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Characterization of peroxidase in buckwheat seed

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Abstract

A peroxidase (POX)-containing fraction was purified from buckwheat seed. The POX consisted of two isozymes, POX I and POX II, that were purified 6.6- and 67.4-fold, respectively. Their molecular weights were estimated to be 46.1 kDa (POX I) and 58.1 kDa (POX II) by gel filtration. While POX I and II each oxidized quercetin, *o*-dianisidine, ascorbic acid and guaiacol, only POX II oxidized ABTS. Kinetic studies revealed that POX I and II had lower K_m values for quercetin (0.071 and 0.028 mM), ABTS (0.016 mM for POX II) and ascorbic acid (0.043 and 0.029 mM) than for *o*-dianisidine (0.229 and 0.137 mM) and guaiacol (0.288 and 0.202 mM). The optimum pHs of POX I and II for various substrates were almost the same, except for quercetin; pH 8.0 for POX I and pH 4.5 for II. Their optimal temperatures were 30 °C (POX I) and 10 °C (POX II), and POX I was more stable than POX II above 30 °C.

Keywords: Buckwheat; *Fagopyrum esculentum*; Polygonaceae; Characterization; Peroxidase; Seed

1. Introduction

Plant peroxidase (EC 1.11.1.7) (POX) is widely distributed in higher plants (Van Huystee and Cairns, 1982). These enzymes are involved in a variety of functions, such as control of cell elongation (Ahmed et al., 1995), defense mechanisms (Bradley et al., 1992; Kolattukudy et al., 1992) and lignification (Blee et al., 2003). On the other hand, POX also plays important roles in food quality, including deterioration of color and flavor (Ashie et al., 1996). In soybean, aldehydes and ketones are the major contributors to 'beany' and 'green' flavors. They are mainly generated by lipid peroxidation, and the activities of enzymes such as lipoxygenase (EC 1.13.11.12) and POX are related to the generation of these flavors (Matoba et al., 1975, 1985; Anli and Tilak, 2004). In butterbur (*Petasites japonicus*), POX plays important roles in deteriorations in flavor and taste (Ibaraki et al., 1988, 1989).

Buckwheat (*Fagopyrum esculentum* Moench) is considered a healthy food. In Japan, buckwheat flour is used mainly for making noodles, and its flavor and color are important factors in its quality. However, buckwheat flour readily deteriorates (Tohyama et al., 1982; Muramatsu et al., 1986), and enzymatic activities are thought to play an important role in this deterioration (Kondo et al., 1982; Ohinata et al., 1997; Suzuki et al., 2004).

Kondo et al. (1982) partially characterized POX in buckwheat seed, and suggested that POX affects the oxidation of flavonoids in buckwheat seed. However, the characteristics of POX such as substrate specificity, thermal stability and organ distribution, which are important for clarifying the roles of POX, have not yet been determined. In this study, we purified and characterized POX in buckwheat seed.

2. Results and discussion

2.1. Purification and molecular weight of POX

POX consisted of two isozymes, POX I and POX II (Table 1), that were separated by ion-exchange chromatography (Fig. 1). We purified them 6.6- and 67.4-fold,

Abbreviations: PMSF, phenylmethyl sulfonyl fluoride; ABTS, 2,2′-azino-bis-(3-ethylthiazoline-6-sulfonate).

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Table 1
Purification of POX I and II from buckwheat seed

	Purification step	Total protein (mg)	Total activity units[a]	Specific activity (units/mg protein)	Yield (%)	Fold
	Crude extract	40,000.0	175,000[b]	4.38	100.00	1.0
	Concentration	17,400.0	182,000[b]	10.50	104.00	2.4
POX I	CM-Sepharose	70.8	321	4.53	0.18	1.0
	Sephacryl S-200	6.3	180	28.70	0.10	6.6
POX II	CM-Sepharose	97.2	12,100	124.00	6.91	28.3
	Sephacryl S-200	6.8	1990	295.00	1.14	67.4

[a] Changes in absorbance at 430 nm/min × 10^3 enzyme = 1 unit. POX activity was measured using *o*-dianisidine as a substrate.
[b] Activity was measured as a mixture of POX I and II.

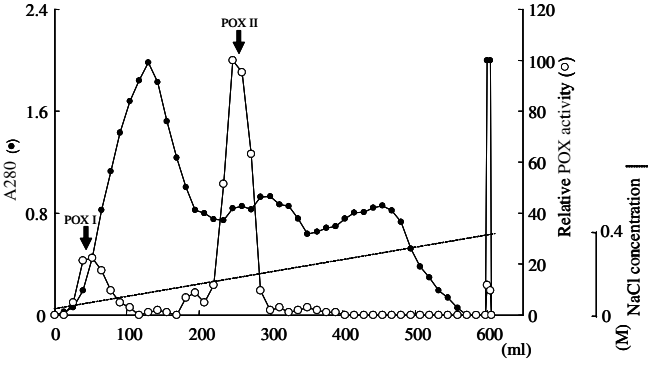

Fig. 1. Elution profile of POX activity from a CM-Sepharose column.

respectively, at a specific activity relative to crude protein extract. The yield of the enzyme was very low (0.1% for POX I and 1.14% for POX II; Table 1), and this is likely due to our purification strategy; we gave priority to the purification-fold over yield. Thus, we collected only a few fractions that had maximum activity, and discarded those around them. In this study, we purified POX from the soluble protein fraction. Kondo et al. (1982) reported that the addition of 1% (v/v) Tween 80 increased the extraction rate of POX in buckwheat flour, but we did not observe this in our experiments.

To investigate the profile of the POX isoforms in buckwheat seed, we tried both anion-exchange chromatography (DEAE-column) and cation-exchange chromatography (CM-column). We found two major peaks of POX activity in cation-exchange chromatography (Fig. 1) whereas no major peaks of POX activity were found in anion-exchange chromatography (data not shown). In addition, in each purification step, we found no POX activity except for POX I and II. These results suggest that POX I and II are the major POX in the soluble protein fraction of buckwheat seed. The molecular weights of POX I and II were 46,100 and 58,100 kDa by gel filtration. These values are similar to those of other peroxidases (Sakharov et al., 2000; Seok et al., 2001).

2.2. Kinetic constants and optimal pH of POX

The results are shown in Table 2. The K_m values for the various substrates tested (Fig. 2) were different for POX I and II. POX II had higher affinity than POX I for all of

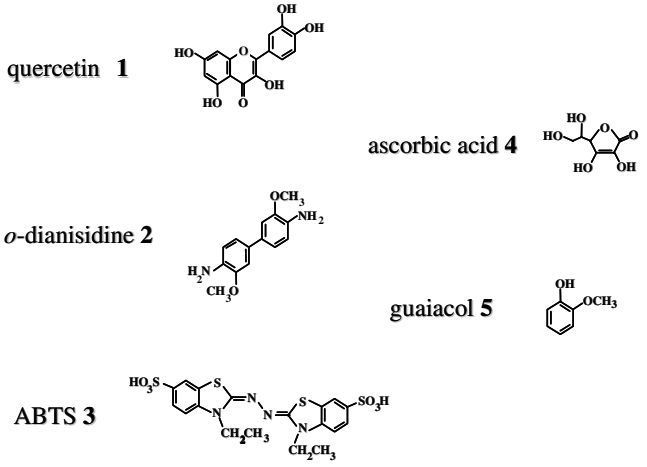

Fig. 2. Chemical structures of substrates.

Table 2
Kinetic constants and optimum pH of POX in buckwheat

	K_m (mM)		Optimum pH	
	POX I	POX II	POX I	POX II
Quercetin (**1**)[a]	0.071	0.028	8.0	4.5
o-Dianisidine (**2**)	0.229	0.137	6.0	5.0
ABTS (**3**)	n.d.[b]	0.016	n.d.	3.0
Ascorbic acid (**4**)	0.043	0.029	7.0	7.0
Guaiacol (**5**)	0.288	0.202	9.0	9.0

[a] Compound number.
[b] Not detected. Data are average of three independent experiments.