

Dopa oxidase

Introduction

The conversion of L-dopa to dopachrome is a reaction that is rarely encountered in schools. It is simple, reliable, interesting, safe, colourful and inexpensive and can be used to introduce the concept and properties of enzymes to secondary pupils or to carry out sophisticated studies of enzyme kinetics.

L-dopa (3,4-dihydroxy-L-phenylalanine) is a colourless metabolite synthesised from the amino acid tyrosine. It is enzymatically converted to dopaquinone by the oxidation of two hydroxyl groups followed by spontaneous conversion of dopaquinone to red dopachrome which slowly and spontaneously converts to melanin.

Background

Phenol oxidase enzymes that catalyse this reaction are ubiquitous in plants, animals and fungi. It is not entirely clear how many enzymes and polymorphs are involved. The enzyme system is essentially the same as catechol oxidase.

The advantages of this system are:

- students can easily extract the enzyme for themselves
- the reaction can be carried out quickly using simple methods and is wonderfully reliable and predictable
- there are no safety issues
- the cost is negligible
- for more advanced studies the reaction can be followed by continuous colorimetry so that rates of reaction can be determined and sophisticated investigations of enzyme kinetics carried out
- it has relevance to a number of important and interesting reactions and is a very good way of introducing the concept of interlinked metabolic pathways.

Suggestions for investigations

The system is ideal for qualitative demonstrations of enzyme action, effect of boiling, addition of acid or alkali to show the effect of pH, etc. Using the colourimeter detailed studies of optimum temperature, pH, enzyme kinetics, effects of inhibitors, are all relatively straightforward.

Enzyme extraction

The enzyme can be prepared from a variety of vegetable sources. Banana and potato work well, the yield from potato peelings being greater than from the flesh. The tissue simply needs to be crushed with cold water, (2cm³ of water per gram of tissue). For preference it should be filtered through muslin. For advanced work careful preparation with measured quantities, centrifugation, etc may be desirable.

Reaction mixture

One of the reasons why this is such a good system is the ease with which the reaction can be carried out. L-dopa can be dissolved in water; the maximum solubility at 25 °C is 0.5g in 100cm³, (25mM). [*L-dopa* M.Wt =197.2] 0.1cm³ of enzyme extract in 3cm³ of 5mM dopa will give a good reaction at room temperature.

In solution dopa will slowly decompose and darken, particularly at pH above 7. Prepare solutions fresh before use and store them cold.

More details, suggestions for investigations and sample results can be viewed on the *Mystrica* website, www.mystrica.com/Experiment.aspx?PageId=59