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Isolation of angiotensin I converting enzyme (ACE) inhibitor from fermented oyster sauce, *Crassostrea gigas*

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Abstract

Angiotensin I converting enzyme (ACE) inhibitor was isolated from fermented oyster sauce (FOS) and purified. Oyster was fermented with 25% NaCl (w/w) at 20 °C for 6 months. FOS was passed through a 40-mesh sieve, desalted using an electrodialyzer and then lyophilized. ACE inhibitory activity of FOS was investigated, and the IC_{50} value was determined to be 2.45 mg/ml. ACE inhibitor from FOS was purified using SP-Sephadex C-25 ion exchange chromatography, Sephadex G-50 gel chromatography, high-performance liquid chromatography (HPLC) on a gel permeation chromatography (GPC) column and reversed-phase HPLC on a C_{18} column. The purified inhibitor had an IC_{50} value of 0.0874 mg/ml, and it exhibited competitive inhibition against ACE. The purified peptide was evaluated for its antihypertensive effect in spontaneously hypertensive rats (SHRs) following oral administration. Rat blood pressure significantly decreased after inhibitor injection.

Keywords: Fermented oyster; ACE inhibitor; Antihypertensive effect; Competitive inhibition

1. Introduction

Hypertension is one of the most common cardiovascular diseases. It is a worldwide problem of epidemic proportions, which affects 15–20% of all adults. It is the most common serious chronic health problem because it carries a high risk factor for arteriosclerosis, stroke, myocardial infarction and end-stage renal disease. It is suggested that hypertension is closely related to food components, and antihypertensive peptides, controlling hypertension, may be associated with the presence of an antihypertensive peptide motif.

Angiotensin I converting enzyme (EC 3.4.15.1; ACE) plays an important physiological role in regulating blood pressure (Skeggs, Kahn, Kahn, & Shumway, 1957). ACE belongs to the class of zinc proteases and is located in the vascular endothelial lining of lungs. ACE acts as an exopeptidase that cleaves dipeptides from the C-terminus of various oligopeptides (Curtiss, Chon, Vrobel, & Francious, 1978; Yang, Erdös, & Levin, 1971). ACE catalyzes the hydrolysis of angiotensin I to generate a potent vasoconstrictor, angiotensin II, and inactivates bradykinin, which has a depressor action. Since the discovery of ACE inhibitors in snake venom, many studies have been directed toward the attempted synthesis of ACE inhibitors, such as captopril, enalapril, alacepril, and lisinopril, which are currently used extensively in the treatment of essential hypertension and heart failure in humans (Ondetti, 1977; Patchett et al., 1980). However, these synthetic drugs are believed to have certain side effects, such as cough, taste disturbances and skin rashes (Atkinson & Robertson, 1979). Therefore, a search for ACE inhibitors from foods has become a major area of research. In recent years, many ACE inhibitory peptides have

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been isolated from various food proteins, such as cheese whey (Abubakar, Saito, Kitazawa, Kawai, & Itoch, 1998), casein (Kohmura et al., 1989; Maeno, Yamamoto, & Takano, 1996; Maruyama et al., 1989), zein (Miyoshi et al., 1991; Yano, Suzuki, & Funatsu, 1996), tuna muscle (Kohama et al., 1988), sardine (Ukeda et al., 1992), corn gluten (Suh & Whang, 1999) and bovine blood plasma (Hyun & Shin, 2000). In addition, some ACE inhibitors have also been reported in some fermented foods, such as soy sauce (Kinoshita, Yamakoshi, & Ikuchi, 1993), soybean (Okamoto, Hanagata, Kawamura, & Yanagida, 1995a, 1995b) and milk (Gobbetti, Ferranti, Smacchi, & Goffredi, 2000).

Fish and shellfish sauce are widely used in southeast and east Asian countries. In Korea, the production of oyster was estimated to be 182,229 ton in 2002, and only a few fish and shellfish sauces have survived in local areas in Korea. However, fish and shellfish sauces have recently been rediscovered because of increased consumer interest in their taste and flavour.

From this point of view, the present study intended to isolate an ACE inhibitor derived from fermented oyster sauce (FOS) and to characterize the purified inhibitor with respect to ACE inhibitory activity. Moreover, the antihypertensive action of the purified inhibitor, by oral administration in spontaneously hypertensive rats (SHRs), was also investigated.

2. Materials and methods

2.1. Materials

Oyster, *Crassostrea gigas*, was purchased from a local shellfish market (Samchunpo, Korea). ACE (from rabbit lung) and substrate peptide (hippuryl-histidyl-leucine) of ACE, SP-Sephadex C-25 and Sephadex G-50 were purchased from Sigma Chemical Co. (St. Louis, MO). Ohpak SB-800 HQ (2.5×250 mm), for gel permeation chromatography (GPC), was purchased from Showa Denko K.K. (Tokyo, Japan) and a Nucleosil 100-3 ODS C_{18} column (4.6×250 mm) was from Macherey-Nagel (Middleton Cheney OX17 2PA, UK). All other reagents used in this study were reagent grade chemicals.

2.2. Preparation of FOS

Oyster was washed with water to remove salt and other materials, and then fermented with 25% NaCl (w/w) at 20 °C for 6 months. Fermentation was terminated by boiling at 95 °C for 10 min. The FOS was desalted by an electrodialyzer (Micro Acilyzer Model G3, Asahi Chemical Industry Co., Tokyo, Japan) with a 100 Da MWCO (molecular weight cut off) membrane (Asahi Chemical Industry Co., Tokyo, Japan). The desalted FOS was lyophilized and stored at −20 °C until used.

2.3. Assay for ACE inhibitory activity

The ACE inhibitory activity was measured by the method of Cushman and Cheung (1971) with slight modifications. A sample solution (50 μl) with 50 μl of ACE solution (25 munits/ml) was pre-incubated at 37 °C for 10 min, and the mixture was incubated with 150 μl of substrate (8.3 mM Hip-His-Leu in 50 mM sodium borate buffer containing 0.5 M NaCl at pH 8.3) for 30 min at the same temperature. The reaction was terminated by the addition of 250 μl of 1 M HCl. The resulting hippuric acid was extracted with 0.5 ml of ethyl acetate. After centrifugation (800*g*, 15 min), 0.2 ml of the upper layer was transferred into a test tube, and evaporated at room temperature for 2 h in a vacuum. The hippuric acid was dissolved in 1.0 ml of distilled water, and the absorbance was measured at 228 nm using a UV-spectrophotometer (Cary 1C, Varian Inc., Australia). The IC_{50} value was defined as the concentration of inhibitor required to inhibit 50% of the ACE activity.

2.4. Purification of ACE inhibitor form FOS

The lyophilized FOS (20 g) was loaded onto a SP-Sephadex C-25 ion-exchange column (4.0×40 cm) equilibrated with 20 mM sodium acetate buffer (pH 4.0) and eluted with a linear gradient of NaCl (0–2 M) in the buffer at a flow rate of 60 ml/h. Active fractions were collected, desalted using an electrodialyzer and lyophilized immediately. For further purification, a Sephadex G-50 gel filtration column (2.5×98 cm), equilibrated with distilled water, was employed. The column was eluted with distilled water and 5.0 ml fractions were collected at a flow rate of 60 ml/ h. The fraction with the highest ACE inhibitory activity was dissolved in distilled water and separated by high-performance liquid chromatography (HPLC) on a GPC column. The separation was performed with distilled water at a flow rate of 60 ml/h. Fractions showing ACE inhibitory activity were pooled and lyophilized. Further purification of ACE inhibitor was carried out by reversed-phase HPLC (RP-HPLC) using a Nucleosil 100-3 ODS C_{18} column. A linear gradient of acetonitrile from 0% to 11% containing 0.1% trifluoroacetic acid (TFA), was maintained at a flow rate of 1 ml/min. Elution peaks were monitored at 215 nm, and their ACE inhibitory activities were measured using the method previously described.

2.5. Determination of molecular weight

Molecular weight of the purified peptide was determined by a GPC column at a flow rate of 1 ml/min. Standard molecular weight makers used were as follows: cytochrome C (MW 12,900 Da), aprotinin (MW 6500 Da), angiotensin I (MW 1296.5 Da) and pentaphenylalanine (MW 753.9 Da).