Isolation of Angiotensin Converting Enzyme Inhibitory Peptide from Beef Bone Extract Hydrolysate

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Angiotensin converting enzyme (ACE) inhibitor was isolated from beef bone extract hydrolysate. After hydrolysis of beef bone extract with a commercial protease, ACE inhibitory peptide was purified by using ultrafiltration, gel permeation chromatography, and reverse-phase high pressure liquid chromatography. The purified ACE inhibitor was a pentapeptide, Gly-Pro-X-Gly-Pro.

Key words : *angiotensin converting enzyme, beef bone extract, protein hydrolysates.*

ACE (Angiotensin converting enzyme, peptidyldipeptide hydrolase, EC 3.4.15.1) converts angiotensin I into angiotensin II by cleaving C-terminal dipeptide (His-Leu) of angiotensin I and also inactivates bradykinin which depresses blood pressure. Thus, ACE inhibitor acts on the inhibition of ACE and causes a decrease in blood pressure. It has been screened from protein hydrolysates of various food sources.[1-4] Beef bone extract is widely used as food ingredient or additive in Asian countries. It has been considered as nutritious and healthy food. In this study the isolation of ACE inhibitors from beef bone extract hydrolysate is to be reported.

Materials and Methods

Materials. Beef bone was obtained from the slaughterhouse. All the chemicals used were of analytical grade.

Preparation of beef bone extract. Beef bone was defatted, washed, cut into small pieces and wet-ground. Beef bone extract was prepared according to the modified method of Lilja.[5] After wet-grinding of beef bone, water was added and the pH of the slurry was adjusted to 3.5 by adding 1 M phosphoric acid and heated upto 110°C for 15 min. And then the pH was adjusted to 5.5 by adding 1 M calcium hydroxide and was filtered. The filtrate was concentrated by vacuum evaporation.

Isolation of ACE inhibitors from beef bone extract hydrolysate. Beef bone extract was hydrolyzed with 0.5 % Flavorzyme (Novo Co.) at 50°C for 24 h and the hydrolysate was filtered by using PM-10 membrane (Amicon Co.). The membrane-filtered solution was loaded onto Sephadex G-25 column (1.5 cm × 120 cm) equlibrated with 20 mM phosphate buffer (pH 7.0). The eluate was monitored for peptides by measuring absorbance at 214 nm. Using the most ACE inhibitory fraction of gel filtration profile, reverse-phase HPLC with ProRPC column (C_8, 5 mm × 10 cm, Pharmacia Chemical Co.) was performed on the condition of buffer A (0.1% trifluroacetic acid, TFA) and buffer B (acetonitrile containing 0.1% TFA), having gradient of 0% of B to 60%.

TNBS assay. Concentration of peptide was determined according to the modified TNBS method.[6]

ACE assay. ACE activity was measured by the modified method of Cushman and Cheung.[7] The reaction mixture contained 150 μl of 5 mM Hip-His-Leu as a substrate, 50 μl of rabbit lung ACE powder (1 unit will produce 1 mole of hippuric acid from hippuryl-His-Leu per min) in 50 mM sodium borate buffer (pH 8.3), and 50 μl of sample solution. The reaction was carried out at 37°C for 30 min, and terminated by the addition of 250 μl of 1 N HCl and 1 ml of ethylacetate. After centrifugation, the absorbances of the supernatants were measured at 228 nm.

Peptide sequencing. ACE inhibitory peptide was analyzed by using a protein sequencer (Applied Biosystem 476-A).

SDS-PAGE. SDS-PAGE was performed by the method of Laemmli.[8]

Results and Discussion

Beef bone extract was obtained by the partial hydrolysis of beef bone, which involved breakdown of the col-

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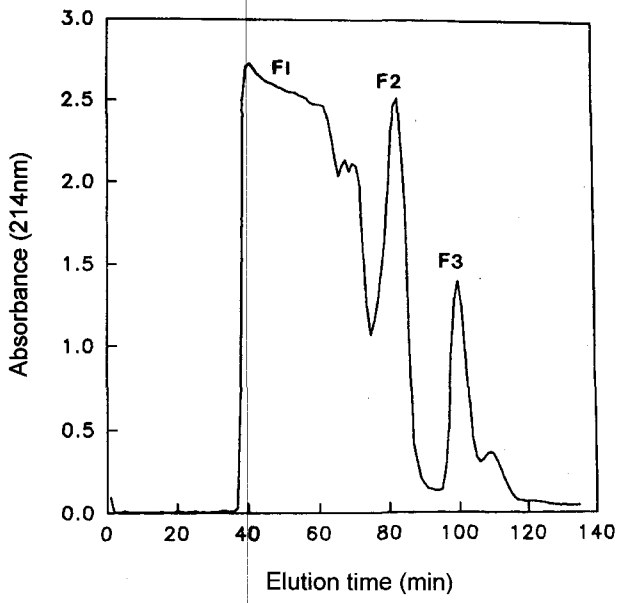

Fig. 1. Elution profile of beef bone extract hydrolysate on Sephadex G-25.

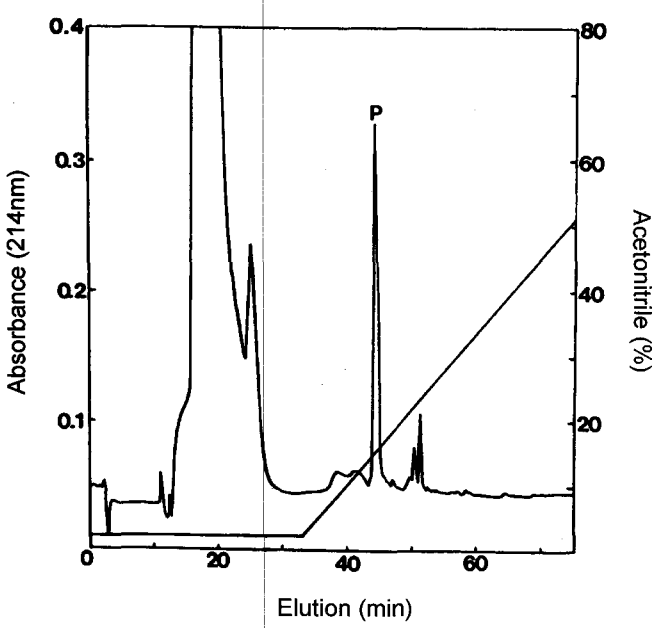

Fig. 2. Reverse-phase HPLC chromatogram of beef bone extract hydrolysate. F2 fraction (Fig. 1) from Sephadex G-25 chromatgraphy was loaded onto the column.

lagen backbone chain and rupture of cross-links. Although there were many ways to prepare beef bone extract, more convenient and time-saving method was considered. Instead of long-time heat treatment, heating at 110°C for 15 min was taken. Extracted molecules of gelatin were shown as degraded pattern with various molecular weight distribution by SDS-PAGE (not shown). There were four major bands of 65, 45, 40, 34 kDa with several minor bands although it did not provide distinct band patterns due to degraded pattern of gelatin molecules, also, it should be mentioned that molecular weight distribution could be changed by extraction condition.

After studying the time dependence of ACE inhibitor formation, beef bone extract was digested with a commercial protease (Flavorzyme) at 50°C for 24 h. After beef bone extract was hydrolyzed with a protease, the hydrolysate was filtered by using PM-10 membrane. After membrane-filtration of beef bone extract hydrolysate, small molecular weight molecules possessing ACE inhibitory activity were fractionated by gel filtration chromatography. There were three major fractions (Fig. 1). The F2 fraction having the most inhibitory activity was selected and the fraction was loaded onto reverse-phase HPLC for further purification. HPLC clearly separated one major peak and two minor peaks during the gradient elution (Fig. 2). The major peak isolated by HPLC contained the most inhibitory peptide having 31 μM as IC_{50} value according to ACE assay. And the sequence of F1 fraction was identified as a pentapeptide, Gly-Pro-X-Gly-Pro, (X assumed as HyP), by using a protein sequencer. This result was in a good comparison with Oshma's report[9] of which studied peptide inhibitors of ACE in digests of gelatin by bacterial collagenase. It was known that collagenase had the specificity to hydrolyze the X-Gly bond in a sequence of Pro-X-Gly-Pro.[10] Therefore, two hexa peptides, Gly-Pro-HyP-Gly-Pro-HyP and Gly-Pro-HyP-Gly-Ala-HyP were identified. Different sample and different hydrolysis condition should be accountable for the different results, also, its variation might have arose from many other factors since there should be many ACE inhibitors among beef bone extract hydrolysate. Considering that collagen is composed of repetitious tripeptide sequence, Gly-X-Y, in which X is frequently a Pro and Y is frequently a HyP, the ACE inhibitory peptide we isolated was thought to be a common hydrolysate of collagen.

Since beef bone extract is a kind of valueless material after appropriate demand of cows, isolation and utilization of ACE inhibitors from beef bone extract hydrolysate is very useful in terms of developing a new food additive. Especially, in Korea, beef bone extract is considered as a nutritious and healthy food. Therefore, utilization of beef bone extract hydrolysate as a food additive is very promising.

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