Enzymes I: Determination of acid phosphatase activity

by Tracey Kuit

Experiment Overview

This experiment looks at the area of enzyme kinetics using an acid phosphatase from potato. Students measure the breakdown of p-nitrophenyl phosphate by phosphatase, by measuring one of the products, p-nitrophenol (PNP). PNP can be measured spectrophotometrically as it is coloured (in alkaline solution). The other product is inorganic phosphate.

In the first part of the experiment students prepare a series of standard solutions containing different amounts of PNP and measure their extinction at 400 nm in the spectrophotometer. Students graph the extinction values against the amount of *p*-nitrophenol and later use this to determine the amounts of *p*-nitrophenol released by enzyme action.

In the second part of the experiment students prepare an enzymatic reaction by mixing the enzyme and buffer with the *p*-nitrophenyl phosphate substrate. At intervals after the start of the reaction, students remove aliquots of the reaction mixture and add them in turn to each of the tubes containing NaOH. The NaOH will stop the reaction, and students then determine the amount of coloured *p*-nitrophenol product released as a function of time by measuring the extinction of the contents of these tubes in a spectrophotometer and interpolating the results from the standard curve.

Students finally graph the amount of *p*-nitrophenol produced in the reaction against time and use this graph to calculate the initial rate of the reaction, and then calculate the specific activity of the enzyme.

Learning Experience

This experiment provides students with an introduction to enzymes, how they work and the steps necessary to perform an enzyme assay. It is a very simple assay which is easy to measure and interpret. The enzyme is quite robust and this experiment works successfully across the student groups. Students once again get hands on use of the spectrophotometer and the important skill of graphing. By preparing standards, students also test their pipetting skills and visualise the importance of accurate and reproducible pipetting. Students must also complete some pre-lab questions, many mathematical involving concentrations and amounts. Many second year students still struggle with these important calculations. This is a skill learnt best through practice. We believe this is a good initial experiment in the area of enzyme kinetics.

Aims and Objectives

The objectives of the experiment are:

1. To construct a standard curve for *p*-nitrophenol (the coloured product of the enzyme-catalysed reaction).
2. To use a spectrophotometer to measure the increase in *p*-nitrophenol produced, as a function of time, for a known concentration of enzyme.
3. To use the standard curve to convert the changes in absorbance measured to amounts (in mol) of *p*-nitrophenol produced.
4. To plot the amount of *p*-nitrophenol produced versus time and to use this plot in calculating the specific activity of acid phosphatase under these conditions.

Level of Experiment

This practical is aimed at second year first semester introductory biochemistry students. This is the first of 2 experiments on enzyme kinetics. The second experiment goes on to include analysis of reaction velocities and determination of Km and Vmax and construction of a Lineweaver-Burk plot.

Keyword Descriptions of the Experiment

Domain
Biological chemistry
Specific Descriptors
enzymes, kinetics, spectrophotometry, phosphatase

Course Context

This experiment is conducted half-way through the session. Prior to this practical, student's have had four hours of lectures on enzymes, including one hour on enzyme kinetics. They have also had a one hour tutorial which covers some pre-lab information and revises the lecture material.

Prerequisite Knowledge and Skills

In order to complete this practical students need the required skills of completing basic mathematical calculations, involving concentrations etc, and graphing. Both of these skills were a focus in the first year subject Biol103 (the pre-requisite subject for this course). Students have already completed practicals using the spectrophotometers but can re-fresh in the prac time.

Time Required to Complete

Prior to Lab: 1-2 hours for pre-lab questions and prac prep.
In Laboratory: 3 hours
After Laboratory: none

Experiment History

This experiment has been used in this subject for more than 10 years.
This experiment has a long history in the School of Biological Sciences at the University of Wollongong; whilst the authors listed in section (1.9) are responsible for the educational analysis of this experiment, their submission of it to ASELL is done on behalf of all academic staff.