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Novel Antioxidant Peptide Derived from the Ultrafiltrate of Ovomucin Hydrolysate

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ABSTRACT: The techno-functional properties of ovomucin as a gel-forming agent and its biological properties are well-known. The aim of the present study was to investigate antioxidant activity in ovomucin hydrolysate using radical scavenging assays. Electrophoresis showed that ovomucin isolated from whole egg was well separated. Ovomucin hydrolysis was carried out using microbial protease according to different incubation times. These ovomucin hydrolysates exhibited 85% antioxidant activity as measured by the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) assay after a 2 h incubation with protease and retained 90% activity until 24 h. At an incubation time of 4 h, the activity of ovomucin hydrolysates reached approximately 90%, corresponding to 115 μM gallic acid equivalent, regardless of the proteases used. The partially purified fraction of the hydrolysate by ultrafiltration and reverse-phase high-performance liquid chromatography was collected and then analyzed by liquid chromatography electrospray ionization mass spectrometry. Two peptides, LDEPDPL and NIQTDDFRT, in this fraction were identified. The antioxidant activities of these two synthesized peptides were measured to be 51.8 and 24.7% by the 2,2-diphenyl-1-picrylhydrazyl assay.

KEYWORDS: *egg white protein, ovomucin, antioxidant peptide, hydrolysis, ABTS assay, DPPH assay*

INTRODUCTION

Egg white or albumen, the substance surrounding the egg yolk, constitutes 60% of the total egg weight. It contains proteins such as ovalbumin, ovotransferrin, ovomucin, and lysozyme that are used as ingredients in food processing because of their functional properties.[1] Among these proteins, ovomucin, a glycoprotein representing approximately 2−4% of the total egg albumen protein, is a key component for maintaining the viscous nature of egg white.[2] The protein ovomucin is composed of an α-subunit and a β-subunit, connected by disulfide bonds. The molecular weights of these subunits are 220 and 400 kDa, and they contain 10−15 and 50−65% carbohydrate, respectively.[3,4]

The techno-functional effects, such as its foaming and emulsifying activities, of ovomucin have been reported to be excellent, but they depend upon viscosity and surface hydrophobicity.[5] In addition, these effects resulted in attenuation of hypocholesterolemia by suppressing cholesterol absorption and inhibiting the reabsorption of bile acids in the ileum, thus lowering serum cholesterol levels in rats and humans.[6−8] Ovomucin is also an antimicrobial agent against *Helicobacter pylori*.[9]

Furthermore, some studies have reported on the biological activities of peptides derived from ovomucin. Two highly glycosylated peptides (220 and 120 kDa) isolated from Pronase-treated ovomucin were shown to inhibit the growth of tumor cells,[10] and another peptide (70 kDa) derived from the α-subunit after Pronase treatment was also shown to exhibit antitumor activity.[11] Other studies on bioactive peptides were reported by Majumder and Wu,[12,13] who isolated peptides exhibiting inhibitory activity against angiotensin I-converting enzyme (ACE) from the simulated gastrointestinal digestion of proteins contained in cooked egg. Hydrolysates of ovotransferrin have also been shown to exert an inhibitory effect on ACE.[14,15] The hydrolysate of ovalbumin also has an antihypertensive effect.[16]

Among the various bioactive peptides derived from food proteins, those having antioxidant properties are important because free radicals produced by oxidative reactions might result in different disorders such as diabetes mellitus, arthrosclerosis, cancer, and coronary heart disease,[17−19] in addition to causing allergies and aging[20−22] due to damage to cellular components.[23,24] Antioxidants are chemical compounds that are capable of inhibiting oxidative reactions and play a role in the destructive activity of free radicals. Although the artificial antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) is effective, its use is limited and not sufficient to prevent these oxidative damages, however, the antioxidant agents (or peptides) obtained from food protein are provided as a supplement to inhibit oxidative reactions and to protect the human body against free radical damage.[25,26]

Antioxidant peptides have been identified from hen egg white lysozyme hydrolysate by hydrolysis with papain, trypsin, and a combination of these two enzymes[26] as well as from ovotransferrin hydrolysate following enzyme treatment.[27] Thus far, antioxidant activity has not been reported in the case of

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ovomucin or its hydrolysate. It is of interest to identify novel antioxidant peptide from ovomucin or its hydrolysate. Thus, the aim of the present study was to investigate the antioxidant activity of ovomucin hydrolysate obtained after microbial protease treatment and to identify the antioxidant peptides derived therefrom.

MATERIALS AND METHODS

Ovomucin Preparation. Egg was obtained from the market in Korea. Egg white protein ovomucin was separated from whole egg according to the method previously described by Omana and Wu[28] with minor modifications. First, egg white was obtained from whole egg using an egg separator and then homogenized by stirring for 30 min at 22 °C. After adding 3 volumes of 0.1 M NaCl, the pH of the egg white solution was adjusted to 6.0 using 0.1 N HCl with stirring for 30 min at 22 °C. The egg white solution was then incubated overnight at 4 °C and subsequently centrifuged at 15300*g* for 10 min at 4 °C. The precipitate obtained was solubilized in 0.5 M NaCl, stirred for 4 h after the pH had been adjusted to 6.0, and then incubated overnight at 4 °C. The egg white solution was then centrifuged at 15300*g* for 10 min at 4 °C. The precipitate obtained was then dialyzed for 24 h and lyophilized. This sample was stored at −20 °C.

Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) was performed as previously described[29,30] using a 5% stacking gel and a 15% separating gel.

Ovomucin Hydrolysis. The purified ovomucin was directly subjected to hydrolysis by treatment with the microbial proteases Protamex, Flavourzyme, and Alcalase, which were purchased from Novozymes (Bagsvaerd, Denmark). A 2 mg/mL ovomucin solution was prepared in 10 mM sodium phosphate buffer, pH 7 for Protamex and Flavourzyme and pH 8 for Alcalase, and the enzyme-to-substrate ratio was 2.5/1. The reaction mixtures were incubated at 50 °C. Enzymatic hydrolysis was stopped by heating for 5 min in boiling water, and an aliquot was retrieved immediately at each incubation time (2, 4, 6, and 24 h). Samples were filtered through 0.45 μm filters (Whatman, Kent, UK) and stored at −20 °C.

Antioxidant Activity: ABTS Radical Scavenging Assay. $ABTS^{\bullet+}$ (Sigma-Aldrich, St. Louis, MO, USA) radical scavenging activity of the ovomucin hydrolysate was determined, according to a previously described method with minor modifications.[31,32] The ABTS and potassium persulfate were prepared at 7 and 2.45 mM final concentrations, respectively, and stored overnight in the dark at room temperature. The $ABTS^{\bullet+}$ radical solution was adjusted to an absorbance of 0.70 ± 0.02 at 734 nm on a spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) by dilution with distilled water. The reaction mixture was prepared to be 1 mL containing 950 μL of the $ABTS^{\bullet+}$ radical solution plus 50 μL of ovomucin hydrolysate. Absorbance was measured at 734 nm after 30 min of incubation at 30 °C in dark conditions. All assays were performed in triplicate, and the values represent the means. Using various concentrations (0, 1, 20, 40, 60, 80, 100, 120, and 150 μM) of gallic acid, a standard curve was prepared. The antioxidant scavenging activity expressed as percent of ovomucin and its hydrolysates was calculated according to method previously described[32] and also expressed as a μmoles gallic acid equivalent (GE) calculated from equation of standard curve.

Antioxidant Activity: DPPH Radical Scavenging Assay. Another method to measure antioxidant activity is to use 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH, Sigma-Aldrich). The DPPH assay was carried out according to a method previously described,[33] with minor modifications. A 0.2 mM DPPH solution was prepared in methanol. An aliquot of the sample was mixed with an equal volume of DPPH solution in a 96-well clear flat-bottom microplate (SPL, Gyeonggi-Do, Korea) and placed for 30 min in dark conditions. The absorbance was measured at 517 nm by using a microplate reader (Molecular Devices). All assays were carried out in triplicate; the reported values represent the means. A standard curve was prepared using various concentrations (0−30 μM) of gallic acid. The percentage of antioxidant scavenging activity was calculated as follows: scavenging activity (%) = $\{1 - [(A_{sample} - A_{blank})/A_{control}]\} \times 100$, where $A_{control}$ represents the initial DPPH absorbance. A_{blank} represents the methanol absorbance.

Fractionation of Hydrolysates. Fractions with a molecular weight cutoff of 3 kDa were separated using an ultracentrifugal filtration membrane system (Millipore, Billerica, MA, USA). To collect each fraction of ovomucin hydrolysate in this <3 kDa fraction, the sample was passed through an analytical column ZORBAX 300SB-C18 (3.0 × 150 mm, diameter = 3.5 μm, Agilent, Santa Clara, CA, USA) connected to a high-performance liquid chromatography (HPLC) system (Jasco, Easton, MD, USA). Fifty microliters of sample was injected into the column and eluted at room temperature using a linear gradient of 5−50% of solvent B (100% acetonitrile) within 15 min; solvent A consisted of 10 mM sodium phosphate buffer (pH 8.0). The flow rate was 0.25 mL/min, and peptides were detected by UV absorption at 215 nm. Fractions were collected using a fraction collector (Bio-Rad, Hercules, CA, USA) at 5 min intervals.

Peptide identification by LC-ESI-MS/MS. Mass analysis was performed at the National Instrumentation Center for Environmental Management (NICEM) of the Seoul National University in Korea. This experiment using nano liquid chromatography−electrospray-mass spectrometry (nano LC-ESI-MS/MS) system carried out according to the method previously described.[32] This MS system consisted of an autoswitching nano pump, autosampler (Tempo nano LC system; MDS SCIEX, Concord, ON, Canada), and a hybrid quadrupole-time-of-flight (TOF) mass spectrometer (QStar Elite; Applied Biosystems, Foster City, CA, USA) equipped with a nanoelectrospray ionization (ESI) source and fitted with a fused silica emitter tip (New Objective, Woburn, MA, USA). Sample of 2 μL of fraction injected and were first trapped on a ZORBAX 300SB-C18 trap column (300 μm i.d × 5 mm, 5 μm particle size, 100 Å pore size, Agilent Technologies, part 5065-9913) equilibrated for 6 min with 98% solvent A [water/acetonitrile (98:2, v/v), 0.1% formic acid] and 2% solvent B [water/acetonitrile (2:98, v/v), 0.1% formic acid] at a flow rate of 5 μL/min, and then separated on a ZORBAX 300SB-C18 capillary column (75 μm i.d × 150 mm, 3.5 μm particle size, 100 Å pore size, part 5065-9911) at a flow rate of 300 nL/min. A linear gradient of 2 to 35% solvent B over 30 min was performed and then from 35 to 90% over 10 min, followed by 90% solvent B for 5 min, and finally 5% solvent B for 15 min. The resulting peptides were electrosprayed through a coated silica tip (FS360-20-10-N20-C12; PicoTip emitter, New Objective) at an ion spray voltage of 2000 eV. Mass data were acquired automatically using Analyst QS 2.0 software (Applied Biosystems) with the 200−2000 range of m/z.

Statistical Analysis. Data were analyzed by ANOVA followed by Tukey's Honestly Significant Difference (HSD) Test using Statistical Analysis System software (SAS version 9.13, SAS Institute, Cary, NC, USA). Significant differences were set at a 5% level ($P < 0.05$).

RESULTS AND DISCUSSION

Egg white hydrolysate or egg proteins are known to possess antioxidant activity. Egg white protein hydrolysate derived from treatment with gastrointestinal enzymes also exhibits antioxidant properties.[34,35] We have previously observed in our laboratory that among the egg white proteins such as ovotransferrin, ovalbumin, lysozyme, and ovomucin, egg white ovomucin (2 mg/mL) exhibited the highest radical scavenging activity, with approximately 30% greater antioxidant activity under the same conditions in comparison to the others (personal communication). Ovomucin is therefore an attractive candidate for the identification of an antioxidant peptide, because there is no such report of an antioxidant peptide from ovomucin.

Ovomucin was separated according to a method described by previous authors.[28] To verify whether ovomucin from whole egg obtained from the market in Korea is well isolated, SDS-PAGE (Figure 1) was selected. As shown in Figure 1, the α-

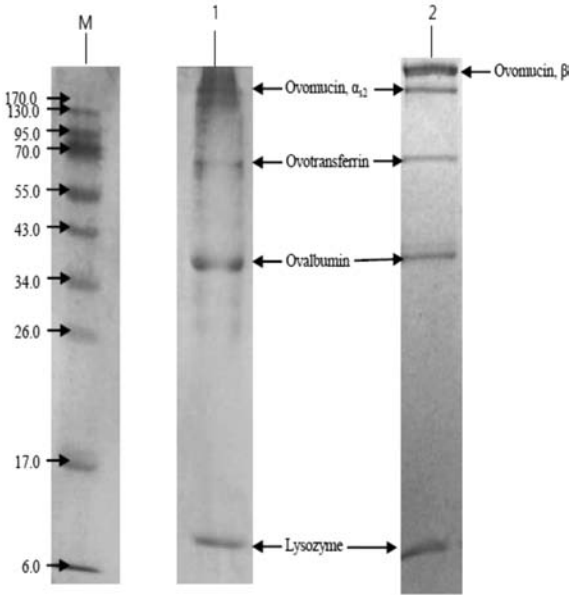

Figure 1. SDS-PAGE patterns of ovomucin prepared from whole egg. Lanes: (M) standard marker; (1) ovomucin separated in present study; (2) ovomucin separated by Omana and Wu.[27].

subunit form of ovomucin (~210 kDa) was well separated, in comparison to the results of Omana and Wu.[28] Although the other egg white proteins (ovotransferrin, ovalbumin, and lysozyme) were examined in the study, this fraction containing ovomucin was chosen for enzymatic digestion. Through MS analysis, we were able to identify the protein from which each hydrolyzed peptide was derived.

Enzymatic Digestion of Ovomucin and Antioxidant Activity Assay. Hydrolysis of ovomucin by microbial proteases such as Protamex and Flavourzyme was accomplished at 50 °C, pH 7, with various incubation times (2, 4, 6, and 24 h). The antioxidant activities of the hydrolysates were measured directly by evaluating their ABTS free radical scavenging activity (Table 1). From the equation of the standard curve (y = 0.7539x + 4.0707, with an R value of 0.9912), the IC_{50} value of gallic acid was 62 μM.

Color interference was generated when the DPPH solution mixed with ovomucin hydrolysates. Thus, ABTS assay was applied in the present study.

The effect of radical scavenging improved according to reaction times (Table 1). The antioxidant activity of untreated ovomucin was 16.6 and 17.3%. Its activity improved to 85.4 and 85.9% after 2 h of treatment with Protamex and Flavourzyme and remained nearly unchanged until 24 h. After 4 h of digestion with Protamex and Flavourzyme, the antioxidant activities were 90.4 and 89.4%, respectively.

Lysozyme remained contained in our separated fraction, and it was known as the antioxidant protein. In relation to this, one paper was cited as following because of the same hydrolysis time and same assay to test antioxidant in the present study. Memarpoor-Yazdi et al.[26] have reported that lysozyme hydrolysate obtained by a 2 h treatment showed relatively high antioxidant activities, which were 37.2, 50.4, and 64.2% for trypsin, papain, and trypsin plus papain treatment, respectively, in the DPPH radical assay. Their trolox equivalent (TE)/mg protein values in the ABTS free radical assay showed 1.91, 2.57, and 2.82 μM for trypsin, papain, and trypsin plus papain hydrolysates, respectively.[26] However, it is necessary to confirm through MS analysis because of incomplete purification of ovomucin in separated fraction. We discuss this further under Mass Spectrometry Analysis.

The antioxidant activity of ovomucin was already high at 2 h regardless of the enzyme selected. This could be explained by the fact that the enzyme used reacts sensitively to the ovomucin substrate. This observation is similar to that reported by Hiidenhovi et al.,[36] who found that the degree of hydrolysis of ovomucin hydrolyzed by 10 enzymes, including Protamex, Flavourzyme, and Alcalase, was high after 1 h of reaction. Thus, ovomucin appears to be easily degraded by enzymes. We fixed the reaction time at 4 h because the activity was high at this time. Under the same conditions, ovomucin was hydrolyzed by Protamex, Flavourzyme, and Alcalase. As shown in Table 2, the free radical scavenging activity of ovomucin hydrolysates was high, at 90.4, 89.4, and 88.6%, for Protamex, Flavourzyme, and Alcalase treatment, respectively. On the contrary, the activity of untreated ovomucin was 17.3%. The GE values of ovomucin hydrolysates (115.9, 114.6, and 113.4 μM GE) were approximately 6-fold higher than that of untreated ovomucin (18.9 μM GE).

Indeed, some papers[37−39] postulated that the antioxidant activity of protein hydrolysates is dependent on their amino acid composition, for example, acidic (Asp and Glu) and basic (Arg and Lys) amino acids. That finding may be attributed to the fact that these amino acids play an important role in metal ion chelation.[40,41] Protein source and hydrolysis conditions such as enzyme, temperature, pH, reaction time, and enzyme-to-substrate ratio can affect their amino acid compositions.[42] In addition, they also showed high ion chelating activity and antioxidant activity. Thus, Memarpoor-Yazdi et al.[26] reported that these amino acids existing within the hydrolysate could be effective in antioxidant activity.

Peptide Fractionation and Investigation of Antioxidant Activity. For further fractionation, ultracentrifugal filtration was performed to retrieve the <3 kDa fraction, and

Table 1. ABTS Free Radical Scavenging Activities of Ovomucin Hydrolysates after Different Reaction Times with Microbial Proteases[a]

		Ovo	2 h	4 h	6 h	24 h
1	A_{734}	0.645 ± 0.008a	0.163 ± 0.032b	0.127 ± 0.008c	0.117 ± 0.004c	0.112 ± 0.005c
	activity[b] (%)	16.6	85.4	90.4	91.9	92.7
2	A_{734}	0.638 ± 0.005a	0.157 ± 0.006b	0.132 ± 0.004c	0.125 ± 0.003c	0.129 ± 0.000c
	activity[b] (%)	17.3	85.9	89.4	90.4	89.9

[a]Mean values in rows show statistically significant differences in Tukey's Studentized Range Test ($P < 0.05$). Ovo, ovomucin; 1, Protamex treatment; 2, Flavourzyme treatment. [b]ABTS free radical scavenging activity (%) = $\{(A_{control} - A_{sample})/A_{control}\} \times 100$, where $A_{control}$ represents the initial ABTS absorbance.

Table 2. Antioxidant Activities Using the $ABTS^{+\cdot}$ Radical Cation of Ovomucin Hydrolysates after 4 h of Reaction with Microbial Proteases[a]

	Ovo	1	2	3
A_{734}	0.638 ± 0.005a	0.127 ± 0.008b	0.132 ± 0.004b	0.138 ± 0.014b
activity[b] (%)	17.3	90.4	89.4	88.6
GE (μM)	18.9	115.9	114.6	113.4

[a]Mean values in rows show statistically significant differences in Tukey's Studentized range test ($P < 0.05$). GE, gallic acid equivalent, calculated from the standard curve equation; Ovo, ovomucin; 1, Protamex treatment; 2, Flavourzyme treatment; 3, Alcalase treatment. [b]ABTS free radical scavenging activity (%) = $\{(A_{control} - A_{sample})/A_{control}\} \times 100$, where $A_{control}$ represents the initial ABTS absorbance.

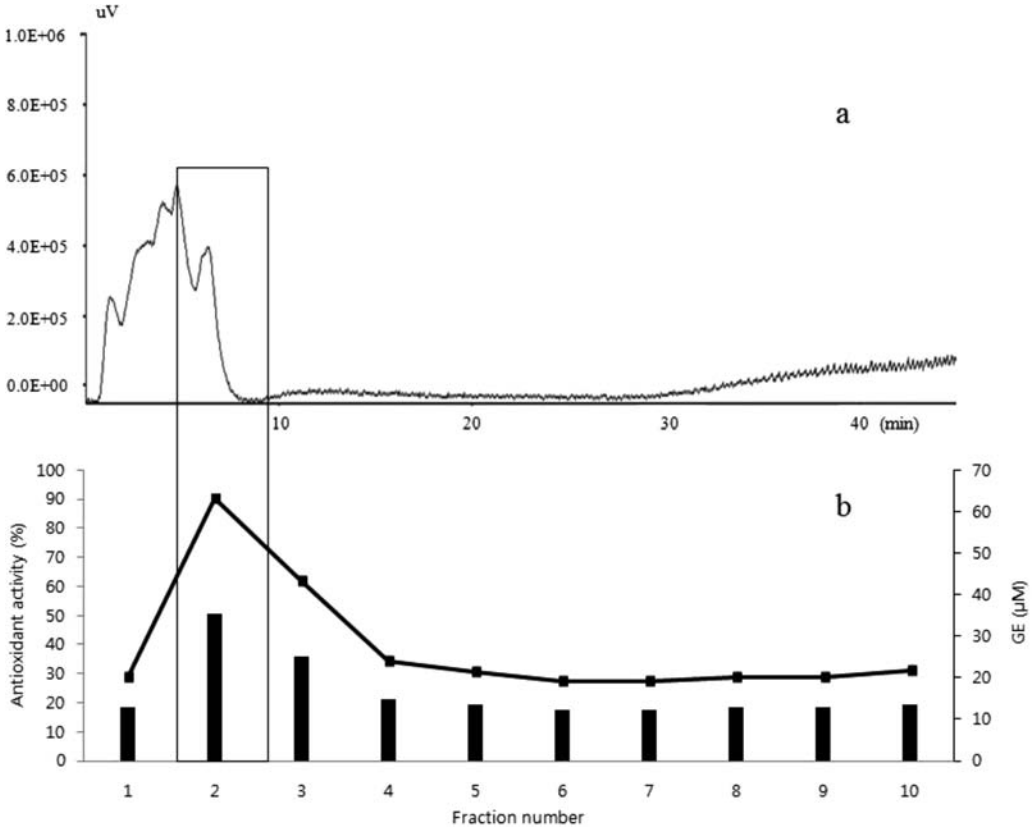

Figure 2. RP-HPLC chromatogram for the ultrafiltrate obtained using a 3 kDa membrane from ovomucin hydrolysate generated by hydrolysis with Protamex (a) and its ABTS free radical scavenging activity (b). The rectangle indicates the F2 fraction containing the peptides LDEPDPL and NIQTDDFRT, which has the highest activity of all fractions collected. The graph bar chart and line chart represent the percentage and gallic acid equivalent (GE) values of antioxidant activity, respectively. Antioxidant activity was evaluated and expressed as percent and GE value.

then the permeate was injected into a ZORBAX 300SB-C_{18} column to verify whether the permeate contains the peptides. The separated profiles showed numerous peaks indicating that ovomucin was well degraded. Fractions of the peaks were collected to verify their antioxidant activity. No antioxidant activity was measurable in the ABTS assay in the presence of 0.1% trifluoroacetic acid (TFA) in the fraction. This lack of antioxidant activity in this study (data not shown) may be due to changes in the amino acid charges of the peptide generated in the acid environment of the fraction by TFA. Therefore, we changed solvent A to 10 mM sodium phosphate buffer (pH 8.0) and solvent B to acetonitrile without TFA. Consequently, the profile showed that the main peaks eluted at an early elution time, within 15 min (Figure 2a). The antioxidant activities in all of the fractions collected at 5 min intervals corresponding to this peak are represented in Figure 2b. The fractions were numbered sequentially. As shown in Figure 2b, the range of GE values measured using the ABTS assay was 19.1−63.1 μM GE for ovomucin hydrolysates after ultrafiltration using a 3 kDa membrane. This corresponds to 17−50% radical scavenging activity. Among all of the fractions collected on RP-HPLC, the activity was the highest in the F2 fraction, at approximately 50%, followed by the F3 fraction showing approximately 35% activity. Their GE values correspond to 63.1 and 43.4 μM, respectively. However, the other fractions showed similar antioxidant activity. The F1 fraction showed weaker antioxidant activity than F2 despite having the same profile on HPLC. In addition, the F3 fraction did not exhibit a large peak compared to the F1 fraction, but its antioxidant activity was stronger than that of the F1 fraction. This phenomenon could be explained by the presence of some salt in the F1 fraction.

Identification of Peptides Generated from Ovomucin. To identify which peptides have antioxidant activity in the F2 fraction, we performed MS analysis via LC-ESI-Q-TOF. Two peptides, LDEPDPL (f686−692) and NIQTDDFRT (f539−

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Table 3. Radical Scavenging Activity of Synthesized Peptides Identified by MS Analysis in the F2 Fraction Collected after RP-HPLC[a]

	ABTS		DPPH	
	1	2	1	2
absorbance	0.534 ± 0.002b	0.659 ± 0.002a	0.220 ± 0.004b	0.344 ± 0.012a
activity[b] (%)	19.7	1.0	51.8	24.7

[a]Fractions were collected following RP-HPLC of the ultrafiltrate obtained using a 3 kDa cutoff membrane from the 4 h hydrolysate with microbial protease treatment. Mean values in rows for each assay show statistically significant differences in Tukey's Studentized range test ($P < 0.05$). Absorbance: A_{734} for ABTS assay, A_{517} for DPPH assay. 1, peptide LDEPDPL; 2, peptide NIQTDDFRT. [b]ABTS assay (%) = $\{(A_{control} - A_{sample})/A_{control}\} \times 100$, where $A_{control}$ represents the initial ABTS absorbance, DPPH assay (%) = $\{1 - [(A_{sample} - A_{blank})/A_{control}]\} \times 100$, where $A_{control}$ represents the initial DPPH absorbance. A_{blank} represents methanol absorbance.

547), from the ovomucin α-subunit were identified with high accuracy. This indicates that the antioxidant activity in the hydrolysate of ovomucin separated in the present study resulted from ovomucin and not from other proteins (ovotransferrin or lysozyme).

To investigate whether the peptides identified by MS analysis have antioxidant activity, these two peptides were synthesized and the ABTS assay was carried out. As shown in Table 3, the amino acid sequence of ovomucin peptide 1 was LDEPDPL (f686−692), and this showed higher activity than that of peptide 2, fragment NIQTDDFRT (f539−547). Their activities were 19.7% (corresponding to 22 μM GE) and 1%, respectively. However, this activity (19.7%) of peptide 1 was weaker than the 50% activity of the F2 fraction (Figure 2b). This observation might be because some other substance is present in the F2 fraction, because the amino acid composition of peptide 1 was affected by the ABTS reagent or because the antioxidant activity of <3 kDa during elution (separation process) on HPLC was lost.[26,42,43]

In addition, NIQTDDFRT (f539−547) had almost no activity, unlike the F3 fraction (35% activity) as mentioned above (Figure 2b). This could be due to the nature of the compounds in the sample. Therefore, we used a different assay method, the DPPH assay, which is a simple and inexpensive method to test antioxidant activity. Table 3 shows that the peptide LDEPDPL (f686−692) exhibited antioxidant activity of 51.8% (corresponding to 9.9 μM GE) as determined by the DPPH assay. On the contrary, the peptide NIQTDDFRT showed radical scavenging activity of 24.7% (corresponding to 4.1 μM GE), unlike that seen in the ABTS assay.

The amino acid compositions, for example, acidic and basic or hydrophobic, of LDEPDPL and NIQTDDFRT are different, and this affects their antioxidant properties.[40,43−45]

The peptide LDEPDPL contains two hydrophobic (Leu) residues and three acidic (2 Asp and 1 Glu) residues. Byun et al.[44] and Gimenez et al.[45] have reported that acidic and basic amino acid residues play a role in metal ion chelating activity, which is related to antioxidant activity. The presence of hydrophobic amino acid residues increases radical scavenging activity, as reported by Rajapakse et al.,[40] Ren et al.,[43] and Chen et al.[46] Thus, the peptide LDEPDPL could be effective in radical scavenging activity. This result is supported by the results of Sarmadi and Ismail,[19] who reported that aromatic amino acids, which belong to the class of hydrophobic residues, can donate protons to electron-deficient radicals and therefore enhance radical scavenging activity.

A similar result was reported by Memarpoor-Yazdi et al.,[26] who found the peptide NTDGSTDYGILQINSR obtained from egg white hydrolysates through MALDI-TOF MS analysis. This peptide, NTDGSTDYGILQINSR, which contains one basic (Arg) amino acid residue and two acidic (Asp) amino acid residues, showed radical scavenging activity. Similar results were also obtained by Shen et al.[27] They identified two novel tetrapeptides, WNIP and GWNI, from ovotransferrin hydrolysate generated by thermolysin treatment; each of these also contain two hydrophobic (Trp and Ile) and one acidic (Asn) amino acid residue, indicating that these amino acid residues are responsible for high antioxidant activity. Common characteristics reported by Shen et al.,[27] Ren et al.,[43] and Chen et al. [46] show that nonpolar amino acids (Pro and Gly) except for the amino acid residues being hydrophobic and nonpolar were contained in the peptides identified. The peptide in this study, LDEPDPL, contains two nonpolar amino acids, Pro. This indicates the peptide containing nonpolar amino acid residues along with hydrophobic residues could be effective for antioxidant activity. On the contrary, peptide NIQTDDFRT in the present study showed low free radical scavenging activity, even if the peptide NIQTDDFRT contains also the hydrophobic (Ile and Phe) and acidic (two Asp) residues as peptide LDEPDPL. The difference between the two peptides was the absence of nonpolar amino acid residues such as Pro, Cys, and Gly. However, there is no nonpolar amino acid in the case of peptide NIQTDDFRT, whereas the Pro residue is contained in the case of peptide LDEPDPL. Another difference was the basic amino acid. Unlike peptide LDEPDPL, the peptide LDEPDPL contains one basic (Arg) amino acid residue. Ren et al.[43] have reported that basic peptides had greater capacity to scavenge hydroxyl radical than acidic or neutral peptides. Even though this peptide, NIQTDDFRT, contains one basic (Arg) amino acid residue, its free radical scavenging property could not be affected in the present study. Thus, the low free radical scavenging activity of NIQTDDFRT could be due to the absence of nonpolar amino acids, despite its ion-chelating activity and hydroxyl radical scavenging activity due to one basic and two acidic acid residues. The antioxidant activity is related to ion chelation, radical scavenging, and lipid peroxidation inhibition.[19]

There have been no previous reports of the antioxidant activity of these peptides from ovomucin hydrolysates. In the present work, however, we identified a novel antioxidative peptide from ovomucin hydrolysate after hydrolysis using microbial proteases, as was our objective. Further investigation into the antioxidant activity of such peptides is an attractive line of research for the potential application of such peptides in the food industry as antioxidant agents or food additives.

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Notes

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■ ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl; GE, gallic acid equivalent; ESI, electrospray ionization; TOF, time of flight; MS, mass spectrometry

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