Purification of Polyphenoloxidase from the Purple-fleshed Potato (Solanum tuberosum Jasim) and its Secondary Structure

J. JANG AND K.B. SONG

ABSTRACT: Polyphenoloxidase (PPO) was purified from purple-fleshed potatoes (Solanum tuberosum Jasim) using membrane concentration, ammonium sulfate fractionation, Resource Q ion exchange chromatography, and Sephacryl S-200 HR gel permeation chromatography. PPO was purified 78-fold from a crude extract. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis results showed that the purified enzyme has a major subunit molecular weight of 40 kDa. To elucidate the secondary structure of the purified PPO, circular dichroism (CD) was performed. The CD spectrum of the purified enzyme showed that PPO contains 35% α-helix, 30% β-turn, and 35% random coil structure.

Keywords: purple-fleshed potato, polyphenoloxidase, purification, SDS-PAGE, circular dichroism

Introduction

Polyphenoloxidase (PPO) is responsible for browning of damaged fruits and vegetables by catalyzing oxidation of phenolic compounds and is widely distributed in plants (Mayer and Harel 1979; Vamos-Vigyazo 1981). PPO is a copper-containing enzyme that catalyzes 2 types of oxidative reactions: hydroxylation of monophenols to o-diphenols (monophenol monooxygenase: EC 1.14.18.1) and oxidation of o-diphenols to o-quinones (diphenol oxidase: EC 1.10.3.2) in the presence of oxygen (Kavrayan and Aydemir 2001; Nagai and Suzuki 2001). Because enzymatic browning by PPO causes deterioration of nutritional quality and affects appearance, inactivation of PPO is desirable for the preservation of foods (Hendrickx and others 1998). Therefore, PPO inhibition for quality control during processing and storage of food is required.

“Jasim” is one variety of purple-fleshed potatoes, bred and produced in Korea, that has an accumulation of anthocyanins in the flesh (Figure 1). Anthocyanins are responsible for red, purple, and blue color of many fruits and vegetables, and they have been known to have antioxidant activity (Suda and others 1997) as well as antimutagenicity (Yoshimoto and others 1999). Physiological functions of anthocyanins have been extensively studied (Bettini and others 1985; Vincieri and others 1992; Saija 1994). Demand for purple-fleshed potatoes is increasing in Korea because they are considered a functional food.

Several PPOs isolated from grape, banana, apple, pear, peach, papaya, and yam have been reported (Wong and others 1971; Rivas and Whitaker 1973; Anosike and Ayaebene 1981; Galeazzi and others 1981; Yokotsuba and others 1988; Zhou and others 1993; Cano and others 1996). The most likely function for PPO present in plants is involvement in resistance against diseases and insects (Ray and Hammerschmidt 1998). Purification of PPO from purple-fleshed potatoes has not been reported yet. Therefore, we report here the purification of PPO from purple-fleshed potatoes as the 1st step for its characterization.

Materials and Methods

Materials

Purple-fleshed potatoes (Solanum tuberosum Jasim) were obtained from Korea Rural Development Administration. Resource Q column (6 mL, anion exchange column) was purchased from Amer sham Biosciences (Uppsala, Sweden). Sephacryl S-200 HR, cate chol, and standard marker proteins for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Protein assay agent was purchased from Bio-Rad Laboratories Inc. (Hercules, Calif., U.S.A.). YM-10 membrane (Mw 10000 cutoff) and YM-3 (Mw 3000 cutoff) were obtained from Millipore Co. (Bedford, Mass, U.S.A.). All other chemicals used were of analytical grade.

Enzyme purification

Purple-fleshed potatoes (1.5 kg) were peeled, sliced, and homogenized, using a blender, in 2.3 L of 10 mM sodium phosphate buffer (pH 7.0) containing 30 mM ascorbic acid. The homogenate was filtered through 4 layers of cheesecloth. The filtrate was centrifuged at 11590 × g at 4 °C for 1 h. The supernatant was taken and concentrated using YM-10 and YM-3 membranes. To the membrane-concentrated solution, solid ammonium sulfate was added slowly at saturation of 80%. After stirring for 30 min, the precipitate was recovered by centrifugation at 24900 × g at 4°C for 1 h. The precipitate was redissolved in 10 mM sodium phosphate buffer (pH 7.0) and dialyzed against 20 volumes of 0.01 M sodium phosphate buffer (pH 7.0) at 4 °C overnight with exchange of buffer twice. After dialysis, the enzyme solution was lyophilized and redissolved in 10 mM Tris–HCl buffer (pH 8.0). The enzyme solution was then purified using fast protein liquid chromatography (FPLC) with a Resource Q column. After loading the enzyme solution (1 mL) onto an anion exchange column equilibrated with 0.01 M Tris–HCl buffer (pH 8.0), PPO was eluted with a linear gradient of Tris–HCl buffer containing 1 M NaCl at 1 mL/min. Major fractions were pooled and each fraction was assayed for PPO activity. The fraction having the highest specific activity was lyophilized and further purified using a Sephacryl S-200 HR column (1.5 × 120 cm) pre-equilibrated with

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10 mM sodium phosphate buffer (pH 7.0). After adding the enzyme solution to the column, PPO was eluted with 10 mM sodium phosphate buffer (pH 7.0) at 1 mL/min. Fractions from the column were collected and then assayed for PPO activity.

Enzyme assay
Activities of PPO were determined using a spectrophotometer (Milton Roy Co., Rochester, N.Y., U.S.A.) according to the method of Flurkey and Jen (1980) with a modification (Sun and Song 2003). One mL of 50 mM potassium phosphate (pH 6.5) buffer and 0.3 mL of 0.2 M catechol solution as a substrate in the phosphate buffer were added to 0.05 mL of the enzyme solution. One unit of PPO activity was defined as an increase in the absorbance of 0.001/min at 420 nm and 25 °C. Enzyme activities were measured 3 times.

Protein assay
Protein concentration was determined according to the method of Bradford (1976) with bovine serum albumin as standard.

SDS-PAGE
SDS-PAGE was performed according to the method of Laemmli (1970). Equal amounts of the protein samples (10 μg) were loaded onto each lane. Proteins were resolved on a 12.5% acrylamide gel and stained with Coomassie Brilliant Blue. The following molecular weight markers were used: bovine serum albumin (66 kDa), egg albumin (45 kDa), rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (36 kDa), bovine erythrocytes carbonic anhydrase (29 kDa), bovine pancreas trypsinogen (24.1 kDa), soybean trypsin inhibitor (20.1 kDa), and bovine milk α-lactalbumin (14.2 kDa).

Circular dichroism (CD) measurements
CD spectroscopy was performed at 25 °C with a JASCO-720 spectropolarimeter (JASCO, Tokyo, Japan) according to the method reported previously (Lee and Song 1997; Sun and Song 2003). A 10-mm-pathlength cell was used. The reported CD spectra were the averages of 5 scans and were smoothed by the polynomial curve-fitting program and analyzed by the method of Chang and others (1978). CD data were expressed as molar ellipticity in degree · cm² · dmol⁻¹.

Results and Discussion
A crude extract of purple-fleshed potatoes was membrane-concentrated and subjected to anion exchange chromatography to purify PPO. Purification of PPO from lettuce and Chinese cabbage by anion exchange chromatography has been reported (Chazarra and others 2001; Nagai and Suzuki 2001). Therefore, FPLC with a Resource Q anion exchange column was performed as a key purification step. An elution profile of the column showed that there were 6 major fractions (F1 to F6) (Figure 2). Each fraction was assayed for PPO activity. Assay results revealed that F4 had the highest specific activity (Table 1). F4 was pooled and then lyophilized for further purification.

The concentrated F4 fraction was subjected to Sephacryl S-200 HR gel permeation chromatography (Figure 3). Two major fractions were obtained from the column. Among them, P1 had the highest specific activity (Table 1). The results of a purification experiment of PPO using the crude extract of purple-fleshed potatoes are summarized in Table 1. PPO was purified 22-fold by Resource Q ion exchange chromatography and further purified 78-fold from crude extract of purple-fleshed potatoes by Sephacryl S-200 gel permeation chromatography as a final purification step.

![Figure 1—Transverse section of a purple-fleshed potato](image1)

![Figure 2—Elution profile of purple-fleshed potatoes extract using Resource Q anion exchange chromatography](image2)

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<th>Table 1—Purification of PPO from purple-fleshed potatoes</th>
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To check the homogeneity of the purified PPO, SDS-PAGE was performed. A typical molecular weight profile of fractions obtained at each purification step is shown in Figure 4. SDS-PAGE results showed that the major subunit molecular weight of the purified PPO is 40 kDa, with minor impurities that could not be removed by further purification using a Mono Q ion exchange column chromatography (data not shown). Native-PAGE also had a similar pattern, where it had a single major band (data not shown). Mazzafera and Robinson (2000) have reported that proteolysis can occur during purification of PPO in the absence of protease inhibitors, resulting in 40- to 45-kDa PPO having full enzyme activity. This could explain the numerous reports of PPO of 40 to 45 kDa purified from various plants (Marques and others 1994).

Regarding PPO isolated from potato tuber, Sanchez-Ferrer and others (1993) reported the partially purified PPO, which appeared as a single activity band in PAGE when DOPA was used as substrate. Kwon and Kim (1996) also reported the purification of PPO, where concanavalin A Sepharose column chromatography was used. However, the molecular weight of the PPO was 64 kDa, different from our results. The difference could be due to the different cultivars.

There have been many studies on the purification of PPO isolated from plant sources (Cano and others 1996). Molecular weights of PPOs have been reported: spinach (40 kDa), banana (41 kDa), sunflower seeds (42 kDa), cabbage (39 kDa) (Vaugham and others 1975; Raymond and others 1993; Fujita and others 1995; Yang and others 2000). Our results are in good agreement with those reports in terms of molecular weight of PPO.

To elucidate the secondary structure of PPO, the CD spectrum of purified PPO was obtained using a spectropolarimeter. The far-UV CD spectrum of the purified PPO is shown in Figure 5. The CD spectrum of the purified PPO showed that it has an ordered structure; there are typical negative ellipticity values between 220 and 210 nm. In particular, it has a distinctive negative minimum at 221 and 208 nm, which is a good indicator of α-helix content. Estimation of secondary structure content of the purified PPO according to the method of Chang and others (1978) showed that PPO contains 35% α-helix, 30% β-turn, and 35% random coil structure.

Conclusions

PPO was purified from the crude extract of purple-fleshed Jasmin potatoes, using consecutive chromatography including FPLC. This was the 1st report on the purification of PPO from purple-fleshed potatoes. The purple-fleshed potato has unique characteristics, and the role of PPO might be interesting because it is different from normal potatoes due to excess anthocyanin content. Therefore, this study provides the useful information regarding knowledge about purple-fleshed potatoes.

References

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Figure 3—Elution profile of Sephacryl S-200 HR gel permeation chromatography using F4 in Figure 2

Figure 4—SDS-PAGE profile of PPO fractions. Molecular weight marker (1); crude extract (2); membrane-concentrated solution (3); 80% (NH₄)₂SO₄ precipitate (4); Resource Q peak, F4 (5); Sephacryl S-200 HR peak P1 (6).

Figure 5—CD spectrum of purified PPO isolated from purple-fleshed potatoes
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