End-product inhibition of the enzyme phosphatase - revisited

The SQA Course Specifications for Higher Biology and Higher Human Biology each suggest, as a context for the study of metabolic pathways, a learning activity where students might, "Carry out experiments based on end-product inhibition using phosphatase and phenolphthalein phosphate" [1].

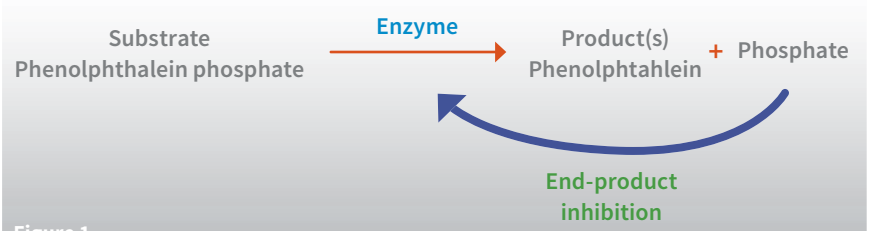

Figure 1

One form of the enzyme phosphatase is easily extracted from mung bean shoots (bean sprouts) and SSERC has produced several protocols based on investigating the activity of this enzyme [2].

The protocol suggested here is based on one produced by the Biotechnology Scotland Project, SAPS and the Higher Still Development Unit [3], which in turn is based on the work of Dr Barry Meatyard [4].

Background

Phosphatase enzymes are found in a wide range of plant and animal tissues. They are key enzymes in cell metabolism because they release the phosphate groups required for synthesis of, for example, ATP, phospholipids and nucleotides.

There are two main groups of phosphatase enzymes. These are acid, or alkaline depending on the pH at which they work optimally. In this protocol we will use an acid phosphatase (pH 5) which is simply and cheaply extracted from germinating mung beans (beansprouts).

For the enzyme assay proposed here, an artificial substrate phenolphthalein bisphosphate (PPP) is used. Phosphatase reacts with PPP to produce phenolphthalein (PP) and a free phosphate group. Increased levels of phosphate inhibit the enzyme (Figure 1).

This protocol uses a range of concentrations of sodium dihydrogen phosphate in a citric acid buffer solution to provide the enzyme-inhibiting phosphate group. The higher the concentration of phosphate present, the greater the inhibition of the enzyme. However, in these acidic conditions, the products of the reaction are colourless, so after a suitable incubation time an alkaline solution (sodium carbonate) is added. This serves two functions: it causes any free phenolphthalein to turn pink and it stops the reaction by denaturing the phosphatase enzyme.

Two different salts of sodium and phosphate are used:

- Disodium hydrogenphosphate (Na_2HPO_4) is a component of the pH 5 citric acid background buffer.
- Sodium dihydrogenphosphate (NaH_2PO_4) is added in varying amounts to the background buffer to provide a range of concentrations of enzyme inhibitor (Table 1).

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Cuvette number	1	2	3	4	5
Concentration NaH_2PO_4 (M) in background buffer	0	0.05	0.10	0.20	0.30

Table 1

	20 minutes at 30°C				
Inhibitor concentration (M)	0	0.05	0.10	0.20	0.30
Absorbance	0.133	0.112	0.093	0.075	0.064

Table 2

Method

In outline, the basic method is as follows:

- Prepare solutions of inhibitor in buffer.
- Extract phosphatase from bean sprouts: grind using a mortar and pestle; filter; centrifuge.
- Set up reaction mixtures in each of 5 cuvettes: 1.5 cm^3 appropriate inhibitor + buffer solution; 0.3 cm^3 PPP; 0.3 cm^3 enzyme extract.
- Incubate for 20 minutes at 30°C.
- Stop the reaction: add 1.5 cm^3 sodium carbonate (makes PP turn pink).
- Measure absorbance using colorimeter (550 nm or green filter).

A detailed teacher/technical guide is available on the SSERC website [5].

Our experiments

Using the method and reaction mixtures described above we set out to measure the effect of varying inhibitor concentration on enzyme activity as indicated by the absorbance of phenolphthalein in the solutions.

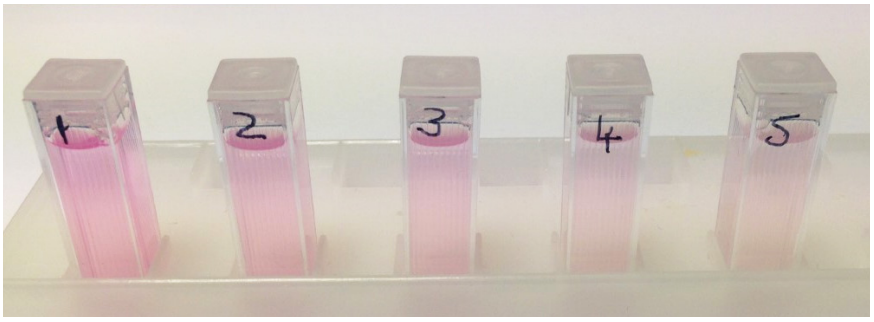

Figure 2 - Phenolphthalein phosphate, phosphatase and a range of concentrations of inhibitor (Table 1). After 20 minutes the addition of sodium carbonate alters the pH thus denaturing the enzyme and causing the phenolphthalein to become pink.

	10 minutes at 20°C				
Inhibitor concentration (M)	0	0.05	0.10	0.20	0.30
Absorbance	0.050	0.050	0.049	0.048	0.043

Table 3

	20 minutes at 20°C				
Inhibitor concentration (M)	0	0.05	0.10	0.20	0.30
Absorbance	0.083	0.065	0.055	0.053	0.040

Table 4

We used a Mystrica colorimeter [6] using the green diode. We used water as a blank. Initially the cuvettes were incubated by floating them in a water bath at 30°C for 20 minutes (Table 2 and Figure 2).

With school-based investigative work in mind, we were interested to see if this procedure could be repeated at room temperature within a typical teaching period, thereby negating the necessity for a water bath. Tables 3 and Figure 3, and Table 4 and Figure 4 contain results of reactions carried out at room temperature for 10 and 20 minutes respectively.

We concluded that it is possible to use the basic experimental protocol with the suggested reaction mixtures and get clearly visible and measurable results which illustrate end-product inhibition of phosphatase at room temperature ~20°C.

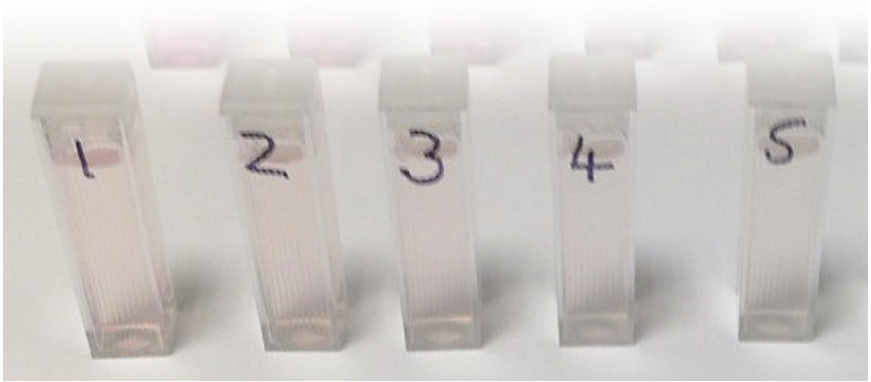

Figure 3

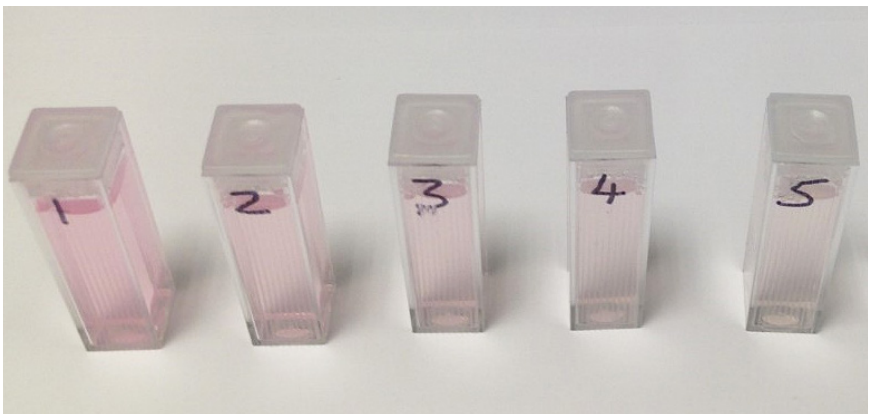

Figure 4

For visual comparison, Figure 5 shows cuvette number 1 from each of our experiments.

Investigation ideas

- Investigate enzyme properties by varying temperature – see also [2].
- Investigate enzyme properties by varying pH [2].
- Investigate the effect of varying substrate concentration.
- Investigate the rate of reaction by varying the times at which the reaction is stopped [2].
- Investigate the inhibiting effect of other phosphates, or of a general enzyme inhibitor.
- Compare phosphatase activity in different plant species. <<

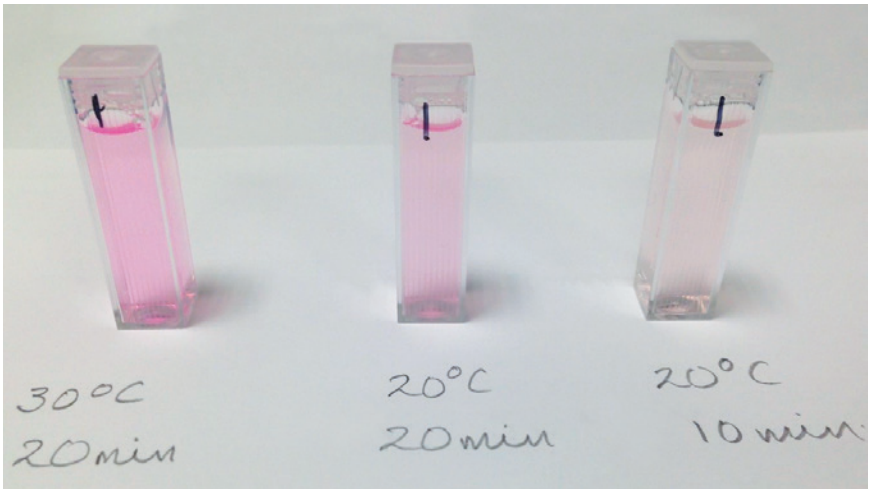

Figure 5

Further information

Further student support and information relating to this protocol, and to phosphatase enzymes can be found on the SAPS website:

- Phosphatase enzymes in plants https://www.saps.org.uk/secondary/teaching-resources/292-student-sheet-14-phosphatase-enzymes-in-plants.
- https://www.saps.org.uk/?category=0&text=phosphatase&option=com_qflarticlesfilter&view=articles&Itemid=0&qfl-search=1&modulename=Article+Filter+Right+Module.

References

[1] SQA (2018) Higher Biology Specification available at https://www.sqa.org.uk/files_ccc/HigherCourseSpecBiology.pdf (accessed November 2019).
SQA (2018) Higher Human Biology Specification available at https://www.sqa.org.uk/files_ccc/HigherCourseSpecHumanBiology.pdf (accessed November 2019).

[2] Fun with Phosphatase, *SSERC Bulletin*, **251**, 6-8, available at https://www.sserc.org.uk/wp-content/uploads/2015/05/SSERC251_p6-8.pdf and SSERC website, Higher Biology available at https://www.sserc.org.uk/subject-areas/biology/higher-biology/enzymes/.

[3] Advanced Higher Practical Activities (2000), Higher Still Development Unit.

[4] Meatyard, B (1999), Phosphatase enzymes from plants: a versatile resource for post-16 students. J Biol. Ed., 33, 109-112.

[5] Link to SSERC website teacher/ technical guide available at https://www.sserc.org.uk/subject-areas/biology/higher-biology/enzymes/ or https://www.sserc.org.uk/subject-areas/biology/higher-human-biology/cell-metabolism-4/.

[6] Mystrica website www.mystrica.com.