Phosphorylase - moving from N5 to AH

Phosphorylase, extracted from potatoes, is an enzyme that has been used in many classrooms to exemplify a synthesis reaction. The enzyme, found in several higher plants, catalyses the reversible transfer of glucosyl units from glucose-1-phosphate (substrate) to the non-reducing end of α-1,4-D-glucan chains with the release of phosphate, forming starch (Figure 1). The production of starch can be monitored by the addition of iodine, forming a blue-black complex.

This practical activity is particularly relevant at National 5 Biology level, with the SQA course specifications outlining a requirement for learners to understand that "enzymes can be involved in degradation and synthesis reactions" (Figure 2).

In this bulletin, we provide progression for learners working at Advanced Higher level through the inclusion of colorimetry and the production of a standard curve to estimate the concentration of starch synthesised during the reaction, supporting Key Area 1b of Cells and Proteins (Figure 2).

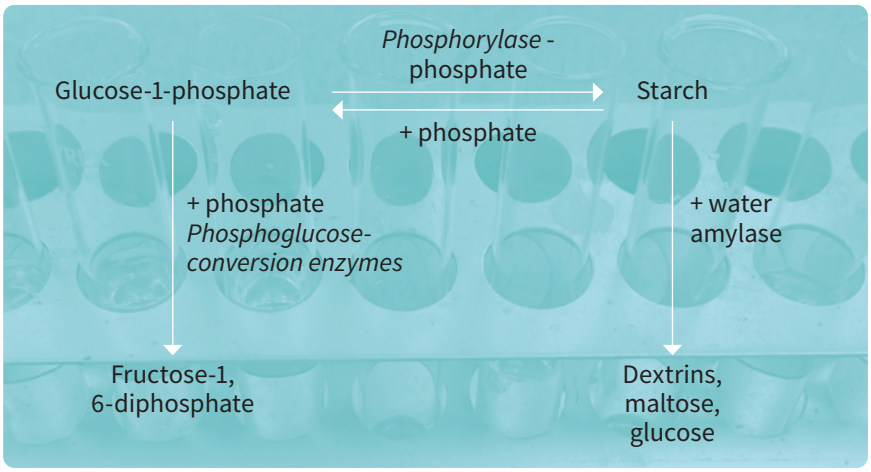

Figure 1 - An overview of the role of phosphorylase in metabolism. Image adapted from (1).

Aim

The aim of this investigation is to extract an enzyme from biological material and investigate the enzymatic synthesis of starch.

Materials and method

In the first part of this investigation, phosphorylase must be extracted from potatoes. This requires the materials outlined in Figure 3.

Approximately 50 g chopped and peeled potato must be blended with sufficient water to form a slurry. After passing through muslin, the filtrate must be centrifuged (> 3000 rpm for 5 minutes) and the supernatant collected (contains the enzyme). Centrifugation separates the enzyme from starch granules; this should be confirmed by taking a drop of supernatant and combining

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National 5 Biology

Candidates must become familiar with the techniques to measure enzyme activity.

Key area 4b: Required knowledge includes "enzymes can be involved in degradation and synthesis reactions".

A suggested learning activity is to "investigate the action of potato phosphorylase".

Advanced Higher Biology

In the course specification, Key Area 1b states that the learner must understand:

★ the method and uses of a colorimeter

★ know how to produce a standard curve to determine an unknown.

SQA

Figure 2 - Curriculum links relevant to this investigation.

Materials

- potato
- knife, chopping board, vegetable peeler
- blender
- muslin
- beaker
- centrifuge
- centrifuge tubes & rack
- container to store extract
- 3 cm^3 plastic pipettes
- 0.01 M iodine solution
- dimple tile

Figure 3 - Materials required for part 1.

it with a drop of iodine on a dimple tile. If there is a blue/black colour, centrifuge the sample for longer. The supernatant will discolour over 24 hours so should be prepared fresh.

The next part of the investigation focuses on the phosphorylase assay. The following steps are required:

1) Set up 7 cuvettes. To cuvette 1, add 3 cm^3 water (to zero the colorimeter). To cuvette 2-7, add 1 cm^3 water and 1 cm^3 iodine.
2) Collect 2 test tubes. To test tube 1 (colorimetric blank), add 1 cm^3 enzyme and 1 cm^3 water. To test tube 2, add 5 cm^3 enzyme and 5 cm^3 1% glucose-1-phosphate. Immediately start the stopwatch and incubate reactions at room temperature.
3) After 2 minutes, transfer 1 cm^3 from test tube 2 to cuvette [3].
4) After 60 s, transfer 1 cm^3 from test tube 2 to cuvette 4.
5) Repeat step 4 every 60 s.
6) Measure the absorbance of the samples. Using the red diode (or approximately 600 nm), zero the absorbance reading using the cuvette of water. To cuvette 2, add 1 cm^3 of the colorimetric blank (test tube 1). This should be recorded as 0 s time point.
7) Measure the absorbance of each sample.

Sample results for this investigation are shown in Figure 6. The assay was carried out in triplicate and

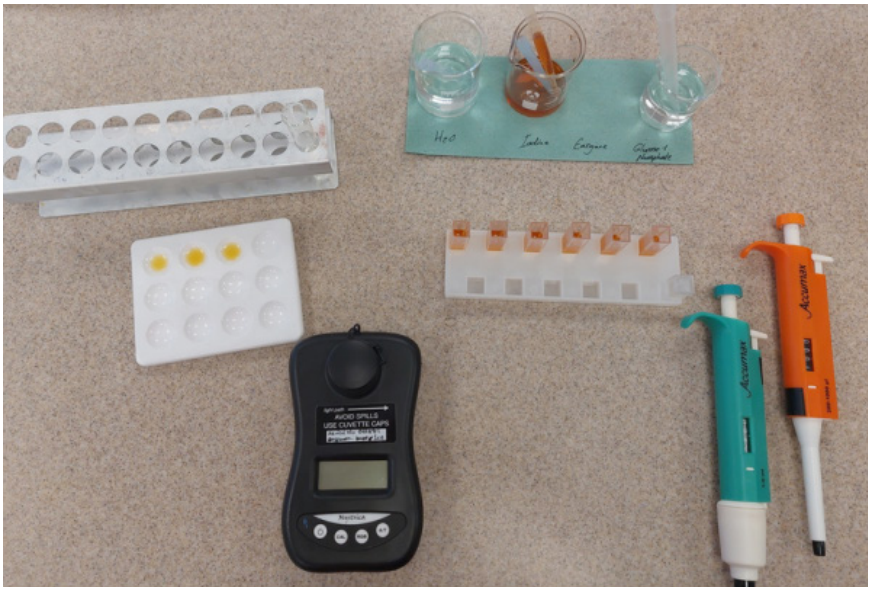

Figure 4 - Materials required for Part 2 of the investigation.

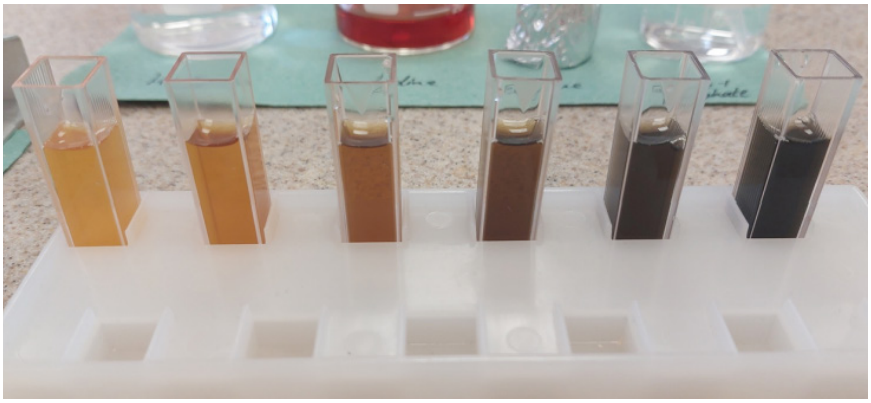

Figure 5 - Synthesis of starch from glucose-1-phosphate by phosphorylase over 5 minutes.

mean values calculated. The mean absorbance value obtained at 0 s was subtracted from remaining absorbance values since this reflected the absorbance of the enzyme alone, not the production of starch, and would be present in all samples.

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Time (s)	Absorbance				
	Trial 1	Trial 2	Trial3	Mean	Corrected mean
0	0.206	0.203	0.207	0.207	0.000
120	0.229	0.232	0.240	0.240	0.033
180	0.327	0.334	0.336	0.336	0.129
240	0.567	0.514	0.533	0.538	0.331
300	0.769	0.769	0.783	0.780	0.573
360	1.082	1.065	1.032	1.060	0.853

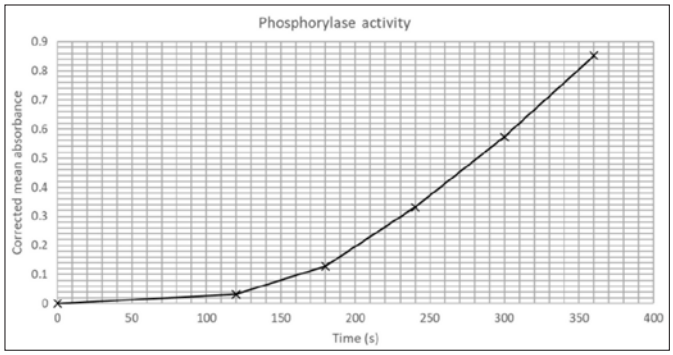

Figure 6 - Results obtained from the phosphorylase assay.

Materials

- 0.01 M iodine
- colorimeter (red diode)
- 9x cuvettes and rack
- 8x test tubes and rack
- 0.1 mg/ml starch suspension
- 0.2 mg/ml starch suspension
- 0.3 mg/ml starch suspension
- beaker of distilled water
- 1 cm³ automatic pipette and tips
- 10 cm³ measuring cylinder
- 3 cm³ plastic pipettes

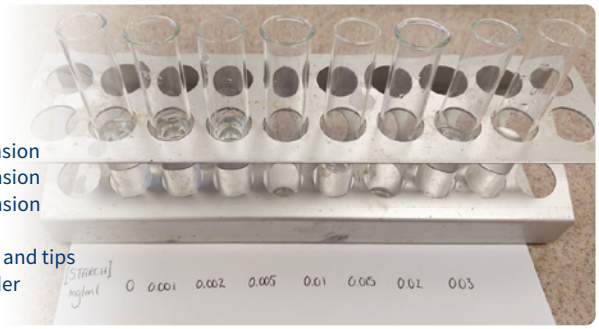

Figure 7 - Materials required to produce a starch standard curve.

Production of a standard curve

To extend this investigation for learners working at Advanced Higher level, a standard curve of starch can be plotted from which estimates of starch concentration synthesised during the phosphorylase assay can be made. Learners can develop their skills using measuring instruments to accurately prepare a dilution series of starch. These are then combined with iodine and the absorbance measured (Figure 7).

Learners should prepare a dilution series of starch to include concentrations of 0.001, 0.002, 0.005, 0.01, 0.015, 0.02 and 0.03 mg/ml starch. Once prepared, the following steps should be taken:

1) Collect 8 cuvettes: to cuvette 1, add 3 cm³ water (to zero the colorimeter); to cuvette 2-8, add 1 cm³ water and 1 cm³ iodine.
2) To cuvettes 2-7: add 1 cm³ appropriate starch suspension, working from the most dilute to the most concentrated.
3) Set the colorimeter to the red diode (or approximately 600 nm). Zero the colorimeter using the cuvette of water and then measure the absorbance of each solution.

Plot the results on graph paper or using a suitable graphing program, such as Excel. Add a line of best fit (hand-drawn graphs) or trendline (Excel). See Figure 9.

The data collected during the phosphorylase assay can be processed to estimate the starch concentration in each sample, as a result of phosphorylase action on the substrate glucose-1-phosphate. This can be done using the equation of the trendline of the standard curve.

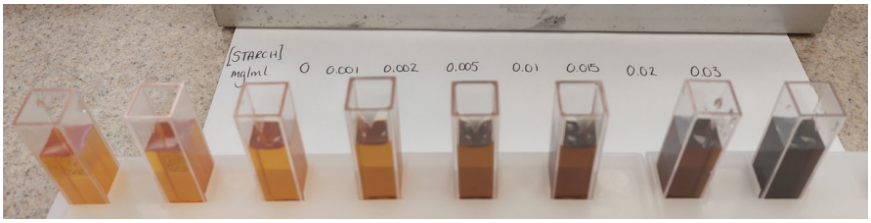

Figure 8 - A standard curve for starch can be produced from a suitable dilution series.

Starch Concentration (mg/ml)	Absorbance
0	
0.001	
0.002	
0.005	
0.01	
0.015	
0.02	
0.03	

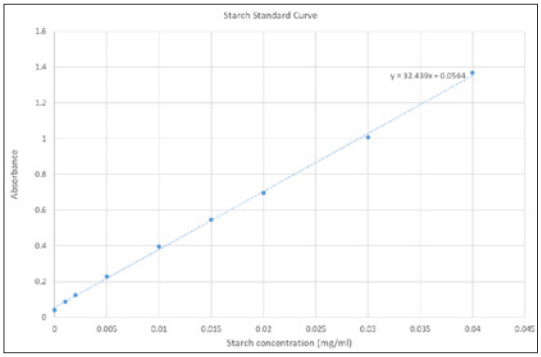

Figure 9 - Standard curve for starch.

Figure 10 provides sample results obtained at SSERC.

This protocol is available via the SSERC website, providing a breakdown of relevant calculations, support with the dilution series and technical support [2].

Time (s)	Starch concentration (mg/ml)	Starch concentration (µg/ml)
0	0	0
120	0	0
180	0.002	2.2
240	0.008	8.5
300	0.016	15.9
360	0.025	24.5

Figure 10 - Concentration of starch synthesised by phosphorylase from glucose-1-phoshate over 6 minutes.

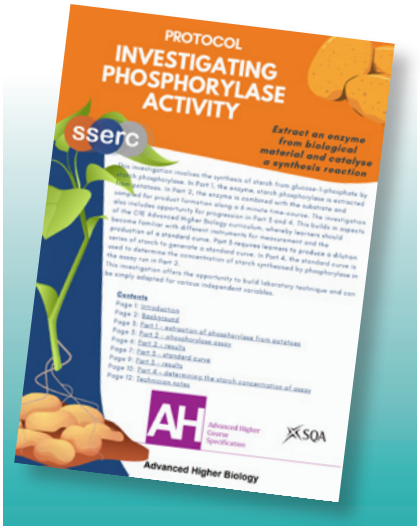

Figure 11 - SSERC's latest phosphorylase assay resource.

References

[1] Hanes, C.S. (1940), The reversible formation of starch from glucose-1-phosphate catalysed by potato phosphorylase, The Royal Society Publishing, available at https://royalsocietypublishing.org/doi/pdf/10.1098/rspb.1940.0035.

[2] SSERC (2023), Investigating phosphorylase activity, available at https://www.sserc.org.uk/wp-content/uploads/2023/08/Protocol-Phosphorylase-Activity.pdf.