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ISOENZYMES OF BANANA POLYPHENOL OXIDASE

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Abstract—Polyacrylamide-gel electrophoresis revealed 9 polyphenol oxidase (PPO) isoenzymes in the interior of banana pulp, 8 in the exterior pulp, and 10 in the peel. With the exception of the absence of one isoenzyme in the exterior pulp, the isoenzymes in the exterior and interior pulp had similar relative mobilities (R_m). Two isoenzymes from the banana peel and from the pulp showed similar R_ms and substrate and inhibitor specificities. Banana PPO were most active with *o*-diphenols and showed a decrease in activity as the substituent group on the ring was increased in size. Sodium diethyldithiocarbamate, cysteine, 2-mercaptoethanol, 1,2 dithiolpropane and sodium cyanide inhibited all the banana PPO isoenzymes. Sodium metabisulfite inhibited some but not all isoenzymes.

INTRODUCTION

Palmer [1] demonstrated the presence of a polyphenol oxidase (PPO; *o*-diphenol:O_2 oxidoreductase E.C. 1.10.3.1) in bananas which oxidized *o*-diphenols but not monophenols. This enzyme was inhibited by reducing agents, chelating agents and substrate analogues [2,3]. Recently, a tyrosine hydroxylase, which was activated by ascorbic acid, has been reported in banana pulp [4]. The PPO systems of several fruit have been shown to contain numerous isoenzymes with various substrate and inhibitor specificities [5–8]. The present work reports the substrate and inhibitor specificities of banana PPO isoenzymes.

RESULTS

Preliminary experiments using the interior banana pulp (the tissue immediately surrounding the immature seeds) showed that both polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG, 20 M) increased the extractability of PPO activity. About 15% more activity was extracted with PVP than with PEG. Using catechol as the substrate, polyacrylamide-gel electrophoretic patterns of extracts made with PVP were more distinct and showed less smearing than those made with PEG or without a phenolic binder. No differences in the number or location of the bands in the electrophoretic patterns were noted when various levels of PVP or PEG were used in preparing the extracts.

Effect of ripening and location of PPO in banana

Activity of PPO was determined on extracts of the interior of the pulp, exterior of the pulp and peel of bananas as the fruit ripened. Results (Table 1) show that the activity, in general, decreased in the pulp and increased in the peel during ripening. Although banana peel contains considerably less PPO activity than the pulp, the extracts from the peels were browner than the extracts from the pulp. This was probably due to the higher concentration of dopamine in the peel of the bananas than the pulp [9]. PPO activity of the banana was more concentrated in the interior of the fruit in the tissue adjacent to the degenerated ovules and the immature seeds.

Electrophoretic patterns of PPO of the interior of the banana pulp (Fig. 1) reveals 9 bands when catechol was used as substrate. The exterior pulp

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Table 1. Changes in polyphenol oxidase activity of banana during ripening. The substrate was catechol.

Day no.	Stage of ripening	Activity (units*/ml of extract) Interior of pulp	Exterior of pulp	Peel
1	Green tip	46300	17800	1800
2	Slight green tip	43300	14000	1500
3	Yellow	40200	16500	2100
5	Brown spot	34900	12900	3800
8	Overripe	29800	13300	4400

* One unit = ΔA of 0·001 at 415 nm.

contained 8 bands of PPO activity with the same substrate (Fig. 2). Comparison of the relative mobilities (R_m) of the bands show that the R_ms of the isoenzymes are identical for the 8 bands, and that the exterior pulp did not contain the fastest migrating component of the interior pulp at R_m of 0·92. In confirmation of the lower activity in the exterior pulp, the bands from extracts of this portion of the banana were not as dark as those from the interior pulp. Extracts of banana peel revealed 10 components of PPO with catechol as a substrate (Fig. 3). Two of these bands (R_ms of 0·77 and 0·83) had the same R_ms as two bands from the interior and exterior pulp. This suggests that these two isoenzymes from the peel may be similar to those found in the banana pulp. No changes in the electrophoretic patterns of PPO isoenzymes were noticed as the bananas ripened.

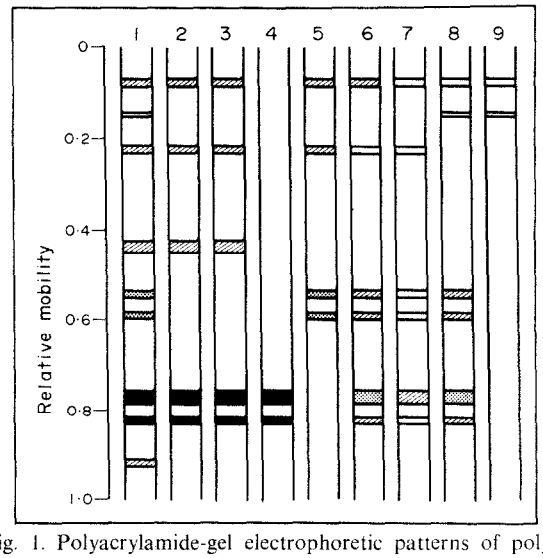

Fig. 1. Polyacrylamide-gel electrophoretic patterns of polyphenol oxidase of interior banana pulp. Substrates: 1, catechol; 2, 4-methyl catechol; 3, dopamine; 4, pyrogallol; 5, *d*-catechin; 6, caffeic acid; 7, chlorogenic acid; 8, DL-DOPA; 9, L-tyrosine. Darker bands indicate higher activity.

Substrate specificity

Activities of the PPOs from the three parts of the banana fruit toward 9 substrates are shown in Table 2. Caution should be used in interpreting these data since the extinction coefficients of the products of oxidation are probably different at 415 nm. However, some general statements about the substrate specificity of banana PPO may be made. L-Tyrosine was oxidized at a very slow rate by extracts of banana pulp and was not oxidized by peel extracts, which would indicate that the banana polyphenol oxidases were more specific for *o*-diphenols. The *o*-diphenols which appeared to be the best substrates (catechol, 4-methyl catechol, pyrogallol, and dopamine) either lack or have a short side chain on the ring in the 4 position. The difference in the activity between dopamine and DOPA indicates that the presence of the carboxyl group of DOPA has an influence on the action of

Table 2. Substrate specificity of banana polyphenol oxidase

	Concentration (mM)	Interior activity (units*/ml)	Exterior activity (units*/ml)	Peel activity (units*/ml)
Catechol	10	34000	5500	4400
4-Methyl catechol	10	28400	5800	4400
Dopamine	10	23100	3900	2800
Pyrogallol	5	18500	7500	4000
d-Catechine	5	6500	770	850
Caffeic acid	10	2000	230	90
Chlorogenic acid	5	2600	350	90
DL-DOPA	5	3900	300	410
L-Tyrosine	2·5	140	50	0

* *One unit* = ΔA of 0·001 at 415 nm.
Bananas were in the yellow to light brown spot stage of ripening.

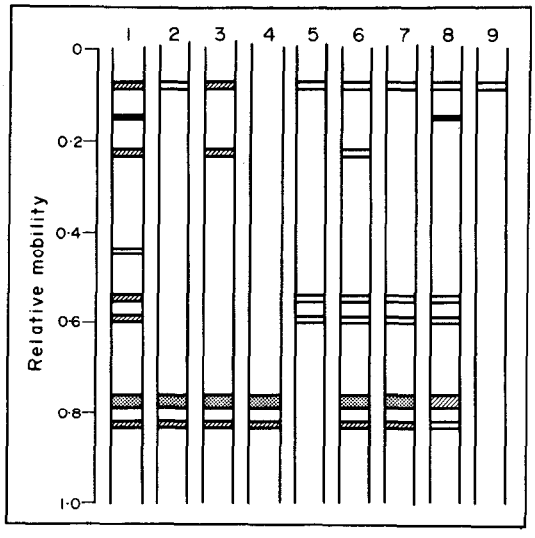

Fig. 2. Polyacrylamide-gel electrophoretic patterns of polyphenol oxidase of exterior banana pulp. Legend is the same as for Fig. 1.

PPO on this substrate. A similar effect of increased side-chain length is also apparent in comparing the activities of 4-methyl catechol and caffeic acid.

Substrate specificities of the PPO isoenzymes from the three parts of bananas are presented in Figs. 1–3, gels 1–9. The two most active bands (R_m 0·77 and 0·83) of the banana pulp oxidized all the substrates except *d*-catechin and tyrosine, and were responsible for all the activity of the pulp towards pyrogallol. The two slowest migrating bands with R_m of 0·08 and 0·15 appear to be capable of oxidizing both the monophenol and diphenol. Diphenols with larger groups in the 4 position of the ring (*d*-catechin, caffeic acid, chlorogenic acid, and DL-DOPA) and the triphenolic, pyrogallol, were not oxidized by the isoenzyme at R_m 0·44. Isoenzymes at R_m 0·55 and 0·59 oxidized catechol and the substrates with the bulkier substituent groups, but not the substrates with the smaller substituent groups on the ring. The fastest migrating band (R_m 0·92) oxidized only catechol of the substrates used in this study.

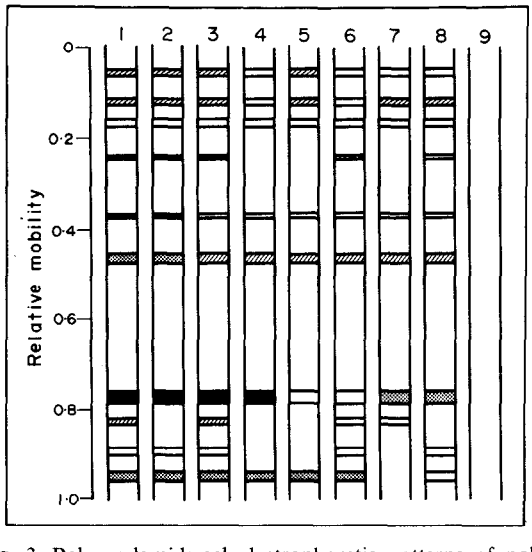

Fig. 3. Polyacrylamide-gel electrophoretic patterns of polyphenol oxidase of banana peel. Legend is the same as for Fig. 1.

Isoenzymes of the banana peel PPO showed no activity towards tyrosine and revealed differences in substrate specificities from the PPO of the banana pulp. The fastest migrating component (R_m of 0·95) did not oxidize chlorogenic acid. Pyrogallol was oxidized by more isoenzymes of the peel PPO than of the pulp PPO. Several of the minor isoenzymes had differences in substrate specificities.

Inhibitor specificity

Sodium diethyldithiocarbamate, cysteine, sodium metabisulfite, 2-mercaptoethanol and 1,2-dithiolpropane caused a delay in the increase in absorbance. Activity was determined by the rate of change after the delay. If the delay was longer than 2 min, the assay was considered to have zero activity. The known inhibitors of PPO also inhibit the banana pulp and peel PPO (Table 3). Compounds which did not show inhibition of the banana PPO were *p*-hydroxymercuribenzoate, *p*-coumaric acid, ferrulic acid, picryl sulfate, quinine sulfate and DL-6,8-thioctic acid amide.

Electrophoretic patterns show that some isoenzymes of banana PPO were not as sensitive to certain inhibitors used in this study. Of particular interest are bands at R_m 0·44, 0·55 and 0·59 in the pulp extract and at R_m 0·36 and 0·46 in the peel extract which were not inhibited by sodium metabisulfite, but were partially or completely inhibited by the other inhibitors.

DISCUSSION

Although Walker and Hulme [10] reported the inhibition of apple peel PPO by PVP and Badran and Jones [11] found that PEG was better than PVP for extraction of PPO from banana tissue, the

Table 3. Effect of inhibitors on banana polyphenol oxidase activity. The substrate was catechol.

Inhibitor	Concentration (mM)	Per cent inhibition Interior of pulp	Exterior of pulp	Peel
Sodium diethyldithio-carbamate	1·0	100	100	100
	0·1	49	49	88
	0·01	5	4	27
L-Cysteine	1·0	100	100	100
	0·1	79	82	100
	0·01	7	6	14
Sodium cyanide	1·0	88	86	100
	0·1	45	58	77
	0·01	17	21	44
Sodium metabisulfite	1·0	100	100	100
	0·1	48	78	100
	0·01	10	16	9
2-Mercaptoethanol	1·0	100	100	100
	0·1	37	74	100
	0·01	10	6	6
1,2-Dithiolpropane	1·0	100	100	100
	0·1	57	75	100
	0·01	3	5	6

Bananas were in the yellow to light brown spot stage of ripening.

results of this study showed that PVP was superior to PEG for extracting banana PPO. The differences may have been due to the manner in which the PVP was used or in the properties of the PVP. Since various levels of PVP and PEG did not change the electrophoretic pattern of banana PPO when compared to patterns prepared from extracts made without the phenolic binders, the isoenzymic pattern of banana PPO did not appear to be affected by the presence of phenolic substances.

The general decrease in PPO activity in ripening banana pulp is difficult to explain. The 5% increase in moisture content of the pulp [3] would not account for the 35% decrease in activity. Brady *et al.* [12] reported no change in protein content and Young [13] found no change in the activities of several banana enzymes as the banana ripened. However, the activity of PPO of tea leaves decreased during maturation [14]. Changes in the phenolic compounds [3] may have caused this decrease in PPO activity. The increase in activity in the banana peel was probably due to the dehydration of the peel during ripening [3]. Concentration of the PPO activity in tissue adjacent to the immature seeds suggests the PPO may be associated with formation of polyphenolics in the seed coat.

Substrates with smaller substituent groups on the ring of diphenols were oxidized by banana PPO at a faster rate than those with larger substituent groups. Similar results were reported with cherry [6] and wheat [15] PPO. However, two purified isoenzymes of peach PPO were most active with *d*-catechin [5]. The ability of banana pulp to oxidize tyrosine confirms the report of tyrosine hydroxylase activity in banana pulp [4], however, no activity was found in the peel in the present work.

The sensitivity of banana PPO to inhibition by cysteine suggests that this amino acid may be used to prevent enzymic browning in processed banana products. Cysteine has been suggested to prevent enzymic browning in apple products [16] and potatoes [17]. Banana PPO isoenzymes that were not inhibited by metabisulfite may be similar to the isoenzyme of peach PPO reported by Wong *et al.* [5] to be resistant to inhibition by sodium bisulfite, ascorbic acid, and glutathione. Sulfite [18] and cysteine [19, 20] have been shown to inhibit PPO by the formation of addition products with *o*-quinones. Hence, the products of oxidation formed by these resistant isoenzymes appear to differ from those formed by the other PPO of bananas. Further research is required to determine the composition of the products of these isoenzymes resistant to sulfite inhibition.

EXPERIMENTAL

Bananas (*Musa cavendishii* cv Nanica) were obtained from a local market and, except in the experiments on ripening, were used in the yellow to light brown spot stage of ripening.

Extraction of polyphenol oxidase activity. To 15 g of banana tissue were added 3·75 g PVP and 60 ml of cold 0·05 M KPi buffer (pH 7) in a 0·5 l. blender jar. This mixture was blended for 15 sec, mixed by hand to incorporate the larger particles and blended for an additional 10 sec. Centrifugation at 20000 *g* for 10 min at 0° resulted in a clear supernatant soln which was used as the enzyme source. All preparations were kept in crushed ice until used, and were used within 5 hr of preparation.

Polyphenol oxidase activity was determined by measuring the rate of increase in A at 415 nm at 30°. Sample cuvettes contained 2·9 ml of substrate dissolved in 0·2 M KPi buffer (pH 7) and 0·1 ml of the enzyme solution. The substrate concentrations used are shown in Table 2. Reference cuvette contained 2·9 ml of the same substrate soln and 0·1 ml of 0·05 M KPi buffer at pH 7. Concentrations of the enzyme extracts were adjusted with the above KPi buffer so the change in A was between 0·05 and 0·2 per min. The linear portion of the curve between 15 and 45 sec after addition of the enzyme soln was used for determining enzyme activity. One unit of enzyme activity was defined as the amount of enzyme that caused a change in A of 0·001 per min. In the experiments with inhibitors, 2·8 ml of the substrate soln, 0·1 ml of inhibitor soln were mixed immediately before the addition of 0·1 ml of enzyme soln.

Polyacrylamide-gel electrophoresis was performed by the method of ref 6 with the exception that gels were in glass tubes (0·5 × 10 cm) and a 7% running gel was used. The electrophoresis apparatus was similar to that described by Davis [21]. The apparatus was placed in a household refrigerator (4°) and 75 V (*ca* 2 mA per tube) was applied until the bromophenol blue had reached the running gel. Then the V was increased to 130 V for the remainder of the run.

After the run the gels were removed from the tubes and placed in 10 ml of substrate solns containing 0·05% *p*-phenylenediamine for 1 hr before treatment with 10 ml of 1 mM ascorbic acid. The color of the bands was stable for 2 hr before noticeable fading occurred. Substrate concentrations and pH of buffer were the same as those used in the enzyme assay. In the inhibitor studies the gels were exposed to 10 ml of 0·1 mM inhibitor soln in 0·2 M KPi buffer (pH 7) for 1 hr before treatment with 10 mM catechol for 1 hr followed by ascorbic acid. Extracts were applied to the gels within 0·5 hr after preparation and best results were obtained when 250–500 units of PPO activity were applied to each tube in 25 μl of soln.

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