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Isolation of Angiotensin Converting Enzyme Inhibitors from Ripe *Cucurbita moschata* Duch

– Research Note –

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Abstract

Angiotensin converting enzyme (ACE) inhibitor acts on the inhibition of ACE and causes a decrease in blood pressure. There have been several reports on screening of ACE inhibitors from natural food products and protein hydrolysates of various food sources. Ripe *Cucurbita moschata* Duch has been used as an oriental medicine in Korea. To isolate ACE inhibitors, crude water extracts of the edible portion of ripe *Cucurbita moschata* Duch were obtained after heating in water at 95°C for 2 h. Crude extracts were then filtered using PM-10 and YM-1 membranes. The membrane-filtered solution was loaded onto Sephadex G-15 column equlibrated with a phosphate buffer. Among the four major fractions of gel permeation chromatography, the second fraction had the highest inhibitory activity of 65%. Further purification of the fraction using reversed-phase HPLC with a C_{18} column produced ACE inhibitors, which were identified as a mixture having molecular mass of 222 and 273 by Tandem mass spectrometry.

Key words: ACE inhibitor, *Cucurbita moschata* Duch, mass spectrometry

INTRODUCTION

Cucurbita spp. is an edible annual plant and divided into three groups; *Cucurbita moschata* Duch, *Cucurbita maximum* Duch, and *Cucurbita pepo* L. Among them, *Cucurbita moschata* Duch is dominant in Korea. A decoction of the edible part of *Cucurbita moschata* Duch is widely used as a traditional medicine against various diseases as well as a popular food source (1-3). Although it has pharmacological effects as well as a nutritional value, little knowledge is available on the active compounds having medicinal effect (3).

Angiotensin converting enzyme (ACE, peptidyldipeptide hydrolase, EC3.4.15.1) 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 isolated from various food sources (4-9). Therefore, the objective of this study was to isolate ACE inhibitors from ripe *Cucurbita moschata* Duch and to develop a new functional food ingredient. We here report the isolation of the ACE inhibitory substances from ripe *Cucurbita moschata* Duch.

MATERIALS AND METHODS

Preparation of crude extracts

Crude water extracts of *Cucurbita moschata* Duch were obtained after heating the plant in water at 95°C for 2 h. Crude ethanol extracts were prepared by shaking the plant in ethanol at room temperature for 12 h. Each extract was centrifuged at 5000 ×g for 30 min and the supernatant was filtered using PM-10 and YM-1 membranes.

ACE assay

ACE assay was performed using Hip-His-Leu as a substrate according to the method of Cushman and Cheung (10) with modification (6-9). 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 ethyl acetate. After centrifugation, the absorbance of the supernatant was measured at 228 nm.

Isolation of ACE inhibitors from crude extracts

Isolation of ACE inhibitors from crude extracts was performed according to Fig. 1. Crude water extracts of *Cucurbita moschata* Duch were loaded onto Sephadex G-15 column (1.5 cm × 120 cm) equlibrated with 10 mM sodium phosphate buffer (pH 7.0). The eluates were monitored by measuring absorbance at 214 nm. Using the most ACE inhibitory fraction of gel filtration profile, reversed-phase HPLC with μ-Bondapak C_{18} column (Waters, USA) was performed on the condition of solvent A (0.1% trifluroacetic acid, TFA) and solvent B (acetonitrile containing 0.1% TFA), having gradient of 0% of B to 80%.

Mass spectrometry

Molecular masses of ACE inhibitors were identified by ESI Tendem mass spectrometer (JMS HX-110A, JEOL, Japan).

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Ripe *Cucurbita moschata* Duch
↓
Preparation of crude water extracts
↓
Membrane filtration
↓
Gel permeation chromatography (Sephadex G15)
↓
2nd GPC
↓
Reversed-phase HPLC (C_{18} column)
↓
2nd HPLC
↓
Mass spectrometer

Fig. 1. Purification scheme of ACE inhibitory substances from ripe *Cucurbita moschata* Duch.

RESULTS AND DISCUSSION

Ripe *Cucurbita moschata* Duch was freshly harvested and used for extraction. Crude water and ethanol extracts of *Cucurbita moschata* Duch were compared in terms of ACE inhibition. Crude water extracts had a 58% inhibitary activity, while ethanol extracts had 5%. Therefore, crude water extracts were further purified. The membrane-filtered solution using PM-10 and YM-1 membranes was loaded onto Sephadex G-15 column (1.8 cm×75 cm) equlibrated with 10 mM phosphate buffer (pH 7.0). The eluates were monitored by measuring absorbance at 214 nm. There were four major fractions obtained from the column (Fig. 2). Each fraction was monitored using the ACE assay. F2 fraction only had ACE inhibitory activity of 65%. Therefore, the fraction was pooled and reloaded onto the Sephadex G-15 column. After obtaining a single peak from the column (Fig. 3), the fraction was loaded onto the reversed-phase HPLC having a C18 column. HPLC was performed on the condition of solvent A (0.1% triflur-oacetic acid, TFA) and solvent B (acetonitrile containing 0.1% TFA), having gradient of 0% of B to 100%. There were

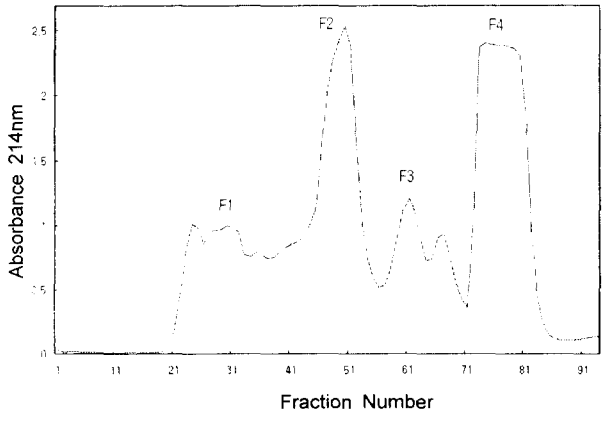

Fig. 2. Elution profile of Sephadex G-15 column chromatography using ripe *Cucurbita moschata* Duch extracts.

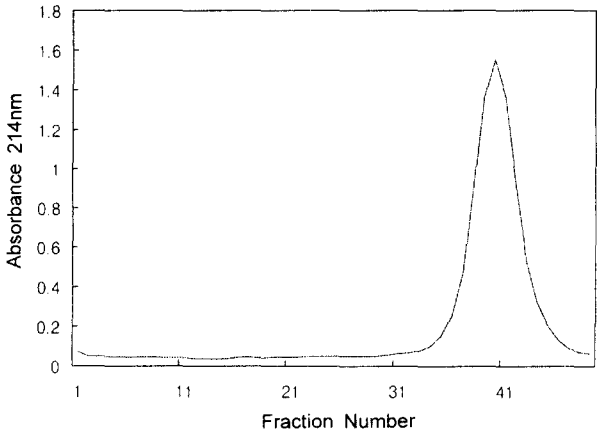

Fig. 3. Elution profile of the second Sephadex G-15 column chromatography using F2 fraction in Fig. 2.

four major fractions separated by HPLC (Fig. 4). Among them, the 4th fraction, F4, had ACE inhibitory activity. Therefore, the fraction was reloaded onto the HPLC on the condition of solvent A and solvent B, having gradient of 0% of B to 30% and elution profile produced four peaks again. F44 had the most inhibitory activity (Fig. 5). The isolated ACE

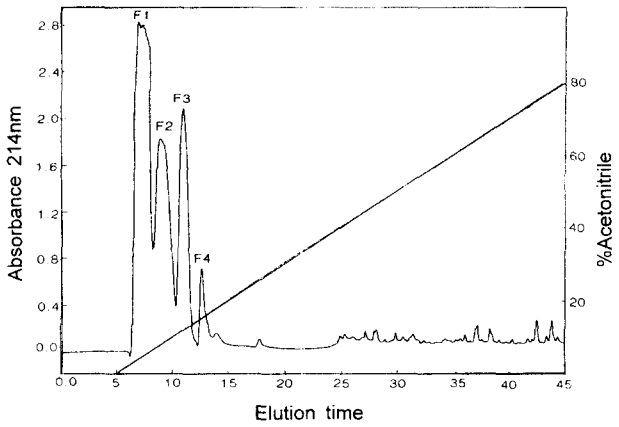

Fig. 4. Elution profile of reversed-phase HPLC using the fraction in Fig. 3.

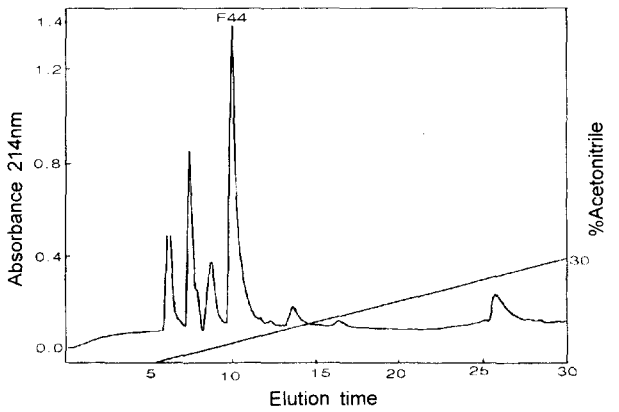

Fig. 5. The second reversed-phase HPLC profile of the peak F4 in Fig. 4.

inhibitor was identified as a mixture having a molecular mass of 222 and 273 by ESI Tendem mass spectrometer (Fig. 6). This is the first report regarding the isolation of an ACE inhibitor from ripe *Cucurbita moschata* Duch. Although the chemical nature of the inhibitor should be further characterized and *in vivo* experiment using spontaneously hypertensive rats (SHR) is needed, this inhibitor is quite promising in terms of manufacturing a drink product using a membrane filtered ripe *Cucurbita moschata* Duch extracts since it is a good candidate containing a functional component. Especially, a simple processing such as membrane filtration of molecular weight 1000 cut-off could produce a product having a functional component. Further characterization of the inhibitor and development of processing of a drink product is currently under study.

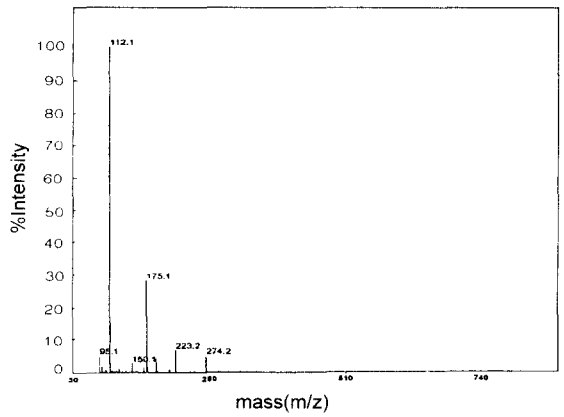

Fig. 6. Mass spectrum of the purified ACE inhibitor, F44.

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