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The biochemical properties of polyphenol oxidase from banana leaf

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Abstract. Polyphenol oxidase from the leaf of the banana (*Musa sapientum* cv, *Giant Cavendishii,* AAA) was purified 7.3 fold in an overall yield of 7.7%. The purification procedure involved two subsequent steps of DEAE-Sepharose CL-6B chromatography. The M. W. was determined by 5-20% gradient PAGE to be 140 kD. The optimum pH with DOPA as substrate was 7.0. The optimum temperature was 40°C. The enzyme was quite active at low temperatures since the enzyme at 4°C retained 87% of maximal activity. Cu^{++} activated the enzyme activity at concentrations lower than 0.6 mM, while inhibiting the activity at higher concentrations. The enzyme was inactivated completely by sodium diethyldithiocarbamate or by cyanide, which suggested that the enzyme required Cu^{++} in its active site. The leaf PPO exhibited unusually high activity toward L-tyrosine, which suggested that it was very important for the monophenol metabolism, and useful for L-DOPA production. The biochemical properties of [illegible] leaf PPO, including the isozyme patterns, its high activity at low temperature, and unusually high activity toward monophenols substrates, were quite different from those of other plant PPO reported in the literature.

measuring the rate of absorbance increase at 475 nm, at 25°C and pH 7.0 (20 mM potassium phosphate buffer) with 3.8 mM L-DOPA as substrate (Shaw *et al.*, 1991). One unit causes a △OD (absorbance change) at 475 nm of 0.001/min under the specified conditions.

The substrate specificity was determined by measuring the O_2 consumption rate with an oxygen electrode (YSI Model 53 oxygen monitor) at 30°C. The reaction was started by adding 0.05 ml PPO to a 4 mM substrate solution (in 25 mM phosphate buffer, pH 6.8) in a total volume of 3.0 ml.

Determination of Isoelectric pH (pI)

The isoelectric pH of PPO was determined by isoelectric focusing on polyacrylamide gel, following the instructions of the Hoefer Scientific Instrument Co. The ampholine used was pH 3.5-10.0 from the Biorad Co. The apparent isoelectric point of PPO was estimated with standard pI marker proteins including amyloglucosidase, soybean trypsin inhibitor, β-lactoglobulin, bovine carbonic anyhydrase B, human carbonic anyhydrase B, myoglobin, horse myoglobin, lentil lectin and trypsinogen (Pharmacia Co.).

Results and Discussion

Liquid nitrogen frozen leaf samples were homogenized with a pestle in a motar and then extracted with six volumes of potassium phosphate buffer (25 mM, pH 6.8; buffer A). The extracts were centrifuged at 10,000 x g for 30 min at 4°C. The supernatant was filtered through Whatman NO.4 filter paper, concentrated and applied to a column (2.6 × 45.0 cm) of DEAE-Sepharose CL-6B equilibrated with buffer A. The absorbed enzyme was eluted with a linear gradient of 0-1.0 M NaCl in buffer A, and 3 ml fractions were collected.

The results are shown in Fig. 1. Three fractions containing PPO activities were eluted: 0.38-0.42 M, 0.68 -0.71 M, and 0.73-0.76 M NaCl. The first fraction (F1) contained most of the PPO activity.

The F1 was concentrated with an ultrafiltration cell (Amicon, U. S. A.) and applied to the 2nd DEAE -Sepharose CL-6B column (2.6 × 45.0 cm), preequilibrated with buffer A, and eluted with a linear gradient of 0.25-0.60 M in buffer A; and 3 ml fractions were collected. The result was shown in Fig. 2. The tubes containing PPO activity were pooled and designated as PPO F*. The results for purification are shown in

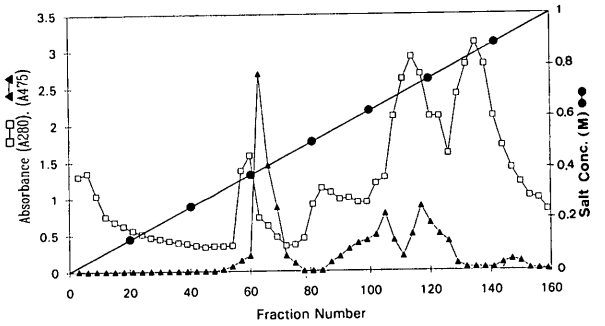


Fig. 1. Column chromatograpy of banana leaf PPO on DEAE -Sepharose CL-6B. See the text for conditions.

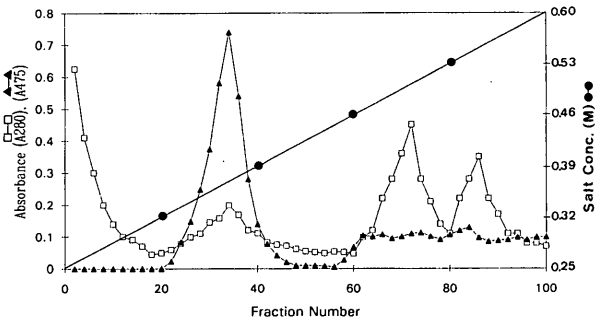


Fig. 2. Column chromatography of banana leaf PPO on 2nd DEAE-Sepharose CL-6B. See the text for conditions.

Table 1. The purification procedure resulted in a 7.3 fold increase in purity with 7.7% recovery. The specific activity of the final preparation (PPO F*) was 84,091 units/mg, and this enzyme was used in subsequent studies.

Analysis of PPO F* by enzyme activity staining following electrophoresis on 5-20% gradient PAGE (Hames, 1981) revealed only one band, and its molecular weight was determined to be 140 kD (Fig. 3), which is much higher than that of banana fruit PPO (60 kD) (Pardon *et al.*, 1975).

The isoelectric point was determined, by isoelectric focusing on polyacrylamide gel containing ampholytes, to be in the range of pH 3.5-10.0. The pH standard marker of the gel after electrofocusing and the section of gel containing the active enzyme are shown in Fig. 4. The isoelectric point for PPO F* was 4. 5. The isoelectric points of the PPO from the fruit of banana cultivars *Musa cavendishii, Dwarf cavendishii* and *Red Skin* were 5.2, 4-5.5, and 4.2-5.8, respectively (Galeazzi *et al.*, 1981; Thomas and Janave, 1986).

Table 1. *Summary of purification procedure of banana leaf PPO*

Steps	Volume (ml)	Protein conc. (mg/ml)	Total protein (mg)	Specific activity (unit/mg)	Recovery (%)	Purification (fold)
Crude extract	220	1.080	237.6	11530	100.0	1.0
1st DEAE	F1 84	0.210	17.6	36950	23.8	3.2
Sepharose CL-6B	F2 48	0.500	24.0	2790	2.5	
	F3 42	0.980	41.2	1939	2.9	
2nd DEAE	F* 45	0.055	2.5	84091	7.7	7.3
Sepharose CL-6B						

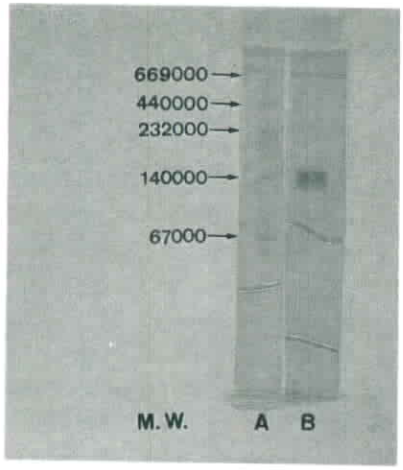


Fig. 3. Estimation of molecular weight of banana leaf PPO by 5–20% gradient polyacrylamide gel electrophoresis. (A) molecular weight calibration proteins stained with coomassie brilliant blue R-250; (B) the activity stain of banana leaf PPO.

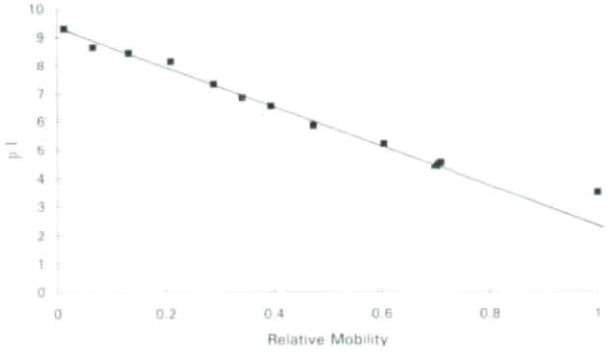


Fig. 4. Estimation of isoelectric point of banana leaf PPO by IEF-PAGE.

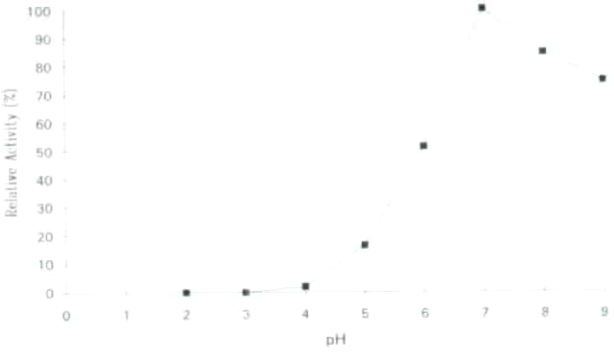


Fig. 5. Effect of pH on the activity of banana leaf PPO. The buffers used were BICINE, CAPS, sodium acetate, and Bis-Tris propane mixtures (0.1 M each) adjusted to various pH using either 5N NaOH or 6N HCl.

Therefore, the pI of banana leaf PPO is similar to that of fruit PPO.

The pH-activity profile for the oxidation of L-DOPA by the leaf PPO (Fig. 5) shows that it is active over a broad pH range (6.5–9.0) with an optimum at pH 7.0. The optimum pH of PPO from the fruit of banana cultivars *Musa Cavendishii* and *Musa Acuminate* were also reported to be 7.0 (Jayaraman *et al.*, 1982 and Palmer, 1963). Therefore, the optimum pH of PPO from leaf and fruit are the same. The pH-activity profile suggests that histidine is involved in the active site as a potential ligand to Cu^{++} of PPO similar to that proposed in *Neurospora* tyrosinase (Pfiffner and Lerch, 1981).

The temperature-activity profile is shown in Fig. 6. The optimal temperature of PPO F* was 40°C. It appeared that the enzyme activity was insensitive to the change of temperature. The activity of PPO F* at 4°C still retained 87% of maximum activity. The high activity of PPO at low temperature for a tropical plant such as the banana has never been reported in the literature. This suggests that the leaf banana PPO might have an important physiological function under cold stress conditions.

The effect of various salts on PPO F* was shown

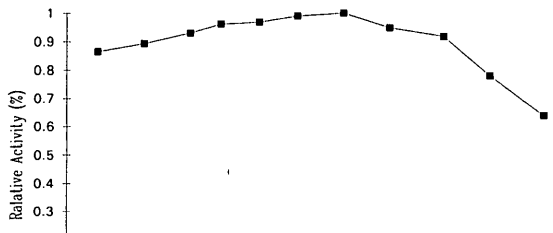
in Table 2. At 4.9 mM, $MnCl_2$ and $CuCl_2$ strongly inhibited, while $CoCl_2$, $NiCl_2$, $CaCl_2$ and $PbCl_2$ activated, the leaf banana PPO F*. The effect of salts might have been greatly changed as the concentration was varied. As shown in Fig. 7, Cu^{++} activated PPO activity at concentrations below 0.6 mM. The maximal activation was 228% at 0.16 mM Cu^{++}. Since inhibitor studies sug-

Cu^{++} is the cofactor of the enzyme similar to other PPO reported.

The activities of the PPO F* from the banana leaf toward 5 substrates are shown in Table 4. It is very striking that banana leaf PPO F* showed the highest activity toward L-tyrosine among the substrates tested. Generally plant PPO showed low or no activity

gested that Cu^{++} was a cofactor of the banana leaf PPO F* (Table 3), the inhibitory effect of Cu^{++} at high concentrations is possibly due to its binding to an allosteric site.

The effect of some common inhibitors on PPO in the presence of L-DOPA as substrate was shown in Table 3. The inhibition of PPO F* by different organic compounds were according to the following order: sodium diethyldithiocarbamate = sodium cyanide > 4-chlororesorcinol > sodium azide > thiourea. Since sodium diethyldithiocarbamate and cyanide are specific chelators of Cu^{++}, the strong inhibitory effect of these compounds on banana leaf PPO F* suggest that



toward L-tyrosine (Mayer and Harel, 1979). Fruit

Table 2. *Effect of various salts on polyphenol oxidase activity*

Salts	Final conc. (mM)	Relative activity (%)
None	-	100
$CoCl_2$	4.9	320
$MnCl_2$	4.9	0
$NiCl_2$	4.9	239
$CaCl_2$	4.9	219
$CuCl_2$	4.9	35
$PbCl_2$	4.9	215
$BaCl_2$	4.9	102
NaCl	245.0	55

Table 3. *Effect of various organic compounds on polyphenol oxidase activity*

Compounds	Final conc. (mM)	Relative activity (%)

PPO from the banana cultivars *Musa cavendishii* and

banana (*Musa cavendishii* L). J. Food Sci. **46:** 150–155.

香蕉葉中多酚氧化酶之生化特性

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多酚氧化酶由香蕉 (*Giant Cavendishii*, AAA) 之葉中抽出，經過兩次 DEAE-Sepharose CL-6B 管柱層析中分出具有單一活性之多酚氧化酶，稱爲 F*，純化 7.3 倍，總回收率爲 7.7%，以 5-20%梯度膠體電泳測得分子量爲 140 kD，pI 值爲 4.5。以 DOPA 爲基質，測得最適酸鹼值爲 7.0。最適溫度爲 40°C，且此酵素對溫度改變非常穩定，即使於 4°C仍保有 40°C 下之 87%活性。銅離子濃度低於 0.6 mM 時會活化 F*，但在較高濃度下則會抑制。sodium diethyldithiocarbamate 與 cyanide可完全抑制F*之活性中心，推測F*爲含金屬酵素 (metallo enzyme)，其活性中心可能含有銅離子。而F*對L-tyrosine 具有不尋常之高活性，推測 F*於單酚化合物之代謝相當重要，並可應用於 L-DOPA 之合成。香蕉葉多酚氧化酶之生化特性包括同功酶圖譜，在低溫具高活性與對於單酚基質之特別高活性等均與過去文獻所報告之植物多酚氧化酶有很大的差異。