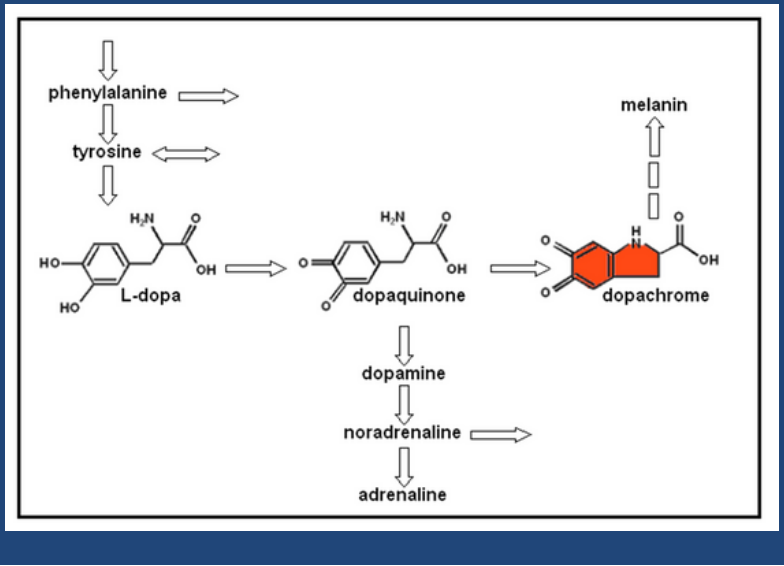
MICROSCALE DOPA OXIDASE

A microscale enzyme study

In the BGE, learners are often introduced to catalase as the classic example of an enzyme. However, dopa oxidase is a fantastic alternative – it is quick and simple to extract from bananas, reinforcing the idea that enzymes are found in all living things. No specialist equipment is required for this experiment (although a centrifuge can be used to make the enzyme extraction "cleaner") and the product is formed very quickly.

In this experiment, dopa oxidase (enzyme) converts L-dopa (substrate) to a product called dopaquinone. This is then sponentaously converted to a coloured product called dopachrome. Dopachrome is orange/red in colour and so its production can be monitored visually.



Dopa oxidase is an interesting enzyme to study because its activity is linked to other reactions including:

- the breakdown of phenylalanine, leading to wider discussions about phenylketonuria (PKU).
- the production of dopamine, an important neurotransmitter.
- the production of melanin, involved in pigmentation.

HEALTH & SAFETY

When starting an experiment with a class, you must ensure you are comfortable and familiar with the risk assessment. What are the hazards, the level of risk and control measures that must be put in place? Is the current risk assessment appropriate for your learners? Do you need to make adjustments? This page on the SSERC website provides a Risk Assessment template and information about Dynamic Risk Assessments.

HAZARD	RISK	CONTROL MEASURES
Dopa oxidase	Since this enzyme has been extracted from a natural source (rather than a powder), there is no risk associated with it.	No control measures required.
L-dopa	At the concentration and volume used in this protocol, L-dopa presents no hazards.	No control measures required.
Copper sulfate SSERC hazardous chemical database	At the concentration and volume used in this protocol, copper sulfate presents no hazards.	No control measures required.

MATERIALS

This experiment investigates the effect of inhibitors on dopa oxidase activity. Depending on the stage of your learners, this protocol can be adapted to investigate alternative independent variables, e.g. substrate or enzyme concentration or the effect of pH by including a range of buffers (replacing water with buffer). The dependent variable is enzyme activity, measured indirectly by a qualitative assessment of the colour observed after 3 minutes.

AIM

To investigate the effect of inhibitors of dopa oxidase activity.

INDEPENDENT VARIABLE

Type of inhibitor.

DEPENDENT VARIABLE

Dopa oxidase activity, measured indirectly by recording the colour of the product formed.

SAMPLE SIZE

The experiment should be repeated three times. This should only be increased if the results are variable.

SOURCE OF ENZYME

Banana.

MATERIALS REQUIRED (PER PAIR)

Stage 1 - extract the enzyme

- Banana
- plastic bag
- distilled water
- 2x 3ml plastic pipettes
- scissors
- 2x microfuge tubes (optional)
- micro-centrifuge (optional)
- bijou bottle
- marker pen

Stage 2 - the microscale assay

- Enzyme from step 1
- 10 mmol/L L-dopa
- Beaker of green tea (add 1 green tea bag to 50ml boiling water and leave for 2 hours)
- 10 mmol/L copper sulfate
- distilled water
- 5x 3ml plastic pipettes
- Activity board

METHOD

STAGE 1

STEP 1

Break off a small piece of banana and put it into the plastic bag.

STEP 2

Add 5ml cold distilled water to the bag and mash the banana.

STEP 3

Using the scissors, trim the end off the pipette to make step 4 easier. At this point, you can proceed to step 4 and carry out **centrifugation** or you can remove some of the liquid from the bag and pipette into the bijou bottle, labelling the sample "enzyme".

STEP 4 - CENTRIFUGATION (OPTIONAL)

Using the pipette, divide the banana mixture between 2 microfuge tubes.

STEP 5 - CENTRIFUGATION (OPTIONAL)

Centrifuge the tubes for 2 minutes, balancing the tubes in volume **and** by placing them opposite each other in the rotor.

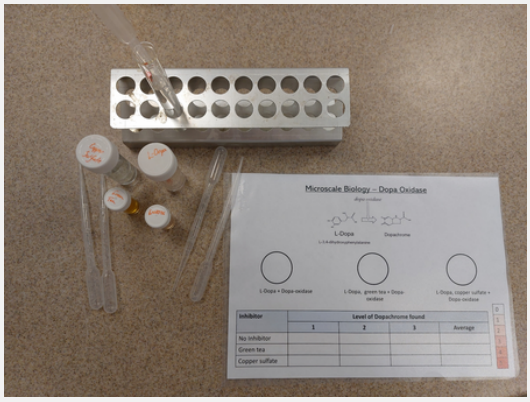
STEP 6

Use a pipette to draw off the supernatant into a clean bijour bottle. Label the bottle "enzyme".

STAGE 2

STEP 7

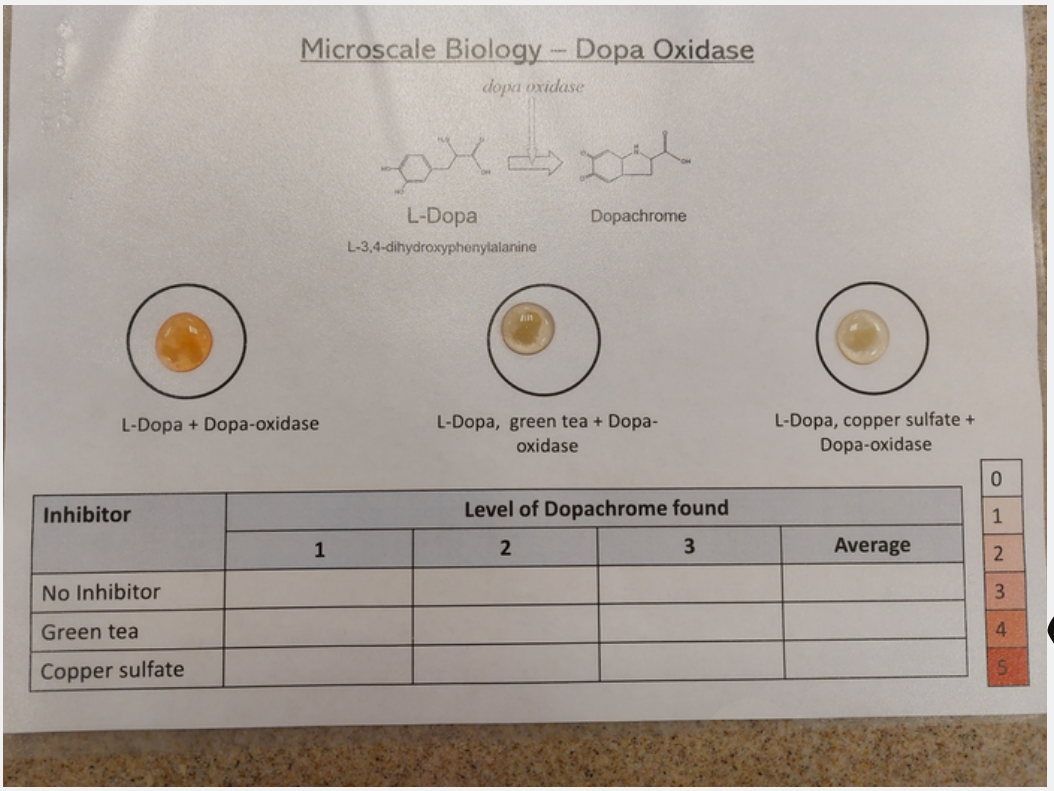
Use the reaction table below to add each reagent to the activity board. Do this in the order listed in the table. Volumes are in "drops".
Start the stopwatch after enzyme addition - leave for 3 minutes.



Reagent	Drops of reagent to add to circle		
	Control	Green Tea	Copper sulfate
L-Dopa	3	3	3
Green tea	-	3	-
Copper sulfate	-	-	3
Water	3	-	-
Enzyme	4	4	4

STEP 8

The appearance of the red colour indicates product formation. use the colour chart to provide a qualitative measure of reaction rate.



Inhibitor	Colour rating after 3 minutes
None	
Green Tea	
Copper sulfate	

0 1 2 3 4 5

STEP 9

Clean the activity board with water and repeat the experiment a further two times. Form a conclusion in response to the aim and evaluate the method.

Did green tea inhibit the reaction? What does this mean?

Discuss what is meant by a positive and negative control.