Phosphatase

Introduction

A phosphatase is an enzyme that releases a phosphate group from its substrate by hydrolysis. The assay method described here is for an acid phosphatase that can be obtained from germinated seeds.

Background

An acid phosphatase (i.e. a phosphatase with an optimum pH below 7) is readily obtained from germinated mung beans, (beansprouts), though other sources could be investigated.
To measure the activity of the enzyme a good substrate is phenolphthalein phosphate which is colourless until it is hydrolysed to phenolphthalein. Phenolphthalein is pink in alkaline solution and the absorbance can be measured using the colorimeter and green light. Adding the reaction mixture to an alkali also serves to stop the reaction.
This reaction is particularly suitable for studying the effects of end product inhibition since the addition of small amounts of phosphate has a marked effect on the rate of the reaction.

Suggestions for investigations

End product inhibition: The action of phosphatase releases phosphate which has a powerful inhibitory effect on the reaction. This is easily demonstrated by adding small amounts of phosphate buffer at the same pH to the citric acid-sodium citrate buffer.

For example:

0.1M Citric acid-Sodium citrate buffer pH6 (cm^3)	0.1M Phosphate buffer pH6 (cm^3)	1% phenolphptalien diphosphate (cm^3)	Enzyme solution (cm^3)	[Phosphate] mM
2.5	0	0.5	0.5	0
2.4	0.1	0.5	0.5	2.8
2.3	0.2	0.5	0.5	5.7

Enzyme extraction

To prepare an extract of acid phosphatase crush 20g of germinated mung beans, (beansprouts), in a pestle and mortar and add 10cm^3 of water. (Alternatively add 10cm^3 of citric acid-sodium citrate buffer pH6. Do not use a buffer containing

phosphate.) Filter the mixture through several layers of muslin and store refrigerated.

Reaction mixture

The following method works well but there are many potential modifications of the volumes, timing and temperature. The end point should turn pink on addition to sodium carbonate.

- 2.5cm^3 citric acid-sodium citrate buffer pH6 0.5cm^3 1%
- 0.5cm^3 1% phenolphthalein diphosphate tetrasodium salt in water
- 0.5cm^3 enzyme preparation

Incubate for 10 minutes at 25°C then add 0.5cm^3 of the reaction mixture to 2.5cm^3 10% sodium carbonate solution.
Read absorbance using green light.

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