise small pieces of potato with 0.1M citric acid-sodium citrate buffer pH6 in the ratio 2g potato : 1cm^3 buffer.
Filter the liquid through four layers of muslin and allow the filtrate to stand to let residual starch settle. Alternatively the liquid can be filtered through filter paper. The resulting liquid can be used as it is or centrifuged to remove suspended particles.

Primer

The synthesis of starch by potato phosphorylase can only proceed if the reaction mix is primed by the addition of small fragments of starch at least four glucose molecules in length. A satisfactory primer can easily be prepared by acid hydrolysis of soluble starch. We have found the following method gives good results.

- Place 2cm^3 of a 10% solution of soluble starch in a test tube.
- In a second test tube put 1cm^3 of 2M hydrochloric acid.
- Stand both test tubes in boiling water for a few minutes to heat up then mix the contents and let the mixture stand in boiling water for 4 minutes.
- Remove the test tube and add 1cm^3 of 2M sodium hydroxide to stop the reactions and neutralise the pH.
- Add 0.1cm^3 of this primer to 5cm^3 of the reaction mixture

Reaction mixture

We have used the reaction mixture given here successfully.

- 4.4cm^3 of 0.01M glucose-1-phosphate in citric acid-citrate buffer, (0.1M, pH6).
- 0.1cm^3 primer
- 0.5cm^3 enzyme
- 0.5cm^3 samples were taken and added to 3cm^3 of iodine solution containing 0.1M HCl,

The reaction can be carried out at room temperature, the results reported here were all obtained at 25°C.

The direction of the reaction, (i.e. synthesis or degradation of starch), depends on the ratio of glucose-1-phosphate to inorganic phosphate. The equilibrium point occurs at a phosphate:G-1-P ratio of about 7:1.
Using a phosphate-free buffer the reaction can be run in the direction of starch synthesis, then by adding a phosphate buffer it can be reversed and the starch begins to be degraded.
Comparing this with the effect of the addition of a phosphate-free buffer acts as a control, showing that in this case the reaction will continue to run in the direction of the synthesis of starch.
The buffers used were 0.1M citric acid-sodium citrate buffer pH6 and 0.1M phosphate buffer pH6.

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