

Lactase

Introduction

Lactase activity can easily be demonstrated using the artificial substrate ONPG which is converted to a yellow product by lactase.

Background

Lactase, is responsible for the hydrolysis of lactose, forming glucose and galactose. It is secreted by the intestinal villi in humans, though the ability to produce the enzyme is lost by many people after childhood and leads to lactose intolerance, an inability to digest lactose.

Lactase, (usually fungal), is readily available in solution; a few drops added to milk help to make it digestible by people with lactose intolerance. This is not quite the same as the mammalian enzyme, being a β -galactosidase.

The method described here uses an artificial substrate, o-nitrophenol-galactopyranoside, (ONPG). Lactase splits colourless ONPG into galactose and o-nitrophenol, (ONP), which is bright yellow.

Suggestions for investigations

The reaction of β -galactosidase, (lactase), with ONPG is particularly good for investigating the effects of varying substrate and enzyme concentration on rate of reaction. Continuous colorimetry can be used to obtain graphs of reaction rates.

Galactose can be used to show end-product inhibition.

The colour of the product ONP is affected by pH so buffering is important and investigations of the effect of pH are not recommended.

Reaction mixture

We have used ONPG dissolved in 0.1M phosphate buffer, pH 7.0 and a 1% solution of a commercial liquid lactase from *Aspergillus*. The buffering is important as the colour of the product (ONP) depends on the pH.

2.9cm³ of the ONPG in buffer solution was added to 0.1cm³ of lactase in a colorimeter cuvette and the absorbance of blue light recorded continuously.

Room temperature (approximately 20°C) was used throughout.

Stored refrigerated the undiluted liquid retained over 60% of its activity after 2 years. Diluted preparations do not keep well but it is easy and inexpensive to prepare fresh dilutions as required.

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