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Original Research Article

Optimization Purification and Characterization of Polyphenol Oxidase from *Plectranthus amboinicus*

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Polyphenol oxidase, *Plectranthus amboinicus* and Anion exchange chromatography.

A B S T R A C T

Polyphenol oxidase (PPO) (EC 1.1.14.18.1) is a copper containing enzyme which oxidizes phenolic compounds. Polyphenol oxidase plays a major role in the browning of plant tissues. Hence in our present study polyphenol oxidase was isolated from *Plectranthus amboinicus* and its activity was optimized with various substrates, temperature, pH and inhibitors. The optimum activity was observed with the substrate dopamine, temperature at 30^0C and pH 7. In the presence of ferrous sulphate, polyphenol oxidase possessed highest enzyme activity. Sodium dodecyl sulphate (SDS) and sodium azide were found to be strong inhibitors for the enzyme polyphenol oxidase. The isolated polyphenol oxidase was further purified using DEAE sephedex A-50 anion exchange chromatography and gel filtration chromatography. The fractions 3 and 10 possesed highest optical density. The purified sample was characterized using PAGE analysis, the fractions 3 and 10 revealed a single band. Thus polyphenol oxidase from *Plectranthus amboinicus* leaf was isolated, purified and characterized, thus enabling us to study many more of its properties in detail.

Introduction

The plant *Plectranthus amboinicus* is a tender fleshy perennials belongs to the family Lamiaceae. The leaves are very thick and succulent, grey green, hairy, aromatic and strongly flavored. The leaves also have many traditional medicinal uses, especially for treatment of coughs, sore throats and nasal congestion (Damanik *et al*., 2001). Polyphenol oxidase (PPO) (EC1.1.14.18.1) is a copper containing enzyme which oxidases phenolic compounds to produce browning during storage.

PPO present in many fruits and vegetables have been studied to prevent the browning that decreases the market value (Khann, 1977; Yang *et al*., 2004; Ding *et al*., 1996). Because of the considerable economic and nutritional loss induced by enzymatic browning in the commercial production of fruits and vegetables, numerous studies have been devoted to the biochemical and catalytic properties of polyphenol oxidase (Herrero *et al*., 2005; Mayer and Harel, 1991). Polyphenol oxidase had been widely

investigated in various plant tissues (Gomez, 2002). This enzyme also influences in browning of wounded tissues and constitutes defensive barriers against diseases (Mohamadi and Kazemi, 2002). This present work was, mainly focused on the studies of polyphenol oxidase in *Plectranthus amboinicus* leaf and further purification and partial characterization of polyphenol oxidase.

Materials and Methods

Collection of Plant Sample

Plectranthus amboinicus leaves were collected from local area of kanyakumari district. The collected leaf samples were brought to the laboratory and subjected for sample preparations.

Sample Preparation

10 grams of *Plectranthus amboinicus* leaf sample was homogenized with 10ml of extraction buffer (0.1M Sodium phosphate bufferpH7.0, 40mM ascorbic acid and 3% tween 80). The homogenate was filtered and the filtrate was centrifuged at 10,000 rpm for 10 minutes. The supernatant was used as stock solution of polyphenol oxidase.

Effect of Substrate on Polyphenol Oxidase Activity

Polyphenol oxidase activity was assayed by measuring the rate of increase inabsorbance at a wavelength of 492 nm. Various substrates such as dopamine, chorogenic acid, phloroglucionol, L-dopa, Gallic acid, pyrogallol and catechel are used for enzyme assay. 50µl of sample with 25 µl of substrate and 75 µl of sodium phosphate buffer added to the micro titre plate. The polyphenol oxidase activity was measured by using micro plate reader (Cyberlab) and the results were tabulated.

Effect of Temperature on Polyphenol Oxidase Activity

The activity of polyphenol oxidase at various temperature range from 10^0C to 50°C was determined. The substrate 20mM dopamine was selected and heated with temperature range from 10^0C to 50^0C. After heating process, 50 µl of sample with 25 µl of heated substrate and 75 µl of sodium phosphate buffer was added to the micro titre plate and the activity was recorded and tabulated.

Effect of pH on Polyphenol Oxidase Activity

Citric acid phosphate buffer with pH 4 to 6 and Tris HCl buffer with pH7 to 10 were prepared. The poyphenol oxidase assay using the dopamine as substrate with different pH was carried out and the results were recorded and tabulated.

Effect of Inhibitors on Polyphenol Oxidase Activity

Various inhibitors such as EDTA, L-ascorbic acid, sodium metathiosulphate, sodium thio sulphate, SDS and L-cystein were used for polyphenol oxidase assay. 50µl of sample with 25 µl of inhibitor and 75 µl of sodium phosphate buffer added to the micro titre plate. The results were tabulated.

Effect of Metal Ions on Polyphenol Oxidase Activity

The effect of different metal ions on ployphenol oxidase activity was tested. They are Sodium chloride, Potassium chloride, Calcium chloride, Copper chloride, Ferrous sulphate and Ferric chloride. 50µl of sample with 25 µl of metal ion and 75 µl of sodium phosphate buffer added to the micro titre

plate. The results were tabulated.

Purification of Polyphenol oxidase

Ion-exchange Column Chromatography

The prepared sample was applied to DEAE sepadex A-50 column. Protein was eluted by stepwise gradient elution using different $Nacl_2$ concentration. The flow rate was set as 250 µl per minute. Polyphenol oxidase activity was carried out for each column fractions. The fractions were measured for absorbance at 492 nm in a UV-spectrophotometer. The values were depicted in a graph.

Gel Filtration Chromatography

0.5ml of purified protein obtained from anion exchange purified fraction was dialysed for overnight with TBS bufferpH7.6. The dialysed samples were applied into 1x5cm glass column that contained sephadex G-75. The flow rate was maintained at 0.3ml per minute and the elutes were monitored with the absorbance at 492 nm. The values were depicted in a graph.

SDS Page

The purified protein was subjected for SDS PAGE for the determination of molecular weight of polyphenol oxidase

Results and Discussion

Polyphenol oxidase from *Plectranthus amboinicus* was isolated and its activity was optimized with different pararmeters, such as substrate, pH, inhibitors, metal ions and temperature. Higher Optimal density value was obtained with the substrate dopamine (0.3853±0.039) (Table 1), with inhibitor SDS (0.1424 ± 0.0126) (Table 2), with metal ion ferrous sulphate (0.72214 ±0.01338) (Table 3), at the pH 7 (0.0972) (Table 4) and with temperature 30°C (0.385) (Table 5). The temperature optimum of polyphenoloxidase in banana was 30°C. The enzyme activity declined rapidly on increased temperature above 40°C.

Table.1 Assay of Polyphenol Oxidase Activity with Different Substrate

SI.No	Substrate	Optical density value for the sample
1	Dopamine	0.3853 ± 0.039
2	Gallic acid	0.3325 ± 0.016
3	Catechol	0.3318 ± 0.010
4	L-Dopa	0.3134 ±0.017
5	Pyrogallol	0.294 ±0.012
6	Phyroglucinol	0.2928 ±0.013
7	Chlorogenicacid	0.1648 ±0.015

Table.2 Assay of Polyphenol Oxidase Activity with Different Inhibitors

Sl.No	Inhibitors	Optical density value for the sample
1	SDS	0.1424 ± 0.0126
2	EDTA	0.167 ±0.0120
4	L-Ascorbic acid	0.1828 ±0.0029
6	Sodium metathiosulphate	0.18667 ±0.019
7	Sodium thisosulphate	0.2028 ±0.0024
8	L-Cystein	0.2104± 0.002

Table.3 Assay of Polyphenol Oxidase Activity with Different Metal Ions

SI.No	Metal ions	Optical density value for the sample
1	Ferrous Sulphate	0.72214 ± 0.01338
2	Calcium Chloride	0.6238 ±0.014291
3	Copper Sulphate	0.55214 ±0.01057
4	Ferric Chloride	0.392±0.015433
5	Potassium chloride	0.24386±0.0147
6	Sodium Chloride	0.22697±0.01338

Table.4 Effects of Ph on the Polyphenol Oxidase Activity

SI.No	pH	Optical density value for the sample
1	4	0.0434
2	5	0.0532
3	6	0.0799
4	7	0.0972
5	8	0.0743
6	9	0.0540
7	10	0.0411

Table.5 Effects of Temperature on Polyphenol Oxidase Activity

SI.No	Temperature	Optical density value for the sample
1	10^{0} C	0.275
2	20^{0} C	0.385
3	30^{0} C	0.385
4	40^{0} C	0.266
5	50^{0} C	0.201
6	60^{0} C	0.128

Ascorbic acid and sodium bisulphate were the most effective inhibitor (Unal, 2007). Yagar and Sagiroglu (2002) reported that 98% and 100% of inhibition in polyphenol oxidase with ascorbic acid. They also observed 52% and 98% inhibition with sodium bisulphate. Yang *et al*., 2000 reported that the optimum pH in banana is 6.6, which compares well with the pH optimum in this study. Purification of polyphenol oxidase using ion exchange chromatography technique was performed. And the fractions were subjected for spectrometric assay. The optical density values plotted on the graph reveals that, highest peak was obtained with 3rd fraction. Hence the 3rd fraction was further selected for gel filtration chromatography. The 11th fraction was found to be high polyphenol oxidase activity. The SDS results also revealed a single band.

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