MECHANISM INVOLVED IN ANTIOXIDATION OF PEPTIDES IN PORCINE MYOFIBRILLAR PROTEIN HYDROLYSATE

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Background

The peroxidation of lipids and fatty acids affects quality of foods. In meat products, the peroxidation causes deterioration of meat quality and limits their shelf-life. Similarly, in cooked meat the peroxidation produces off-flavor or warmed-over flavor during storage[1]. Protection of lipids and fatty acids from peroxidation is, therefore, very important.

To control lipid peroxidation, various antioxidants have been synthesized or found out from bioresources. Alfa-Tocopherol (Vitamin E) and beta-carotene are very famous natural antioxidants. Recently, soybean protein hydrolysates have been reported as antioxidant substances .[2] The antioxidant mechanism of peptides have been suggested to be due to a combination of metal-ion chelation, free radical scavenging and singlet oxygen quenching, which were caused by His residue.[3] On the other hand, there was little information on antioxidation of meat protein hydrolysates obtained by protease treatment. We have found porcine myofibrillar protein hydrolysates possessed antioxidant activity on linolenic acid peroxidation and isolated antioxidant peptides from its hydrolysate[4].

Objectives

In this study, we synthesized several peptides on the basis of the structure of antioxidant peptides from porcine myofibrillar protein hydrolysates by papain, and evaluated their antioxidant activities.

Methods

Preparation of Myofibrillar Protein Hydrolysate.

Myofibrillar proteins were prepared according to the method reported by Yang et al.[5] These proteins (10mg/ml) were incubated with papain, (1/100) at 37 degrees C, pH7.0 for 24h. After adding ethanol (final conc.; 80%) to remove un-hydrolyzed proteins, this solution was centrifuged at 2000×g for 10min, and then ethanol in its supernatant being removed by evaporation at 45 degrees C.

Peroxidation System.

We used this system reported by Chen et al [2] with slight modification. Linolenic acid (10mg) was mixed with 20 microliter of 10mM $FeCl_2$ (as accelerator of oxidation, final conc.; 0.05mM) in 0.2 M K-phosphate buffer (pH7.0) containing Triton X-100 (1%, w/v), then the total volume of this mixture was adjusted to 4 mL with distilled water. A part of mixture was taken into a glass test tube and heated at 80 degrees C for 60min.

Measurement of Antioxidative Activity.

The measurement of antioxidative activity was performed by the ferric thiocyanate method. The antioxidant activity was obtained from the subtraction of values at 0 and 60 min.

Peptide Syntheses.

Peptides were synthesized by the fluorenylmethoxycarbonyl (Fmoc) -strategy using a simultaneous multiple peptides synthesizer (model PSSM-8; Shimadzu, Kyoto, Japan). After being synthesized, peptide were purified on a HPLC using an ODS column (PEGASIL-300, 20×250 mm; Senshu, Tokyo, Japan) with linear gradient from 0 to 50% CH_3CN containing 0.1% trifluoroacetic acid in 100 min (flow rate; 5.0mL/min, monitoring; 220nm). Furthermore, molecular masses of isolated peptides were determined by mass spectrometry.

Result and discussion

Effect of peptide length on antioxidant activities.

Five peptides possessing antioxidative activity were isolated from porcine myofibrillar protein hydrolysate using ion-exchange column chromatography and reversed-phase HPLC. Their N-terminal amino acid sequences were analyzed with a protein sequencer (Hewlett Packard, USA). On the basis of these structures, various peptides were synthesized and characterized.

Among them, 2 kinds of acidic peptides (IEAEGE (No.33) and DAQEKLE (No.35)) exhibited strong antioxidant activity. To clarify the

relationship between peptide length and antioxidant activity, the peptides, whose amino acid was deleted from C-terminus or N-terminus, were synthesized. As shown in Fig. 1, antioxidant activity was gradually decreased as peptide length was shortened. Carnosine (beta-alanyl L-histidine) is known to be one of the antioxidative peptide. An antioxidant activity of the modified carnosine with a long hydrophobic chain at N-terminus is reported to be higher than that of intact one. Hydrophobicity seems to play an important role in antioxidant activity, because it increases accessibility of antioxidative peptide towards fatty acids.

Effect of charged amino acids on antioxidative peptides.

Antioxidative acidic peptides isolated from papain hydrolysate contained two or three acidic amino acids (Asp and Glu) in their sequences. In the oxidation system of this study, Fe (II) was used to accelerate the lipid peroxidation. Acidic amino acids are charged negatively in the neutral pH region. So we assume that anion of acidic amino acid interacts with cation in Fe(II) and inactivates prooxidant, Fe(II). Indeed, the replacement of Glu to Ala in IEAEGE (No.33) caused weakening antioxidative activity of its peptide (Fig.2). The replacement of Lys, Asp and Glu to Ala in DAQEKLE (No.35) slightly lowered its antioxidative activity. This result suggests that charge as well as length of peptides may influence their antioxidant activity.

Conclusion

The relationship between the structure and activity was examined by using peptides synthesized on the basis of the structures of antioxidative peptides in porcine myofibrillar protein hydrolysate. It is concluded that the length and charge of peptides play an important role in its antioxidative activity.

Reference

1) Tims, M.J. *et.al.* Protection of cooked meats with phosphates. Food Technol. **12;** 240-243(1958)

2) Chen, H.M. *et.al.* Structural analysis of antioxidative peptides from soybean B-conglycinin. J. Agric. Food. Chem. **43**; 574-578 (1995)

3) Chen, H. *et.al.* Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. J. Agric. Food. Chem. **44**; 2619-2623 (1996)

4) Saiga, A. *et.al.* Antioxioxidative peptides in porcine myofibrillar protein hydrolysates by protease treatment. in ACS symposium (Hawaii) (2000)

5) Yang, Ryung. *et.al.* Studies on myofibrils from the stored muscle part I. **34**; 1765-1772 (1970)

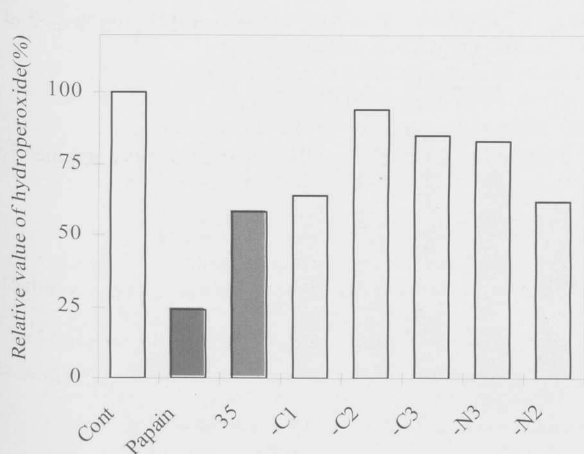

Fig.1. Effect of peptide length on antioxidant activity
Cont;Dw, Papain; hydrolysate by papain, 35;DAQEKLE ,-C1;DAQEKL, -C2;DAQEK, -C3;DAQE, -N3;EKLE, -N2;QEKLE

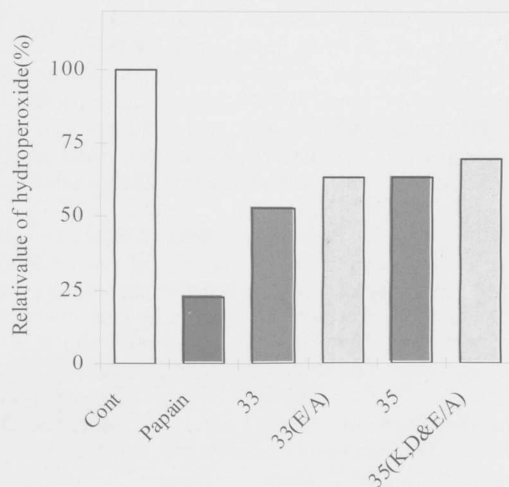

Fig.2. Effect of charged amino acids on antioxidant activity
Cont;Dw, Papain; hydrolysate by papain, 33;IEAEGE, 33(E/A);I AAAGA, 35;DAQEKLE, 35(K,D&E/A);AAQAALA