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Isolation of an Angiotensin Converting Enzyme Inhibitory Substance from *Lycium chinense* Miller

- Research Note -

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Abstract

An angiotensin converting enzyme (ACE) inhibitory substance was isolated and purified from *Lycium chinense* Miller. A crude water extract of *Lycium chinense* Miller was prepared by adding it to water shaking at 25°C for 1 hr, followed by centrifugation at 8000×g for 30 min. The crude extract was then filtered using YM-3 and YM-1 membranes. An ACE inhibitor was isolated using consecutive chromatographic methods including: ion exchange chromatography, gel permeation chromatography, and FPLC. The inhibitor was identified to have a molecular mass of 862 daltons by mass spectrometry.

Key words: *Lycium chinense* Miller, ACE inhibitor, chromatography

INTRODUCTION

Angiotensin converting enzyme (ACE, peptidyldipeptide hydrolase, EC3.4.15.1) converts angiotensin I into angiotensin II by cleaving the C-terminal dipeptide (His-Leu) of angiotensin I. ACE also inactivates bradykinin, a potent vasodilative peptide with hypotensive effects. The first ACE inhibitor was reported as a natural peptide isolated from a Brazilian snake's venom (1,2). Using this peptide as a model system, specific ACE inhibitors such as captopril and enalapril have been synthesized and commercially used in the treatment of hypertension and for the prevention of chronic heart failure. However, these synthetic drugs have side effects such as cough, taste disturbances, and skin rash. Therefore, various food sources that are generally recognized as safe (GRAS) were screened for ACE inhibitory activity (3-10). Extract of *Lycium chinense* Miller is traditionally used in oriental medicine. To assess the efficacy of *Lycium chinense* Miller for the treatment of hypertension, extract of *Lycium chinense* Miller was prepared and its ACE inhibitory activity was measured. We report here the isolation of an ACE inhibitory substance from *Lycium chinense* Miller.

MATERIALS AND METHODS

Materials

Lycium chinense Miller, which was harvested in August, 2002, was obtained in Cheongyang, Korea.

Preparation of crude extracts

A crude water extract of *Lycium chinense* Miller (100 g) was obtained by shaking in water (600 mL) at 25°C for 1 hr, followed by centrifugation at 8,000×g for 30 min.

Isolation of an ACE inhibitor

Crude water extract was filtered using a series of YM-3 (Mw 3000 cut-off) and YM-1 (Mw 1000 cut-off) membranes. The membrane-filtered solution was loaded on a Q-Sepharose ion exchange column (40×150 mm, Amersham Pharmacia Co., Uppsala, Sweden). The column was equilibrated with 10 mM Tris-HCl buffer (pH 8.0) and eluted with a linear gradient of NaCl in the buffer from 0 to 0.5 M at a flow rate of 1.5 mL/min. The eluate was monitored by measuring the absorbance at 254 nm. The highest inhibitory fraction was further purified using a Sephadex G-15 column (1.5×100 cm, Sigma Chemical Co., St. Louis, MO, USA) pre-equilibrated with 10 mM phosphate buffer (pH 7.0). The highest inhibitory fraction was further purified using FPLC with an anion exchange column (Mono Q, Amersham Pharmacia Co., Uppsala, Sweden). The column was equilibrated with 10 mM Tris-HCl buffer (pH 8.0) and eluted with a linear gradient of NaCl in the buffer from 0 to 1 M at a flow rate of 1 mL/min. Finally, to further purify an ACE inhibitor, the highest inhibitory fraction from the ion exchange chromatography was loaded onto the FPLC with a gel permeation column (Superdex GPC, Amersham Pharmacia Co., Uppsala, Sweden) pre-equil-

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ibrated with 10 mM phosphate buffer (pH 7.0).

ACE assay

ACE inhibitory activity was measured by the method of Cushman and Cheung (11) with modifications established in our lab (7,10). The reaction mixture contained 150 μL of 5 mM Hip-His-Leu as a substrate, 50 μL of rabbit lung ACE powder (5 munit, Sigma Chemical Co., St. Louis, MO, USA) in a 50 mM sodium borate buffer (pH 8.3), and 50 μL of the 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 absorbances of the supernatants were measured at 228 nm.

Mass spectrometry

The molecular mass of the purified ACE inhibitor was determined using an ESI mass spectrometer (JMS HX-110A, JEOL, Tokyo, Japan).

RESULTS AND DISCUSSION

A crude water extracts of *Lycium chinense* Miller was obtained and filtered using a series of YM-3 (Mw 3000 cut-off) and YM-1 (Mw 1000 cut-off) membranes. Previous studies showed that most ACE inhibitory substances have molecular weights below or around 1000 daltons (3,12). Therefore, the filtrate from YM-1 membrane of crude extract was concentrated and loaded onto Q-Sepharose ion exchange column, and resolved into 5 fractions (Fig. 1). During washing of the column, unbound fractions did not exhibit ACE inhibition. Among the bound fractions, the F2 fraction exerted the highest inhibitory activity of 47.8%. The F2 fraction was pooled and loaded onto a Sephadex G-15 column for further purification. There were two major peaks eluted from the column (Fig. 2). The F22 fraction, having the highest

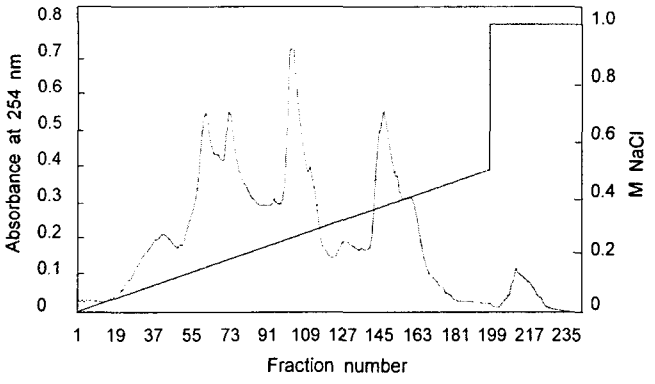

Fig. 1. Elution profile of a Q-Sepharose column chromatography using *Lycium chinense* Miller crude extract. ACE inhibition (%) of each fraction: F1, 4.2; F2, 47.8; F3, 31.5; F4, 0; F5, 0.

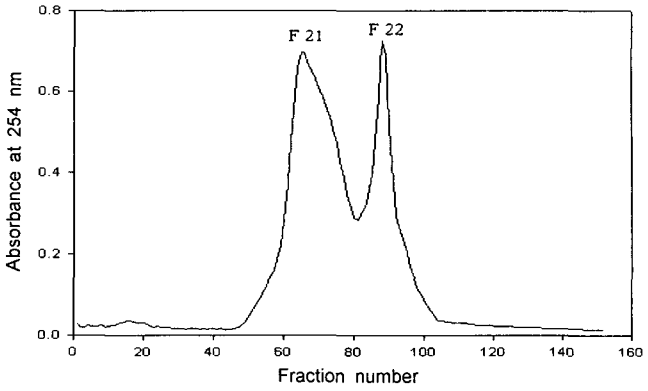

Fig. 2. Elution profile of gel permeation chromatography using F2 in Fig. 1. ACE inhibition (%) of each fraction: F21, 21.6; F22, 53.5.

inhibitory activity (53.5%), was pooled and subjected to anion exchange chromatography with Mono Q column. The F223 fraction, having the highest inhibitory activity (58.4%), from the column was then pooled (Fig. 3) and further purified. Final purification of the ACE inhibitor utilized FPLC with a Superdex GPC column, from which a single peak was obtained (Fig. 4). The ACE inhibitory substance was identified by ESI mass spectrometry. The molecular weight of the ACE inhibitory substance was

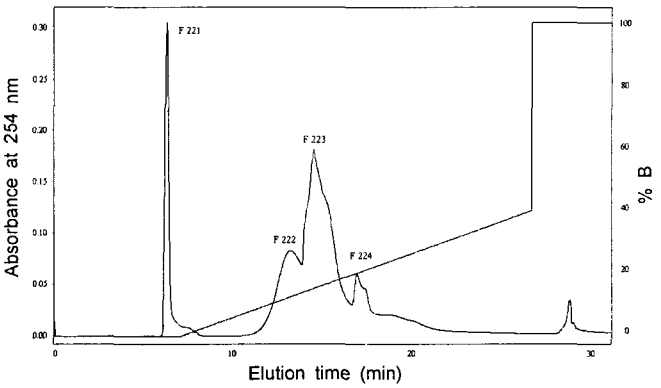

Fig. 3. Elution profile of anion exchange chromatography using F22 in Fig. 2. ACE inhibition (%) of each fraction: F221, 8.0; F222, 5.1; F223, 58.4; F224, 3.0.

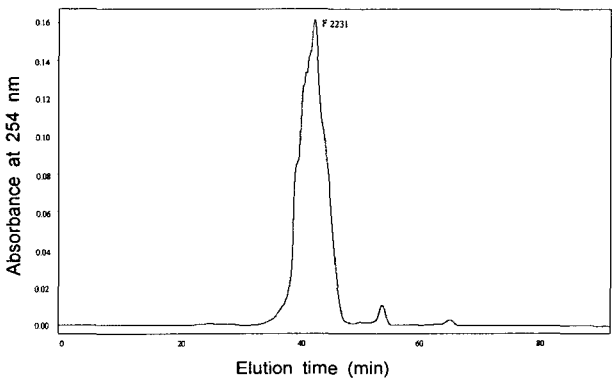

Fig. 4. Elution profile of FPLC using F223 in Fig. 3.

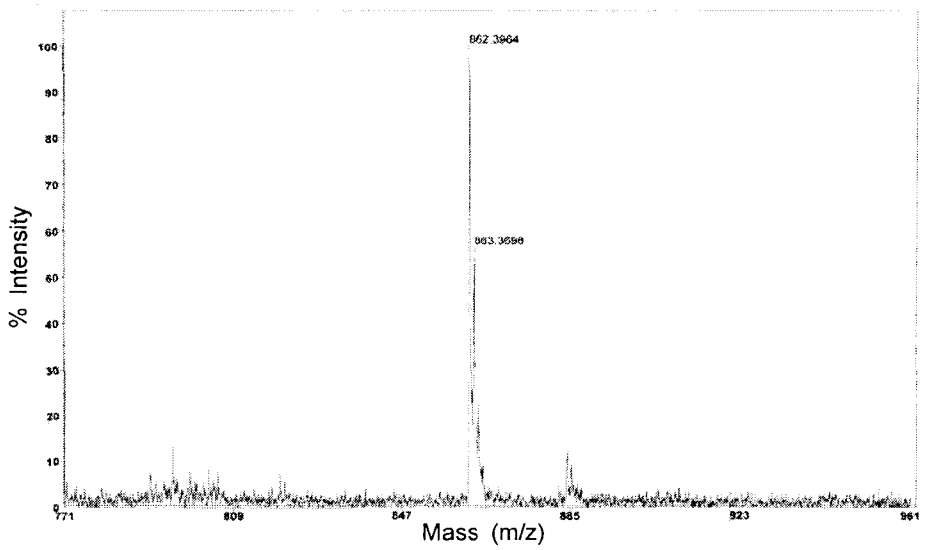

Fig. 5. Mass spectrum of the purified ACE inhibitor.

determined to be 862 daltons (Fig. 5) and had an IC_{50} of 40.9 μg. An additional NMR study is needed to identify the inhibitor. This is the first report of the isolation of an ACE inhibitor from *Lycium chinense* Miller. Although the chemical nature of the inhibitor should be further characterized, this small molecular weight inhibitor is promising in terms of manufacturing a functional food product.

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