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Partial Purification and Some Properties of Polyphenol Oxidase from *Aster scaber*

Seung-Shi Ham†, Eun-Hee Hong, Sang-Young Lee, Gwi-Gun Park and Hirohisa Omura*

Dept. of Food Science and Technology, Kangwon National University, Chuncheon 200-701, Korea
**Dept. of Food Science and Nutrition, Nakamura Gakuen College, Fukuoka, Japan*

Abstract

A polyphenol oxidase from the crude extract of *Aster scaber* was partially purified by $(NH_4)_2SO_4$ precipitation and subsequent Sephadex G-150 chromatography. The final preparation showed five peaks of enzyme activity. Optimum pH and temperature for the activity of polyphenol oxidase were 7.0 and 30°C, respectively. Enzyme activity was stable at 40°C for 5min in pH 7.0 reaction mixture but ceased completely at 60°C for 30min and 70°C for 5min at pH 7.0. The polyphenol oxidase has good activity on chlorogenic acid but was inactive on DL-dopa. The apparent Km for catechol was about 17.6mM.

Key words : *Aster scaber*, polyphenol oxidase

INTRODUCTION

Polyphenol oxidase(o-diphenol : O_2 oxidoreductase, E.C. 1.10.3.1) is a copper-containing enzyme which catalyzes either one or two reactions involving molecular oxygen. The first type of reaction is hydroxylation of monophenols leading to formation of o-dihydroxy compounds. The second type of reaction is oxidation of o-dihydroxy compounds to quinones[1].

Polyphenol oxidase is also known as phenol oxidase, tyrosinase, o-diphenol oxidase, catechol oxidase, phenolase and chlorogenic acid oxidase.

Brown discoloration of an edible mountain herb and its concentrate was found to be related to the enzymatic browning that takes place before or during processing[2,3].

We previously reported some properties of polyphenol oxidase from *Spuriopimpinella bracycarpa*[4] and *Ligularia fischeri*[5]. Since polyphenol oxidase in *Aster scaber* has not been adequately investigated, the purpose of this study was to purify the polyphenol oxidase complex of *Aster scaber* and to investigate some of its properties and specificity.

MATERIALS AND METHODS

Materials

The Edible mountain herb, *Aster scaber* were purchased on a local market. Particulate fractions studied were generally obtained in the following manner : *Aster scaber* washings were cut into slices, rinsed with tap water, and then chilled to 4℃.

For the extraction, the mixtures were homogenized in a waring blender for 2min with McIlvaine buffer solution (pH 6.0). The homogenates were filtered and treated with cold acetone, and then obtained white acetone powder. The powder was frozen and kept at −20℃.

† To whom all correspondence should be addressed

Protein determination

The protein in effluents of gel filtration was estimated at 280nm, assuming that the absorbance at 280nm at the concentration of lmg per ml is 1.0. The protein concentration in polyphenol oxidase solutions was determined by the method of Lowry[6], using bovine serum albumin as the standard.

Measurement of enzyme activity and enzyme unit

Enzyme activity was determined by measuring the rate of the increase in absorbance at 420nm at 25℃ in a model of the CECIL universal automatic scanning spectrophotometer. The reaction mixture contained 2ml of 0.1M catechol solution, 1ml of distilled water and 20μl of the enzyme solution. The reference cuvette contained only substrate solution and distilled water. One unit of polyphenol oxidase activity is defined as the amount of enzyme that causes a 0.01 extinction change in absorbance 420nm per min at 40℃(Josyln and Ponting's method)[7].

Purification of the enzyme

All the procedures were carried out in a chromato-chamber at about 4℃. After addition of powdered $(NH_4)_2SO_4$ to 80% saturation, the precipitate was collected, dissolved in 20ml of McIlvaine buffer solution(pH 6.0), dialysed for 12hrs against water, and the remaining undissolved precipitate removed by centrifugation.

The resulting solution (2ml) of clear supernatant (30ml) was immediately applied, without further concentration, to a column of Sephadex G-150. A column (2×90cm) filled with Sephadex G-150 was conditioned with a 0.02M phosphate buffer solution (pH 6.0) containing 0.1M sodium chloride. The enzyme solution (2ml) was then applied to the column and eluted with the same buffer solution at the flow rate of 10ml per hr.

Enzyme preparation

lg of frozen acetone powder (−20℃) was homogenized in 80ml of McIlvaine buffer solution (pH 6.0). The homogenate was centrifuged at 10,000 rpm for 30min. Solid $(NH_4)_2SO_4$ was added to the supernatant to give 80% saturation. The solution was stirred for 30min, and the precipitation recovered and dissolved in 0.02M phosphate buffer solution(pH 6.0) and dialyzed against the same buffer overnight.

RESULTS AND DISCUSSION

Purification of the enzyme

Five polyphenol oxidase were isolated from the crude extract of a *Aster scaber* by gel filtration on Sephadex G-150 (Fig. 1). The final preparation thus obtained showed five peaks of enzyme activity. Our previous paper[4,5] reported that polyphenol oxidase from *Spuriopimpinella bracycarpa* and *Ligularia fischeri*. Three and four polyphenol oxidases were partially isolated from the crude extract of a *Spuriopimpinella bracycarpa* and *Ligularia fischeri* by Sephadex G-150 chromatography.

Table 1 summarizes the increases in the specific activity of polyphenol oxidase and the yield during

Table 1. Summary of the purification of polyphenol oxidase from *Aster scaber*

Purification steps	Volume (ml)	Activity (units/ml)	Total units	Protein (mg/ml)	Specific activity (units/mg)	Activity recovery	Fold purification
Crude extract	50.00	74.00	3700.00	64.40	1.14	100.00	1.00
$(NH_4)_2SO_4$ precipitate(P)	20.00	140.00	2800.00	87.30	1.60	75.67	1.40
Chromatography on Sephadex G-150 of (P)							
Ⅰ	14.00	10.54	147.56	1.45	7.26	3.98	6.36
Ⅱ	16.00	12.32	197.12	1.12	11.00	5.32	9.64
Ⅲ	12.00	9.63	115.12	1.86	5.17	3.12	4.53
Ⅳ	10.00	8.76	87.60	2.81	3.11	2.36	2.72
Ⅴ	14.00	15.30	124.20	2.21	6.92	5.78	6.07

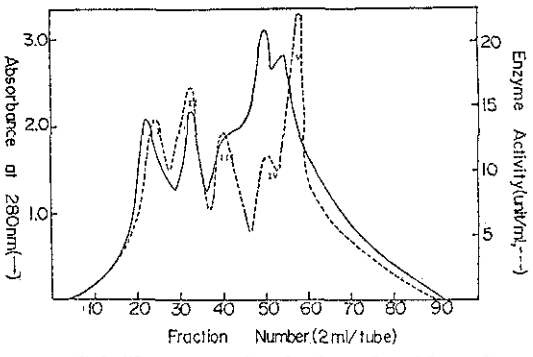

Fig. 1. Gel filtration of polyphenol oxidase from *Aster scaber* on a Sephadex G-150 column.

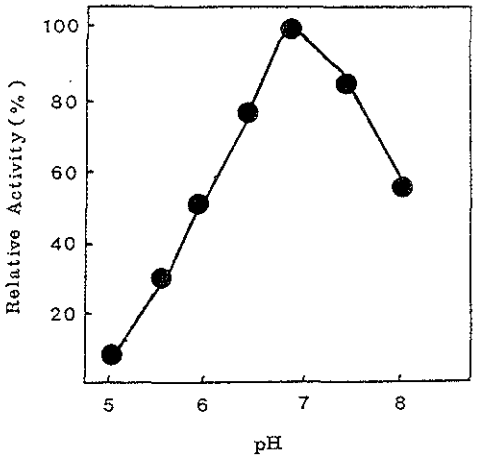

Fig. 2. Effect of pH on polyphenol oxidase activity of *Aster scaber*. The polyphenol oxidase activities were determined in 0.1M citrate-0.2M phosphate buffer solution.

the purification. After the $(NH_4)_2SO_4$ treatment, a purification fold of 6.0 was obtained and the specific activity was 6.9 units/mg (peak V). Peak V, which contained 214.2 units of polyphenol oxidase activity was the main component.

Optimum pH and temperature

The polyphenol oxidase activity was measured at various pHs from 5 to 8.5, using a 0.1M citric acid-0.2M phosphate buffer solution. The optimum pH for the activity was found to be around 7.0 (Fig. 2). The enzyme activity was measured at various temperature, from 20℃ to 50℃. The optimum temperature for the activity was arround 30℃(Fig . 3).

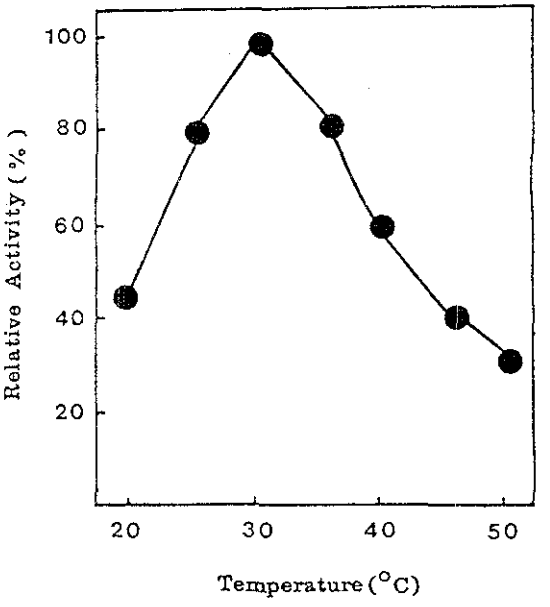

Fig. 3. Effect of temperature on polyphenol oxidase activity of *Aster scaber*. The polyphenol oxidase activity was determined at various temperature between 20°C and 50°C for 10min.

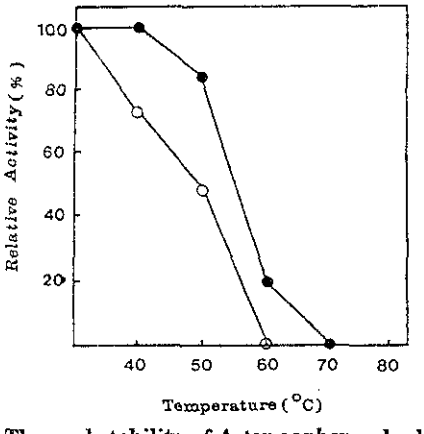

Fig. 4. Thermal stability of *Aster scaber* polyphenol oxidase. The enzyme solutions were heated at various temperature(40~80°C)for 5 and 30min. After heating, the remaining enzyme activities were determined with catechol as subtrate at pH 7.0 and 30°C. ●; 5min ○ ; 30 min.

Thermal stability

The enzyme treated in a water bath at various temperatures from 40℃ to 80℃ for 5 and 30min at pH 7.5 in the 0.1M citric acid-0.2M phosphate buffer solution, and then remaining activity was

determined with catechol as substrate. The enzyme was stable at 40℃ when examined at pH 7.0 for 5min, and completely in active at 60℃ for 30min and 70℃ for 5min at pH 7.0 (Fig. 4). In comparison with the values reported for the polyphenol oxidase from *Spuriopimpinella bracycarpa* and *Ligularia fischeri*, the values of these enzymes were similar to one another, though small differences were found among them.

Substrate specificity and enzyme kinetics

The purified polyphenol oxidase showed good activity on chlorogenic acid but was inactive on DL-dopa(Table 2). The apparent Km for catechol was about 17.6mM(Fig. 5). In comparison with the substrate specificity and Km value of the enzymes from *Spuriopimpinella bracycarpa* and *Ligularia fischeri*, *Spuriopimpinella bracycarpa* enzyme has good activity on catechol and 3,4-dihydroxy toluene but was strongly inactivated on pyrogallol, dopamine and DL-dopa. Also, *Ligularia fischeri* enzyme is inactive on dopamine. And the Km value of *Aster scaber* enzyme is close to that of *Ligularia fischeri* enzyme.

Table 2. Substrate specificity of *Aster scaber* polyphenol oxidase

Substrate (10mM)	Relative activity (%)
Catechol	45
Chlorogenic acid	100
3,4-Dihydroxytoluene	36
Pyrogallol	27
Hydroxyhydroquinone	20
Dopamine	12
DL-Dopa	0

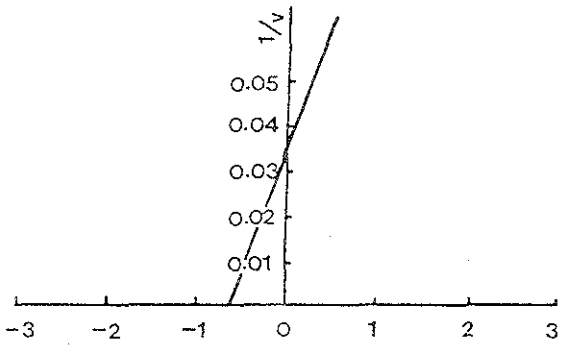

Fig. 5. Lineweaver-Burk plot of *Aster scaber* polyphenol oxidase activity.

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참취로부터 추출한 Polyphenol Oxidase의 부분정제 및 성질

함승시 · 홍은희 · 이상영 · 박귀근 · 오무라 히라히사*

강원대학교 농과대학 식품공학과
*일본 나까무라학원대학 식품영양학과

요 약

참취로부터 추출한 polyphenol oxidase의 부분정제를 $(NH_4)_2SO_4$ 처리 및 Sephadex G-150 column chromatogaphy에 의해 분리하였다. Polypenol oxidase의 최적pH와 최적온도는 각각 7.0과 30℃였으며, 효소활성은 60℃에서 30분간 처리시와 70℃에서 5분간 처리시 완전실활하였다. 기질특이성에서는 chlorogenic acid의 기질이 높은 특이성을 나타낸 반면 DL-dopa는 활성을 억제하였다. Polyphenol oxidase의 Km값은 17.6mM이었다.