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Antioxidant Activity of Peptides Obtained from Porcine Myofibrillar Proteins by Protease Treatment

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Hydrolysates obtained from porcine myofibrillar proteins by protease treatment (papain or actinase E) exhibited high antioxidant activity in a linolenic acid peroxidation system induced by Fe^{2+}. Hydrolysates produced by both papain and actinase E showed higher activities at pH 7.1 than at pH 5.4. The antioxidant activity of the papain hydrolysate was almost the same as that of vitamin E at pH 7.0. These hydrolysates possessed 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity and chelating activity toward metal ions. Antioxidant peptides were separated from the papain hydrolysate by ion exchange chromatography. The acidic fraction obtained by this method exhibited higher activity than the neutral or basic fractions. Antioxidant peptides in the acidic fraction were isolated by high-performance liquid chromatography on an ODS column and shown to possess the structures DSGVT, IEAEGE, DAQEKLE, EELDNALN, and VPSIDDQEELM. The DAQEKLE peptide showed the highest activity among these peptides.

KEYWORDS: Antioxidant; papain; myofibrillar protein hydrolysate; peptide

INTRODUCTION

It is well-known that lipid peroxidation occurring in food products causes such deteriorations of food quality as rancid flavor, unacceptable taste, and shortening of shelf life. Consuming oxidative foods is thought to cause serious diseases such as hepatomegaly (*1*) or necrosis of epithelial tissues. The factors involved in these diseases are lipid peroxides and low molecular weight compounds produced during the late stage of the oxidative reaction (*2*). Uchida and Stadman (*3*) reported that the 4-hydroxynonenal (4-HNE), which is a secondary breakdown product of lipid peroxides, can react with proteins, phospholipids, and nucleic acids to produce modifications and cross-linking of proteins or peptide molecules. Furthermore, cancer, coronary heart disease, and Alzheimer's diseases are also reported to be caused in part by oxidation or free radical reactions in the body (*4*−*7*). To prevent foods from undergoing such deterioration and to provide protection against serious diseases, it is very important to inhibit lipid peroxidation occurring in foodstuffs and the living body (*8*). Lipid oxidation is inhibited by antioxidant agents. Artificial antioxidants (BHA, BHT, and *n*-propyl gallate) exhibit strong antioxidant activity against several oxidation systems. However, as they pose potential risks in vivo, the use of artificial antioxidants in foodstuffs is restricted or prohibited in some countries. Therefore, it is necessary to develop safer antioxidants from natural sources. α-Tocopherol (*9*), carotenoids from brightly colored vegetables, catechins in tea (*10*, *11*), and polyphenol compounds in red wine (*12*) are well-known popular natural antioxidants. These natural antioxidants are almost all derived from plants and nonprotein compounds. Recently, some protein hydrolysates have been reported to exhibit antioxidant activity (*13*−*16*). However, there is little information about antioxidants derived from animal foods.

In the present study, we investigated the antioxidant activity of porcine myofibrillar protein hydrolysates obtained by enzymatic treatment in a peroxidation system of aqueous linolenic acid induced by Fe^{2+} and examined its antioxidant mechanism by measuring their DPPH radical scavenging activity and metal chelating activity. Furthermore, antioxidant peptides were isolated from the hydrolysates and characterized.

MATERIALS AND METHODS

Materials. Fresh pork muscles (*longissimus dorsi* muscle from the hybrid pigs; Landrace × Large white × Duroc) were obtained from Nippon Meat Packers, Inc. (Osaka, Japan). Triton X-100 (peroxide and carbonyl free) and papain were purchased from Sigma Chemical Co. (St. Louis, MO). Actinase E was from Kaken Pharmaceutical Co. (Tokyo, Japan). All other chemicals were of reagent grade.

Preparation of Myofibrillar Protein Hydrolysates. Fresh pork muscles were stored at 4 °C for 5 days after slaughter. Myofibrillar proteins were prepared from these fresh pork muscles according to the method of Yang et al. (*17*). The myofibrillar proteins were incubated at a 100 to 1 (w/w) ration with actinase E (reagent grade) or papain (double-crystallized product) in distilled water (pH 7.0) at 37 °C for 24 h. After they were incubated, four volumes of ethanol (99.5%) were added to the reaction mixture to remove unhydrolyzed proteins and the solution was centrifuged at 2000*g* for 10 min. The supernatant after centrifugation was concentrated by evaporation at 45 °C and adjusted to an adequate concentration using distilled water.

Amino Acid Analysis. Amino acid analysis was performed according to the method described by Fujiwara et al. (*18*). Proteolytic hydrolysates of myofibrillar proteins were hydrolyzed in 6 N HCl at

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110 °C for 24 h. Amino acid analysis of the hydrolysates was performed with an amino acid analyzer (Shimadzu Co., Kyoto, Japan) by the method of *o*-phthalaldehyde derivatives. The amino acid composition of peptides was determined by subtraction of free amino acids from total amino acids in the hydrolysate. The following amino acid analyses showed that the concentrations of peptides in both hydrolysates with papain and actinase E treatment were 94 and 75%, respectively. The residual compounds in both hydrolysates were free amino acids.

Measurement of Hydroperoxides in a Peroxidation System. To assay antioxidant activity, we used the peroxidation system reported by Chen et al. (*13*) with slight modification. Ten milligrams of linolenic acid in 4 mL of 0.1 M K-phosphate buffer (pH7.0) containing 0.5% TritonX-100 (w/v) and 0.05 mM $FeCl_2$ (accelerator of oxidation) was sonicated with a sonicator at 40 °C for 4 min. One hundred microliters of peptide sample in water or vitamin E in 99.5% ethanol was added in the sonicated mixture, and 100 μL of distilled water or 99.5% ethanol was added in the other mixture as control. Then, they were heated in a water bath at 80 °C for 60 min. The hydroperoxides in this peroxidation system before and after heating for 60 min were measured according to the method of Osawa and Namiki (*19*). That is, 100 μL of reaction mixture was mixed with 4.5 mL of 75% ethanol, 100 μL of 30% ammonium thiocyanate, 200 μL of 1 N HCl, and 100 μL of 20 mM ferrous chloride in 3.5% HCl. The hydroperoxides in this mixture were measured at 500 nm. The antioxidant activity was estimated as the rate of inhibition of hydroperoxide production.

Measurement of DPPH Radical Scavenging Activity. DPPH radical scavenging activity was measured based on the method of Bersuder et al. (*20*). Test samples in 4 mL of water were mixed with 1 mL of 99.5% ethanol containing 0.02% DPPH. This mixture was shaken and kept at room temperature for 60 min, and then, the absorbance of the mixture was measured at 517 nm. The residual radicals were calculated as follows:

$$\text{residual radicals (\%)} = 100 - \frac{(\text{DPPH blank} + \text{control sample}) - \text{DPPH sample}}{\text{DPPH blank}} \times 100$$

where the DPPH blank is the value of 4 mL of water/1 mL of ethanol including 0.02% DPPH, the DPPH sample is the value of 4 mL of sample solution/1 mL of ethanol including 0.02% DPPH, and the control sample is the value of 4 mL of sample solution/1 mL of ethanol.

Measurement of Metal Ion Chelating Activity. Metal ion chelating activity was evaluated by chelate titration using pyrocatechol violet (PV) as the metal chelating indicator (*21*). One milliliter of 2 mM $CuSO_4$ was mixed with pyridine (pH 7.0) including 20 μL of PV. The complex of PV with $CuSO_4$ was blue, and the color turned to yellow when PV dissociated a Cu ion in the presence of chelating agents. The change in color of the PV solution was measured at 400−900 nm.

Purification of Antioxidant Peptide. A sample (1.4 g) of the papain hydrolysate was dissolved in 0.05 N ammonium acetate buffer (pH 6.0) and applied to an AG 50W-X2 column (100−200 mesh, hydrogen form, ϕ 2 cm × 50 cm; Bio-Rad, Hercules, CA) equilibrated with the same buffer. After it was washed, the adsorbed peptides were eluted with 0.2 M ammonia solution. The unadsorbed fraction containing the acidic and neutral peptides was concentrated and then applied to an AG 1-X4 column (100−200 mesh, chloride form, ϕ 2 cm × 18 cm; Bio-Rad) equilibrated with 0.05 M ammonium acetate buffer (pH 3.5). After it was washed, the adsorbed fraction comprised of the acidic peptides was eluted with 1.0 M HCl. The acidic peptide fraction was neutralized with NaOH, and the NaCl was removed by ultrafiltration.

Antioxidant peptides in the acidic fraction were further purified by HPLC on an ODS column (VP-318-1251, ϕ 4.6 mm × 25 mm, Senshu Scientific Co., Tokyo, Japan) using a liner gradient of acetonitrile (0−30%) containing 0.1% trifluoroacetic acid (TFA) at a flow rate of 1.0 mL/min for 100 min. The peptides were detected at 220 nm.

The molecular masses of the purified antioxidant peptides were determined with an ESI mass spectrometer (LC-Q, Thermo Finnigan, San Jose, CA). The amino acid sequences of the antioxidant peptides were analyzed with a protein sequencer G1005A (Hewlett-Packard Co., Wilmington, DE). Each antioxidant peptide fraction isolated on a HPLC was dried in vacuo and reconstituted in 0.1% TFA−50% acetonitrile solution or distilled water for the mass spectrometer and protein sequencer, respectively. The sequence data were compared with a homology database site provided by the SWISS-PROT protein database from available public source (DDBJ, http://www.nig.ac.jp).

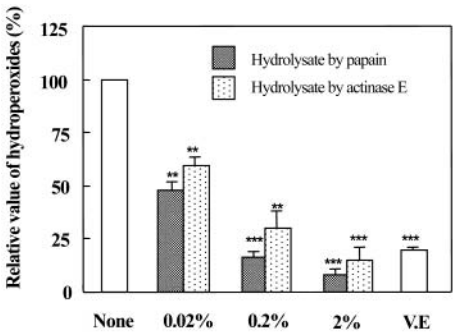

Figure 1. Effect of hydrolysate concentration on the antioxidant activity. VE (vitamin E) was added at 0.2%. Dark bars and dotted bars represent hydrolysates prepared by papain treatment and actinase E treatment, respectively. Error bars show the standard deviations. Values are the means of triplicate analyses. *, **, ***, significantly different from the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, with Student's *t*-test.

Peptide Syntheses. Peptides were synthesized with a simultaneous multiple peptide synthesizer (model PSSM-8; Shimadzu) according to the method (fluorenyl methoxycarbonyl (Fmoc) strategy) reported by Nokihara et al. (*22*). The synthesized peptides were then purified by HPLC on an ODS column (PEGASIL-300, ϕ 20 mm × 250 mm; Senshu Scientific Co.) with a linear gradient from 0 to 50% CH_3CN containing 0.1% TFA in 100 min (flow rate, 5.0 mL/min; monitoring, 220 nm). The molecular masses of the isolated peptides were determined by mass spectrometry.

Statistical Analysis. All assays were carried out in triplicate. Data were expressed as means with standard deviations. Student's *t*-test ($p < 0.05$) was calculated to compare the mean of each sample with that of the control (represented as none in the figures) sample.

RESULTS AND DISCUSSIONS

Antioxidant Activities of Protein Hydrolysates. The antioxidant activities of the hydrolysates were measured in a linolenic acid peroxidation system. The addition of 0.02, 0.2, and 2% of either hydrolysates exhibited antioxidant activity in this system. The antioxidant activity of the hydrolysates increased with increasing concentrations (**Figure 1**). At all concentrations, the papain hydrolysate exhibited higher activity than the actinase E hydrolysate.

Although the major constituent amino acids of both hydrolysates were Glx, Asx, and Lys, the amino acid composition of the papain hydrolysate was different from that of the actinase E hydrolysate (**Table 1**). The hydrophobic amino acid content in the papain hydrolysates was greater than that in the actinase E hydrolysate. Murase et al. (*23*) reported that *N* (long chain acyl)-histidine and *N* (long chain acyl)-carnosine showed stronger antioxidant activity than intact histidine and carnosine. The increase in the antioxidant activity of the modified compounds seems to be caused by the increase in their hydrophobicity, that is, solubility in lipid. The hydrophobic amino acid content in the papain hydrolysates was greater than that in the actinase E hydrolysate. The calculation of the hydrophobic indices (hydrophobicity) using $H\Phi$ (amino acid side chain hydrophobicity; kcal/amino acid residue) by Bigelow (*24*) showed that the hydrophobicity of the papain hydrolysate (average value; 101.0 kcal/amino acid residue) was larger than that of the actinase E hydrolysate (78.7 kcal/amino acid residue). Therefore, the papain hydrolysate containing more hydrophobic

Table 1. Amino Acid Composition of Each Hydrolysate (mol %) of Myofibrillar Protein

amino acid	papain treatment	actinase treatment
Asx	9.98 ± 0.85	10.95 ± 0.70
Thr	6.05 ± 0.51	6.97 ± 0.45
Ser	5.43 ± 0.16	6.14 ± 0.49
Glx	16.00 ± 2.14	20.06 ± 1.45
Pro	4.37 ± 0.78	4.68 ± 1.26
Gly	7.12 ± 0.98	7.53 ± 0.34
Ala	8.82 ± 0.18	11.12 ± 0.83
Val	5.72 ± 0.42	5.37 ± 0.25
Cys	0.23 ± 0.22	0.22 ± 0.13
Met	2.40 ± 1.20	1.27 ± 0.99
Ile	5.22 ± 0.88	4.31 ± 0.23
Leu	8.50 ± 0.49	2.49 ± 3.28
Tyr	2.74 ± 0.38	1.92 ± 0.77
Phe	3.18 ± 0.46	0.79 ± 1.00
His	1.99 ± 0.07	1.94 ± 0.55
Lys	8.01 ± 0.27	9.49 ± 1.48
Arg	4.24 ± 0.08	4.75 ± 1.17

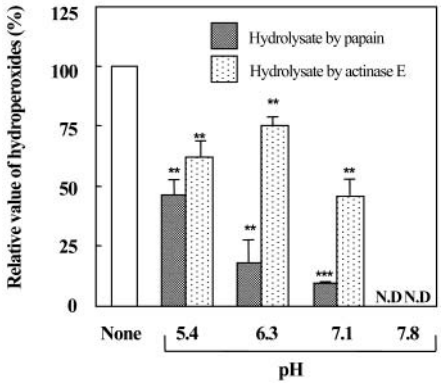

Figure 2. Effect of pH on the antioxidant activity of the hydrolysates. Dark bars and dotted bars represent hydrolysates prepared by papain treatment and actinase E treatment, respectively. Error bars show the standard deviations. Values are the means of triplicate analyses. *, **, ***, significantly different from the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, with Student's *t*-test. ND, not determined.

amino acids might be expected to exhibit higher antioxidant activity than other hydrolysates.

To compare the activities of the protein hydrolysates with a well-known antioxidant agent, α-tocopherol, the antioxidant activity of α-tocopherol was also measured in the same system. The papain hydrolysate showed the same activity as α-tocopherol. There is no information concerning the antioxidant activities of protein hydrolysates from edible meat. Our results are the first report that hydrolysates of myofibrillar proteins produced by proteases possess antioxidant activity.

Effect of pH on the Antioxidant Activity of Protein Hydrolysates. The antioxidant activities of the actinase E and papain hydrolysates at pH 5.4−7.8 were examined (**Figure 2**). Both hydrolysates suppressed the production of hydroperoxides more strongly as pH was increased. At higher pH, carboxyl residues of acidic amino acids (Asp, $pK_a = 3.86$; Glu, $pK_a = 4.25$) are charged to form anions (*25*). These residues should be involved in the formation of complexes with metal ions and thus suppress lipid peroxidation. A constituent amino acid mixture of a hydrolysate showed lower antioxidant activity than the hydrolysate (data not shown). Therefore, the peptides in the hydrolysates seem to be important for high antioxidant activity.

Scavenging of DPPH Radical. The radical scavenging activities of the hydrolysates produced by actinase E and papain were measured in order to clarify the mechanism of the suppression of lipid peroxidation. α-Tocopherol was used as a positive control radical scavenger. As shown in **Figure 3**, both hydrolysates showed radical scavenging activities lower than that of α-tocopherol.

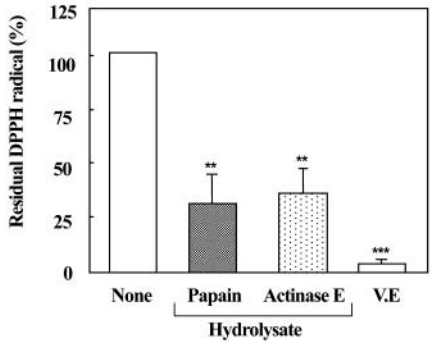

Figure 3. DPPH radical scavenging activity of the hydrolysates and VE. Error bars show the standard deviations. Values are the means of triplicate analyses. *, **, ***, significantly different from the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, with Student's *t*-test.

α-Tocopherol immediately showed a strong effect as a radical scavenger (96.2% decomposition of DPPH radical) with a gradual decrease in activity over 1 h (data not shown). On the other hand, the hydrolysates continued to possess antioxidant effects even after 1 h. A protein hydrolysate is a good antioxidant compound with radical scavenging activity for use over a long time, although the activity is lower than that of α-tocopherol.

Some amino acids, such as His, Tyr, Met, and Cys, have been reported to show antioxidant activity (*26*−*29*). Especially, histidine exhibits strong radical scavenging activity due to the decomposition of its imidazole ring (*30*). Carnosine (β-alanyl-L-histidine) is a famous antioxidant peptide in muscle protein, and its activity has been suggested to be due to the radical scavenging activity (*31*, *32*) and the quenching of singlet oxygen species (*33*) by His. As shown in **Table 1**, both hydrolysates contain His, Tyr, and Met. Therefore, the radical scavenging activities of both hydrolysates seem to be caused by these amino acids in the hydrolysate peptides.

Chelation of Metal Ion. Transition metals, such as Fe^{2+} and Cu^{2+}, can catalyze the generation of reactive oxygen species such as hydroxyl radical (•OH) and superoxide anion (O_2^-) (*34*). Especially, Fe^{2+} generates •OH by the Fenton reaction, by which the lipid peroxidation chain reaction is accelerated. Therefore, the chelation of metal ions contributes to antioxidation. In this section, the chelating activities of the hydrolysates produced by actinase E and papain were measured using PV and Cu^{2+}.

Figure 4 shows the Cu^{2+} chelating activity of both hydrolysates. Ethylenediaminetetraacetic acid (EDTA) was used as a positive control of a metal chelating agent. The complex of PV and Cu^{2+} absorbs blue light at 632 nm, while PV dissociated with a metal ion does not show this absorption. As a control, a PV complex containing 2mM Cu^{2+} had an absorbance at 632 nm of 1.3. The addition of an equivalent amount of EDTA (0.045% EDTA) to the PV and Cu^{2+} mixture resulted in a disappearance of the blue color and a decline in the absorption at 632 nm from 1.3 to 0.08. Both hydrolysates also showed metal chelating activity, with the activity of the hydrolysate produced by actinase E larger than that of papain produced hydrolysate. Although the amino acid compositions of both hydrolysates are similar, the chelating activities differ. This difference may be caused by differences in the structure and length of the peptides in the hydrolysates.

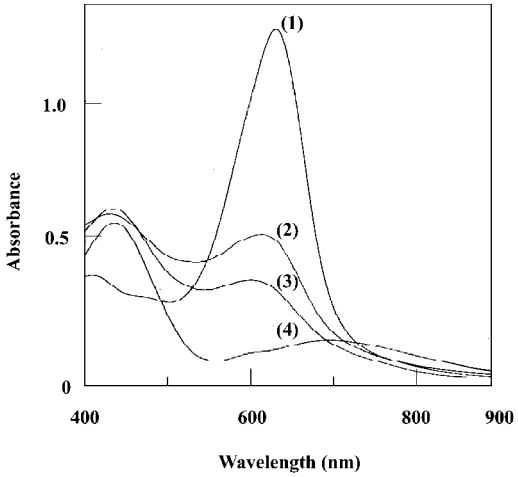

Figure 4. Metal ion chelating activity of the hydrolysates and EDTA. (**1**) Without metal chelating agent, (**2**) 1.28% papain hydrolysate, (**3**) 0.14% actinase E hydrolysate, and (**4**) 0.045% EDTA.

Some proteins have metal ion chelating activities. For example, Fe ion in hemoglobin is coordinated by the nitrogen in the imidazole ring of His, and some enzymes retain metal ions by metal chelation by their amino acid residues (*35*). Peptides as well as proteins have chelating activity. It is thought that acidic and/or basic amino acids play an important role in the chelation by peptides of Fe^{2+} and Cu^{2+}. Especially, carboxyl and amino groups in branches of the acidic and basic amino acids, respectively, are involved in metal chelation by peptides.

Separation of Antioxidant Peptides. To isolate and characterize the antioxidant peptides in the proteolytic hydrolysates, we tried to separate peptides in the papain hydrolysate by ion exchange column chromatography, because the papain hydrolysate exhibited higher antioxidant activity than the actinase E hydrolysate.

Cation exchange column (AG 50W-X2) chromatography provided four peptide fractions from this hydrolysate, with fractions I−III containing acidic and neutral peptides and fraction IV containing basic peptides (**Figure 5**). All fractions inhibited 30−90% of lipid peroxidation in a control sample (no peptides). Fraction I showed the strongest activity among all fractions.

The peptides in fraction I were further separated by anion exchange (AG 1-X4) column chromatography. Three neutral peptide fractions (fractions I-1−3) and one acidic peptide fraction (fraction I-4) were obtained (**Figure 6**). Fraction I-4 was neutralized and applied to an ultrafiltration membrane (MW < 500, Millipore Co., Bedford, MA) to remove small compounds and the salt produced by neutralization. The antioxidant activity of fraction I-4 was highest among these fractions. This fraction also exhibited higher antioxidant activity than a papain hydrolysate before separation.

Fraction I-4 was applied onto a reversed-phase HPLC column to isolate antioxidant peptides. As shown in **Figure 7**, more than 80 peaks were detected. The antioxidant activities of these peptides were measured in the linolenic acid peroxidation system. As shown in **Figure 8**, 26 peptides inhibited lipid peroxidation by more than 20%. The increase of hydroperoxide production by the addition of peptides such as 57, 60, and 76−80 was observed. Because we did not analyze these peptides, we cannot explain the reason for this result. In some experimental conditions, some amino acids such as Gly, Met, or Trp have been reported to accelerate an oxidation (*27*, *36*) even if they have been accepted as an antioxidant. Therefore, some peptides containing these amino acids in the fractions might exist and accelerate oxidation in this assay system. Analysis of peptides in these fractions is another important problem to be resolved. On the basis of the activity and amounts of these peptides, the N-terminal amino acids of peptides possessing large specific activity were analyzed with a protein sequencer. Among them, the N termini of five peptides in the fraction nos. 4, 33, 36, 61, and 81 were found to possess the structures DSGVT, IEAEGE, DAQEKLE, EELDNALN, and VPSIDDQEELM (**Table 2**). One peptide, DAQEKLE, showed the highest activity among these peptides. Acidic amino acids such as Asp or Glu were commonly detected in all of the sequences. These peptides were synthesized on the basis of the sequence of isolated five peptides in **Table 2**, and the antioxidant activity of these peptides was measured. All synthesized peptides possessed antioxidant activities (data not shown).

It is well-known that peptides that include basic amino acid residues such as His and Lys possess high antioxidant activities. This has been reported to be the case for VNPHDHQN,

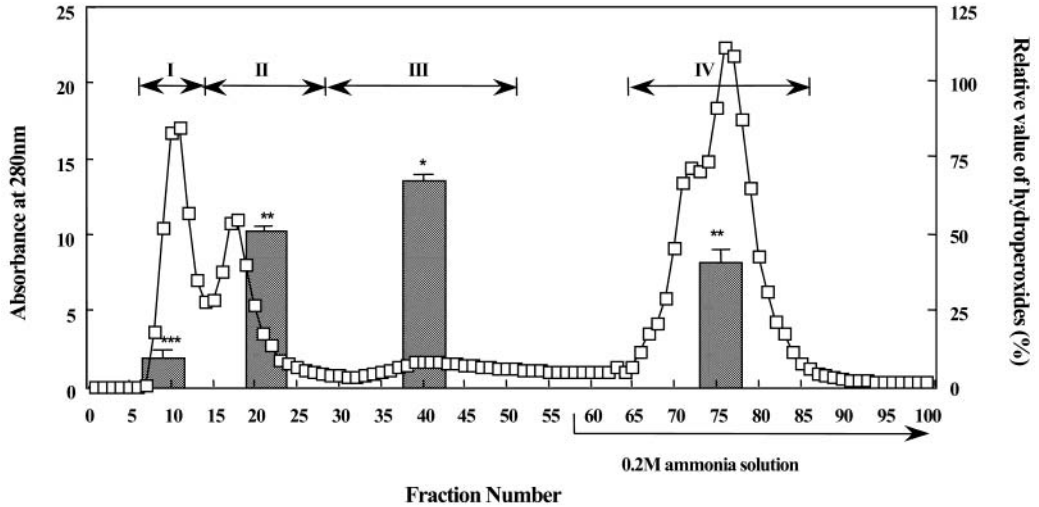

Figure 5. AG 50W-X2 column chromatography of the papain hydrolysate. The column was equilibrated and eluted with 0.05 M ammonium acetate (pH 6.0). Adsorbed peptides were eluted with 0.2 M ammonia solution (fraction no. 58∼). Antioxidant activity was measured by the ferric thiocyanate method. Final concentration of peptides was 0.2%. Bars represent a comparison of the antioxidant activity of each fraction. Error bars show the standard deviations. Values are the means of triplicate analyses. *, **, ***, significantly different from the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, with Student's *t*-test.

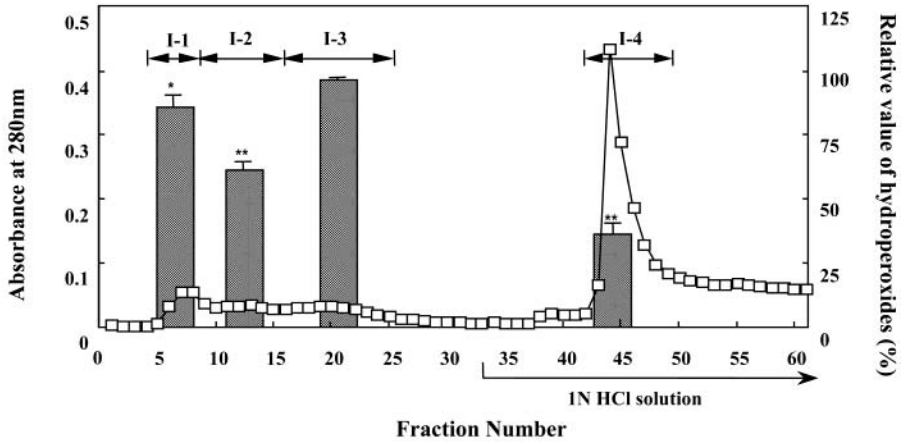

Figure 6. AG 1-X4 column chromatography of fraction I from AG 50W-X2 chromatography. The column was equilibrated and eluted with 0.05 M ammonium acetate (pH 3.5). Adsorbed peptides were eluted with 1 N HCl (fraction no. 33∼). Antioxidant activity was measured by the ferric thiocyanate method. The final concentration of peptides was 0.05%. Bars represent a comparison of the antioxidant activity of each fraction. Error bars show the standard deviations. Values are the means of triplicate analyses. *, **, ***, significantly different from the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, with Student's *t*-test.

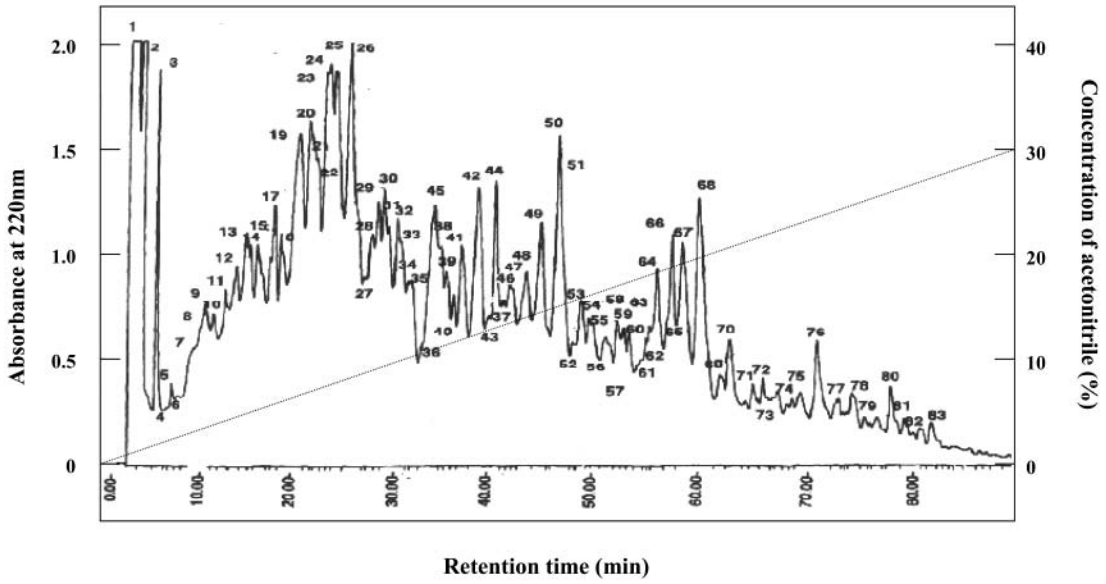

Figure 7. HPLC profile of fraction I-4 from AG 1-X 4 chromatography. Details are provided in Materials and Methods.

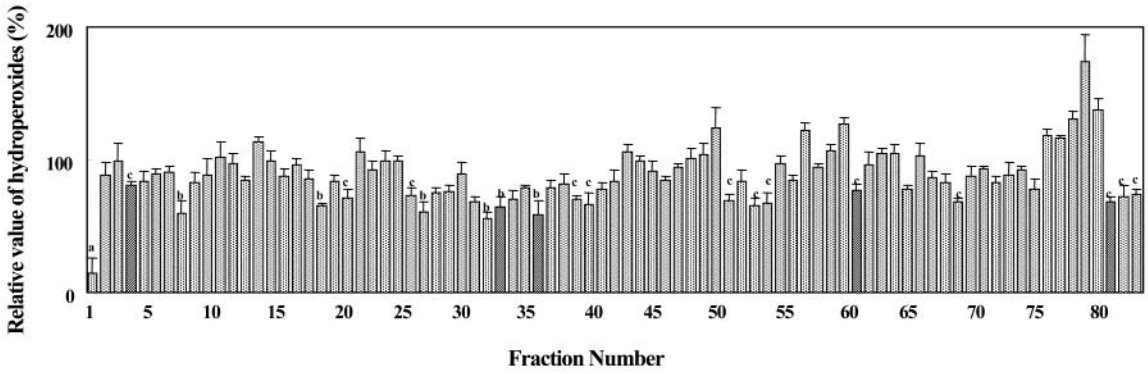

Figure 8. Comparison of the antioxidant activity of each peptide isolated by HPLC. Each fraction was dried in vacuo and reconstituted in the same volume of distilled water. Concentrations were less than 0.0001% (w/v). The amino acid sequences of the fractions indicated by dark bars were analyzed. Error bars show the relative standard deviation. Values are the means of triplicate analyses. a–c, significantly different from the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, with Student's *t*-test.

LVNPHDHQN, LLPHH, LLPHHADADY, and LNSGDALRVPSGTTYY, isolated from soybean hydrolysate (*13*), and AH, VHH, and VHHANEN from egg white albumin hydrolysate (*37*).

In this study, however, an acidic peptide fraction obtained by anion exchange column chromatography has also been shown to possess high antioxidant activity. The isolated peptides contained mainly not basic amino acids but mainly acidic amino

Table 2. Sequences of Antioxidant Peptides in Acidic Peptide Fraction I-4

peak nos.	MW	sequence	origin
4	650.3	Asp-Ser-Gly-Val-Thr	actin
33	646.4	Ile-Glu-Ala-Glu-Gly-Glu	*a*
36	832.5	Asp-Ala-Gln-Glu-Lys-Leu-Glu	tropomyosin
61	916.9	Glu-Glu-Leu-Asp-Asn-Ala-Leu-Asn	tropomyosin
81	1275.0	Val-Pro-Ser-Ile-Asp-Asp-Gln-Glu-Glu-Leu-Met	myosin heavy chain

[a] Peak no. 33 (Ile-Glu-Ala-Glu-Gly-Glu) was not matched with any proteins in the database.

acid residues. This study provides the first report that acidic peptides as well as basic peptides possess high antioxidant activity. As the isolated peptides except for no. 81 contained no Tyr or Met as well as no His and contained 5−11 amino acid residues, they seem to exhibit antioxidant activity due to the metal chelating activity of the acidic amino acid residues in the sequences. Also, antioxidant activity of peptide no. 81 containing Met residue seems to be caused by both the metal ion chelating activity of acidic amino acids and the action of Met.

Dietary intake of foods that contain large amounts of polyphenol compounds reduce the risk of cardiovascular disease and certain types of cancer (*38*). They are thought to suppress damage to human tissues such as the stomach and/or liver caused by radicals and active oxygen. Antioxidant peptides might also play an important role in preventing damage caused by oxidant compounds. Meat protein is thought to be a good resource from which to obtain antioxidant peptides, because the major constituent amino acids in porcine myofibrillar proteins are Glu, Asp, and Lys (14, 9.5, and 8%, respectively). These amino acids may interact with metal ions through their charged residues and inactivate oxidant activity of metal ions. Furthermore, because meat proteins, in general, contain essential amino acids and possess high availability for us, they also seem to be highly nutritious. Hydrolysates obtained by protease treatment might be used as a good source of antioxidants for use in meat products. The next study is to investigate whether the isolated antioxidant peptides possess the ability to prevent body tissues from oxidation, what quantity of peptides is necessary to produce a sufficient antioxidant activity in food, and what kind of effects on food taste are caused by addition of peptides.

ABBREVIATIONS USED

DPPH, 1,1-diphenyl-2-picrylhydrazyl; BHA, butyl hydroxyl anisole; BHT, butyl hydroxyl toluene.

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