Feeding a growing world: Observing the synthesis of starch from reducing sugar

Student activity sheet

Introduction

You are aware that potato leaves make sugars during photosynthesis. Sugars are translocated to the stem tubers where much of the sugar is converted to starch. This synthesis of larger molecules from small monomers is catalysed by an enzyme, potato phosphorylase, present in potato tuber cells.

Many enzyme experiments you have carried out involve hydrolysis (digestion) of large molecules to small ones. These reactions are parts of catabolic pathways. Starch synthesis is a good example of an **anabolic pathway**. Given that the first step in the digestion of starch to the monosaccharide glucose involves its digestion to the disaccharide maltose, you might expect synthesis of starch to involve maltose. However, the best substrate for starch synthesis is glucose-1-phosphate, a compound found inside cells. Hydrolysis of the glucose-1-phosphate provides the energy needed to synthesise the starch molecules.

Practical investigation

Aim

The aim of this investigation is to observe the synthesis of starch from reducing sugar, catalysed by an enzyme extracted from potato tuber tissue.

Method

Safety

Carry out a risk assessment with your teacher. What hazards do you predict, and how will you control them?

Wear eye protection.

Take care when using scalpel blades and when handling microscope slides and coverslips.

Use the centrifuge as shown by your teacher.

Equipment

For making the extract:

- medium-sized potato tuber
- scalpel
- white tile

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- mortar and pestle
- sharp sand
- distilled water
- glass rod
- syringe or 5 mL measuring cylinder
- 4 × 1 mL syringes
- muslin/old tights
- 2 × small/medium beakers
- centrifuge tubes
- centrifuge

For the synthesis:

- test tube and rack
- timer/watch
- 2 × spotting tiles
- dropper pipette
- iodine/potassium iodide (KI) solution and dropper pipette
- 1% glucose-1-phosphate solution, 10 mL
- distilled water
- eye protection
- marker pen
- microscope and slides

Note: If you have extract left over from the main investigation, you can use the surplus during the same lesson. In this case, you can skip straight to step 5.

1. Wear eye protection.
2. Cut four cubes of tissue, without skin, from a raw potato tuber. Grind in a mortar with a little sharp sand using a pestle, for just long enough to break up the potato tissue.
3. Add 3 mL distilled water and stir gently with a clean glass rod.
4. Quickly pour the liquid through a single layer of muslin/nylon tights material into a beaker to strain it.
5. Transfer the strained liquid into two centrifuge tubes, putting equal volumes in each tube. Using the centrifuge as shown to you by your teacher, centrifuge these tubes at the highest speed for five minutes. This should separate the starch grains, which will be at the bottom of the tubes.
6. With a pipette, take a small drop of the clear supernatant liquid from the centrifuge tubes and place it on a white tile. Add one drop of iodine/KI solution. If there are any black specks present, spin the liquid again in the centrifuge. Test the supernatant again for the presence of starch. Once the extract is starch-free, you can proceed.
7. Carefully pour the supernatants into one test tube, labelled with your initials.
8. If you are working in pairs, while one of you carries out steps 1–4 the other can set up the spotting tiles. Label the rows as shown in Figure 1.
9. Row A: each well has 0.5 mL 1% glucose-1-phosphate solution.

   Row B: each well has 0.5 mL distilled water.

   Row C: each well has 0.5 mL glucose-1-phosphate solution and 0.5 mL distilled water.

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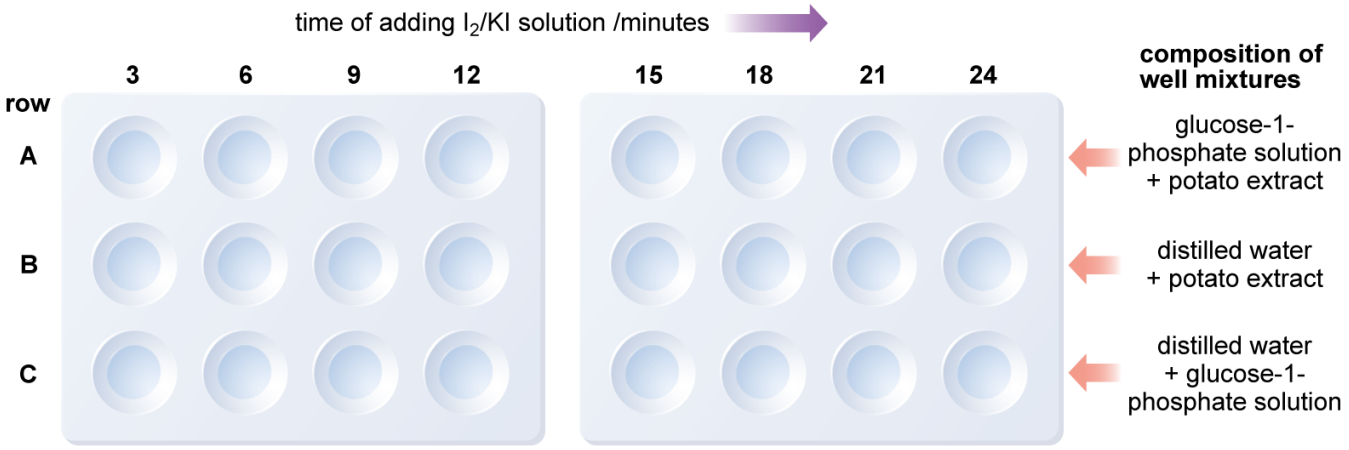

Figure 1 How the spotting tiles are set up.

10. Note the time and put one drop of the starch-free potato extract into each of the wells in rows A and B of the tiles. Do NOT add any extract to the wells of row C.

11. After three minutes, add one drop of iodine/KI solution to the first well in each of rows A, B and C. Note any colour changes. Iodine/KI solution inhibits the enzyme, so once it is added any reaction being catalysed will stop.

12. After a further three minutes add one drop of iodine/KI solution to the second well in each of rows A, B and C and note any colour changes.

13. Repeat step 11 at three-minute intervals, adding the iodine/KI solution to wells in rows labelled 9, 12, 15, 18, 21 and 24 minutes.

14. Has starch been formed, and if so, when?

15. Place a drop of solution from a well in which starch has formed onto a microscope slide and examine under low and high power. You should observe starch grains of 4–10 μm length.

Questions

1. What is necessary for starch to form?

2. There is a lag phase observed in this protocol. It takes a long time for starch to begin to be synthesised, but once begun the reaction rate increases. What does this suggest about the catalysis of this reaction?

3. How could you make this investigation quantitative?

4. How could you investigate the hypothesis that starch synthesis in potato tubers does not involve conversion of maltose to starch?

5. Suggest why potato tubers store much of their carbohydrate as starch.

6. Suggest why some of the carbohydrate in potato tubers is present as reducing sugars.

7. Suggest and explain under what circumstances you would expect more of the stored starch to be hydrolysed to reducing sugars within the cells of the potato tubers.

8. Explain how and when potato tubers can be both sources and sinks.

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