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Antioxidative Characteristics of Dihydroxyphenylalanine, Melanin and Enzymatic Browning Reaction Products of Tyrosine in a Model System

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Abstract

Antioxidative characteristics of dihydroxyphenylalalnine (DOPA), melanin and enzymatic oxidation products of tyrosine (EOPTs) were studied in a model system. EOPTs were prepared by the tyrosine-tyrosinase reaction at pH 6.5 and 25° C at various time intervals (0~120min). All EOPTs were brown in varied intensities with increased absorption at 200~210, 280, 310~320nm, and 450~490nm. EOPTs obtained at the early stage of the reaction (1~3min especially) showed a higher antioxidative activity than those from the later stage on the inhibition of peroxide, conjugated dienoic acid and malonaldehyde formations in linoleic acid autooxidation. Additionally among the substances of tyrosine, DOPA and melanin, DOPA showed the highest antioxidative activity while that of tyrosine was the lowest during the linoleic acid autooxidation. It was observed that DOPA and melanin had the ability of free radical scavenging, which may partly contribute to their antioxidative activity.

Key words : tyrosine, DOPA, melanin, tyrosinase, antioxidant, enzymatic browning products

INTRODUCTION

Tyrosine related enzymatic browning in foods and other biological substances is mainly caused by the oxidative reaction of tyrosine catalyzed by tyrosinase (E.C. 1.14.18.1) in the tissues. Tyrosinase initiates the oxidation of tyrosine to dihydroxyphenylalanine (DOPA) and leads to melanin, brown pigments. Some of these reaction products have revealed specific chemical characteristics depending on the reaction conditions (1,2). The antioxidant activity of non-enzymatic browning products has been extensively studied (3-5) while those of enzymatic browning products are now beginning to be studied. A few select researches on this type of research of apples have been reported (6-8). However, there was no report on this antioxidative activity of the products from specific polyphenol compounds such as tyrosine.

Tyrosine, one of the major amino acids found in many kinds of foods, is also the principal phenolic compound in biological substances (9,10). On the other hand, tyrosine itself is the major cause of enzymatic browning acting as a good substrate for tyrosinase in animal tissue and in many kinds of food products including mushroom and fruits (11,12).

The present study was carried out to identify the antioxidative activity of enzymatic oxidation products of tyrosine (EOPTs), DOPA, and melanin in a model system.

MATERIALS AND METHODS

Reaction products and their absorption spectra

Enzymatic oxidation products of tyrosine (EOPTs) were prepared as follows (13). A sample of 19.5ml of tyrosine solution (2mM) in Mc'Ilvaine's citric acid-phosphate buffer (0.02M, pH 6.5) was mixed with 0.5ml tyrosinase (E.C. 1.14.18.1 ; 1000unit/ml) solution in the buffer and kept at 25° C in a water bath while stirring. Samples of the EOPTs were taken at 0, 1, 3, 6, 15, 30, 60 and 120 min of reaction time. Some samples were used directly to determine the antioxidant activity while other samples were freeze-dried for the later use. The absorption spectra (190~700 nm) of EOPTs were measured using a spectrophoto-

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meter (Shimadzu UV 2100, Japan).

Determination of antioxidative activity

Inhibitory effect, expressed as an antioxidative activity, of EOPTs, tyrosine, DOPA and melanin on the lipid oxidation was measured by determining the level of oxidized products formed from the linoleic acid oxidation system. For this reaction system 2.5ml of EOPTs (or 7.5μg of tyrosine, DOPA or melanin in 2.5 ml of ethanol) was mixed with linoleic acid soluton (15mg in 2.5ml ethanol) and kept at 37°C for 2~4 days for autooxidation.

The formed peroxides were then determined using a ferric thiocyanate method (14,15). And also the formed malonaldehyde and conjugated dienoic acid were determined by TBA method and AOCS Official method, respectively (16,17).

Measurement of free radical scavenging property

Antioxidant characteristics in terms of free radical scavenging were measured by using α,α'-diphenyl-β-picrylhydrazyl (DPPH) method (18). DPPH (16mg) was dissolved in 100ml ethanol and diluted to 200ml with distilled water and then filtered using Whatman filter paper No. 2. This DPPH solution (5ml) was mixed with 1ml of EOPTs and the absorbance decrease at 528nm was recorded.

Other materials and statistical analysis

Enzymes used in this experiment were from Sigma Chemical Co. (St. Louis, MO, USA). Tyrosine, DOPA, melanin and other chemicals were purchased from Fluka Chemical Corp. (Ronkonkoma, NY, USA). Data were analyzed by analysis of varience ($p<0.01$) and mean separation was conducted using Duncan's multiple range test (19).

RESULTS AND DISCUSSION

Browning characteristics and absorption spectra of EOPTs

During the tyrosinase catalyzed oxidation of tyrosine in the model system, the absorption spectra of EOPTs are shown in Fig. 1. The browning degree expressed as an absorbance at 420~520nm of EOPTs was slow at the beginning, up to the 15min reaction time, and then increased slowly at the steady rate reaching a maximum absorption at 120min reaction time. It was reported that catechine was oxidized within the first 15min and more than 50% of original substrate in the model system of polyphenoloxidase-catechine reaction has been converted to the enzymatic browning products within the first 12min of the reaction (20). However, browning rate of EOPTs slowed during the first 15min.

It was noted that tyrosine (control) showed an maximum absorption at around 200 and at 280nm. EOPTs obtained at the early stages of the reaction showed a similar spectral pattern but the reaction products obtained later (after 15min) showed increased absorbance at 400~510nm. A rapid color change was observed at 15min which was coincided with the increased in absorption at 200nm, 320 and 460~490nm. This increase in absorbance progressed with the reaction time. It has been reported that the characteristics of brown color in the enzymatic browning reactions depend on the nature of the reactants, the rate of reaction, and the photo absorption characteristics of the reactants (21).

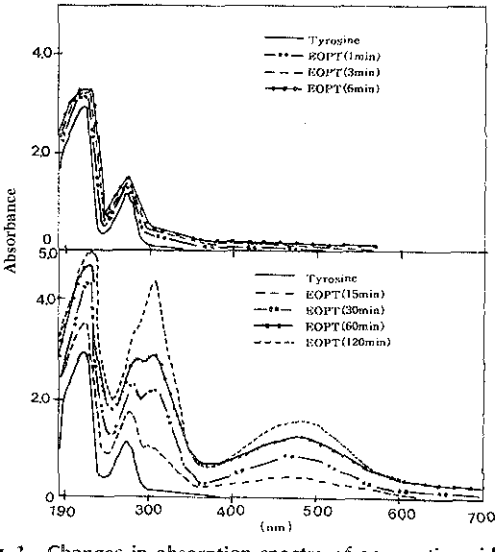

Fig. 1. Changes in absorption spectra of enzymatic oxidation products of tyrosine (EOPTs).
EOPTs were obtained from the reaction system, which was prepared with 19.5ml of 2mM tyrosine solution and 0.5ml of tyrosinase solution (1000unit/ml), and reacted at pH 6.5 and 25°C for 0~120min.

Antioxidative activity of EOPTs

Antioxidant activity of EOPTs, measured by the inhibitory effect on the peroxides formation in the linoleic acid autooxidation system, is shown in Table 1. The control (linoleic acid alone) produced peroxides very rapidly, reaching a maximum absorbance after 48hr, and then decreased thereafter, while the absorbance of samples to which tyrosine or EOPTs added was lower than control due to their antioxidative activity. There were significant differences ($p<0.01$) in the antioxidant activity among tyrosine and EOPTs obtained from various reaction times. The EOPTs obtained from the early stages of the reaction (3min) showed the highest level of antioxidative activity compared to these of the other EOPTs or tyrosine, but those EOPTs obtained at 60 and 120min of reaction time exhibited lower antioxidative activity that decreased with increased reaction time. This tendency of antioxidative characteristics of EOPTs were confirmed by the inhibitory effects on the formation of the conjugated dienoic acid (Fig. 2) and malonaldehyde (Table 2) in the linoleic acid autooxidation system.

And also all EOPTs showed a higher antioxidative activity compared to that of tyrosine alone. Thus antioxidant activity of EOPTs was different from the results of catechine products (20). Recently, the chemical characteristics of phenolic compounds as a hydrogen donor and free radical scavenger have been reviewed

Table 1. Comparison of the antioxidative activity of enzymatic oxidation products of tyrosine (EOPTs) by the various reaction times[1]

Treatment	Relative antioxidative activities[2]	
	2 days	4 days
Control[3]	1.69±0.02[d5]	1.40±0.05[e]
Tyrosine[4]	1.46±0.05[c]	1.65±0.01[d]
EOPT		
1min	0.09±0.01[a]	0.12±0.01[ab]
3min	0.06±0.01[a]	0.11±0.01[a]
6min	0.10±0.01[a]	0.13±0.01[ab]
15min	0.10±0.01[a]	0.14±0.01[ab]
30min	0.10±0.01[a]	0.15±0.01[bc]
60min	0.15±0.01[b]	0.18±0.01[c]
120min	0.16±0.01[b]	0.18±0.01[c]

[1] For EOPTs preparation, see the legend of Fig. 1

[2] Relative antioxidative activities are expressed as an absorbance at 500nm, which represents the amounts of peroxides produced during autooxidation of linoleic acid at 37° C during 2 and 4 days

[3] Citric acid-phosphate buffer of pH 6.5 was added to the reaction system

[4] Tyrosine (7.5μg in 2.5ml ethanol) was added to the reaction system instead of EOPTs

[5] The different letters in 2 days and 4 days are significantly different at 0.01 level of Duncan's multiple range test (n=3)

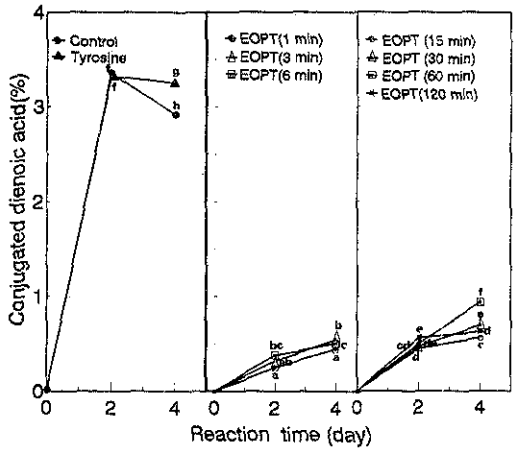

Fig. 2. Comparison of antioxidative activity of enzymatic oxidation products of tyrosine (EOPTs) by various reaction times.
Antioxidative activity was compared with the contents of conjugated dienoic acids during the autooxidation of linoleic acid. For the EOPTs preparation, see legend of Fig. 1. The control and tyrosine groups were the same as these in Table 1. The different letters on the curve in figure are significantly different at the 0.01 level of Duncan's multiple range test (n=3).

Table 2. Comparison of the antioxidative activity of enzymatic oxidation products of tyrosine (EOPTs) by the various reaction times[1]

Treatment	Relative antioxidative activities[4]	
	2 days	4 days
Control[2]	0.74±0.03[c5]	0.97±0.07[e]
Tyrosine[3]	0.75±0.04[c]	0.97±0.05[e]
EOPT		
1min	0.25±0.02[ab]	0.39±0.01[b]
3min	0.26±0.01[ab]	0.41±0.01[b]
6min	0.26±0.02[ab]	0.31±0.02[a]
15min	0.26±0.02[ab]	0.41±0.02[b]
30min	0.28±0.01[ab]	0.46±0.01[bc]
60min	0.30±0.01[b]	0.55±0.02[d]
120min	0.29±0.01[ab]	0.52±0.02[cd]

[1-3] For the preparation of EOPTs, control, and tyrosine, see the legend of Fig. 1

[4] Relative antioxidative activities are expressed as an absorbance at 532nm, which represents the relative content of malonaldehyde (MA) produced during autooxidation of linoleic acid at 37° C for 2 and 4 days. Initial value (0 day) of reaction mixture of linoleic acid was 0.04±0.01

[5] For the statistical analysis, see the legend of Table 1

extensively (22). However, information on the antioxidant activity of the phenolic compounds-polyphenol oxidase reaction products is limited to a small amount (7-9).

Antioxidative activity of tyrosine, DOPA and melanin

Fig. 3 focuses on the antioxidant activities of tyrosine, DOPA, and melanin on the peroxide formation of linoleic acid autooxidation. DOPA showed the highest antioxidative activity while that of tyrosine was the lowest. These indicated that intermediate product such as DOPA had higher antioxidant activity than that of the original substrate, tyrosine or final product, melanin.

Although the mechanism of enzymatic browning of phenolic compounds has not been understood completely, the general pathway of browning by tyrosine-tyrosinase is reported. Tyrosine is at first converted into DOPA, which is then oxidized to the corresponding dopa quinone, and then finally to the brown melanins through intermolecular rearrangement or further oxidation (2-4). Tyrosine, one of amino acids, was known to have a low level of antioxidative activity (8,9). While DOPA or melanin, oxidized products from tyrosine, exhibited a relatively higher antioxidative activity comparing to tyrosine itself in the present study. This observation is in accordance with the results of EOPTs in Table 1 and suggests that the EOPTs of early stage of reaction or DOPA may have relatively higher antioxidative activity.

Free radical scavenging ability of EOPTs

Fig. 4 shows the free radical scavenging activities of tyrosine, DOPA and melanin using a DPPH method. This method has been used often to determine the antioxidative activity, ability of hydrogen donor, or free radical scavenging ability (8,19). The present study indicated that a varied range of scavenging activity was shown among tested materials. Melanin or DOPA was found to have a higher free radical scavenging activity than that from tyrosine. However, melanin showed the highest activity among them. It is considered that the antioxidative characteristics of EOPTs may be partly explained by their ability to free radical scavenging. However, further research in this area is deemed necessary.

On the other hand, it was reported a varied range of free radical scavenging activity among enzymatic oxidation products of catechin (EOPCs) obtained at

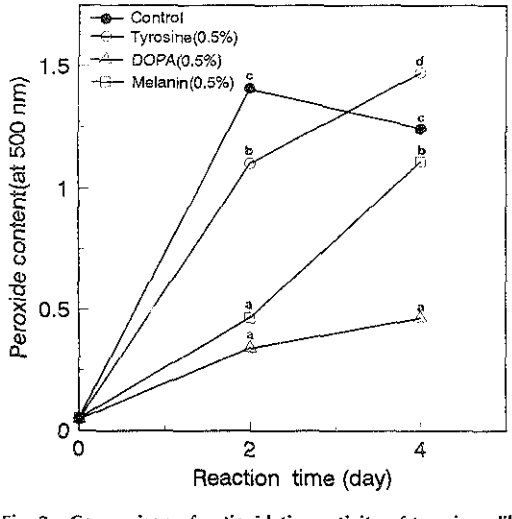

Fig. 3. Comparison of antioxidative activity of tyrosine, dihydroxyphenylalanine (DOPA) and melanin.
Antioxidative activity was compared with the peroxide contents produced during the autooxidation of linoleic acid (see the legend of Fig. 2).

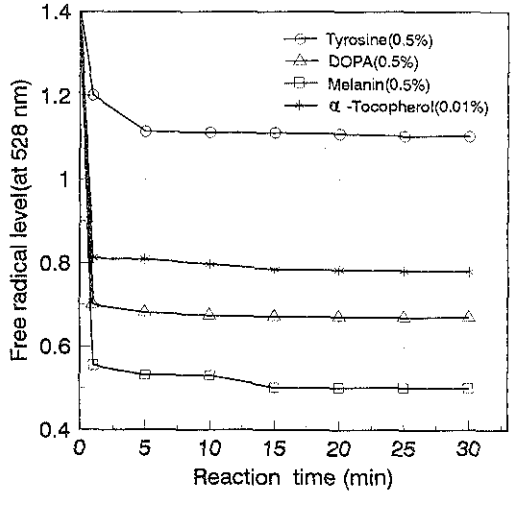

Fig. 4. Effect of tyrosine, dihydroxyphenylalanine (DOPA) and melanin on the changes in the free radical level of DPPH solution.
Free radical levels were determined by, α,α'-diphenyl β-picrylhydrazyl (DPPH) method and expressed as an absorbance at 528nm.

the various reaction time and EOPCs collected from the early stages of reaction had a higher activity (20).

REFERENCES

1. Cheigh, H. S. : Inhibition of enzymatic oxidation and browning of plant phenolic compounds. *Life Science (Korea)*, **3**, 115 (1993)
2. Mason, H. S. : Comparative biochemistry of the phenolase complex. *Adv. Enzyme.*, **16**, 136 (1955)
3. Cheigh, H. S. and Lee, C. Y. : Antioxidative and antimutagenic characteristics of melanoidin related products. *J. Korean Soc. Food Nutr.*, **22**, 246 (1993)
4. Cheigh, H. S., Lee, J. S. and Lee, C. Y. : Antioxidative characteristic of melanoidin related products fractionated from fermented soybean sause. *J. Korean Soc. Food Nutr.*, **22**, 570 (1993)
5. Cheigh, H. S., Lee, J. S. and Lee, C. Y. : Antioxidative characteristic of browning products fractionated from fermented soybean sause. *J. Korean Soc. Food Nutr.*, **22**, 565 (1993)
6. Omura, H., Sonda, T., Asada, Y. and Tachibana, H. : Antioxidative activity of the browing system with apple enzyme. *Nippon shokuhin Kogyo Gakkaishi*, **22**, 387 (1975)
7. Omura, H., Sonda, T., Asada, Y., Muranaka, M. and Tachibana, H. : Effect of amino acid on the antioxidative activity of the browning system of apple enzyme and catechol. *Nippon Shokuhin Kogyo Gakkasihi*, **22**, 395 (1975)
8. Nicolas, J. J., Richard-Forget, F. C., Goupy, P. M. and Aubert, S. Y. : Enzymatic browning reaction in apple and apple production. *Crit. Rev. Food Sci. Nutr.*, **34**, 109 (1994)
9. Rhodes, J. M. and Wooltorton, L. S. C. : The biosynthesis of phenolic compounds in wounded plant storage tissues. "*Biochemistry of Wounded Plant Tissues*", Kahl, G.(ed.), Walter de Gruyter & Co., Berlin, p.244 (1978)
10. Mathew, A. G. and Parpia, H. A. B. : Food browning as a polyphenol reaction. In "*Advances in Food Research*" Chichester, C. O., Mark, E. M. and Stewart, G. F. (eds.), Academic Press, Vol. 19, New York, p.104 (1971)
11. Joslyn, M. A. and Ponting, J. D. : Enzyme catalyzed oxidative browing of fruit products. In "*Advances in Food Research*" Mark, E. M. and Stewart, G. F. (eds.), Academic Press, Vol Ⅲ, New York, p.22 (1951)
12. Constantindes, S. M. and Bedford, C. L. : Multiple forms of phenoloxidase. *J. Food Sci.*, **32**, 446 (1967)
13. Oszmianski, J. and Lee, C. Y. : Enzymatic oxidative reaction of catechin and chlorogenic acid in a model system. *J. Agric. Food Chem.*, **38**, 1202 (1990)
14. Inatani, R., Nakatani, N. and Fuwa, H. : Antioxidative effect the constituent of rosemary and their derivatives. *Agric. Biol. Chem.*, **47**, 521 (1983)
15. Stine, C. M., Horland, H. A., Coulter, S. T. and Jenness, R. : A modified peroxide test for detection of lipid oxidation in dairy products. *J. Dairly Science*, **37**, 202 (1954)
16. Osawa, T. and Namiki, M. : A novel type of antioxidant isolated from leaf wax of eucalyptus leaves. *Agric. Biol. Chem.*, **45**, 735 (1981)
17. A.O.C.S. : *A.O.C.S.* Official Method. A.O.C.S., Illinois, p.365 (1973).
18. Blois, M. S. : Antioxidant determination by the use of a stable free radical. *Nature*, **26**, 1199 (1958)
19. Steel, R. G. D. and Torrie, J. H. : "*Principles and Procedure of Statistics*", Magraw-Hill Kogakusha, Ltd., Tokyo, p.96 (1980)
20. Cheigh, H. S., Um, S. H. and Lee, C. Y. : Antioxidant characteristics of melanin related products from enzymatic browning reaction of catechin in a model system.(in press ; at "*ACS Symposium Series*" on "Enzymatic Browning and Its Prevention", 1995)
21. Masom, H. S. : The chemistry of melanin. Ⅲ Mechanism of the oxidation dihydroxyphenylanine by tyrosinase. *J. Biol. Chem.*, **172**, 83 (1948)
22. Gordon, M. H. : The mechanism of antioxidant action *in vitro*. In "*Food Antioxidants*" Hudson, B. J. F. (ed.), Elsevier Applied Science, London, p.1 (1990)

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Dihydroxyphenylalanine, Melanin 및 Tyrosine의 효소적 산화반응생성물질의 항산화 특성

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요 약

모델시스템에서 얻어진 tyrosine의 효소적 산화반응생성물(enzymatic oxidation products of tyrosine; EOPTs) 그리고 동 반응의 중간 또는 최종 물질로 알려진 두가지의 표준 물질 즉, dihydroxyphenylalanine (DOPA)과 melanin에 대한 일련의 항산화 특성을 살펴보았다. Tyrosine-tyrosinase 반응시스템(pH 6.5, 온도 25°C, 시간 0~120분)에서 얻어진 EOPTs는 전반적으로 200~210, 280, 310~320 그리고 450~490mm에서 정점을 갖는 광학적 흡수특성을 보였으며 15분 후에는 450~520nm에서 강한 흡수특성을 나타냈다. EOPTs의 리놀레산에 대한 산화반응 억제 효과는 다 같이 최초 반응물질인 tyrosine 보다는 현저히 높고 반응초기(1~3분)의 것이 후기의 것들 보다 높았다. 또한 tyrosine, DOPA 및 melanin 등의 표준물질에 있어서는 tyrosine 보다는 다른 두 물질에서 항산화 효과가 더 높았고 그 중 DOPA의 항산화력이 가장 높게 나타났다. 한편 이들의 유리기 소거능은 tyrosine 보다는 다른 두 표준물질에서 높았으며 이러한 산화반응 물질들은 다양한 항산화 특성을 나타내고 있으며 특히 최종 갈변생성물 보다는 중간 물질에서 더 높은 효과를 보이고 있다. 그러나 아직은 그 작용 메카니즘이 분명치 않으며 앞으로 더 규명되어야 할 것으로 생각된다.