Dopa oxidase -

The advent of the Revised and *CfE* Highers in Biology and Human Biology, and Revised and *CfE* Advanced Higher in Biology [1-4] has prompted the Biology Team in SSERC to consider different enzyme systems for use in practical work and/or investigations.

At Higher/Advanced Higher levels, enzyme practical work should meet a number of criteria. Our belief is that practical work, as far as is possible, should in no order of priority:

- be robust;
- involve affordable (and readily available) substrates and enzymes;
- be versatile and offer opportunities for investigative work;
- be reliable;
- allow students to extract enzymes from 'living things';
- incorporate an assay that is simple to follow;
- produce results in short timescales;
- offer opportunities for kinetic studies.

We hope to show you that the enzyme-catalysed conversion of L-dopa to dopaquinone followed by conversion to dopachrome meets most if not all of the criteria in the bulleted list above.

The enzyme system

The enzyme studied here is called dopa oxidase although it is known by a myriad of other names (see Table 1 for a comprehensive list) including tyrosinase and catechol oxidase. Dopa oxidase, a copper containing metalloenzyme [5], is involved in a number of metabolic reactions including melanin biosynthesis.

• *N-acetyl-6-hydroxytryptophan oxidase*	• *o-diphenol: O_2 oxidoreductase*
• *o-diphenol oxidase*	• *monophenol monooxidase*
• *o-diphenol oxidoreductase*	• *monophenol oxidase*
• *monophenol dihydroxyphenylalanine: oxygen oxidoreductase*	• *monophenol, dihydroxy-L-phenylalanine oxygen oxidoreductase*
• *o-diphenol: oxygen oxidoreductase*	• *monophenolase*
• *o-diphenolase*	• *phenol oxidase*
• ***catechol oxidase***	• *phenolase*
• *catecholase*	• *polyaromatic oxidase*
• *chlorogenic acid oxidase*	• *polyphenol oxidase*
• *chlorogenic oxidase*	• *polyphenolase*
• *cresolase*	• *pyrocatechol oxidase*
• *diphenol oxidase*	• ***tyrosinase***
• ***dopa oxidase***	

Table 1 *- Alternative names for dopa oxidase [Enzyme Commission Number 1.14.18.1]. Common names are highlighted; information taken from [5].*

A key substrate for the enzyme is L-dopa (alternatively known as 3,4-dihydroxyphenylalanine) (Figure 1) and it is closely related to the amino acid tyrosine (Figure 2) from which it is snythesised in humans.

In humans a related product of tyrosine metabolism is dopamine (Figure 3) which is released at a number of sites in the brain and is reported to exhibit a range of effects on sleep, mood, attention and learning [6].

It appears that L-dopa can cross the blood-brain barrier and be converted to dopamine and this has led to L-dopa being used in the treatment of Parkinson's disease.

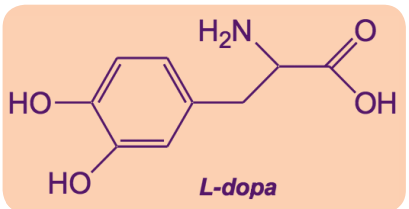

Figure 1 *- L-dopa.*

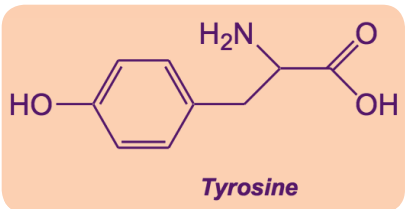

Figure 2 *- Tyrosine.*

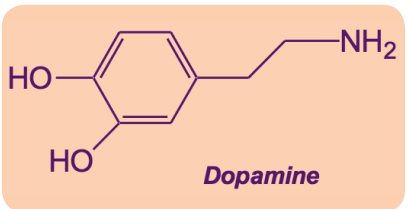

Figure 3 *- Dopamine.*

a perfect enzyme?

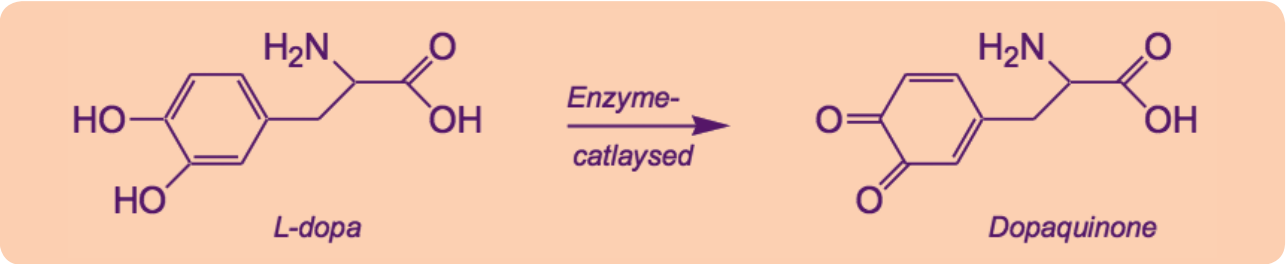

Figure 4.

A key reaction in melanin biosynthesis is the oxidation of L-dopa to dopaquinone (Figure 4).

Both substrate and product of the above reaction are colourless and so monitoring the conversion of L dopa to dopaquinone is not possible using conventional colorimetry. However, dopaquinone is spontaneously converted to dopachrome which is orange/red in colour with an absorption peak at about 470 nm (Figure 5).

Sources of tyrosinase for experimental work

Dopa oxidase can be conveniently extracted from animals, plants and fungi. Readily available sources include bananas and potato peelings.

The enzyme assay

The following is an adaptation of the method available on the Mystrica website [7]; a more detailed protocol will be published shortly on the SSERC website [8].

Extracting the enzyme

1) Weigh out about 2.5 g of banana.

2) Add 5 cm^3 of **cold** distilled water and crush the banana to a pulp.

3) The enzyme mixture can be filtered through muslin or (preferably) centrifuged to remove cellular debris. The filtrate will contain dopa oxidase.

Following the reaction

4) The formation of dopachrome can be conveniently followed using a colorimeter. For a 'standard assay' we find the following mixture is convenient:
   - L-dopa (we used L-dopa from Sigma-Aldrich) in distilled water at a concentration of 2.5 x 10^{-2} mol dm^{-3} (2 cm^3)
   - dopa oxidase extract from step 3 (0.5 cm^3)
   - distilled water (0.5 cm^3)

   Both substrate and enzyme extract will slowly degrade at room temperature and so it is best to keep these solutions on ice when they are not being used in the assay. (It is worth noting that solid L-dopa is relatively stable and has a long 'shelf-life'.)

5) If the above mixture is prepared in an absorption cuvette the reaction can be followed at 470 nm. Over a 6 minute period we typically find that the absorbance of the mixture changes from 0.0 to 0.7 (see Figure 6). Using a colorimeter such as that produced by Mystrica (www.mystrica.com) allows continuous monitoring of absorbance changes coupled with potential kinetic analysis.

It is worth noting that the effect of both substrate and enzyme concentration on the rate of dopachrome production can be easily studied [7].

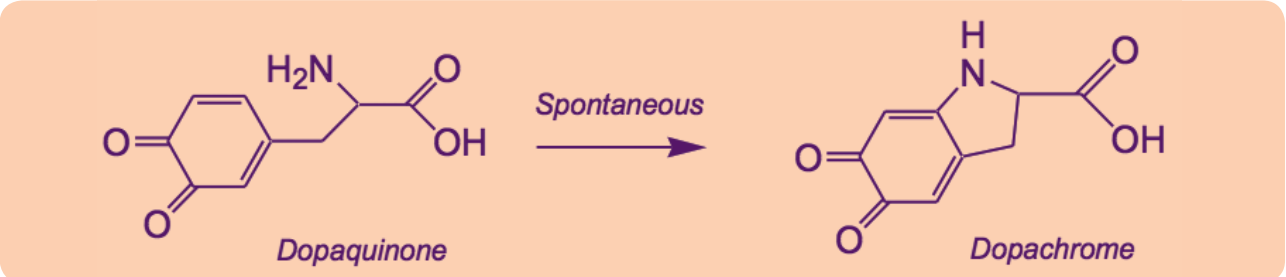

Figure 5.

Conclusion

So, does dopa oxidase live up to the title of this article and meet the criteria in terms of the desirable characteristics for an enzyme assay? We acknowledge that the substrate, L-dopa, is not cheap; for example the current cost from Sigma-Aldrich [catalogue number D9628-5G] is £27.20 for 5 g. That said, only small amounts are used in each assay and an investment of £35 (to include postage and packing) will last a significant proportion of us to retirement age and beyond....

Whilst the substrate is not cheap from 'conventional' suppliers an on-line search reveals a number of possible other sources although we have not explored these in any depth. Against the other criteria, the enzyme performs pretty well.

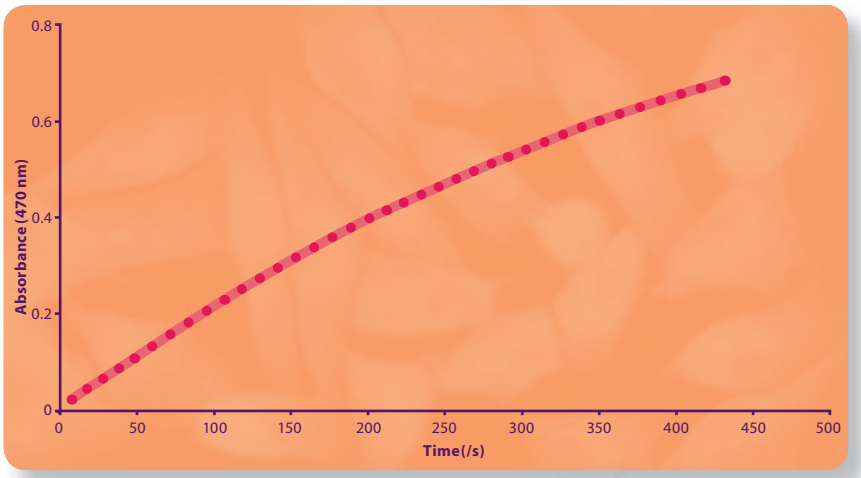

Figure 6 *-The effect of a crude preparation of dopa oxidase (produced by crushing banana in distilled water) on the absorbance of a solution of L-dopa (final concentration 1.67 x 10^{-2} mol dm^{-3}) at 470 nm. The sample was contained in a cuvette of pathlength 1 cm.*

There would seem to be lots of scope for investigative work given the ubiquitous nature of the enzyme in different plant materials. ◀

References

[1] SQA (2010) Biology (revised) Higher - the Arrangements Document can be downloaded at www.sqa.org.uk/sqa/files_ccc/HigherBiologyCourseSpec.pdf

[2] SQA (2012) Higher Biology Course Support Notes - can be downloaded at www.sqa.org.uk/files_ccc/CfE_CourseUnitSupportNotes_Higher_Sciences_Biology.pdf

[3] SQA (2010) Human Biology (revised) Higher - the Arrangements Document can be downloaded at www.sqa.org.uk/sqa/files_ccc/HigherHumanBiologyCourseSpec.pdf

[4] SQA (2012) Higher Human Biology Course Support Notes - can be downloaded at www.sqa.org.uk/files_ccc/CfE_CourseUnitSupportNotes_Higher_Sciences_HumanBiology.pdf

[5] Park, Y-D., Lee, J-R., Park, K-H., Hahn, M-J. and Yang, J-M. (2003), A new continuous spectrophotometric assay method for DOPA oxidase activity of tyrosinase, J. Prot. Chem., 22, 473 480.

[6] Reece, J.B., Minorsky, P.V., Cain, M.L., Wasserman, S.A., Jackson, R.B. and Urry, L.A. (2011) Campbell Biology (9th edition), Pearson Education.

[7] Dopa oxidase - available at www.mystrica.com/Experiment.aspx?PageId=59 and www.mystrica.com/files/Dopa%20oxidase.pdf

[8] The SSERC website is available at www.sserc.org.uk
Please note that to access all resources on the website you will need a log-on ID and password.