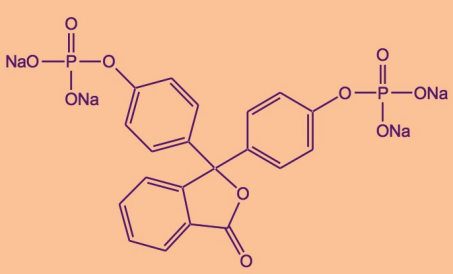
Fun with Phosphatase

In a previous issue of the SSERC Bulletin [1] we explored how the enzyme dopa oxidase might be used to support learning and teaching across a range of curriculum levels. Phosphatase is one of the enzyme systems specifically mentioned in both of the recently implemented *CfE* Highers in Biology [2] and Human Biology [3]. A particular emphasis is the use of phosphatase in '*experiments on product inhibition*'.

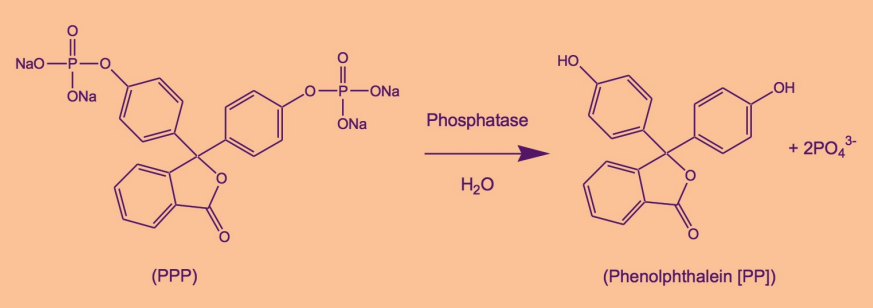
Within SSERC we believe that assays of phosphatase activity are useful in a variety of protocols and some of these are detailed here. The experiments described here are not new and are, in large part, based on the work of Meatyard [4].

Phosphatase enzymes are involved in a range of metabolic reactions. A key function of these enzymes is to release phosphate groups into the metabolic pool thereby increasing their availability for use in a range of processes including ATP synthesis and membrane construction. Acid phosphatases (those with a pH optimum below 7.0) can be extracted from a range of plant tissues with germinating mung beans being a particularly cheap and reliable source (see section marked 'Experimental' for more details).

The substrate used here for phosphatase is phenolphthalein bisphosphate (abbreviated as PPP):



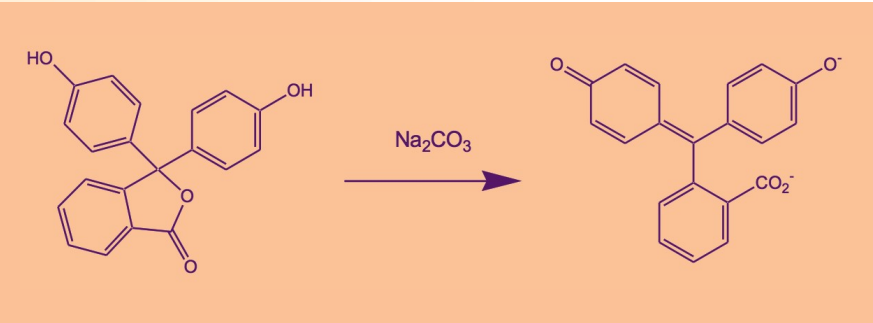
Phenolphthalein bisphosphate (PPP)



(PPP) (Phenolphthalein [PP])

Under suitable conditions the enzyme phosphatase catalyses the following reaction as shown above.

At neutral or acidic pH the products of the above reaction (phenolphthalein [PP] and phosphate) are both colourless and their presence is, therefore, difficult to detect. This can be overcome through the addition of sodium carbonate which has 2 major effects: (i) raising the pH of the solution to above 10 with consequent cessation of enzyme activity and, (ii) converting (colourless) phenolphthalein to its anionic form with its characteristic pink colour in solution:



A detailed protocol (together with a supporting PowerPoint) for 'a standard assay' is available on the SSERC website (http://www.sserc.org.uk/index.php/biology-2/biology-resources/higher-biology-revised/metabolism-a-survival/3426-metabolism-is-essential-for-life) but briefly our preferred assay system can be described as:

a) Place 1 cm^3 of the sodium carbonate solution into each of 7 cuvettes.
b) Prepare enzyme extract and substrate solutions and store them at a given temperature.
c) Mix enzyme extract with buffer of desired pH.
d) Add 1 cm^3 of the mixture in (c) above to the first cuvette and use this solution as your blank.
e) Add substrate solution to the mixture of enzyme extract and buffer.
f) At 1 minute intervals remove 1 cm^3 of the mixture in (e) above and add it to one of the cuvettes containing sodium carbonate solution.
g) Measure the absorbance of the solutions at 550 nm.

As can be seen in Figure 1 an increase in the concentration of phenolphthalein is readily observed after a few minutes and changes can be readily observed using a colorimeter.

Investigations

With a working assay one might think about how this could be incorporated into a student investigation. As noted above phosphatase is inhibited by increased concentrations of phosphate and so is often used as an example of end-product inhibition. Other areas which are worthy of investigation include:

- *Effect of temperature.*
- *Effect of pH.*
- *Phosphatase levels from different species.*
- *Phosphatase levels in different locations of the same species.*

Figure 1 *- Changes in colour observed after 1 minute intervals as described in step (f) above.*

In particular Meatyard [4] highlights the pH and temperature profiles of phosphatase extracted from mung beans (shown in Figures 2 and 3) and these are worthy of further exploration.

Experimental

1 PREPARATION OF ENZYME EXTRACT

1.1 Mung bean germination

Take 2 g of mung bean seed for each group of students and place in a dark container with a lid (e.g. margarine tub or similar). Make sure that the container is sufficiently large so as to ensure that that there is only a single layer of seed. Cover the seed with tap water, replace the lid and leave for 24 h at a temperature of 30°C - this can usefully be achieved by floating the container in a water-bath. After 24 h drain off the water and rinse with fresh tap water. Replace the lid and return to the water-bath. From this time on seeds should be kept moist but

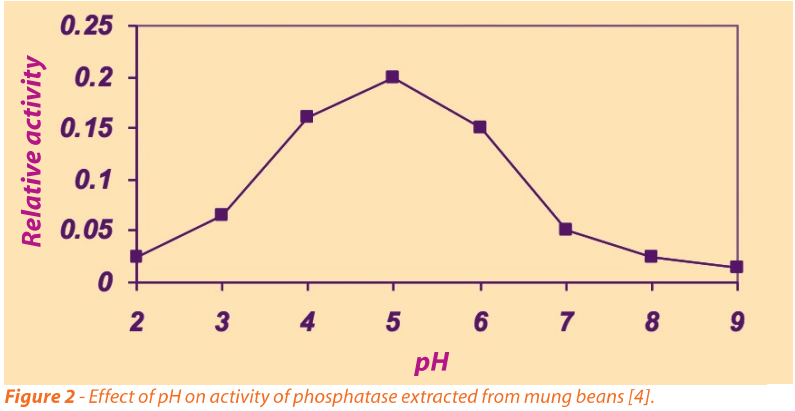


Figure 2 *- Effect of pH on activity of phosphatase extracted from mung beans [4].*

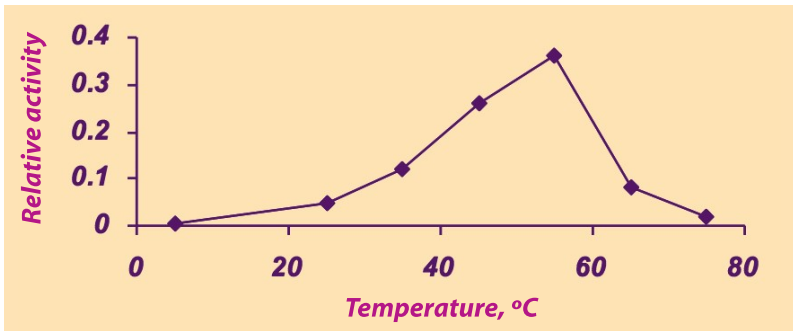


Figure 3 *- Effect of temperature on activity of phosphatase extracted from mung beans [4].*

not soaking in water. Repeat this rinsing procedure at 48 h and 72 h. The optimal size of seed is reached when the radicle (seed root) has reached a length of 3 - 4 cm.

1.2 Extraction of enzyme

Enzyme extracts lose activity over time and it is strongly recommended that enzyme extracts are prepared on the day of use. For each group of students select 12 seedlings (3 - 4 cm in length) and place them into a mortar. If the green testa (seed case) is still attached it is important that it is removed and discarded. Add approximately 5 cm^3 of tap water and grind the seedlings with a pestle until a smooth paste is obtained. Add a further 10 cm^3 of tap water to the paste. The crude extract should be centrifuged for at least 5 min at a minimum of 2500 g. Carefully remove the supernatant and dispense into small bottles.

If you do not have access to a suitable centrifuge, enzyme extracts can be prepared by using 10 cm^3 of water rather than 15 cm^3 of water for each group of 12 seedlings and allowing the extract to settle at 4°C overnight. Carefully remove the supernatant and dispense into small bottles as above.

1.3 Extraction of enzyme from bean-sprouts

Many supermarkets offer bean sprouts for sale and these can be used in place of mung beans

Homogenisation of approximately 100 g of bean sprouts (no additional water should be added) should produce approximately 50 cm^3 of crude extract. This crude extract should be filtered through muslin and then centrifuged as above. Carefully remove the supernatant and dispense into small bottles.

2 MATERIALS

PPP is available from Sigma-Aldrich (http://www.sigmaaldrich.com/) as the tetrasodium salt - catalogue P9875-1G; cost £14.30 g-1.

References

[1] Dopa oxidase - a perfect enzyme? SSERC Bulletin, 242, 8-10.
[2] SQA (2014) Higher Biology Course Support Notes - available at www.sqa.org.uk/files_ccc/CfE_CourseUnitSupportNotes_Higher_Sciences_Biology.pdf.
[3] SQA (2014) Higher Human Biology Course Support Notes – available at www.sqa.org.uk/files_ccc/CfE_CourseUnitSupportNotes_Higher_Sciences_HumanBiology.pdf.
[4] Meatyard, B (1999), Phosphatase enzymes from plants: a versatile resource for post-16 students. J Biol. Ed., 33, 109-112.

Year of Light

EnLightenment: build it, see it, show it

To celebrate the UN International Year of Light 2015, Heriot-Watt University and the Wellcome Trust are launching a smartphone microscope competition for schools and the public!

In collaboration with SSERC, we have developed smartphone microscope kits, which we will be distributing to secondary schools across Scotland. S1 pupils can build the microscopes and take their own images of life in miniature; these images can be entered into a competition to win a prize presented by Prof. Jim Al-Khalili at the Scottish closing event of the International Year. The public will also be able to enter the competition at various science festivals during 2015.

Schools can register their interest at http://eepurl.com/bhX2uP