ABSTRACT

1-Aminocyclopropane-1-Carboxylic Acid (ACC)

Oxidase from Carica papaya: Isolation, Characterization, Role in Ethylene Biosynthesis and fruit Ripening.

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1-Aminocyclopropane-1-carboxylic acid (ACC) oxidase catalyses the conversion of ACC into ethylene, a phytohormone associated with the ripening process of climacteric fruits. Most of the studies done on the enzyme were carried out using in vivo techniques due to its difficulty of extraction. It was not until 1991 that an in vitro isolation was done successfully by Ververidis and John, using the melon fruit.

This thesis is an embodiment of the analysis and description of many in vitro assays that were conducted on the enzyme extracted from Carica papaya fruit. The enzyme exhibited a Km of 37 μM and was inhibited by n-propyl gallate (0.24 mM); sodium dithionite (0.220 mM); sodium metabisulphite (0.240 mM) and cobalt sulphate (0.100 mM). The enzyme activity increased with ripening then declined as the fruit became overripe.
The enzyme is very labile in nature, losing total activity within 4 days when stored at 14 °C. Storage of the crude homogenate at -15 °C prolonged its shelf life beyond an 8 day period. In the initial extraction process, the papaya tissue had to be freeze-dried with liquid nitrogen to maintain the enzyme activity. Isolation and purification were subsequently achieved using ammonium sulphate precipitation, followed by gel filtration using Sephadex G 100 - 120 and then by anion exchange chromatography using DEAE - Sephadex anion exchanger 40 - 120 μ. Gel electrophoresis of the purified enzyme gave a single band which corresponded to a $M_r$ of 27.5 kDa.

The enzyme also displayed an optimum temperature and pH of 30 °C and 7.5 respectively. Interestingly, the enzyme can also utilize a second substrate, ACC methylester, an analogue of ACC.

The amino acid content of the enzyme was also determined which showed a relatively high percentage of valine (12.69 %) and it also contained cysteine residues (5.87 %). The latter made the enzyme highly sensitive to sulphydryl-reactive reagents such as dithiothreitol. At low concentrations (3 mM), this compound enhanced the enzyme activity.
Some compounds that were present in the assay medium also enhanced the enzyme activity; these included bicarbonate ion (30 mM), oxygen and iron II ions (80 μM).

ACC oxidase activity in the fruit of *Carica papaya* is relatively high. As a result of this it was not necessary to subject the fruit to ethylene prior to enzyme assay.