Food Chemistry **24** (1987) 203–217

Studies on the Purification of Banana Polyphenoloxidase

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(Received 2 June 1986; revised version received 19 June 1986; accepted 18 September 1986)

ABSTRACT

Studies on the extractability of polyphenoloxidase (PPO) from the pulp of five banana cultivars revealed a varietal difference in the nature of binding of the PPO in the cell, with the enzyme being entirely in the soluble fraction in one and partly associated with the cell wall in others, necessitating use of a detergent to release it from the latter. Partial purification by acetone precipitation and chromatography using a DEAE-cellulose column yielded two major fractions DE-I and DE-II with purifications of 4- and 16·3-fold and activity recoveries of 38·2 and 43·3% respectively. Further gel filtration of the two fractions on a Sephadex G-100 column improved the purifications to 44- and 50-fold respectively with full activity recovery. Polyacrylamide gel electrophoretic studies showed the two fractions to be composed of isoenzymes differing in pattern. The purified enzyme showed maximum absorption at 275 nm.

INTRODUCTION

In common with many fruits and vegetables, banana contains the polyphenoloxidase (PPO) (E.C. 1.10.3.1, O-diphenol:O_2 oxidoreductase) enzyme system which catalyses the oxidation of phenolic substrates by

Food Chemistry 0308-8146/87/$03·50

molecular oxygen, resulting in the formation of intense brown pigments and is therefore susceptible to enzymatic browning when cut or injured. Earlier work by Palmer (1963) has shown that banana PPO is absorbed or structurally associated with the cell wall and use of a non-ionic detergent was necessary to solubilise the enzyme for extraction. Later work showed that inclusion of PEG and PVP in the extracting buffer increased extractability of the enzyme from the pulp (Badran & Jones, 1965; Montgomery & Sgarbieri, 1975).

Palmer (1963) reported a 10- to 12-fold purification of the PPO from Gros-Michel banana with 12% recovery by acetone precipitation followed by DEAE-cellulose column chromatography, while Galeazzi *et al.* (1981) obtained a single PPO fraction with 38·8-fold purification and 27·9% recovery from Dwarf Cavendish banana by a combination of techniques involving acetone precipitation, storage at −40°C, Sephadex G-100 column chromatography and polyacrylamide gel electrophoresis. Mowlah *et al.* (1982) separated partially purified banana PPO into eight peaks by column isoelectric focusing and six to seven peaks by a Sephadex G-100 column.

In an earlier publication (Jayaraman *et al.*, 1982) we reported differences in the susceptibility to browning observed in five commercially important local cultivars of banana as influenced by PPO activity and other endogenous factors. In an attempt to further characterise the enzyme, we carried out studies on the isolation and partial purification of PPO from five banana varieties to identify any varietal differences and studies on the elution profile of the partially purified enzyme from the most active variety, i.e., 'Pachabale' (*M. cavendishii*) independently on Sephadex gel and DEAE-cellulose columns. We also studied further purification of the enzyme using DEAE-cellulose and Sephadex G-100 column chromatography applied in sequence and characterisation of the purified fractions by polyacrylamide gel electrophoresis. Results of these studies are reported in this paper.

MATERIALS AND METHODS

Bananas

In studies on the extractability and partial purification of the enzyme to identify varietal differences, the pulp tissue from the following five commercially important varieties of fully ripe banana (skin colour yellow with brown flecks) obtained from the local market were used: (i) *Musa cavendishii* cv. 'Pachabale' (genome AAA); (ii) *M. paradisiaca* cv. 'Poojabale' (AAB); (iii) *M. sapidisiaca* cv. 'Rasabale' (AAB); (iv) *M. paradisiaca* cv. 'Kadubale' (AAB); and (v) *M. paradisiaca* cv. 'Puttabale' (AB). These were

the varieties in which we had earlier shown (Jayaraman *et al.*, 1982) susceptibility to browning upon cutting to vary in the order: 'Pachabale' > 'Poojabale' > 'Kadubale' > 'Rasabale' = 'Puttabale' which was influenced mainly by PPO concentration and ascorbic acid content. Thus varieties which exhibited low browning rate had low PPO concentration and high ascorbic acid content while the high browning ones were *vice versa.*

In experiments on fractionation using Sephadex gel and DEAE-cellulose and further purification of the enzyme, only the pulp tissue from the variety 'Pachabale' (*M. cavendishii*), with the highest concentration and activity of PPO among the five varieties studied, was used.

Extraction of PPO

The PPO was extracted by homogenising the pulp in 0·1 M potassium phosphate buffer, pH 7·0, containing 1% non-ionic detergent (Tween 80, Sigma Chemical Co., USA) at 0°C for 15 min using a glass tissue grinder with a teflon pestle at a speed of 5000 rev/min. The homogenate was directly centrifuged at 20 000 × *g* for 15 min at 0°C using a refrigerated centrifuge (Sorvall, USA). The supernatant containing the enzyme was maintained at 0°C in crushed ice until used. All operations were carried out at 0°C unless otherwise stated.

In the investigation on subcellular location of the PPO, such as its binding to different cell organelles to identify any possible varietal differences in the nature of binding, the crude homogenate obtained with plain 0·1 M phosphate buffer, pH 7·0, was fractionated sequentially by centrifugation at 500 × *g* for 10 min followed by 17 000 × *g* for 15 min, by the method suggested for isolation of mitochondria and chloroplasts (Axelrod, 1955) and the pellets and supernatants were assayed for activity.

Enzyme activity and protein assay

The PPO activity in the enzyme extracts was routinely assayed by the method of Palmer (1963) by measuring the rate of increase in absorbance at 475 nm at 25°C in a Perkin Elmer Model 124 double beam grating spectrophotometer or a Bausch and Lomb Spectronic 20 colorimeter using dopamine as substrate. Since linearity was maintained for 2 min under the conditions of the experiment, the increase in OD which occurred within the first 2 min of the reaction time was taken as rate of reaction. One unit of PPO activity was defined as the amount of enzyme that caused unit change in absorbance per minute at 475 nm. The protein content was estimated by the method of Lowry *et al.* (1951) using a colorimeter (Klett-Summerson, USA)

and bovine serum albumin as the standard. Specific activity was expressed as units/mg protein.

Acetone precipitation

From the supernatant obtained by centrifugation of the crude detergent buffer extract, the enzyme was precipitated by slow addition of 1·6 volumes of cold AnalaR acetone at −20°C and stirred for about 10 min, maintaining temperature at −10°C using a bath containing salt-ice freezing mixture. The precipitate was collected by centrifugation at 15 000 × *g* for 10 min at −10°C and dissolved in 0·01 M potassium phosphate buffer, pH 7·0. After standing overnight at 0°C, the solution was centrifuged at 20 000 × *g* for 20 min to remove the inactive residue and the clear supernatant used as partially purified enzyme. It was maintained at 0°C until used.

Chromatography on Sephadex G-100 and G-200 columns

The partially purified PPO was chromatographed on Sephadex G-100 (Sigma Chemical Co., USA) using a 2·5 × 38 cm column which was prepared and equilibrated with 0·05 M potassium phosphate buffer, pH 7·0, as described by Reiland (1971). Two ml of the enzyme containing about 6 mg protein was applied to the column. Elution was carried out with 0·05 M phosphate buffer, pH7·0 (300 ml) at the rate of 0·5 ml/min and 3 ml fractions were collected using a fraction collector (ISCO, Inc., USA, Model 1220). An aliquot from each fraction and from pooled fractions comprising the activity peaks was assayed for enzyme activity. Protein in each fraction was monitored spectrophotometrically by recording the absorbance at 280 nm using a Perkin Elmer Model 124 spectrophotometer. It was assayed in pooled fractions by the method of Lowry *et al.* (1951). The column was maintained at 0°C throughout the experiment by circulation of ice water. The bed void volume was measured using blue dextran 2000 as described by Reiland (1971). Chromatography on Sephadex G-200 was also performed as described above for G-100 but using a column of dimensions 1·5 × 30 cm.

Chromatography on DEAE-cellulose column

Preliminary experiments on the chromatography of the partially purified banana PPO on DEAE-cellulose (microgranular, Whatman DE-52, preswollen, with a capacity of 1·0 meq/g) were carried out using a 1·5 × 15 cm column. The DEAE-cellulose was prepared and filled in the column as per manufacturer's instructions. The column was equilibrated first with the starting buffer, 0·01 M potassium phosphate, pH 8·0.

Five ml of the partially purified PPO containing 8·5 mg protein and dissolved in 0·01 M phosphate buffer, pH 7·0, was applied to the column and eluted with 0·01 M phosphate, pH 8·0 (100 ml), to wash all proteins not absorbed to the cellulose. This was followed by a stepwise elution using 0·08 M potassium phosphate buffer, pH 8·0 (150 ml), or a linear gradient elution consisting of 0·05 M (100 ml) and 0·2 M (100 ml) potassium phosphate buffer, pH 8·0. A flow rate of 50–60 ml/h was used and 5 ml fractions were collected using a fraction collector. The column was maintained at 0°C by the circulation of ice water throughout the experiment. An aliquot from each fraction and from pooled fractions comprising the activity peaks was assayed spectrophotometrically for PPO activity and protein as stated above for gel chromatography.

Further purification of DEAE-cellulose fractions by Sephadex G-100 column chromatography

Ten ml of the partially purified enzyme containing 31·2 mg protein dissolved in 0·01 M phosphate buffer, pH 7·0, was first applied to a 2·5 × 40 cm DEAE-cellulose column prepared and equilibrated with the starting buffer, 0·01 M phosphate, pH 8·0, and washed with the same buffer (300 ml). The column was then eluted stepwise with 0·08 M phosphate buffer, pH 8·0 (500 ml). A flow rate of 50–60 ml/h was used and 10 ml fractions were collected. The major DEAE-cellulose pooled fractions obtained were then separately dialysed against 2 litres of 0·001 M phosphate buffer, pH 7·0, for 24 h with two changes, concentrated to a small volume (2–3 ml) using a lyophiliser (Virtis, USA), applied individually to a 2·5 × 38 cm Sephadex G-100 column prepared and equilibrated with 0·05 M phosphate buffer, pH 7·0, and eluted with the same buffer at the rate of 0·5 ml/min; 3 ml fractions were collected. The active tubes were pooled together, dialysed against 0·001 M phosphate, pH 7·0, and concentrated by lyophilisation to obtain the purified fractions. The whole operation was carried out at 0°C.

Polyacrylamide gel electrophoresis

Polyacrylamide disc gel electrophoresis was carried out by the method of Davis (1964) using 7·5% polyacrylamide in 0·375 M Tris-HCl, pH 8·9, as the resolving gel (Gabriel, 1971) and 10 mM Tris-glycine buffer, pH 8·3, as the electrode buffer at 0–4°C. While the partially purified enzyme extract was used as such for electrophoresis, the different active fractions from DEAE-cellulose and Sephadex G-100 column chromatography and the purified enzyme were concentrated by lyophilisation prior to application on the gels.

After electrophoresis the gels were stained for PPO activity by

equilibrating for 30 min, first with 0·1% *p*-phenylenediamine in buffer followed by 10 mM catechol in a buffer. Proteins were stained using Coomassie Brilliant Blue (Gabriel, 1971).

Absorption spectrum

The absorption spectra of the partially purified and DEAE/Sephadex G-100 purified enzymes were obtained using a Perkin-Elmer Model 124 double beam grating spectrophotometer.

RESULTS AND DISCUSSION

Extraction of PPO

Experiments carried out on the extraction of PPO from the pulp tissue of five banana cultivars using buffer and detergent buffer showed that, while a negligible amount of activity was extracted with a plain buffer in the varieties having low PPO concentration (namely, 'Kadubale' and 'Puttabale'), activity extracted was increased two- to four-fold by inclusion of a non-ionic detergent (either Tween-80 or Triton X-100) in the buffer at 1% level in all the varieties except 'Poojabale' (Table 1). In the 'Poojabale' variety the enzyme could be solubilised and extracted with a plain buffer and use of detergent extracted only more inactive proteins, thereby resulting in less specific activity.

Data obtained with banana varieties by differential centrifugation showed again that, with the exception of 'Poojabale', in all other varieties only a fraction of the activity was extracted by a plain buffer into the crude homogenate and a considerable amount was sedimented at $500 \times g$. There was insignificant activity sedimentation at $17\,000 \times g$ (normally used for isolation of mitochondria and chloroplasts), thereby showing that, in these varieties, the PPO was partly in the soluble fraction and partly associated with the cell wall. In 'Poojabale', in contrast, almost the entire PPO activity was extracted into the crude homogenate obtained with a plain buffer with no sediment at $500 \times g$ and negligible activity sedimenting at $17\,000 \times g$; therefore in this variety the enzyme was apparently not bound to the cell wall or any other cell organelle but was entirely in the soluble fraction.

Results of our study are generally in agreement with those of Palmer (1963) in that use of detergent was necessary to increase the extractability of the enzyme, especially from the low active varieties. However a varietal difference in the nature of binding of the PPO in the cell was apparent from the present studies, the enzyme being entirely in the soluble fraction in one variety and partly associated with the cell wall in others. Padron *et al.* (1975)

TABLE 1
PPO Activity and Protein Extracted by Plain Buffer and Detergent Buffer from the Pulp of Five Varieties of Ripe Banana

Banana variety	*Mode of extraction*[a]	*Activity (units/ml)*	*Protein (mg/ml)*	*Specific activity*	*Fold extraction (activity)*
Pachabale	Plain buffer	1·25	0·56	2·23	
	Detergent buffer	5·00	1·38	3·62	4·00
Poojabale	Plain buffer	3·75	0·76	4·97	
	Detergent buffer	3·75	1·32	2·84	1·00
Rasabale	Plain buffer	1·17	0·57	2·06	
	Detergent buffer	3·00	1·20	2·50	2·56
Kadubale	Plain buffer	0·65	0·60	1·08	
	Detergent buffer	1·50	1·40	1·09	2·30
Puttabale	Plain buffer	0·03	0·44	0·07	
	Detergent buffer	0·95	1·06	0·91	—

[a] 2 g pulp homogenised at 0°C with 20 ml buffer (0·1 M phosphate, pH 7·0) or 1% detergent (Tween-80) buffer, centrifuged at 20 000 × *g* for 15 min at 0°C and the supernatant used for assay.

likewise reported extraction of the enzyme from *M. cavendishii* using a buffer without the need for detergent.

Partial purification

Preliminary investigations on fractionation of a detergent buffer extract using ammonium sulphate revealed that about 37% of the activity was

TABLE 2
Activity, Protein and Specific Activity of Partially Purified PPO[a] from Five Banana Varieties

Banana variety	*Activity (units/ml)*	*Protein (mg/ml)*	*Specific activity*[b]	*Recovery (%)*	*Fold purification*
Pachabale	21·25	2·1	10·1 (4·8)	93·0	2·1
Poojabale	14·75	1·5	9·8 (4·2)	92·2	2·3
Rasabale	8·20	1·6	5·1 (2·4)	90·1	2·1
Kadubale	5·10	1·7	3·0 (1·0)	91·0	3·0
Puttabale	2·90	1·5	1·9 (0·9)	89·8	2·1

[a] 10 g pulp homogenised with 50 ml 1% detergent buffer, centrifuged, supernatant precipitated using 80 ml acetone at −20°C and residue obtained by centrifugation dissolved in 25 ml 0·02 M phosphate buffer, pH 7·0.

[b] Figures in parentheses represent specific activity of the corresponding crude detergent buffer extract.

precipitated in the 30–90% fraction with an increase of only about 20% in specific activity while, in the 40–75% range, only 20% of the activity was precipitated with a 60% increase in specific activity compared with the crude enzyme. In contrast, acetone precipitation of the detergent extract increased the specific activity of the enzyme in all the varieties by a factor of two to three with 90% recovery (Table 2).

These results differ from those of Padron *et al.* (1975) who reported a 2·5-fold purification of banana PPO in the supernatant after 50% saturation with ammonium sulphate with 60% recovery, while Galeazzi *et al.* (1981) obtained 120% recovery by re-extraction of the acetone precipitate with a detergent buffer, the higher recovery being attributed to possible reactivation by the buffer or removal of inhibitory substances.

Gel filtration on Sephadex G-100 and G-200

When the partially purified PPO was chromatographed directly on a Sephadex G-100 column (2·5 × 38 cm) the elution profile revealed two distinct active fractions, designated S-I and S-II, comprising 18·0 and 29·1% of the total activity fed onto the column respectively and a third inactive fraction, S-III, comprising the contaminating inert proteins as revealed by

TABLE 3
Chromatography of Partially Purified PPO from Banana (*Cv. Pachabale*) on Sephadex G-100 Column (2·5 × 38 cm)[a]

Purification step	*Volume (ml)*	*Total activity (units)*	*Total protein (mg)*	*Specific activity*	*Recovery (%)*	*Purification (fold)*
1. Detergent extract	8	88·0	17·92	4·9	100·0	1·0
2. Acetone precipitate	2	79·3	6·77	11·7	90·1	2·4
3. Sephadex G-100 column						
(a) S-I						
Peak (Fn 25)	3	2·7	0·17	15·8	—	3·2
Pooled (Fn 23–28)	18	12·6	1·03	12·3	14·3	2·5
(b) S-II						
Peak (Fn 38)	3	3·1	0·11	27·6	—	5·6
Pooled (Fn 35–42)	24	20·4	0·91	22·4	23·2	4·6

[a] Void volume of the column = 60 ml. Volume collected per fraction = 3 ml.

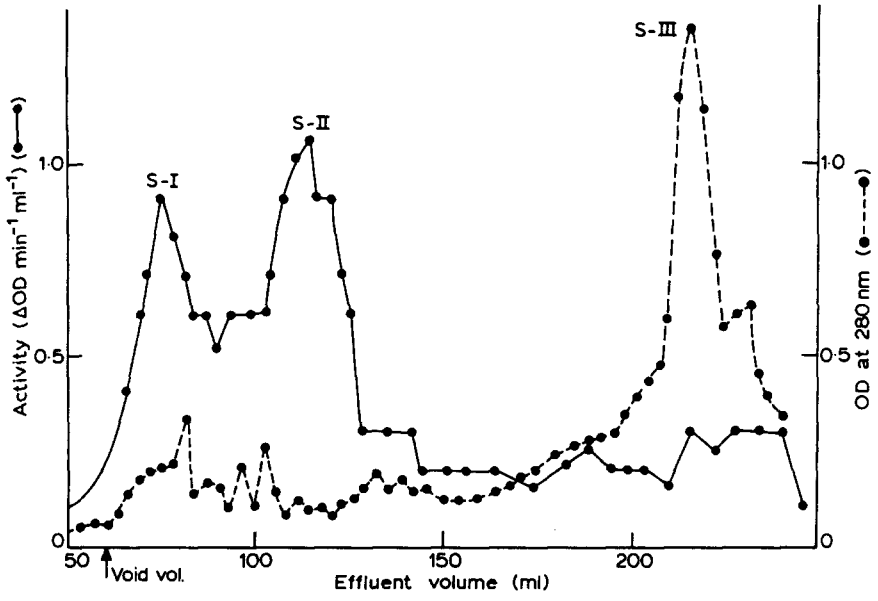

Fig. 1. Elution profile of partially purified banana PPO var. 'Pachabale' from Sephadex G-100 column ●——● PPO activity; ●----● protein.

the peak at 280 nm (Fig. 1). While pooled fractions of S-I yielded no significant improvement in specific activity, those of S-II showed an almost 100% increase over that of the acetone precipitate (Table 3). Total activity recovered in the two fractions was low.

All the three Sephadex G-100 fractions were eluted after the void volume (Fig. 1), thereby indicating the molecular weights of these fractions to be less than 150 000, the exclusion limit of Sephadex G-100. The elution volumes of the fractions were in the order S-I < S-II < S-III and their molecular weights in the reverse order, since it has been shown for a number of proteins that their rates of elution from a column of Sephadex G-100 are inversely proportional to their molecular weights (Reiland, 1971).

Chromatography of the acetone precipitate on Sephadex G-200 using a 1·5 × 30 cm column gave a similar elution profile as on G-100 and confirmed the presence of two distinct active fractions differing in molecular size which were eluted after the void volume. There was again no significant improvement in specific activity or degree of purification, and the percentage recovery was low.

These results are similar to those of Benjamin & Montgomery (1973) on cherry PPO and compare with those of Padron *et al.* (1975) who obtained two fractions from banana PPO by Sephadex G-100 gel filtration of the ammonium sulphate fraction. They differ, however, from those of Galeazzi *et al.* (1981) who reported a single major peak with 25-fold purification and

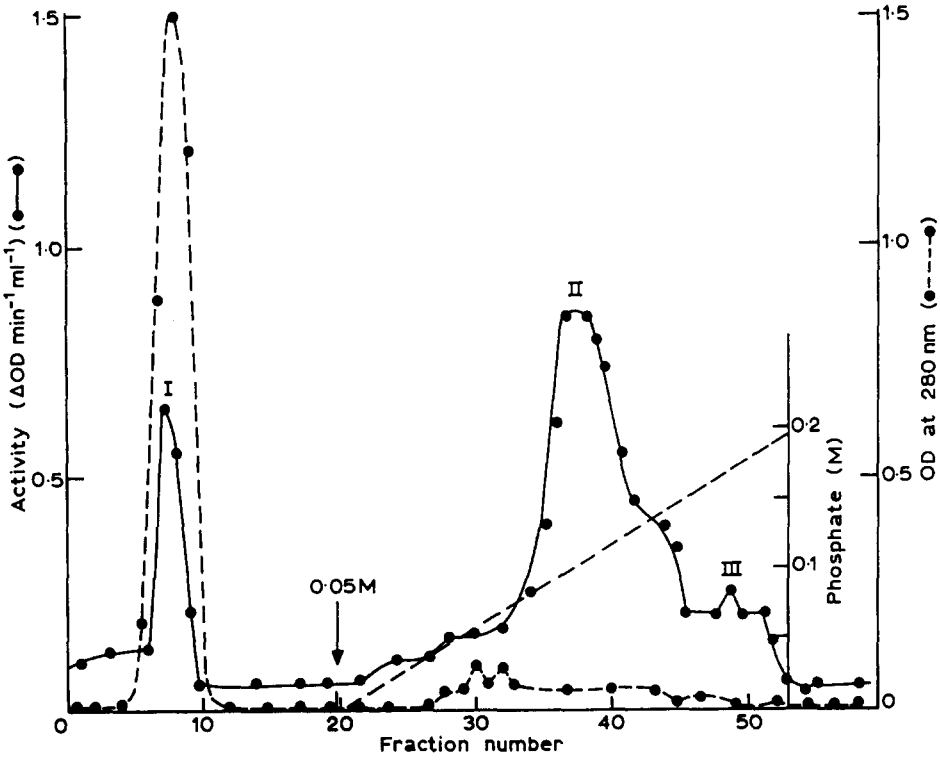

Fig. 2. Elution profile of partially purified banana PPO var. 'Pachabale' from DEAE-cellulose column by linear gradient elution ●——● PPO activity; ●----● protein.

63·7% activity recovery by gel fractionation of the acetone precipitate and from those of Mowlah *et al.* (1982) who resolved partially purified banana PPO into six to seven peaks by Sephadex G-100 chromatography.

Ion-exchange chromatography on DEAE-cellulose

Preliminary experiments on chromatography of the partially purified PPO on DEAE-cellulose using a 1·5 × 15 cm column by stepwise elution yielded two major fractions, one unbound and the other bound, designated DE-I and DE-II respectively and a third minor bound fraction, DE-III.

Fraction DE-I, comprising about 27% of the original activity fed onto the column, was eluted sharply (in 10 ml) with the initial starting buffer along with some of the contaminating inert proteins, as evident from the peak at 280 nm, thereby yielding a fraction with a specific activity and degree of purification less than that of the original acetone precipitate. This fraction was eluted even when the molarity of the starting buffer was reduced to as low at 0·001 M and therefore may be considered unbound to the column.

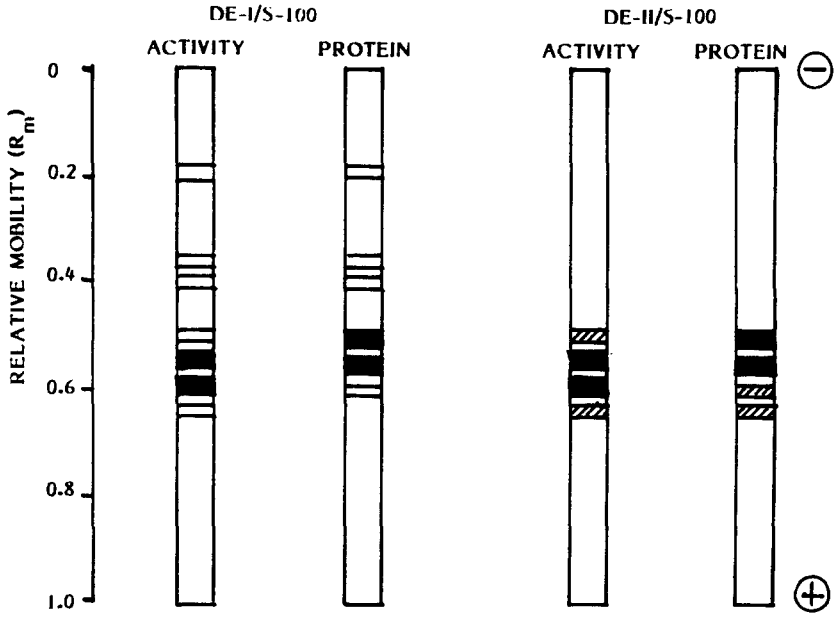

Fig. 3. Polyacrylamide gel electrophoretic activity and protein patterns of DEAE-cellulose/Sephadex G-100 purified banana PPO fractions (band intensity: ■ high; ▨ medium; □ low).

Fractions DE-II and DE-III, containing 56 and 4·6% of the total activity fed onto the column respectively, were eluted with 0·08 M buffer. The most active tube in DE-II yielded a 33-fold purification, while that in DE-III yielded 7·8-fold purification. Pooled fractions constituting DE-II gave a 22-fold purification, while those of DE-III gave a 7·2-fold purification.

Gradient elution performed on the same column yielded similar results (Fig. 3) and indicated that the majority of activity in the bound fraction was eluted between 0·05 and 0·08 M phosphate buffer concentration and there was no further resolution of the peaks.

While DEAE-cellulose chromatography has been used extensively by earlier workers to separate plant PPOs into one or more fractions, work on its use to purify banana PPO is so far confined to that of Palmer (1963), who reported a single bound fraction yielding a 10 or 12-fold purification with 12% recovery compared with two major fractions, one (unbound) with 1·8-fold purification and 22% recovery and the other (bound) with 22-fold purification and 46·7% recovery obtained in the present studies.

Further purification of DEAE-cellulose fractions by Sephadex G-100

The partially purified PPO was first fractionated using a larger (2·5 × 40 cm) DEAE-cellulose column by stepwise elution, as described above, to give the two major fractions DE-I and DE-II, as with the small column. The

TABLE 4
Purification Chart of Banana PPO

Purification step	*Volume (ml)*	*Total activity (units)*	*Total protein (mg)*	*Specific activity (units/mg protein)*	*Recovery (%)*	*Purification (fold)*
1. Detergent extract	40	550	84·8	6·5	100	1·0
2. Acetone precipitate	10	500	31·2	16·0	90·9	2·5
3. DEAE-cellulose column						
(a) DE-I pooled	20	210	8·20	25·6	38·2	4·0
(b) DE-II pooled	140	238	2·24	106·2	43·3	16·3
(c) DE-III pooled	40	14	0·60	23·3	2·5	3·6
4. Sephadex G-100 column						
(a) DE-I/S-100 pooled	50	200	0·70	285	36·4	44·0
(b) DE-II/S-100 pooled	60	230	0·72	320	41·8	50·0

unbound fraction, DE-I, comprising 42% of the total activity fed onto the column, was eluted sharply (in 20 ml) with the starting buffer, along with some of the inert proteins, yielding a slightly improved specific activity and a 4-fold purification (Table 4). The major bound fraction, DE-II, comprising 47·6% of the total activity fed, was eluted with 0·08 M buffer, pH 8·0, yielding a specific activity of 160·0 with 25-fold purification in the peak tube and a specific activity of 106·2 with 16·3-fold purification in the pooled fraction, while the minor bound fraction, DE-III, comprising 2·8% of the total activity fed, gave specific activities of 26·7 and 23·3 and degrees of purification of 4·1 and 3·6 in the peak tube and pooled fraction respectively.

The two major (DE-I and DE-II) pooled fractions were further purified by applying each of them independently, after dialysis and concentration, onto a Sephadex G-100 column (2·5 × 38 cm). Each fraction was eluted as a single active peak with almost 100% recovery and the degree of purification increased from 4·0 to 44 in DE-I and from 16·3 to 50 in DE-II with overall recoveries of 36·4 and 41·8% respectively (Table 4). These two purified fractions were designated DE-I/S-100 and DE-II/S-100 respectively.

Results of our present studies thus show that, by a combination of techniques involving acetone precipitation, DEAE-cellulose and Sephadex G-100 column chromatography applied in a sequence, banana PPO could be resolved into two major fractions, I and II, with degrees of purification of 44 and 50 and percentage recoveries of 36·4 and 41·8 respectively. This is in contrast to a single fraction with 38·8-fold purification and 27·9% recovery obtained by Galeazzi *et al.* (1981) by a combination of acetone precipitation, freezing, Sephadex G-100 gel filtration and polyacrylamide gel electrophoresis.

Gel electrophoresis of Sephadex G-100 and DEAE-cellulose fractions

The gel electrophoretic isoenzyme patterns obtained with the two active fractions, S-I and S-II, from Sephadex G-100 column chromatography and DE-I and DE-II from DEAE-cellulose column chromatography on comparison revealed that the S-I pattern was similar to that of DE-I and the S-II pattern to that of DE-II. The former fraction of higher molecular weight showed two groups, I (one band) and II (two bands), of slow moving isoenzymes, and an intermediate group III (four bands) of isoenzymes compared with the second fraction of lower molecular weight, which was composed mainly of intermediate group III isoenzymes (four bands). Since polyacrylamide gel electrophoresis separates proteins on the basis of size and net charge, the size of the PPO would decrease and the net charge increase from Group I to III.

The visually recorded electrophoretic isoenzyme and protein patterns obtained with the purified fractions DE-I/S-100 and DE-II/S-100 are given in Fig. 3. Again, as seen from the activity patterns, while DE-I/S-100 was composed of seven isoenzymes, comprising the slow moving group I and II and the intermediate group III isoenzyme, DE-II/S-100 was composed only of intermediate group III isoenzymes. The protein patterns generally correlated with the activity patterns.

It may therefore be concluded from the above data that the banana PPO system is composed of two enzyme fractions of different molecular size and charge density both of which were composed of isoenzymes, seven and four respectively. This is in contrast with two isoenzymes reported by Padron *et al.* (1975) in the two purified fractions and four isoenzymes reported by Galeazzi *et al.* (1981) in the single purified fraction obtained by gel filtration of banana PPO. Our data compare with those of Benjamin & Montgomery (1973) who found the two fractions obtained from cherry PPO by DEAE-cellulose chromatography to consist predominantly of slow and intermediate isoenzyme bands in one and of fast-moving isoenzymes in the other by gel electrophoresis.

Enzyme spectrum

The absorption spectrum of the partially purified PPO in the UV region showed a maximum at 265 nm and that of the DEAE/Sephadex purified enzyme at 275 nm. There was no significant absorption above 300 nm. These results are in fair agreement with those reported earlier on PPO from cherry (Benjamin & Montgomery, 1973), egg plant (Sharma & Ali, 1980, potato (Patil & Zucker, 1965) and banana (Galeazzi *et al.*, 1981). Galeazzi *et al.* (1981) however, reported an additional shoulder at 340–350 nm which they attributed to chlorogenic acid linked to the enzyme.

ACKNOWLEDGEMENT

The authors wish to thank Dr T. R. Sharma, Director, Defence Food Research Laboratory, Mysore, for his kind permission to publish this paper.

REFERENCES

Axelrod, D. (1955). Preparation of mitochondria from plants. In: *Methods in enzymology*, Vol. 1 (Colowick, S. P. & Kaplan, N. O. (Eds)). New York, Academic Press, 19–22.

Badran, A. M. & Jones, D. E. (1965), Polyethylene glycol-tannin interaction in extracting enzymes. *Nature*, **206**, 622–4.

Benjamin, N. D. & Montgomery, M. W. (1973). Polyphenoloxidase of Royal Ann cherries: purification and characterization. *J. Food Sci.*, **38**, 799–806.

Davis, B. J. (1964). Disc electrophoresis. II: Method and application to human serum proteins. *Ann. NY Acad. Sci.*, **121**, 404–27.

Gabriel, O. (1971). Analytical disc gel electrophoresis. In: *Methods in enzymology*, Vol. 22 (Jakoby, W. B. (Ed.)). New York, Academic Press, 565–78.

Galeazzi, M. A. M., Sgarbieri, V. C. & Constantinides, S. M. (1981). Isolation, purification and physicochemical characterization of polyphenoloxidase (PPO) from a dwarf variety of banana (*Musa cavendishii*, L.). *J. Food Sci.*, **46**, 150–5.

Jayaraman, K. S., Ramanuja, M. N., Dhakne, Y. S. & Vijayaraghavan, P. K. (1982). Enzymatic browning in some banana varieties as related to polyphenoloxidase activity and other endogenous factors. *J. Food Sci. Tech.* (India), **19**, 181–6.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–7.

Montgomery, M. W. & Sgarbieri, V. C. (1975). Isoenzymes of banana polyphenol-oxidase. *Phytochemistry*, **14**, 1245–9.

Mowlah, G., Takano, K., Kamoi, I. & Obara, T. (1982). Characterization of banana polyphenoloxidase (PPO) fractions with respect to electrophoretic and gel filtration behaviour. *Lebensm. Wiss. Technol.*, **15**, 207–10.

Padron, M. P., Lozana, J. A. & Gonzalez, A. G. (1975). Properties of *o*-diphenol: O_2 oxidoreductase from *Musa cavendishii*. *Phytochemistry*, **14**, 1959–63.

Palmer, J. K. (1963). Banana polyphenoloxidase—preparation and properties. *Plant Physiol.*, **38**, 508–12.

Patil, S. S. & Zucker, M. (1965). Potato phenolases—purification and properties. *J. Biol. Chem.*, **240**, 3938–43.

Reiland, J. (1971). Gel filtration. In: *Methods in enzymology*, Vol. 22 (Jakoby, W. B. (Ed.)). New York, Academic Press, 287–321.

Sharma, P. C. & Ali, R. (1980). Isolation and characterization of catechol oxidase from *Solanum melongena*. *Phytochemistry*, **19**, 1597–600.