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Antioxidant Properties of Branched-chain Amino Acid Derivatives[1,2]

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The antioxidant activities of branched-chain amino acid derivatives, such as dipeptides and amino alcohols, were evaluated by means of the active oxygen method. Dipeptides containing a branched-chain amino acid showed higher antioxidant activities than did those having no branched-chain amino acid. An N-terminal branched-chain amino acid in dipeptides was preferable to a C-terminal one for antioxidant activity. A free N-terminal amino group was also found to be important for the appearance of antioxidant activity.

Keywords——antioxidant; dipeptide; amino alcohol; leucylglycine; lard; safflower oil; active oxygen method

Oxidation is one of the most important and complex deteriorative reactions occurring in foods as well as pharmaceutical materials containing lipids. The effects of amino acids or peptides on the rate of lipid oxidation have been reported by many investigators.[4–8] In the previous papers,[9–10] we reported the antioxidant properties of the browning products obtained by heating amino acids with low-molecular carbonyl compounds in vegetable oil. It was shown that browning products from branched-chain amino acids such as leucine, isoleucine and valine were much more effective than those from other amino acids. The antioxidant effect of a browning product formed from leucine and glucose was demonstrated in potato chips by Kato *et al.*[11]

The purpose of the present study was to investigate the antioxidant effect of derivatives of branched-chain amino acids, such as dipeptides and amino alcohols.

Experimental

Materials——Butylated hydroxyanisol (BHA) and linoleic acid were purchased from Katayama Chemical Co.; safflower oil was from Sigma Co. Lard containing no added antioxidant was obtained from a commercial supplier; a natural tocopherol mixture (tocopherol content: 80%) was from Eisai Co.

Dipeptides listed in Table I were prepared by ordinary peptide synthesis procedures. Besides these free peptides, some N-protected and N- and C-protected peptides were synthesized for antioxidant tests. Amino alcohols derived from branched-chain amino acids, such as valinol and leucinol, were obtained as their oxalates by the method of Seki *et al.*[12] All of these peptides and amino alcohols were shown to be pure by thin-layer chromatographic analysis.

1) This paper was presented at the 5th International Congress of Food Science and Technology, Kyoto, September 1978.
2) Abbreviations for amino acids and their derivatives are those recommended by the IUPAC-IUB Commission of Biochemical Nomenclature: z=benzyloxycarbonyl.
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Table I. Free Peptides used

Group	Peptides
Branched-chain amino acid	Val–Gly, Gly–Val, Val–Val
	Val–His, Val–Tyr
	Leu–Gly, Gly–Leu, Leu–Leu
	Leu–Sar[a], Leu–Met
	Ile–Gly, Gly–Ile
Tryptophan	Trp–Ala, Ala–Trp
	Trp–Asp, Asp–Trp
	Trp–Trp, Trp–His
Miscellaneous	Gly–Gly, His–Gly, Thr–Gly
	Asp–Ala, Pro–Ser, Ser–Phe
	Pro–Phe, Glu–Gly

Amino acids (except for glycine) were all of L-configuration.
a) Leucylsarcosine.

Evaluation of Antioxidant Effect——Antioxidant effect in the oil system was mainly evaluated by the active oxygen method (AOM). Lard and safflower oil were chosen as representative substrates for animal fat and vegetable oil, respectively. The substrates did not contain any added antioxidant. The substrate (20 ml) with additive was placed in each test tube (24 mm × 200 mm) of an AOM apparatus (Kuramochi Kagaku Co., Tokyo). The test tubes were maintained at 97.8±0.1°. Air was bubbled into the oil at a constant rate of 2.33 ml/sec for 8 or 10 hr. The peroxide value of the oil was determined by the conventional KI–$Na_2S_2O_3$ titration procedure. Antioxidant effect was arbitrarily expressed as the ratio (percent) of value of the substrate with additive to that of the control.

Antioxidant effect in an emulsion system containing water was determined by the hemin-catalyzed oxygen uptake test using a Gilson differential respirometer (Gilson Medical Electronics, Wisconsin, U.S.A.).[13] Linoleic acid (0.5 ml), 0.1 M phosphate buffer, pH 7.0 (3.0 ml), 20% Tween 20 (0.5 ml), ethanol (0.1 ml) and water (0.1 ml) were placed in a respirometer flask. Antioxidant samples were dissolved in ethanol or water. The sidearm of the flask contained 0.2 ml of a weakly alkaline solution of hemin (2.5 mg/100 ml). After being equilibrated and emulsified with shaking at 37° for 5 min, the contents of the sidearm were tipped into the main part of the flask. Oxygen uptake per 60 min was taken as a measure of the antioxidant activity.

Results

The effects of dipeptides on the oxidation of lard or safflower oil as determined by the active oxygen method are summarized in Table II. In general, the antioxidant effect of samples was more apparent in lard than in safflower oil. One reason why the relative efficacy of antioxidants varied with the substrate may be the difference in fatty acid composition between lard and safflower oil. Peptides having branched-chain amino acids showed considerably higher antioxidant activities than others in both lard and safflower oil. Tryptophan-containing peptides had rather weak activity under these conditions. Other miscellaneous peptides exhibited little or no antioxidant effect except for proline-containing peptides. Among the branched-chain amino acid peptides, leucylglycine, leucylsarcosine and isoleucylglycine were most effective, followed in decreasing order by leucylleucine, leucylmethionine and valylglycine. It is interesting that valylglycine, leucylglycine, and isoleucylglycine were more effective than glycylvaline, glycylleucine, and glycylisoleucine, respectively. This suggests that the presence of branched-chain amino acids at the N-terminal position is important for the antioxidant activity of these dipeptides.

As leucylglycine was found to have a marked antioxidant activity, the activities of peptides composed of leucine and glycine were compared with those of each constituent amino acid. In this experiment, antioxidant effects were compared on a molar basis.

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Table II. Antioxidant Effects of Dipeptides

Peptide	POV ratio[a]		Peptide	POV ratio[a]	
	Lard	Safflower		Lard	Safflower
None	100	100			
BHA	5	68	Trp–Ala	86	99
Tocopherol	12	99	Ala–Trp	82	98
			Trp–Asp	90	96
Val–Gly	56	78	Asp–Trp	89	94
Gly–Val	71	86	Trp–Trp	88	92
Val–Val	63	81	Trp–His	87	97
Val–His	74	79			
Val–Tyr	80	88	Gly–Gly	93	100
Leu–Gly	26	64	His–Gly	105	102
Gly–Leu	65	80	Thr–Gly	104	101
Leu–Leu	41	75	Asp–Ala	100	99
Leu–Sar[b]	28	63	Pro–Ser	86	81
Leu–Met	51	72	Pro–Phe	82	87
Ile–Gly	32	67	Ser–Phe	111	102
Gly–Ile	63	78	Glu–Glu	108	105

Lard or safflower oil was oxidized for 8 hr under the AOM conditions. Levels of additives were 0.2% for peptides and 0.02% for BHA and tocopherol.
Data are averages of duplicate determinations.
a) Per cent ratio of the peroxide value of the substrate with additive to that of the control.
b) Leucylsarcosine.

Table III. Antioxidant Effects of Peptides Consisting of Leucine and Glycine

Amino acid or peptide	Level of additive (m mol/20 ml lard)	POV (meq/kg)	POV ratio[a]
None	—	80.8	100
Gly	0.2	85.5	106
	0.4	78.7	97
Leu	0.2	69.2	86
	0.4	62.5	77
Gly+Leu	0.2 each	66.8	83
Gly–Gly	0.2	76.8	95
Leu–Gly	0.2	26.0	32
Gly–Leu	0.2	62.1	77
Leu–Leu	0.2	53.9	67

Lard was oxidized for 10 hr under the AOM conditions.
Data are averages of duplicate determinations.
a) See footnote *a*) in Table II.

As can be seen from Table III, leucylglycine, leucylleucine and glycylleucine were more effective than leucine or glycine, or even a mixture of leucine and glycine.

In order to determine whether or not amino or branched moieties in dipeptides are necessary for the antioxidant activity, some protected peptides and amino alcohols were subjected to antioxidant tests. As shown in Table IV, it is clear that neither N-protected peptides nor N- and C-protected ones possessed antioxidant properties. Therefore, the amino group of peptide appears to be indispensable for antioxidant activity. From the results shown in Table V, branched-chain amino alcohols such as valinol and leucinol were found to be effective antioxidants, while alaninol, which possesses no branched group, was almost ineffective. Thus, it seems that a branched group may be important for the activity and that a carboxylic group is not essential.

All the above results were obtained in the oil system. Thus, the antioxidant effects of leucine dipeptides were further evaluated in terms of hemin-catalyzed oxygen uptake of linoleic acid. As shown in Table VI, four peptides consisting of leucine and glycine exhibited little or no antioxidant effect. Determination of an additive blank, using a system without substrate oil, resulted in no oxygen uptake. Thus, the results obtained in an emulsion system containing water were different from those in the oil system. The reasons for this will be discussed below.

Table IV. Antioxidant Effects of Protected Peptides

N-(and C) protected peptide	POV ratio[a)]	
	Lard	Safflower
None	100	100
Leu–Gly	26	64
Z–Leu–Gly	91	98
Z–Gly–Leu	101	96
Z–Leu–Leu	93	102
Z–Val–Val	98	105
Z–Ile–Gly	103	100
Z–Val–GlyOMe	96	107
Z–Val–ValOMe	105	108

Lard or safflower oil was oxidized for 8 hr under the AOM conditions. Levels of additives were 0.2%. The following abbreviations are used: Z=benzyloxycarbonyl, OMe=methyl ester.
Data are averages of duplicate determinations.
a) See footnote *a*) in Table II.

Table V. Antioxidant Effects of Amino Alcohols

Amino alcohol	POV ratio[a)]	
	Lard	Safflower
None	100	100
DL-Valinol oxalate	63	72
DL-Leucinol oxalate	53	69
DL-Alaninol oxalate	90	92

Lard or safflower oil was oxidized under the AOM conditions. Levels of additives were 0.2%.
Data are averages of duplicate determinations.
a) See footnote *a*) in Table II.

Table VI. Effects of Dipeptides on Hemin-Catalyzed Oxygen Uptake of Linoleic Acid[a)]

Additive	Oxygen uptake (μl/60 min)
None	245.1
BHA	46.5
Tocopherol	71.3
Gly–Gly	233.8
Leu–Gly	220.6
Gly–Leu	238.5
Leu–Leu	241.9

Levels of additives were 0.2% for peptides and 0.02% for BHA and tocopherol.
Data are averages of triplicate determinations.
a) Experimental conditions were as described in the text.

Discussion

In recent years, the antioxidant effects of protein hydrolysates or peptides have attracted considerable research attention.[5-7,14] Bishov and Henick[7] demonstrated the antioxidant properties of protein hydrolysates such as hydrolyzed vegetable protein and autolyzed yeast protein. However, these hydrolysates are rather poorly defined mixtures of amino acids and peptides.

Previously we found that synthetic dipeptides containing L-tryptophan as the N-terminal amino acid were quite effective for inhibiting the autoxidation of linoleic acid in an emulsion system.[8] Yamaguchi *et al.*[14] reported the antioxidant activities of dipeptides and their synergistic effect with tocopherol. In the above studies, the antioxidant effects of peptides were evaluated in model systems containing water.

On the other hand, little information can be found in the literature on the antioxidant effects of peptides in an oil system which contains no water. In the present work, antioxidant activities of many synthetic dipeptides were measured mainly by the active oxygen method at 97.8°, using lard or safflower oil as a substrate. Peptides containing branched-chain amino acids were found to have considerable antioxidant effects (Table II). In particular, the activity of leucylglycine was very high. Leucylglycine, however, did not show antioxidant effect in an emulsion system at 37° (Table VI). On the other hand, tryptophan peptides exhibited only a slight activity under the conditions of the active oxygen method (Table II) although they were previously found to be effective antioxidants in an emulsion system.[8]

One reason for inactivity of leucylglycine in an emulsion system may be the presence of water, since it has been shown that water has a marked effect on the efficacy of antioxidants.[15] Another possible explanation is that the activity of leucine peptides may appear during the heat treatment under the conditions of the active oxygen method. In this context, reports on thermal interactions of amino acids with lipids are available, although these studies are not related to antioxidant activity. Lien and Nawar[16] identified a number of decomposition products from a reaction between valine and tricaproin. Sims and Fioriti[17] reported the formation of an N-substituted stearamide derived from leucine and methylstearate. This may possibly be related to our observations that N-protected peptides were inffective as antioxidants (Table IV).

It also seems very interesting that dipeptides having branched-chain amino acids showed antioxidant activity in spite of their insolubility in oil. Further work will be needed to clarify the mechanism of antioxidant action of dipeptides.

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