

**Studies on functional properties of peptides derived from meat proteins
-Antioxidant and antihypertensive activities**

Ai SAIGA

*Graduated School of Biosphere Science, Hiroshima University,
Higashi-Hiroshima 739-8528, Japan*

The morbidity rate of lifestyle-related disease such as coronary heart diseases, cerebral stroke and cancers has been reported to increase gradually. The oxidations of biomolecules and/or hypertension are thought to be main triggers of these diseases. To prevent from them, the regulatory compounds for physiological function in our body have been surveyed from food materials. Recently, peptides derived from proteins in foods have been shown to regulate physiological functions in the alimentary, neural, and circulation systems. Meat is a good source for investigation of functional peptides, because its protein content is very high (about 20% in wet weight).

This study was performed to survey and clarify the antioxidant and antihypertensive activities of peptides derived from meat proteins.

Chapter I; Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment

Hydrolysates were obtained by two kinds of protease treatment (papain or actinase E). The antioxidant activities of the hydrolysates were measured in a linolenic acid peroxidation system induced by Fe (II). At all concentrations (0.02-2% addition), the hydrolysate obtained by treatment with papain (papain hydrolysate) exhibited higher activity than the hydrolysate obtained by treatment with actinase E (actinase hydrolysate). The major constitutional amino acids of both hydrolysates were Glx, Asx, and Lys, however, the hydrophobic amino acid content in the papain hydrolysate was greater than in the actinase hydrolysate. It has been reported that the hydrophobicity of compounds affected the antioxidant activity, that is, solubility in lipid. Therefore, papain hydrolysate may exhibit the strong antioxidant activity than actinase hydrolysate. Both hydrolysates showed higher activities at pH 7.1 than at 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 DPPH radical scavenging activity and chelating activity toward metal ions. Transition metals catalyze the generation of reactive oxygen species and accelerate the lipid peroxidation chain reaction. The charged amino acids in both hydrolysates may affect their metal chelating activity.

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 fraction. Antioxidant peptides in the acidic fraction were isolated by HPLC on an ODS column and shown to possess the structures,

Asp-Ser-Gly-Val-Thr, Ile-Glu-Ala-Glu-Gly-Glu, Asp-Ala-Gln-Glu-Lys-Leu-Glu, Glu-Glu-Leu-Asp-Asn-Ala-Leu-Asn, and Val-Pro-Ser-Ile-Asp-Asp-Gln-Glu-Glu-Leu-Met. The Asp-Ala-Gln-Glu-Lys-Leu-Glu peptide showed the highest activity among these peptides.

The characterized peptides were synthesized based on these sequences. Among them, 2 kinds of acidic peptides, Ile-Glu-Ala-Glu-Gly-Glu and Asp-Ala-Gln-Glu-Lys-Leu-Glu showed strong antioxidant activity. The relationship between peptide length and antioxidant activity was investigated using synthesized peptides. Antioxidant activity was gradually decreased when peptide length was shortened. Replacement of charged amino acids to Ala in these peptides also affected their antioxidative activity. Interaction of acidic amino acid with cation of Fe (II) seemed to contribute to inactivate oxidant activity of Fe (II).

To evaluate antioxidant activity in vivo, papain hydrolysate was administered to water-immersed rats which were given stress. This hydrolysate was shown to significantly suppress the occurring of gastric ulcer in stressed rats.

Chapter II; Angiotensin I-converting enzyme (ACE) inhibitory peptides in meat protein hydrolysates

Porcine myofibrillar and sarcoplasmic proteins, and gluten were hydrolyzed by papain, trypsin, chymotrypsin and actinase E. All protein hydrolysates exhibited ACE inhibitory activity (3.4-41.8 mg%). In particular, the protein hydrolysate obtained by treatment with papain showed the highest inhibitory activity (3.7-5.3 mg%). The ACE inhibitory activity of gluten hydrolysate obtained with actinase E was mainly due to peptides of less than 500 Da in molecular mass. On the other hand, the ACE inhibitory activity of the myofibrillar protein hydrolysate obtained with papain was due to peptides of both less and more than 500 Da. The blood pressure of spontaneously hypertensive rats (SHR) administered the myofibrillar protein hydrolysate was significantly reduced at 2 hr after administration. Therefore, it was concluded that porcine myofibrillar protein hydrolysate may potentially be useful as antihypertensive food materials.

Next, we investigated the antihypertensive activity of chicken extract. In China, it is thought that chicken soup has physical functions such as improvement of the blood circulation or a weak condition. The chicken extract was prepared from chicken breast muscle by boiling for 3 hr in acidic pH solution (pH3.5). The blood pressure of spontaneously hypertensive rats (SHRs) decreased after oral administration of an extract, falling maximally to 50 mmHg lower than before. This effect continued for at least 4 hr after administration. The peptides possessing hypotensive activity in the chicken extract were examined by measuring the inhibitory activity (IC_{50}) against ACE. The inhibitory activity of the chicken extract was 1060 mg%, while the activity of the extract treated with an *Aspergillus* protease and gastric proteases (trypsin, chymotrypsin and intestinal juice) became stronger, reaching 1.1 mg%. Peptides in this hydrolysate of the extract were isolated by HPLC on a reversed-phase column, and their *N*-terminal sequences were analyzed. Three peptides possessed a common sequence, Gly-X-Hyp-Gly-X-Hyp-Gly-, which was homologous with that of collagen. A synthetic peptide, Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe, showed the strongest inhibitory activity ($IC_{50} = 42\mu M$). This activity was almost the same as Val-Tyr and Val-Pro-Pro, which were derived from sardine muscle and milk protein, respectively. Furthermore it was revealed that N terminus amino acids of this peptide played an important role of ACE inhibitory activity.

Chapter III. Utilization of meat by-products -Angiotensin I-converting enzyme inhibitory activity of hydrolyzed hemoglobin-

Animal blood contains about 20% protein. Blood proteins were known to be a nutritionally well-balanced protein sources based on their amino acid composition. The high content of lysine

in blood protein makes it a good complement for cereal products which is lack of lysine. Therefore, bloods have been used for food products in European countries. Although blood is thought to be beneficial foodstuff, it is not utilized efficiently in the food industry in Japan. Most of the slaughterhouses, indeed, waste this potential food material. In this chapter we investigate the possibility for utilization of blood hemoglobin as a functional food material.

The blood pressure of spontaneously hypertensive rats (SHR) lowered after oral administration of hemoglobin (1 g/kg weight), falling into maximally 30 mmHg lower than before administration. This effect has continued for at least 6 hr after administration. On the other hand, hemoglobin affect no changes in normal blood pressure rat; WKY. The peptides possessing hypotensive activity were separated from hemoglobin hydrolysates treated with various proteases (actinase E, pepsin, trypsin, chymotrypsin and intestinal juice) by an ODS column on reversed-phase HPLC. The inhibitory activity (IC_{50}) against ACE of these peptides was measured. Four fractions showed strong inhibitory activities (IC_{50} ; 7.9~10.0 mg%). The inhibitory peptides in these fractions were isolated on a same column, and their N-terminal sequences were analyzed. Their structures were Thr-Pro-Ala-Val-His, His-Leu-Asp-Asp-Leu-Lys, Val-Asp-Pro-Val-Asn-Phe, and Val-Val-Tyr-Pro-Trp. These peptides were synthesized and their inhibitory activities were evaluated. Val-Asp-Pro-Val-Asn-Phe showed the strongest activity (IC_{50} ; 63 μ M).

It is concluded that peptides in meat protein hydrolysate possessed antioxidant and antihypertensive activities. The peptides derived from hemoglobin, which is a meat by-products, also showed hypotensive activity to spontaneous hypertensive rats. These peptides seemed to be very useful for development of functional foods.

Key words: peptide, protein hydrolysate, antioxidant activity, angiotensin I-converting enzyme inhibitory activity, by-product