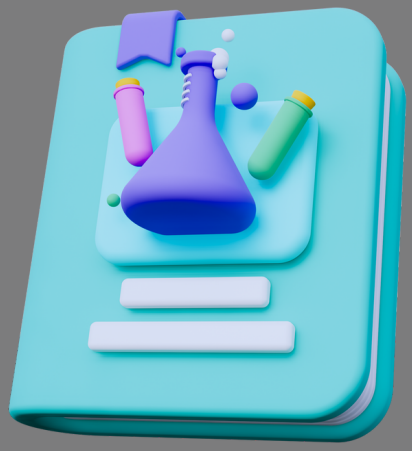
PROTOCOL

INVESTIGATING INHIBITION OF DOPA OXIDASE ACTIVITY

Extract dopa oxidase from bananas and investigate the breakdown of L-dopa

sserc

Page



Background

Enzyme inhibition studies are of curricular importance given the therapeutic success of enzyme inhibitors in the treatment of disease, e.g. statins, prescribed for the treatment of cardiovascular disease, are inhibitors of HMG-CoA reductase, an enzyme involved in cholesterol biosynthesis; similarly, inhibitors of kinase enzymes have led to many successes in cancer treatment [1]. In this assay, the effect of a potential inhibitor of dopa oxidase, green tea, is investigated. Dopa oxidase, also known as catechol oxidase and tyrosinase (among many others), is involved in the biosynthesis of melanin, as shown in Figure 1. Disruption to this biochemical pathway affects melanin formation and is associated with disorders including hyperpigmentation, vitiligo, and skin cancer [2]. Thus, research into dopa oxidase inhibition is of interest to the pharmaceutical and cosmetic industries.

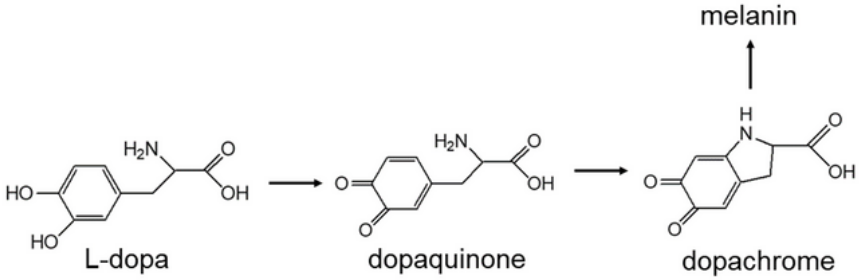


Figure 1: Dopa oxidase reaction. In this reaction, dopa oxidase catalyses the degradation of L-dopa to form dopachrome, through a transient intermediate called dopaquinone. Dopachrome is orange/red in colour.

Dopa oxidase, conveniently extracted from bananas, catalyses the conversion of L-dopa to dopaquinone, which is then spontaneously converted to dopachrome (Figure 1). The orange/red colour of dopachrome enables the qualitative assessment of product formation. In this assay, the potential inhibitory effect of green tea on dopa oxidase activity is explored. Enzyme activity in the presence of green tea is compared to enzyme activity in the absence of any inhibitor (negative control); and compared to the reaction in the presence of copper sulfate (positive control), a known inhibitor of dopa oxidase.

AIM

Aim: To investigate the inhibitory effect of green tea on dopa oxidase activity

This protocol can be adapted to investigate:

- effect of pH on dopa oxidase activity, by using different pH buffers
- effect of substrate concentration on dopa oxidase activity, by using different concentrations of L-dopa
- effect of enzyme concentration on dopa oxidase activity, by using different concentrations of extracted enzyme
- effect of temperature on dopa oxidase activity, using incubating the assay and enzyme at various temperatures using thermostatically-controlled waterbaths.
- effect of different research-informed inhibitor candidates on dopa oxidase activity.

RISK ASSESSMENT

A risk assessment for this activity can be downloaded from the SSERC website. Click here. This should be adapted for your centre, where appropriate.
There are relatively few hazards and most of very low risk. These mainly apply to science technicians during the preparation of any reagents. At Advanced Higher level, it might be anticipated that learners would be preparing these reagents independently and should be aware of the risk assessment.

This is a screenshot and doesn't include all necessary information. Please download to see full document.

SSERC Risk Assessment (revised version March 2018)
(based on HSE's INDG 163 'Risk assessment - A brief guide to controlling risks in the workplace')

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Activity assessed	Inhibition of Dopa oxidase
Date of assessment	27/07/23
*Date of review (**Step 5**)*	27/07/24
School	SSERC
Department	Biology

Step 1	Step 2	Step 3	Step 4		
List Significant hazards here:	*Who might be harmed and how?*	*What are you already doing? What further action is needed?*	*Actions*		
			by whom?	*Due date*	*Done*
Preparation of L-Dopa (from powder) **Eye and skin irritant.**	Technician	Use goggles and wash hands after use. Minimise contact with skin.			
Centrifuge **Electrical and manual handling**	Technician / teachers / learners	Centrifuge must be suitably maintained with a valid PAT test certificate. The design should include an interlock to prevent the lid being opened while the rotor is turning.			

PART 1: EXTRACTING DOPA OXIDASE FROM BANANAS

Materials: the following materials are required per pair/group

- banana
- plastic bag
- distilled water
- 3 cm^3 plastic pipette
- 4x microfuge tubes
- microcentrifuge
- bijou bottle
- marker pen
- scissors

Method:

1. Weigh out 2.5 g banana and add it to the plastic bag.
2. Add 10 cm^3 cold distilled water to the bag and mash the banana.
3. Using the scissors, cut the tip off the pipette to make step 4 easier.
4. Using a pipette, divide the mixture between 4 microfuge tubes.
5. Centrifuge the tubes for 2 minutes.
6. Using a pipette, draw off the supernatant into a clean bijou bottle. Label the bottle "enzyme".

PART 2: DOPA OXIDASE ASSAY - NEGATIVE CONTROL

Materials: the following materials are required per pair/group

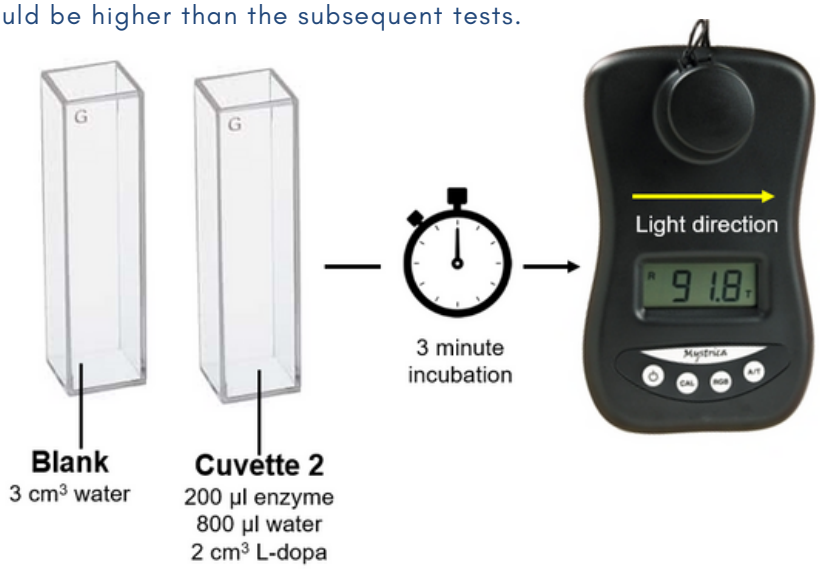
- Extracted enzyme (dopa oxidase)
- 10 mmol/L L-dopa
- 2x cuvettes
- Colorimeter
- Distilled water
- 3 cm^3 plastic pipette
- 1 cm^3 automatic pipette + tips

1. Take a clean cuvette and add approximately 3 cm^3 distilled water. Use this cuvette to zero the colorimeter at 465 nm (blue diode).

2. To a clean cuvette add:
   - 200 µl enzyme
   - 800 µl distilled water

3. To initiate the reaction, add 2 cm^3 L-dopa to the cuvette. Leave at room temperature for 3 minutes and then insert the cuvette into the colorimeter. Measure the absorbance of the solution at 465 nm.

Figure 2: Integrated diagram to show the negative control assay. This shows the activity of L-dopa in the *absence of treatment*. It would be anticipated that this absorbance value would be higher than the subsequent tests.



PART 3: DOPA OXIDASE ASSAY - EFFECT OF GREEN TEA

Materials: the following materials are required per pair/group

- Extracted enzyme (dopa oxidase)
- 10 mmol/L L-dopa
- beaker of green tea (1 tea bag immersed in 50 cm^3 boiled water for 3 hours)
- 2x cuvettes
- Colorimeter
- Distilled water
- 3 cm^3 plastic pipette
- 1 cm^3 automatic pipette + tips

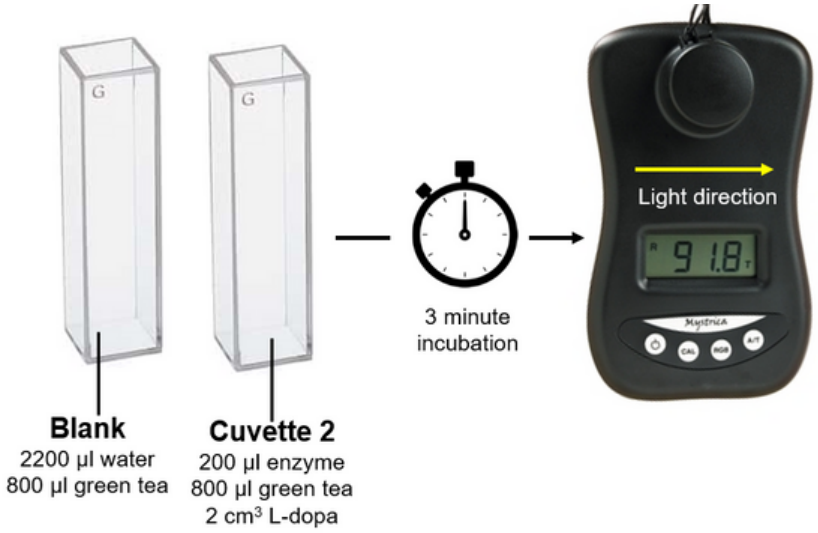
1. For the colorimetric blank, the colour contributed by the green tea must be accounted for. To a clean cuvette, add:
   - 2200 µl distilled water
   - 800 µl green tea

   Use this to zero the colorimeter at 465 nm / blue diode.

2. To a clean cuvette add:
   - 200 µl enzyme
   - 800 µl green tea

3. To initiate the reaction, add 2 cm^3 L-dopa to the cuvette. Leave at room temperature for 3 minutes and then insert the cuvette into the colorimeter. Measure the absorbance of the solution at 465 nm.

Figure 3: Integrated diagram to show assay to test the effect of green tea on dopa oxidase activity. The colorimetric blank must include the same proportion of green tea as in the test sample (cuvette 2) to account for the colour of the solution.



PART 3: DOPA OXIDASE ASSAY - POSITIVE CONTROL (COPPER SULFATE)

Materials: the following materials are required per pair/group

- Extracted enzyme (dopa oxidase)
- 10 mmol/L L-dopa
- 10 mmol/L copper sulfate
- 2x cuvettes
- Colorimeter
- Distilled water
- 3 cm^3 plastic pipette
- 1 cm^3 automatic pipette + tips

1. For the colorimetric blank, the colour contributed by copper sulfate must be accounted for. To a clean cuvette, add:
   - 2200 µl distilled water
   - 800 µl copper sulfate green tea

   Use this to zero the colorimeter at 465 nm / blue diode.

2. To a clean cuvette add:
   - 200 µl enzyme
   - 800 µl copper sulfate

3. To initiate the reaction, add 2 cm^3 L-dopa to the cuvette. Leave at room temperature for 3 minutes and then insert the cuvette into the colorimeter. Measure the absorbance of the solution at 465 nm.

Diagram on next page

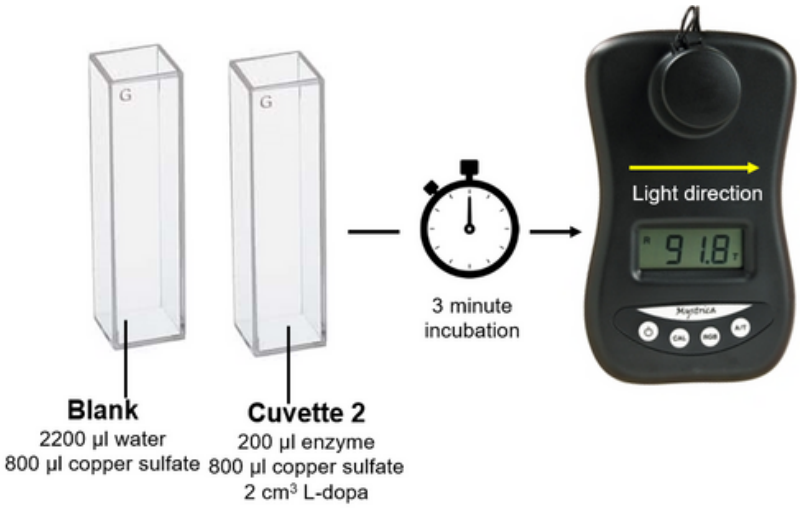


Figure 4: Integrated diagram to show assay to test the effect of copper sulfate on dopa oxidase activity. Copper sulfate has published inhibitory effects on dopa oxidase and, as such, provides a positive control for this investigation. The colorimetric blank must include the same proportion of copper sulfate as in the test sample (cuvette 2) to account for the colour of the solution.

RESULTS

Complete the results table below by inputting the absorbance values obtained in each of the experiments. Each experiment should be carried out in triplicate and mean values calculated.

Treatment	Final absorbance after 3 minutes			
	1	2	3	Average
Water				
Green tea				
Copper sulfate				

SAMPLE RESULTS

Treatment	Final absorbance after 3 minutes			
	1	2	3	Average
Water	0.756	0.784	0.743	0.761
Green tea	0.354	0.364	0.352	0.356
Copper sulfate	0.213	0.257	0.210	0.226

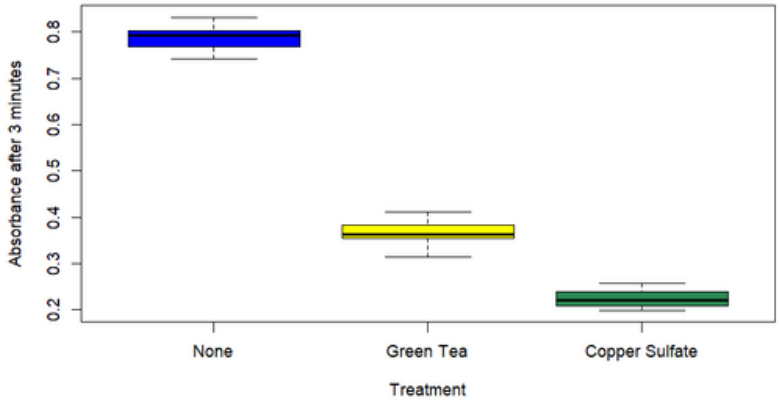


Figure 5: Box plot to show the spread of data values, generated in R Studio. This was based on 10 repeats for each treatment. The code used to generate this box plot is shown below. This shows the median, interquartile range, and whiskers. There are no outliers.

```
water <-c (0.756, 0.784, 0.743, 0.789, 0.812, 0.769, 0.832, 0.800, 0.797, 0.803)
greentea <-c (0.354, 0.364, 0.352, 0.355, 0.363, 0.412, 0.384, 0.315, 0.409, 0.376)
coppersulfate <-c (0.213, 0.257, 0.210, 0.199, 0.239, 0.231, 0.227, 0.201,0.241, 0.217)
boxplot(water, greentea, coppersulfate)
label<-c("None", "Green Tea", "Copper Sulfate")
boxplot(water, greentea, coppersulfate, col=c("blue", "yellow", "seagreen"), names=label,
xlab = "Treatment", ylab="Absorbance after 3 minutes")
```

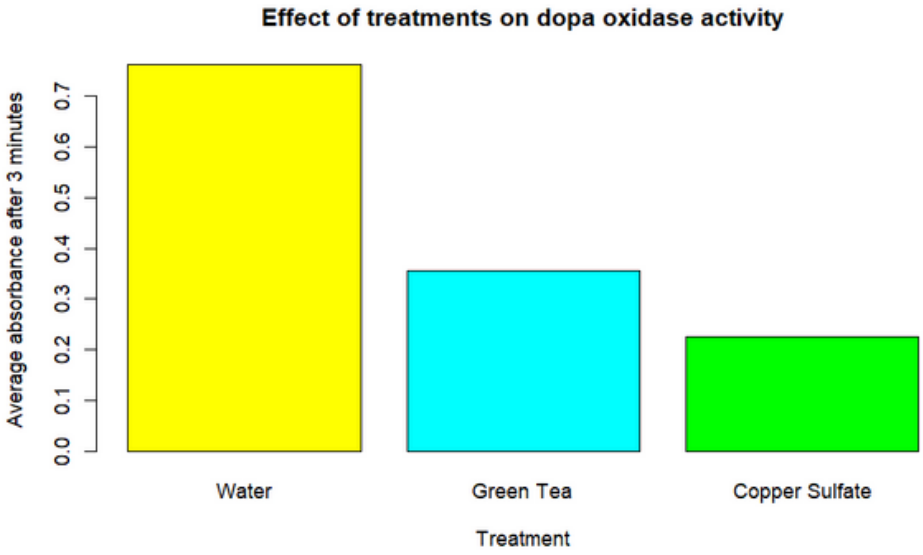


Figure 6: Plot showing the effect of treatments on dopa oxidase activity, indirectly via the measurement of absorbance of light through assay solutions. Treatments included water (negative control), green tea (test), and copper sulfate (positive control). This shows that green tea inhibits dopa oxidase activity, but to a slightly lesser extent compared to copper sulfate.

SAMPLE RESULTS

[1] Holdgate, G.A., Meek, T.D., Grimley, R.L. (2018), Mechanistic enzymology in drug discovery: a fresh perspective, Nature Reviews Drug Discovery, 17, pp115-132.

[2] Deri, B., Kanteev, M., Goldfeder, M., Lecina, D., Guallar, V., Adir, N., Fishman, A., (2016), The unravelling of the complex pattern of tyrosinase inhibition, Nature Scientific Reports, 6, article number 34993.