Peroxidase-Polyphenol Oxidase Association in *Dioscorea esculenta*

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A crude enzyme extract from *Dioscorea esculenta* var. *fasiculata* tissue subjected to ion exchange chromatography on DEAE-Sephadex A-50 column. This procedure resolved the extract into two main protein peaks one of which eluted through the column relatively unbound while the other protein peak which remained bound to the column was eluted with 1.0 M NaCl. Both protein peaks contained polyphenol oxidase (PPO) and peroxidase (POD) activities. The non-binding protein peak was resolved by gel filtration on Sephadex G-200 into distinct PPO and POD activities and by virtue of their apparent molecular weights of 95.5 Kd and 38.0 Kd for PPO and POD respectively were determined to be the typical enzymes. The PPO activity was completely inhibited invitro by 5 mM polyvinyl pyrrolidone (PVP). The binding protein peak was not resolved by gel filtration. It contained PPO activity which was not inhibited by PVP and a POD activity which was completely inhibited by dithiothreitol (DTT) This ionic protein peak contained 60% of total POD in the tissue, has an apparent molecular weight of 56 Kd and is suggested to be a strongly anionic peroxidase which also exhibits polyphenol oxidase activity.

Introduction

Peroxidase (POD, donor: H_2O_2 – Oxido – reductase, EC. 1:11.1: 7) is an intracellular enzyme which has been implicated in diverse cellular functions. In relation to edible vegetables, POD has been considered to play a role in the unfavourable changes in the flavour and colour of underblanched vegetables (Cain, 1967), but in this regard it is not considered to be exclusively responsible for the effects. (Vamos–Vigyazo, 1981). Polyphenol oxidase (PPO, monophenol-monooxygenase, EC. 1:14:18:1) is an intracellular enzyme concerned with various biological oxidations, and perhaps respiration and fermentation (Mathew and Parpia, 1971). The wide and similar range of substrates oxidised by both PPO and POD suggested that the two enzymes complement each other in living systems. De-Biasi and Badiani (1990), reported the presence of some POD enzymes with PPO activity indicating a very close association between the two enzymes. The importance of the close association between the two enzymes lies in the need to control their activities in food processing/preservation techniques. In yams and other tubers, the role of PPO in post-harvest browning phenomenon has been in focus of several workers (Omidiji and Okpuzor, 1996; Bachem *et al.*, 1994). The possible involvement of POD in such process has been largely ignored. This study is designed to investigate the nature of occurrence of PPO and POD in the tubers of *Dioscorea esculenta* in order to understand the role of POD in the browning phenomenon.

Abbreviations: POD, Peroxidase; PPO, Polyphenol oxidase; PVP, Polyvinyl pyrrolidone; DTT, Dithiothreitol; cv. Cultivar.

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Materials and Methods

Plant Sample

The mature tubers of *D. esculenta* L. var. *fasiculata* were obtained from the Biological garden, University of Lagos, Nigeria. After a period of curing for 14 days, the tubers were stored in the refrigerator (8 ± 2 °C) . Tubers were stored for 5–8 days before assays.

Enzyme source and PPO/POD assays

Freshly peeled tuber (25 g) was homogenised for 3 minutes in 100 ml of cold buffer (50 mM so-

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dium phosphate, pH 7.0) in a mini blender. The homogenate was centrifuged at 20,000×*g* (4 °C, 10 min) and the supernatant represented the crude enzyme source which was loaded directly onto the DEAE Sephadex A-50 ion exchange column. Crude enzyme extract from yam tissues for PPO and POD assays were obtained as previously described (Omidiji and Okpuzor, 1996). Catechol was used as substrate for PPO activity and enzyme assay was as described by Adamson and Abigor (1980). The assay for POD was carried out according to Mujer *et al.* (1983) using O-dianisidine as substrate. One unit of enzyme activity in each case is expressed as the amount of enzyme that caused a change of 0.001 absorbance unit per second under the assay condition.

Enzyme fractionation

The crude enzyme was subjected to ion exchange chromatography in a DEAE-Sephadex A-50 column (2 x 46 cm; bed volume, = 132 ml). The column was eluted with a linear gradient of NaCl (0–1.2 M) at the rate of 60 ml h^{-1} with sodium phosphate buffer (50 mM, pH 7.0); 10 ml fractions were collected.

Gel filtration

Enzymatically active peaks from the ion exchange stage were pooled and concentrated separately in an Amicon ultrafiltration unit (molecular weight cut off = 10 Kd) and eluted through a Sephadex G-200 column (Pharmacia, 2.5 x 45 cm; bed volume = 196 ml). Elution with 50 mM sodium phosphate buffer, pH 7.0 was at the rate of 18 ml h^{-1} and 3 ml fractions were collected.

Protein determination

The protein content of the column eluates were estimated by measuring the O.D at 280 nm. The protein content of the pooled, concentrated column eluates was determined according to Lowry *et al.* (1951) using bovine serum albumin (BSA, 0–250 mg) as standard.

pH Studies

Enzyme activity was determined in 50 mM sodium citrate buffer (pH range = 3.0–5.2) and 50 mM sodium phosphate buffer (pH range = 5.4–8.0) as previously described.

Temperature Studies

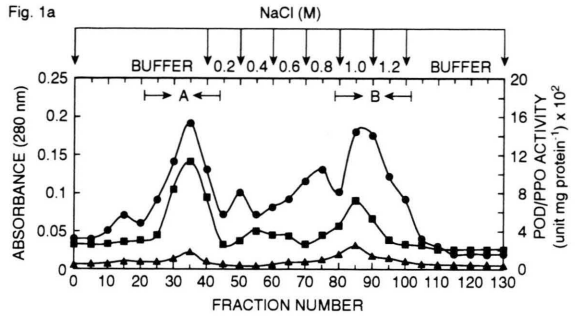
This was carried out as described by Simpson *et al.* (1987) over a temperature range of 10–70 °C.

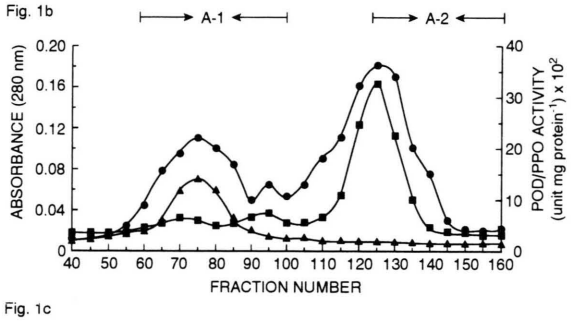
Inhibition Studies

Compounds known to inhibit the two enzymes were used at 5 mM concentration by incorporating into the reaction medium before addition of the enzyme.

Results and Discussion

Ion exchange chromatography on DEAE-Sephadex A-50 resolved the crude enzyme preparation into two enzymatically active peaks, each containing PPO and POD activities. The first active peak (sample A, Fig. 1a) which eluted through the column freely contained 80% of total PPO and 40% of total POD activities, while the second peak which was bound to the column (sample B, Fig. 1a.) contained 60% of total POD and 20% of total PPO activities. It is likely that previous reports on PPO of yam extracts cv. *rotundata* (Anosike and Ikediobi, 1983) focused on our sample A which eluted freely through the ion exchange column and which contained most PPO but limited POD activities. The sample B which bound tightly to the column probably remained in the column in those previous reports which focused mainly on the PPO of yams. The pooled sample A was further resolved by gel filtration as shown in Fig. 1b. Two protein peaks which eluted were associated with distinct PPO and POD activities. The PPO eluted first (protein peak A-1) while most of the POD activity eluted later (protein peak A-2). The PPO has an apparent molecular weight of 95.5 Kd, it is inhibited strongly by 5 mM PVP and is on this basis similar to other PPOs (Ikediobi and Obasuyi, 1982). The POD has an apparent molecular weight of 38.0 Kd, it is inhibited by 5 mM DTT but not by PVP. The strongly binding fraction (fraction B, Fig. 1a) was not resolved further by the gel filtration step. The elution profile (Fig. 1c) shows that the major protein peak contained both PPO and POD activities. The PPO activity was not PVP-sensitive but the POD activity





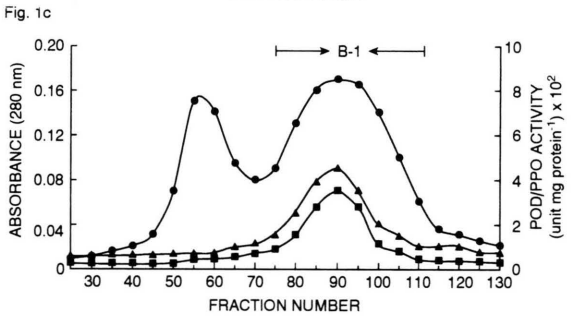


Fig. 1a. The elution profile of crude enzyme extract of *Dioscorea esculenta* on DEAE-Sephadex A-50 column. The crude enzyme extract was loaded directly onto the equilibrated column (2 x 46 cm, bed volume = 132 ml) and eluted with 400 ml of 50 mM sodium phosphate buffer, pH 7.0, and further with 100 ml portion of buffer containing 0.2–1.2 M NaCl. Elution was at the rate of 60 ml h^{-1} ; 10 ml fractions were collected. (●), protein content; (▲), peroxidase activity ; (■), polyphenol oxidase activity.

1b. The elution profile of sample A on Sephadex G-200 column. Concentrated sample A from ion exchange step was fractionated in a Sephadex G-200 column (2.5 x 45 cm; bed volume = 196 ml). Elution was with 50 mM sodium phosphate buffer, pH 7.0 at 18 ml h^{-1}; 3 ml fractions were collected. (●), protein; (▲), peroxidase activity; (■), polyphenol oxidase activity.

1c. The elution profile of sample B on Sephadex G -200. Legend is as for figure 1b.

was inhibited by DTT (Table I). The fraction is thus predominantly a POD. The apparent molecular weight of this fraction (56 Kd), also supports the suggestion that it is a POD (Murphy and O'Heocha, 1973). It is noteworthy to observe that the gel filtration step enhanced the POD activity over that of PPO by a factor of 3 : 1 from the ion exchange stage to gel filtration. This suggests an unmasking of apparent POD activity during progression from ion exchange to gel filtration.

Table I. The effect of various inhibitors on the activities of peroxidase and polyphenol oxidase of *Disocorea esculenta.*

	Enzyme activity (unit mg protein $^{-1}$)		Inhibition (% control)	
Compound	POD	PPO	POD	PPO
Catechol	n.d.	1470	n.d.	0
Poly ethylene glycol	308	1575	50	0
EDTA	n.d	1590	n.d	0
Citric acid	383	1297	38	12
Dithiothreitol (DTT)	0	1155	100	21
Thioglycollate	132	630	79	57
L-cysteine	433	593	30	60
Sodium azide	550	577	11	61
Potassium cyanide	500	463	19	69
Polyvinylpyrrolidone (PVP)	n.d	0	n.d	100
O-dianisidine	618	n.d	0	n.d

- All inhibitors were used at 5 mM.
- n.d. = not determined
- Each reading is the average of two determinations.
- One unit of enzyme activity is the amount of enzyme that caused a change in absorbance of 0.001 per second under the assay condition.

We observe in this study that 60% of yam peroxidase (cv. *esculenta)* may exist in ionic form. MacNicol (1966) had obtained four POD isoenzymes (three cationic and one neutral) from Alaska peas. The reasons for the differential distribution of POD types is not clear but may be of physiological importance. It may be related to differences in ability of plant tissue to resist infection (Lojkowska and Holubowska, 1992). An association of POD and PPO in the seedlings of *Aestivum triticum* has been reported (De Biasi and Badiani, 1990). Our assumption for the existence of a PPO-like POD is based mainly on the selective inhibition of yam PPO by PVP and POD by DTT. We therefore offer the suggestion that yam tissues contain the strongly ionic POD which has a potential for PPO action and may on that basis participate in tissue browning of yams.

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Adamson I. and Abigor R. (1980), Transformations associated with catecholase in *Dioscorea alata* during storage. Phytochemistry **19**, 1593 1595.

Anosike E. O. and Ikediobi C. (1983), Purification and some properties of polyphenol oxidase from yam tubers *Dioscorea bulbifera*. Phytochemistry **20**, 2625–2628.

Bachem C. W. B., Speckman G. J., Linde P. C. G., Vander – Verhegen F. M .T., Hunt M. D., Steffen J. C., Zabeau M. and Van-der-Linde P. C. G. (1994), Antisence expression of polyphenol oxidase inhibits enzymatic browning in potato tubers. Biotechnology **12**, 1101–1105.

Cain R. F. (1967), Water soluble vitamins. Changes during processing and storage of fruits and vegetables. Food Technology **21**, 70–89.

De-Biasi M. G. and Badiani M. (1990), The phenol oxidase-like activity of purified peroxidase from *Triticum aestivum* L. seedling leaves. Plant Science **67**, 29–37.

Ikediobi C. O. and Obasuyi H. N. (1982), Purification and some properties of O-diphenolase from white yam tubers. Phytochemistry **21**, 2815–2820.

Lojkowska E. and Holubowska M. (1992), The role of polyphenol oxidase and peroxidase in potato resistance soft rot caused by *Erwinia carotovora* J. Phytopathology **136**, 319–328.

MacNicol P. K. (1966), Peroxidases of the Alaska peas *Pisum sativum* L. Arch. Biochem. Biophys. **117**, 347–356.

Mathew A. G. and Parpia H. A. B. (1971), Food browning as polyphenol reaction. Adv. Food Res. **19**, 75–145.

Mujer C. U., Mendoza E. M. and Ramierez D. A. (1983), Coconut peroxidase isoenzymes: isolation, partial purification and physiochemical properties. Phytochemistry **22**, 1133–1340.

Murphy M. J. and O' Heocha C. (1973), Peroxidase from green alga *Enteromorpha linza* Phytochemistry **12**, 61–65.

Omidiji O. and Okpuzor J. (1996), Time course of PPO-related browning of yams. J. Sci. Food Agric. **70**, 190–196.

Simpson B. K., Marshall M. R. and Otwell S. W. (1987), Phenol oxidase from shrimp (*Penaeus setferus*). Purification and some properties. J. Agric. Food Chem. 35: 918–921.

Vamos-Vigyázo L. (1981), Polyphenol oxidase and peroxidase in fruits and vegetables. Crit. Rev. Food Sci. Nutr. **15**, 49–127.