PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

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Pakistan Journal of Nutrition 5 (5): 478-480, 2006
ISSN 1680-5194

The Contribution of an Ionic Peroxidase Isozyme to Enzyme-Mediated Browning in *Dioscorea esculenta* L. Tubers

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Abstract: The time course of the activity of a strongly ionic peroxidase (POD) isozyme from *Dioscorea esculenta* Var. *fasiculata* tissues correlated with that of tissue browning during incubation at room temperature for 24 hours. Tissue browning was inhibited by 1 mM DTT (a peroxidase-specific inhibitor) by 40%; by about 75 % by 1 mM PVP (a polyphenol oxidase-specific inhibitor) and completely by a combination of DTT and PVP at 1 mM. Moreover, a temperature profile study revealed that POD activity was high at temperatures (up to 60°C) during enhanced browning but not limited to it. This strongly ionic POD isozyme contributes up to 40% of enzyme-related tissue browning in *Diocorea esculenta* especially at temperature above 40°C. This is based on results derived from inhibition by PVP. The results support our view that high temperature enzyme-mediated browning in this tissue is more of a POD activity which should be considered in the overall processing of yam tissues.

Key words: *Dioscorea esculenta var fasiculata*, peroxidase (POD), polyphenol oxidase (PPO), dithiothreitol (DTT), polyvinyl pyrrolidone (PVP), peroxidase isozyme

Introduction

The post-bruise browning phenomenon in plant tissues is a physiological response leading to the generation of specific polymers which may isolate the bruised surface from successful infection by pathogens (Mathew and Parpia, 1971; Vamos-Vigyazo, 1981). Post-bruise browning occurs in all edible yams examined so far and the incident has been associated with the activity of a polyphenol oxidase (Omidiji and Okpuzor, 1996). However, we have suggested that a functional peroxidase may also participate in this event (Okpuzor and Omidiji, 1998). This followed our observation that enzyme-related yam browning is not completely prevented by polyphenol oxidase inhibitors while there is a temperature relationship in enzyme-mediated browning which may be unfavorable to a heat labile polyphenol oxidase (Okpuzor and Omidiji, 1999).

We had also observed the presence of a heat stable peroxidase with potential for polyphenol oxidase activity and this reinforced our suggestion that yam browning involves some role for a peroxidase (Okpuzor and Omidiji, 1998). Peroxidase is a very ubiquitous enzyme in plant physiological activities, with functions related to morphogenic changes in cell division, growth and differentiation (Goleniowski *et al*., 2001). The activities of polyphenol oxidase and peroxidase in plant tissues contribute to the choice of grains in food processing industries (Mamoudou *et al*., 2006). Peroxidase activity has also been demonstrated to confer protection to animal tissues through oxidative mechanism (Al - Jabri, 2005). In this study we observed that an ionic POD from yam (*D. esculenta*) has an activity profile and temperature properties which suggest strongly that it participates actively in post-bruise browning of yam tissue perhaps at elevated temperatures.

Materials and Methods

Plant sample: Mature tubers of *Dioscorea esculenta var fasiculata* from 2000-2001 planting season were harvested from The Biological Garden University of Lagos, Nigeria. They were left for curing for a period of 14 days before storing in a refrigerator (8±2°C).

Peroxidase (POD) and Polyphenol Oxidase (PPO) Assay: Freshly peeled tuber (25 g) were homogenized for 3 minutes in 100 ml of cold 50mM Na-PO_4. buffer (pH 7). The resulting homogenate was centrifuged at 20,000 g for 10 mins at 4°C. The supernatant represented the crude enzyme source. POD assay was carried out as described (Mujer *et al*., 1983) using 2mM O-dianisidine as substrate. PPO assay was as we previously described (Omidiji and Okpuzor, 1996). One unit of enzyme activity is defined as the amount of enzyme that caused a change of 0.001 absorbance units per second under the described assay conditions at room temperature (29±2°C).

Protein determination: The protein content of both the crude and concentrated fractions from column elutes were determined using Lowry reagent (Lowry *et al*., 1951) and bovine serum albumin BSA (0-250 mg) as standard. However, the protein profile in column eluates from ion exchange and gel filtration columns were estimated by measuring the OD at 280nm.

Source of partially purified POD isozyme: The crude enzyme extract obtained as usual (Okpuzor and Omidiji, 1998) was subjected to ion exchange chromatography in a DEAE- Sephadex A-50 column (2.4 × 46 cm, bed volume = 160 ml) and eluted with Na-PO_4 buffer (50mM pH 7.0) containing a linear gradient of NaCl (0 - 1.2 M) at

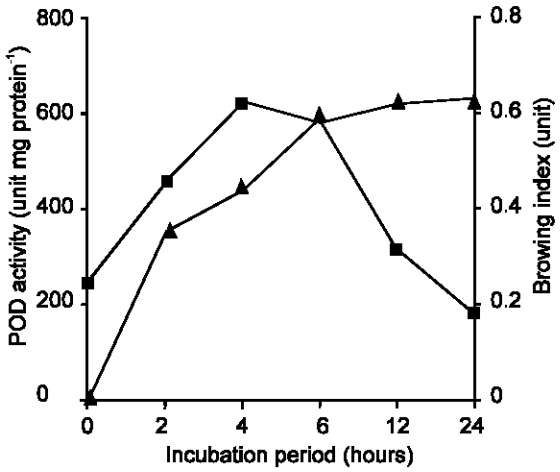

Fig. 1: The time course of browning and POD activity in *D. Esculenta* tissues (■) POD activity, (▲), Browning unit (each reading is the mean of two determinations

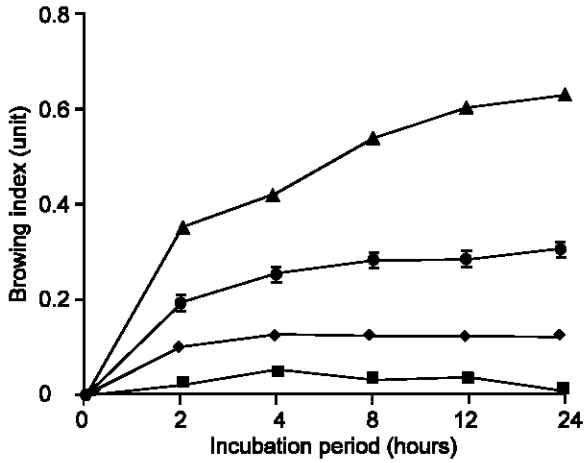

Fig. 2: The inhibition of browning of yam tissue by DTT and PVP. Each reading is the mean of two determinations. (▲), control tissue, no inhibitor, (●), Tissue + 1mM DTT, (◆), tissue + 1Mm PVP, (■), Tissue + 1mMDTT + 1mM PVP.

a rate of 60 ml h^{-1}, 10 ml fractions were collected. Active peaks from ion exchange column containing POD activity were pooled together and concentrated in an Amicon ultra filtration unit with molecular weight cut off of 10kd. This was eluted through Sephadex G-200 column (2.5 × 45, bed volume 196 ml) using the same buffer at a rate of 18ml h^{-1} and 3ml fractions were collected.

Determination of browning potential: The browning potential of peeled yam tissues was as described previously (Omidiji and Okpuzor, 1996). One browning unit is the difference of 0.01 absorbance unit from control per gram dried flour at 420 nm.

Browning and POD inhibitors: The effect of dithiothreitol (DTT) and polyvinyl pyrrolidone (PVP) on the browning of yam tissues was by crushing yam tissues (25 g) in buffer containing 1 mM concentrations of the inhibitors and the browning potential determined as described.

Temperature course of browning: Crushed yam tissues were incubated at various temperatures (20°C-70°C) for 4 hours (period of POD maximum activity), dropped into boiling water to inactivate the enzyme, dried in the oven (60°C) for 24 hours and subjected to browning extraction as described previously. POD activities were also determined after similar treatment for 4 hours.

Results and Discussion

The protein fraction associated with POD activity which was obtained from ion exchange column also showed the potential for PPO activity. This POD isozyme was obtained as we described previously using a neutral phosphate buffer containing 1 mM NaCl. We had calculated that the 60% of total tissue POD activity resided in this fraction (Okpuzor and Omidiji, 1998).

Freshly-peeled tissues of *D. esculenta* incubated at room temperature (29±2°C), would turn brown gradually over a 24 hr period with most of the browning occurring within 8 hours. Similarly, POD activity increased rapidly and climaxed within 4 hours followed by a decline (Fig. 1). The rise in POD activity is thus slower than the 2 hour point which we observed for PPO previously (Omidiji and Okpuzor, 1996) The slight displacement in optimum activity points of PPO and POD in yams does not preclude the relevance of POD to the browning process; especially the later events. An association between the POD activity and the gross process of browning of potato tissues has also been reported (Bachem *et al.*, 1994).

The role of POD in tissue browning in yams may not be easily demonstrated with selective use of substrates because both POD and PPO utilise similar substrates and are also inhibited by similar compounds *in vivo* (Ikediobi and Obasuyi, 1982). We may however consider the selective inhibition of POD by DTT and PPO by PVP to distinguish between the relative involvements of the two enzymes. Table 1 shows the typical time course of inhibition of yam POD by 5 mM DTT during a 24 hour incubation period. Inhibition of POD activity by DTT is total after 2 hours while PVP did not inhibit the enzyme. We have previously reported on the strong inhibition of yam PPO by PVP (Okpuzor and Omidiji, 1998). We exploited this concept as shown in Fig. 2 by determining the browning indices in the presence of 1 mM DTT, PVP and a combination of both inhibitors. The browning was completely prevented in the presence of 1 mM concentration of both DTT and PVP. About 40% of potential browning was prevented by DTT alone, while PVP alone prevented about 80% of potential browning. The enhanced activity of PVP over DTT may be due to its

Table 1: The inhibition of yam POD by 5 mM DTT., PVP. Each reading is the mean of two assays ±1 DM

Incubation) Time (Hours	POD activity (unit mg protein $^{-1}$) Control	+ 5mM DTT	+5 mM PVP
	0	0	0
0	250±21	90±6	230±18
2	460±32	10±1	400±26
4	620±38	5	612
8	610±28	2	620±30
12	320±19	2	315±12
24	180±9	2	200±8

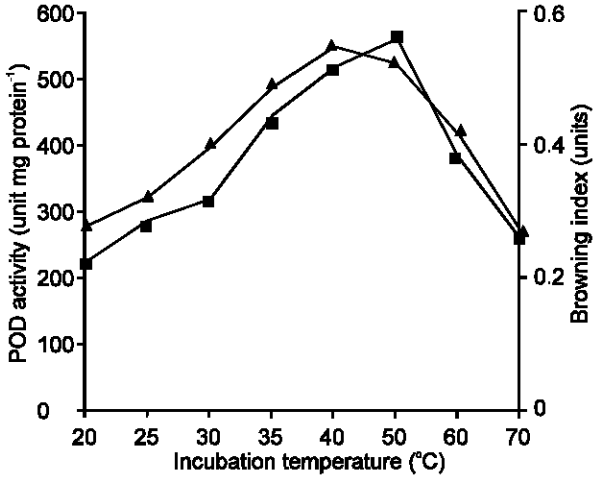

Fig. 3: The effect of incubation temperature on the brwoning and POD activity of yam tissues (●), POD activity, (■), Browning index

inhibition of PPO which is the major browning enzyme in yams. This difference might have been unnoticed if higher doses of the inhibitors with potential for total inhibition of both enzymes had been used. Enzyme - mediated browning of bruised yams had been associated with the activity of PPO and this is not in doubt. The ionic POD reported in this study has the potential for PPO activity which is not inhibited by PVP- a strong PPO inhibitor. Its potential for browning action may reside in its PPO-like subunit or a part of the enzyme with strict similarity to the active portion of a typical yam PPO. These concepts we suggest deserve to be further investigated.

Yam ionic POD is relatively heat stable with optimum temperature of 55°C and 35% maximum activity remaining after exposure to 70°C for 60 minutes (Okpuzor and Omidiji, 1999). It is a more thermostable enzyme than most PPOs (Omidiji *et al.*, 2002, Miller *et al.*, 1990). We investigated these temperature properties in relation to yam browning and POD potential as shown in Fig. 3. Browning was higher at elevated temperatures and POD activity was highest after exposing the tissues to 50°C for 4 hours. However maximum potential for browning occurred at lower temperatures between 30 and 40°C and this is consistent with the involvement of the more heat sensitive PPO in the event as we observed previously. At this stage we associate the enzyme mediated-browning in the tissues of *D. esculenta* at higher temperature (45-60°C) with the activity of this strongly ionic POD isozyme. It is important to investigate if this property inherent in the yam POD is related to any modification of the isozyme moiety at an active site. We envisage that such modification may be reflected in some degree of similarity to a PPO section at a functional site.

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