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Partial Purification of Angiotensin Converting Enzyme Inhibitory Peptide Isolated from Supernatant of Bovine Plasma Treated by Trichloroacetic Acid

– Research Note –

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Abstract

An angiotensin converting enzyme (ACE) inhibitor was isolated and partially purified from bovine blood plasma. Bovine blood plasma was obtained after removing blood cells by centrifugation, followed by the addition of anti-coagulant to whole bovine blood. To precipitate plasma proteins, bovine blood plasma was treated with 4% trichloroacetic acid (TCA) as a final concentration. An ACE inhibitor was isolated from TCA supernatant, using ultrafiltration, gel permeation chromatography, and reverse-phase high pressure liquid chromatography. The ACE inhibitor purified from TCA supernatant had IC_{50} values of 9.4 μM.

Key words: bovine blood, TCA supernatant, ACE inhibition

INTRODUCTION

Considerable amount of blood coming from slaughtered animals as waste materials have caused many problems. Since utilization of bloods released in the slaughter house is scarce except for a few applications where the blood is usually dried and used as food ingredients for sausages and puddings (1-6), the blood is mostly discharged without suitable wastewater treatment. Therefore, disposal of blood causes serious water pollution. To solve this problem encountered in the slaughter house, there have been a few reports regarding the utilization of porcine blood plasma (7-10). In this study, to develop the utilization of waste materials and find out new functional biomaterials from bovine blood, angiotensin converting enzyme (ACE) inhibitory peptide was researched. ACE (peptidyldipeptide hydrolase, EC3.4.15.1) converts angiotensin I into angiotensin II by cleaving the 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 results in the decrease of blood pressure and was screened from protein hydrolysates of various food sources (11-14). We report here the isolation and partial purification of an ACE inhibitory peptide from bovine blood TCA supernatant.

MATERIALS AND METHODS

Preparation of TCA supernatant from bovine blood

Whole bovine blood was freshly collected right after slaughter and immediately used for plasma preparation. Bovine blood was centrifuged at 5000×g for 40 min to remove blood cells after adding ethylenediaminetetraacetic acid (EDTA, 2 g/L) to prevent coagulation. To precipitate plasma proteins, 4% trichloroacetic acid (TCA) as a final concentration was added to bovine plasma. The TCA supernatant was used to isolate the ACE inhibitor in the next step.

Isolation of the ACE inhibitor from TCA supernatant

TCA supernatant was filtered using PM-10 membrane (Amicon Co.) and the membrane-filtered solution was loaded onto Sephadex G-15 column (1.5 cm×120 cm) equilibrated with 20 mM phosphate buffer (pH 7.0). The eluate was monitored for peptides by measuring the absorbance at 214 nm. Using the most ACE inhibitory fraction of gel filtration profile, it was reloaded onto the Sephadex column to obtain a single peak. And finally, to purify the ACE inhibitory peptide, reverse-phase HPLC with ProRPC column (C_8, 5 mm×10 cm, Pharmacia Chemical Co.) was performed under the condition of buffer A (0.1% trifluroacetic acid, TFA) and buffer B (acetonitrile containing 0.1% TFA), having a gradient of 20% of B to 80%.

TNBS assay

Concentration of peptide was determined according to the modified TNBS method (15).

ACE assay

ACE activity was measured by the modified method of Cushman and Cheung (16). The reaction mixture contained 150 μl of 5 mM Hip-His-Leu as a substrate, 50 μl of rabbit lung ACE powder (5 munit) 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 using a protein sequencer (Applied Biosystem 476-A).

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RESULTS AND DISCUSSION

Bovine blood was freshly collected from the slaughter house and used for plasma preparation. To isolate the low molecular weight molecules from bovine blood plasma, plasma proteins were separated by the addition of 4% TCA as a final concentration to the bovine plasma. Most plasma proteins were precipitated and segregated and the major protein of the TCA precipitate was albumin as reported in the previous article (13). This suggests that this should be an efficient way to treat blood plasma with TCA to isolate new functional materials from TCA supernatant. After membrane filtration of the TCA supernatant, small molecular weight molecules were fractionated on gel filtration chromatograhy. There were two major peaks and a few minor peaks (Fig. 1). F1 fraction having the most ACE inhibitory activity was selected and was reloaded onto the same column to get homogeneity. After the second run of Sephadex G-15 column, the main peak was obtained (Fig. 2). And finally, to purify the ACE inhibitory peptide, reverse phase HPLC was used according to the methods reported previously (8-10). A major peak, F, which has ACE inhibition, were separated during the gradient elution (Fig. 3). It came out at 53% acetonitrile solution. The major peak isolated by HPLC contained the most inhibitory peptide having 9.4 μM as IC_{50} value according to ACE assay. Comparing with the reports of porcine blood plasma TCA supernatant (8,9), IC_{50} value of the ACE inhibitory peptide is lower. However, it is still a promising candidate for ACE inhibitor since most ACE inhibitors reported in the literature were isolated after proteolytic hydrolysis and the processing in this study does not need it, which is one of the major advantages. The sequence of F fraction was identified as an octapeptide, Ser (Ala)-Ala (Ser)-Ile-Trp-X-Thr-Met-Glu (Leu), using a protein sequencer. Although it is not completely purified, this fractionation is a good way to utilize wasted bovine blood for new functional materials. And it is a promising result in terms of the fact that much of bovine blood can be screened and utilized for new biofunctional materials as a high value-added product.

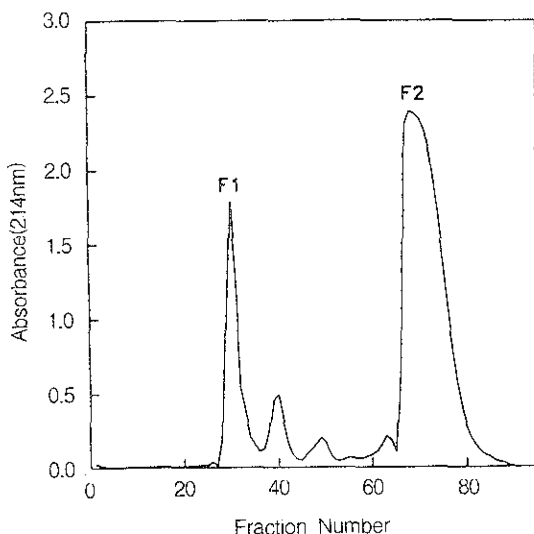

Fig. 1. Elution profile of bovine plasma TCA supernatant from Sephadex G-15.

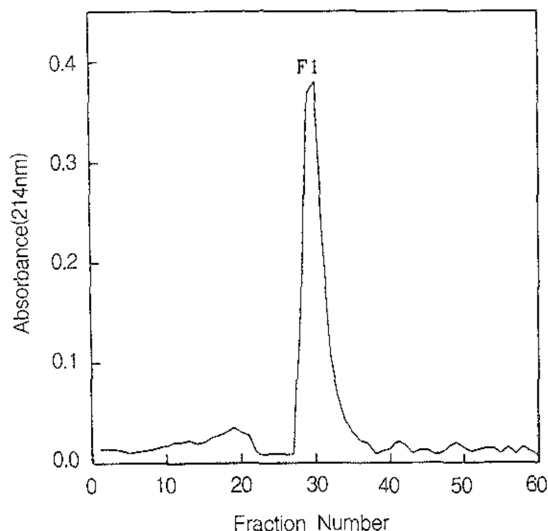

Fig. 2. Elution profile of the fraction #1 (Fig. 1) from Sephadex G-15.

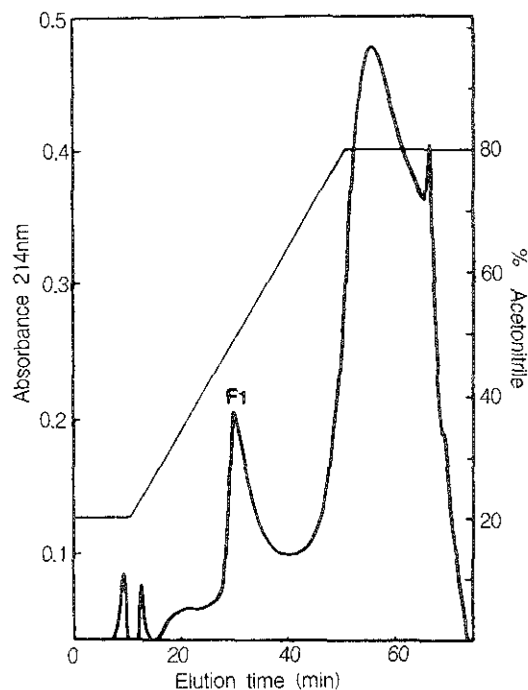

Fig. 3. Elution profile of bovine plasma TCA supernatant from reverse-phase HPLC. The main fraction (Fig. 2) from Sephadex G-15 was loaded onto the column.

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