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TRANSFORMATION ASSOCIATED WITH CATECHOLASE IN *DIOSCOREA ALATA* DURING STORAGE

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Key Word Index—*Dioscorea alata*; Dioscoreaceae; water yam; storage; catecholase; multiple forms.

Abstract—The Michaelis constant of catecholase in 3 cultivars of *Dioscorea alata* was determined. Oxidative browning was related to the activity of the enzyme in the yams. Inhibition by cysteine was competitive, while 2-mercaptoethanol caused an uncompetitive type of inhibition. On DEAE column chromatography a minor peak preceded the major peak in the fresh yam while a minor peak was eluted after the major peak in the stored yam.

INTRODUCTION

The major role ascribed to catecholase, a polyphenol oxidase (PPO), (EC 1.10.3.10) present in plant tissue is the elaboration of a polymerized protective dark-brown melanin product which could resist infection from an injured or cut surface of a plant tissue. The ubiquitous presence of the enzyme even in those plants that do not turn brown when cut has led to the suggestion that the PPO could also be responsible for hydroxylation reactions in phenolic biosynthesis and respiration [1–3]. PPO from several fruits [4–6] and in potato [3] has been investigated, but by contrast, only indirect reports on the presence of polyphenols in *Dioscorea* sp. associated with oxidative browning have been published [7, 8].

Dioscorea alata is the most widely distributed species of yam and ranks third as the staple food of the peoples of the West African zone. Only a very small fraction of the tuber is processed and because the whole tuber is not always consumed, cut tubers can readily be infected with fungi which can destroy the entire tuber in a short time [9]. Storage losses in intact yams are also significant [10]. The activities of some enzymes have been investigated during prolonged storage of some varieties of yam [11] but the role of PPO has not been included. PPO has been shown to undergo changes during development in potato and in fungal fruiting body during ageing [12, 13].

This paper presents results on the enzymic changes which accompany the cut and stored yam from some cultivars of *D. alata*.

RESULTS AND DISCUSSION

Table 1 shows the activities and kinetic constant (K_m) of 3 cultivars of *D. alata*. The higher the activity of the enzyme, the greater the tendency to phenolic oxidation in the cultivars (cvs) examined. The cv Orovbo, with the greatest tendency to phenolic oxidation when cut had the highest catecholase activity while the cv which did not turn brown when cut has *ca* 20% of the activity of Orovbo. Although the phenolic compounds were not determined in this study, dopamine and 2-leucocyanidine are present in high quantity in yam which are responsive to phenolic oxidation by PPO [7, 8].

In its role during oxidative browning, PPO presents culinary problems as the grey or ugly brown polymerized melanin is associated with off-flavours, including bitterness. Prevention of the enzyme activity during processing with safe reagents is important in food technology. Data from inhibition studies may also yield information on the active site of the enzyme.

Of the two sulfhydryl reagents tested which presumably may inactivate the enzyme at its active site (Table 2), 2-mercaptoethanol (2-ME) caused 100% inhibition in all the cvs, however, it could not be considered for the processing of food. L-Cysteine achieved various degrees of inhibition in the cvs tested. The yam that had the least activity was fully inhibited, while only 50% inhibition was achieved with the yam with the greatest tendency to phenolic oxidation. In yam that had a medium tendency to oxidation 75% of the PPO was inhibited by cysteine. While cysteine inhibition could be related to tendency to oxidation, 2-Me showed effective inhibition in all the cut yams investigated. Inhibition by cysteine was competitive, while 2-ME caused an uncompetitive type of inhibition. The

Table 1. Activities and kinetic constants of 3 varieties of *D. alata*

D. alata cv	Tendency to phenolic oxidation	Activity ΔA/min	K_m(M)
Egbenehikhuere	Low	125	1.7×10^{-2}
Ediakpakon	Medium	401	1.2×10^{-2}
Orovbo	High	522	2.3×10^{-2}

Table 2. Effect of inhibitors on catecholase activity in cultivars of *D. alata*

Inhibitor*	% Inhibition a	b	c
2-Mercaptoethanol	100	100	100
L-Cysteine	100	75	53
Sodium cyanide	19	19	8
1,10-Phenanthroline	19	16	4
EDTA†	19	16	8

* 10 mM of each inhibitor was made and added as in Experimental.

† Ethylenediamine tetraacetic acid; cultivars a, Egbenehikhuere; b, Ediakpakon; c, Orovbo.

results, therefore, suggest an important role for the sulphur containing amino acids in the activity of the enzyme. The inhibition resulting from NaCN was unexpectedly low compared to the higher inhibition obtained for PPO from banana [14]. 1,10-Phenanthroline and EDTA did not result in much inhibition (less than 20%), although the enzyme contains copper atoms.

Two forms of catecholase isoenzymes were separated in all the cvs of yam. In the fresh yam, a minor peak was eluted with 0.1 M NaCl while a major peak was eluted with 0.2 M NaCl on DEAE-Sephadex column (Fig. 1). In the yam cut and stored for 21 days two similar peaks were also obtained but with an alteration in profile and a general decrease in enzyme activity (Fig. 2). Unlike the fresh yam no peak was recorded with 0.1 M NaCl, the major peak was eluted first with 0.2 M NaCl while the minor peak was eluted later with 0.3 M NaCl. In both cases, therefore, 0.2 M NaCl eluted the major peak while the minor peak was eluted before in the fresh yam and after the major peak in the stored yam. The significance of this is not known. Patil and Zucker [3] suggested that the resolution of potato phenolase complex into two components on DEAE-cellulose column could represent 'tanning' of the phenolase on the column as was shown for tea leaf phenolase. The potato complex was, however, interpreted to contain two multiple forms as shown by the different K_ms of the two forms. The yam phenolase complex showed that the major peak in both the fresh and stored yam had identical K_m (2.3×10^{-2} M) with the crude enzyme although the minor peak had a different K_m (3.6×10^{-2} M) Conclusive interpretation of resolution into multiple forms, in spite of the gradient DEAE-Sephadex separation, could only be made with a highly purified enzyme. It should be noted that appearance and disappearance of isoenzymic forms have been linked to infection in potato [12]. The elution patterns and the role of the minor peak in *D. alata* should be interesting in the ageing of the tuber, especially since both cysteine and 2-ME caused an irreversible inhibition of the resolved components while inhibition of the crude enzyme was reversible (unpublished).

EXPERIMENTAL

Enzyme preparation. Tissue (15 g) obtained from the tail of peeled freshly harvested yams was quickly chopped with 3.75 g polyethylene glycol [14]. The mixture was added to 60 ml of ice-cold 0.05 M K-Pi buffer (pH 7) and blended for 2 min. The homogenate was filtered through a double layer of cheesecloth and the filtrate centrifuged at 10 000 g for 5 min. The resultant clear supernatant was used as the crude enzyme without further purification.

Enzyme assay. The standard reaction mixture at 45° contained 2.5 ml catechol (10 mM) in 0.1 M K-Pi buffer (pH 6) as substrate to which 20 μl of crude enzyme was added. The reference cuvette contained 2.5 ml of the substrate. At this concn of enzyme, linearity was obtained for product formed in 3 min. The rate of increase in A was measured at 420 nm. One unit of enzyme activity was defined as an increase of 0.001 A unit per min under the optimum conditions.

Kinetic studies. The standard assay procedure at different substrate concs was carried out to determine the K_m. For the

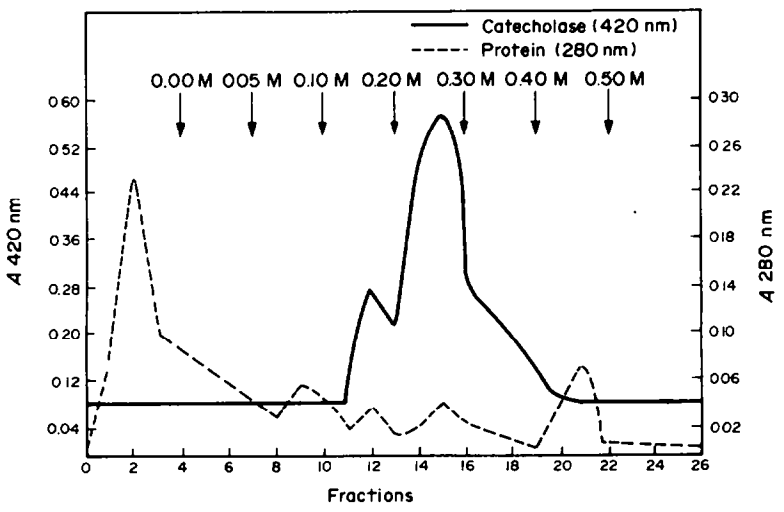

Fig. 1. Fresh yam catecholase profile on DEAE-Sephadex (A.50).

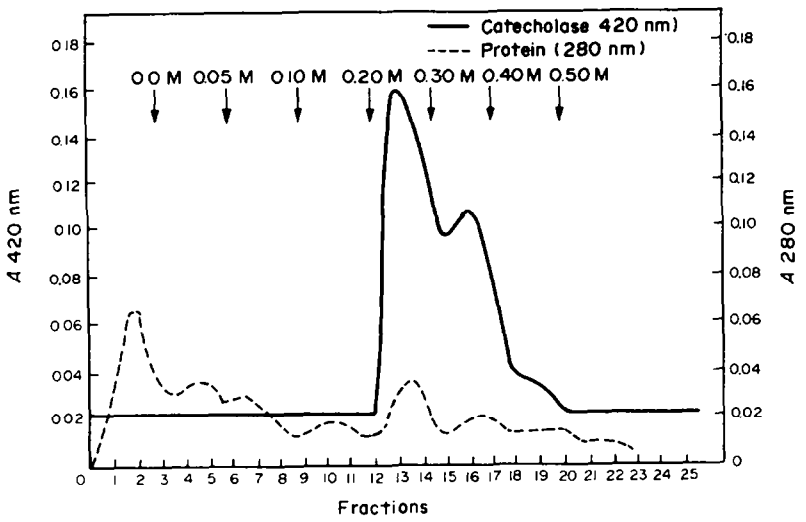

Fig. 2. Stored yam catecholase profile on DEAE-Sephadex (A.50).

inhibition studies the reference cuvette contained 3 ml of substrate (10 mM catechol) while the sample cuvette contained 2.5 ml substrate, 30 μl (10 mM) of the respective inhibitor which were thoroughly mixed before the addition of 20 μl of enzyme to initiate the reaction. The types of inhibition were determined by varying the substrate concns to relieve the inhibition from the added inhibitor. Separated isoenzyme (0.5 ml) was used with appropriate substrate and inhibitors.

Ion exchange chromatography. Enzyme extract (1.5 ml) was diluted to 3 ml with 0.05 M K-Pi buffer (pH 7) and was loaded on to a column (0.7 × 12 cm) containing DEAE-Sephadex (A-50) that had been equilibrated with the same buffer. The column was washed with 13.5 ml of K-Pi buffer and then eluted in a stepwise fashion with a series of solns of NaPi containing increasing concns of NaCl (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 M). Fractions of 4.5 ml were collected and assayed for the enzyme [15]. Protein was estimated spectrophotometrically in each fraction by monitoring at 280 nm. The alterations in catecholase complex were studied by comparing the elution profiles of fresh tubers with those of cut and stored yam at room temp. for 21 days.

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