ANGIOTENSIN I-CONVERTING ENZYME INHIBITORY PEPTIDES IN A CHICKEN BREAST MUSCLE EXTRACT

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Background

It is well known that chicken soup is an extremely nutritious and palatable food. It is also thought in china that chicken soup has physical functions such as improvement of blood circulation or weak constitution. Recently, peptides derived from proteins in foods have been shown to regulate physical functions in alimentary, neural and circulatory systems. Among them, peptides possessing hypotensive activity are thought to be useful as functional food materials for high blood pressure patients. Although many peptides possessing hypotensive or angiotensin I-converting enzyme (ACE) inhibitory activity have been discovered in hydrolysates of food proteins such as zein, tuna muscle and milk, there is little information on the functions of ACE inhibitory peptides derived from animal meat proteins.

Objective

In the present study, we investigated the hypotensive effect of chicken muscle extract in spontaneously hypertensive rats (SHR). ACE inhibitory peptides were isolated from treated chicken extract with proteases, and their structures were clarified. Furthermore, a peptide possessing the largest inhibitory activity was synthesized and characterized.

Methods

Digestion of chicken extract by proteases: A chicken extract was prepared from chicken breast muscle, and then further digested by a protease derived from *Aspergillus* (protease-A) at 50 degrees C, pH 7.0 for 1 hr. In order to isolate peptides possessing ACE inhibitory activity, a chicken extract treated with protease-A was digested with trypsin, chymotrypsin and small intestinal proteases at 37 degrees C, pH 7.0 for 1 and 0.5 hr, respectively.

Measurement of blood pressure and heart-beat counts in SHR: Eight-week-old male SHRs were fed a commercial non-purified diet (AIN-76; Oriental Yeast, Tokyo, Japan) and water for 2 weeks *ad libitum* in a controlled room (23 degrees C, 55% humidity), and then either saline or a chicken extracts (1g/kg weight) dissolved in saline was administered orally. Their tail systolic blood pressure and heart-beat counts were determined at 0, 1, 2, 3 and 4hr after oral administration using a plethysmographic tail apparatus (Softron 98A; Softron Co., Tokyo, Japan).

Assaying of angiotensin I-converting enzyme (ACE) inhibitory activity: The inhibitory activity of chicken extracts or peptides toward rabbit lung ACE was assayed according to the method reported by Cheung et al. (1). The ACE inhibitor concentration required to inhibit 50% of the ACE activity was defined as IC50.

Separation and purification of ACE inhibitory peptides from a chicken extract hydrolysate: Peptides in the chicken extract digested with tyrpsin, chymotrypsin and small intestine proteases were first divided into two groups with a molecular mass of less and more than 1000 Da using the ultrafiltration membrane (Millipore Co. USA). Peptides in the latter group were purified on HPLC by ODS columns (22×250mm and 4.6×250mm, Senshu Scientific Co., Tokyo, Japan) using a linear gradient of CH_3CN (8 to 40% in 40min and 8 to 40% in 64min, respectively) containing 0.1% trifluoro-acetic acid at flow rate of 1.0mL/min. The elution peaks were monitored at 220 nm.

Analysis of N-terminal amino acid sequence of peptides: N-terminal amino acid sequences of isolated ACE inhibitory peptides were determined with a protein sequencer G1005A (Hewlett-Packard, CO, USA).

Results and discussion

Hypotensive activity of chicken extracts in SHR: The blood pressures of SHRs were measured at 0, 1, 2, 3 and 4 hr after oral administration of the treated or non-treated chicken extract. Administration of protease-A-treated and non-treated chicken extract significantly declined the blood pressure of SHRs 1hr later, while no decline of blood pressure was observed in control SHR fed no chicken extract (Figure 1). This effect of both chicken extracts on blood pressure continued for at least 4 hr. This is the first observation that a hypotensive activity was exhibited by an oral administration of animal food material.

Inhibition of ACE Activity by Chicken Extract Hydrolysates: The non-treated chicken extract exhibited a very low inhibitory activity (IC50: 1.06 %). On the other hand, the chicken extract treated with protease-A exhibited high activity (IC50: 1.1mg%). This extract treated with trypsin/chymotrypsin, and small intestine proteases also possessed high inhibitory activity (IC50: 0.8mg%). The peptides with molecular mass of less than 1000 Da showed a higher activity than those with molecular mass of more than 1000 Da. These results suggested that proteins and peptides would exhibit a higher inhibitory activity through hydrolysis by gastric enzyme.

Analyses of the structure of ACE inhibitory peptides: The ACE inhibitory peptides in a chicken extract treated with protease-A and gastric proteases were separated on a HPLC by reversed-phase columns. The structure of the six peptides isolated on a HPLC by ODS column was analyzed with a protein sequencer (Hewlett Packard, USA). The sequences of 4 peptides were clarified as shown in Table 1. Among them, P2, P3 and P4 possessed a unique and repeated sequence, Gly-X-X-Gly-X-X-Gly-X-X (X; amino acid). Homology analysis with known proteins showed that these peptides were derived from collagen. Skeletal muscle contains about 4 % collagen, which is a major constituent in connective tissue, indicating that muscle could be good resource for production of ACE inhibitory peptides.

In order to confirm an ACE inhibitory activity of isolated peptides, Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe was synthesized with a peptide synthesizer (Shimadzu, Kyoto, Japan), and its inhibitory activity was measured. This peptide exhibited the activity 85 μM in IC50, which was almost same activity as that of peptides derived from dried bonito, and higher activity than that of peptides derived from porcine myosin (2). From these results, chicken extract seems to be useful as food material possessing anti-hypertensive activity.

Conclusion

The administration of chicken extract prepared from chicken breast muscle into spontaneously hypertensive rats (SHR) lowered their blood pressure. This effect by chicken extract continued for at least 4 hr after administration. ACE inhibitory peptides isolated from a chicken extract hydrolysate possessed common sequence Gly-X-X-Gly-X-X-Gly-, which was homologue with collagen. Among them, a peptide, Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe showed the strongest inhibitory activity (IC_{50}=87μM).

References

(1) Cheung et al., J. Biol. Chem., 255, 401-407 (1980), (2) Arihara et al., Meat Science, 57, 319-324 (2001).

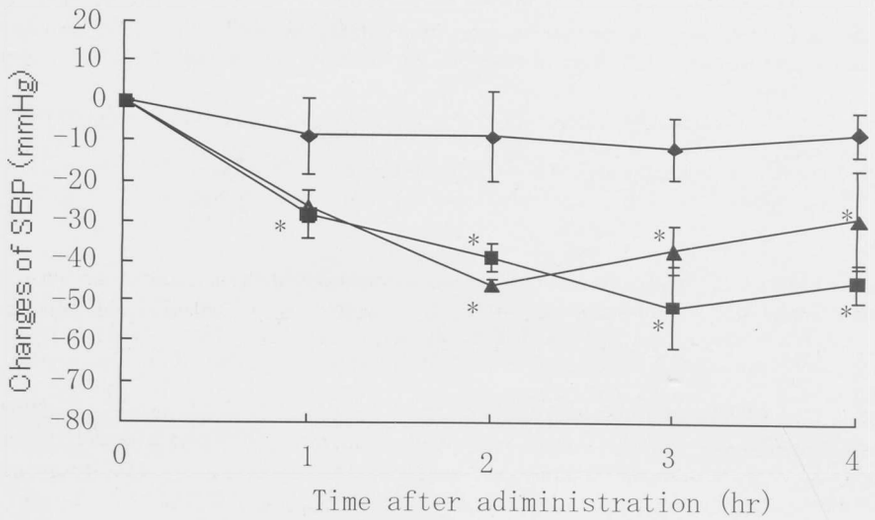

Figure 1. Effect of chicken extracts administration on the blood pressure of spontaneously hypertensive rats (SHR).

The blood pressure of 8 week-old SHRs was measured by the tail-cuff method and averaged. Each protein hydrolysate (1.0g/kg weight) was administrated orally. Values are the mean±standard error (n=5). *; significantly different from control ($p<0.05$). ♦; control (saline), ■; protease-A-treated chicken extract, ▲; non-treated chicken extract.

Table 1 Sequences of ACE inhibitory peptides from chicken extract

Peptide	Sequence
P1	Leu-Phe
P2	Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-X
P3	Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe
P4	Gly-Val-Asn-Gly-Glu-Glu-Gly-Val-Pro-Gly