Pepsin

Introduction

The slow breakdown of albumen proteins gives good results with continuous colorimetry and is well suited for the study of the effect of pH on enzyme activity.

Background

Pepsin is a proteolyic (protein splitting) enzyme produced in the gastric glands lining the stomach of vertebrates. It is produced as pepsinogen, a larger molecule that is activated by hydrochloric acid to the smaller pepsin molecule.
Pepsin can only work in a low pH environment, (optimum pH~1.8) Pepsin does not break the bonds between every amino acid so the products of the reaction are polypeptides of varying lengths.
Pepsin can conveniently be assayed using a cloudy suspension of egg white (albumen), which will slowly clear as the albumen protein is broken down into small, soluble polypeptides.

Suggestions for investigations

It is particularly suitable for demonstrating the effect of pH. Pepsin's low optimum pH is noteworthy and interesting comparisons can be made with other enzymes which usually have much higher pH optima.
The effect of temperature on enzyme activity can also be investigated, though this cannot be done with continuous colorimetry because of difficulties maintaining temperatures very different from ambient in the colorimeter. The results may be affected by changes in pH with changing temperature. Each new temperature should have its pH individually adjusted using a pH meter.

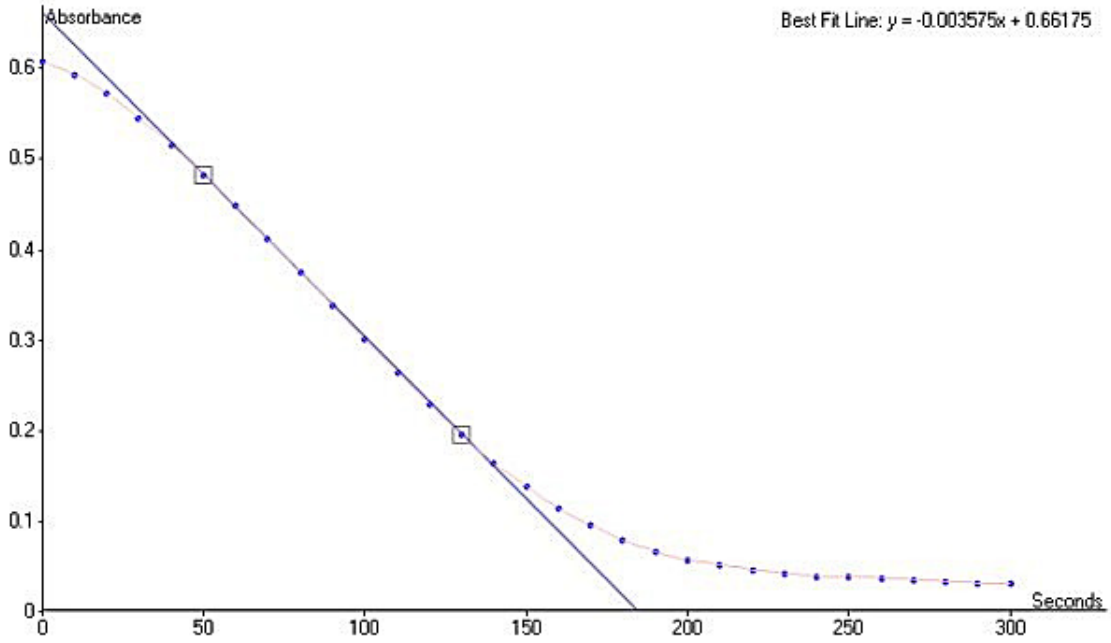
Preparation of an egg white suspension

- separate the white from the yolk – depending on the size of the egg this will probably be about 30cm^3
- add 9 volumes of water – 270cm^3 to a 30cm^3 egg (this seems to work well with a fresh egg, but older eggs may need less water to achieve a good, dense suspension.)
- stir gently to disperse the egg white
- stand the mixture in a bath of hot water, with gentle stirring, for 5-10 minutes, (do not boil this – the suspension will form at around 70°C)
- pour the mixture through 2-4 layers of butter muslin cloth to remove large particles.

You should be left with an opaque suspension. This can be diluted 1:1 with water or buffer for use.

Reaction mixture

The results illustrated below were obtained with 2.8cm^3 egg white suspension adjusted to pH2.1 at 25°C + 0.2cm^3 of 5% pepsin solution. The absorbance was measured using blue light.



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