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ACTION MECHANISM OF ANGIOTENSIN I-CONVERTING ENZYME INHIBITORY PEPTIDES OBTAINED FROM HYDROLYZATE OF CHICKEN BREAST MUSCLE

AI SAIGA*, TOMOYUKI OKUMURA, TOSHIKAZU MAKIHARA, SHINICHIRO KATSUTA, FUMIKI MORIMATSU, SOICHI TANABE And TOSHIHIDE NISHIMURA

R&D Center, Nippon Meat Packers, Inc., Tsukuba, Ibaraki 300-2646, Japan and Faculty of Applied Biological Science, Hiroshima University, Higashi-hiroshima 739-8528, Japan.

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Introduction

Hypertension has raised the risks of stroke, cerebral infarction. Blood pressure is controlled by various regulatory factors in the body and angiotensin I-converting enzyme (ACE) is one such factor. In the previous paper, we reported that administration of hydrothermal extract of chicken breast muscle prevented rises in blood pressure in spontaneously hypertensive rats (SHRs) by 50mmHg compared with the case in which saline was administered(1). We discovered an ACE-inhibitory peptide (Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe; P4 peptide) in the extract digested by gastric proteases (pepsin, trypsin–chymotrypsin, and small intestinal enzymes). The P4 peptide was as active as a lactotripeptide and a sardine peptide, which have been reported to be ACE-inhibitory peptides.

Objectives

The P4 peptide is a promising ingredient for functional foods, and its characteristics should be analyzed in detail. We synthesized a peptide that has this amino acid sequence, examined whether the synthetic peptide suppressed rises in blood pressure, and investigated the inhibition mechanisms and active regions of the peptide.

Methodology

Measurement of Blood Pressure and Heartbeat in SHRs

Eight-week-old male SHRs were fed a commercial diet and water for 2 weeks *ad libitum* in an environment-controlled room (23ºC, 55% humidity), and then either saline or a peptide (30mg/kg weight) dissolved in saline was administered through a tail vain. Their tail systolic blood pressure and heartbeat were determined by tail-cuff method.

Student's *t*-test was used to analyze whether there were significant differences among the data.

Assaying of Inhibitory Activity toward Angiotensin I-converting Enzyme (ACE). The following assay components, in a final volume of 0.25ml, were incubated at 37ºC for 30min: 100mM sodium borate buffer (pH8.3), 5mM Hip-HL, 500mM NaCl, 20 mU of rabbit lung ACE, and peptide. The enzyme reaction was stopped by the addition of a 1N HCl solution. The rate of hydrolysis of Hip-HL was determined by measuring the absorbance of the released hippuric acid at 228nm. The ACE inhibitor concentration required to inhibit 50% of the ACE activity under the conditions described above was expressed as IC_{50}.

Analysis of the N-*terminal Amino Acid Sequences of Peptides*

The N-terminal amino acid sequences of the isolated hypotensive peptides were determined with a protein sequencer G1005A (Hewlett Packard Co. Wilmington, DE).

Determination of ACE Inhibition Manner

Six mM of the peptide was incubated with 10 mU of ACE at 37 °C for 3 h. After the incubation, the mixture was heated to 100 °C for 10 min. The supernatant from centrifugation (10,000*g*, 10 min) was applied on an ODS column and the chromatographs before and after incubation with ACE were compared. The peaks were recovered, and the molecular weights were determined by electrospray-ionization mass spectrometry.

Results & Discussion

Suppression of Rises in Blood Pressure by the P4 Peptide

Changes in blood pressure when the P4 peptide was injected through a vein are shown in **Figure 1**. Rises in blood pressure were not suppressed in the group to which saline was administered. The blood pressure of the group to which the P4 peptide was administered was significantly lower than that of the control group, and this effect continued for over 30 minuets. In the previous paper, we showed that oral administration of chicken breast muscle thermal extract reduced blood pressure by up to 50 mm Hg in 3 hours. The fact that intravenous administration of the P4 peptide caused drops in blood pressure immediately after the administration suggests that the hypotensive peptide is produced within the body by digestive enzymes and reduces blood pressure.

Manner of Inhibition by the P4 Peptide

The inhibitory mechanisms by the P4 peptide was analyzed in detail by calculating the inhibitory constant (K_i). The inhibitory activity was measured by changing the concentration of the substrate (Hip-HL) at a constant concentration of the P4 peptide. The results were plotted (Dixon plot) to determine the mode of inhibition and the K_i. Because the plots obtained by changing the substrate concentration intersect with the X-axis, the inhibition of ACE by the peptide was found to be noncompetitive inhibition. From the

value at which the line intersects with the X axis, the K_i value was calculated to be 11.5 μM. This value is lower than Hip-HL (2.3 mM) and angiotensin I (70 μM). This indicates that the affinity of the P4 peptide to ACE is higher than those of these ACE substrates. We then investigated whether the P4 peptide is decomposed when the peptide is incubated with ACE, by using HPLC. Incubation with ACE resulted in two additional peaks besides the peak for the original peptide. The molecular weight corresponding to peak 2 was 804, which is the same as that of a peptide lacking the Gly-Phe at the C-terminus of the P4 peptide. The peptide solution after incubation with ACE was heated, and the ACE inhibition activity was investigated again. The IC_{50} of the heated solution was 52.5 μM (the IC_{50} before heating was 46 μM). The area of the HPLC plot showed that about 90% of the P4 peptide had been decomposed.

Length of Peptide Chain and ACE Inhibition

Regions in the P4 peptide involved in the expression of ACE inhibition were investigated by preparing synthetic peptides removed amino acids from the C- and N-termini of the P4 peptide and measuring their ACE inhibition (**Table 1**). A peptide consisting of three residues at the C-terminus showed an inhibitory activity 1/20th of that of the original peptide (IC_{50} = 443 μM). On the other hand, Hyp-Gly-Leu-Hyp-Gly-Phe and Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe were as active as the original P4 peptide, suggesting that Hyp-Gly-Leu at the fourth to sixth positions of the C-terminus, is responsible for the affinity with the enzyme. Removal of the C-terminus produced marked drops in activity, and the drop was especially large when Phe was removed from the C-terminus. All the ACE-inhibitory peptides that have already been reported, such as Ile-Pro-Pro (hydrolysates of casein) (2), Val-His-Leu-Pro-Pro (hydrolysates of corn proteins) (3), and Phe-Gln-Pro, Ile-Tyr, Asp-Tyr-Gly-Leu-Tyr-Pro and Ile-Lys-Pro-Leu-Asn-Tyr (hydrolysates of sardine proteins) (4), have an aromatic amino acid at their C-termini, such as Pro, Phe, or Tyr.

Conclusions

Intravenous administration of synthetic P4 peptide resulted in significant drops in the blood pressures of SHRs. This peptide showed noncompetitive manner, and the affinity site was suggested Hyp-Gly-Leu in its sequences.

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Tables and Figures

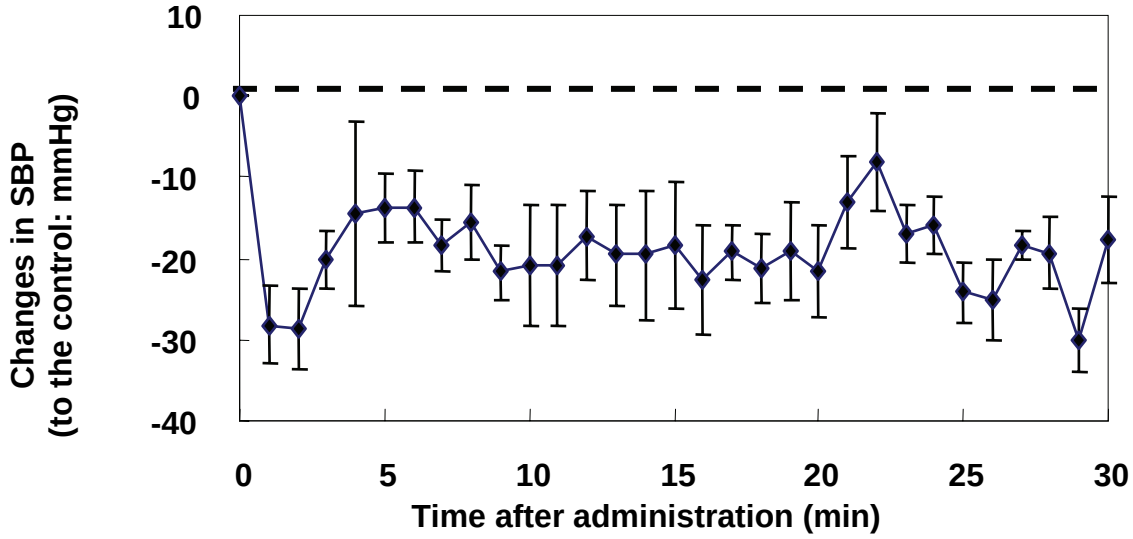

Figure 1. Changes in blood pressure on intravenous administration of the P4 peptide; ■, Synthetic P4; ---, saline (control). The blood pressure of 9-week-old SHRs was measured by the tail-cuff method after intravenous administration of the P4 peptide (30mg/kg of rat weight). Average blood pressure values in five SHRs are shown. Each bar shows the standard error. The values at whole time for 30min except for at 4 and 22min were significantly lower than those of the control (p>0.05).

Table 1. Identification of the Inhibition Activity Site in the P4 Peptide

	Synthetic peptide	IC_{50}(μM)
P4	Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe	26
No.1	Hyp-Gly-Phe	433
No.2	Hyp-Gly-Leu-Hyp-Gly-Phe	10
No.3	Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe	19
No.4	Phe-Hyp-Gly	171
No.5	Phe-Hyp-Gly-Thr-Hyp-Gly	406
No.6	Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly	25000