J. Microbiol. Biotechnol. (2012), **22**(3), 339–342
http://dx.doi.org/10.4014/jmb.1109.09015
First published online December 16, 2011
pISSN 1017-7825 eISSN 1738-8872

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Characterization of New Antihypertensive Angiotensin I-Converting Enzyme Inhibitory Peptides from Korean Traditional Rice Wine

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Received: September 14, 2011 / Revised: October 29, 2011 / Accepted: November 15, 2011

This study describes the characterization of a new angiotensin I-converting enzyme (ACE) inhibitory peptide from a Korean traditional rice wine. After purification of the ACE inhibitor peptides with ultrafiltration, Sephadex G-25 column chromatography, and successively C_{18} and SCX solid-phase extraction, reverse-phase HPLC, and size exculsion chromatography, two types of the purified ACE inhibitors with IC_{50} values of 0.34 mg/ml and 1.23 mg/ml were finally obtained. The two purified ACE inhibitors (F-1 and F-2) were found to have two kinds of novel oligopeptides, showing very little similarity to other ACE inhibitory peptide sequences. The amino acid sequences of the two purified oligopeptides were found to be Gln-Phe-Tyr-Ala-Val (F-1) and Ala-Gly-Pro-Val-Leu-Leu (F-2), and their molecular masses were estimated to be 468.7 Da (F-1) and 357.7 Da (F-2), respectively. They all showed a clear antihypertensive effect on spontaneously hypertensive rats at a dosage of 500 mg/kg.

Keywords: Korean traditional rice wine, angiotensin I-converting enzyme inhibitory peptide, antihypertension

Korean traditional rice wines have long been produced in traditional ways using *nuruk*, cooked non-glutinous rice and flour, yeasts, and some medicinal plants or herbs [3]. Many research groups have been studying the quality of Korean traditional rice wines, as well as the changes in microbes and enzyme activity related to quality during the fermentation, the characteristics of free sugars and organic acids, acceptability, standardization of the manufacturing process, storage, and so on [1, 2, 4, 12, 14, 15]. Since the functionalities in Korean traditional rice wines such as chitooligosaccharides from *koji* or *nuruk* molds were discovered, many functional traditional rice wines have been developed [4]. However, there have been some problems, such as short shelf life, lack of unique characteristics, and inferior acceptability and functionality. Therefore, it is necessary to develop new Korean traditional rice wines with excellent acceptability and functionality with an extension of shelf life. We previously reported on the characteristics of Korean traditional rice wines made by dandelions [5], chamomile [8], acacia [16], and *Paecilomyces japonica* [9]. Furthermore, physiological functionalities of several Korean traditional rice wines were also investigated [10]. The results from these studies suggest that medicinal plants, fruits, or mushrooms are very useful for increasing the physiological functionality of Korean traditional rice wine.

Angiotensin I-converting enzyme catalyzes the production of the active hypertensive hormone, angiotensin II, from the inactive prohormone, angiotensin I, in the renin–angiotensin system [6]. In the kallikrein–kinin system, ACE also inactivates the hypotensive peptide, bradykinin. Therefore, ACE has a very important role in both blood pressure regulation systems. ACE inhibitors have long been used as antihypertensive agents because they inhibit both the ACE in the renin–angiotensin system and that in the kallikrein–kinin system. Ever since the ACE inhibitor was first discovered in snake venom, many antihypertensive ACE inhibitors have been isolated and characterized from various natural sources and microorganisms including cereals and legumes [13], *Saccharomyces cerevisiae* [7], *Ganoderma lucidum* [4], and *Tricholoma giganteum* [11]. ACE inhibitory peptides have also been isolated from foods such as sake and its by-products [17], and from Korean traditional rice wines [3] or from the enzymatic digestion of food proteins including gelatin, casein, fish, fig tree latex, α-zein, *etc.* [6, 11]. Even though many natural and synthetic ACE inhibitors, such as captopril, enalapril, and lisinopril, are remarkably effective as antihypertensive drugs, they show certain disadvantages, with side effects such as coughing, allergies, taste disturbances, and skin

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rashes. Therefore, the development of novel ACE inhibitors that have strong antihypertensive activity without side effects is required. In this paper, we describe the characterization of new ACE inhibitors from a Korean traditional rice wine, which can be used as an antihypertensive agent for preparation of functional alcoholic beverages or foods.

Purification of the ACE Inhibitor from Korean Traditional Rice Wine

The concentrates from the traditional rice wine were ultrafiltrated with a 5,000 MW cut-off filter, and the ACE inhibitory activity of the filtrates was then determined. The ACE inhibitory activity of the filtrates was an IC_{50} of 1.14 mg/ml. After Sephadex G-25 column chromatography of the active filtrates, an active fraction with ACE inhibitory activity of IC_{50} 0.7 mg/ml was obtained. The active fractions were then subjected to a C_{18} solid-phase extraction chromatography from 5% to 100% acetonitrile. The fraction F-1 from the extraction of 25% ACN showed 77.7% (IC_{50}=0.45 mg/ml) of ACE inhibitory activity. After SCX solid-phase extraction chromatography of the active fraction by 10–200 mM ammonium formate, the active fraction (F1-1, IC_{50}=0.39 mg/ml) from the 200 mM ammonium formate extraction was obtained. When subjected to RP-HPLC using a Vydac protein/peptide reverse-phase 218TP column for the active fraction, one active fraction (F1-1A, IC_{50}=0.37 mg/ml) was obtained and then subjected to size-exclusion chromatography. Finally, we obtained the purified ACE inhibitor, showing ACE inhibitory activity with an IC_{50} of 0.35 mg/ml (Fig. 1 and Table 1).

Amino Acid Sequence of the ACE Inhibitor

The purified ACE inhibitor (F1-1A) was analyzed and we obtained two kinds of oligopeptides. The amino acid sequences of the two ACE inhibitory oligopeptides were found to be Gln-Phe-Tyr-Ala-Val (F1-1A-1) and Ala-Gly-Pro-Val-Leu-Leu (F1-1A-2) (Fig. 2). Their ACE inhibitory activities were of IC_{50} values of 0.34 mg/ml and 1.23 mg/ml, respectively. Many various ACE inhibitors have been reported. Furthermore, it is known that almost all of the ACE inhibitors are peptides ranging from dipeptides to oligopeptides [7], except for triterpene from *Ganoderma lucidum* [4]. Saito *et al.* [17] reported on the isolation of an ACE inhibitory oligopeptide from sake and sake lee. However, these peptides from Korean traditional rice wine

Table 1. Purification of an ACE inhibitor from Korean traditional rice wine.

Purification steps	ACE inhibitory activity (IC_{50}: mg/ml)
Ultrafiltration	1.14
Sephadex G-25 chromatography	0.7
C_{18} solid-phase extraction chromatography	0.45
SCX solid-phase extraction chromatography	0.39
RP-HPLC	0.37
Size-exclusion chromatography	0.35

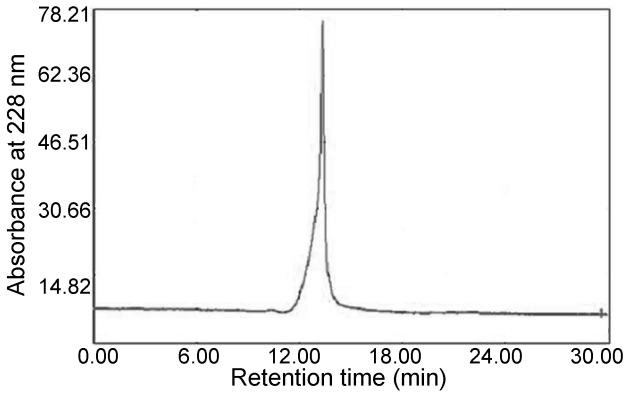

Fig. 1. Size-exclusion chromatogram of the active fraction from RP-HPLC.
Separation was performed with an isocratic of water containing 0.1% TFA at a flow rate of 0.5 ml/min.

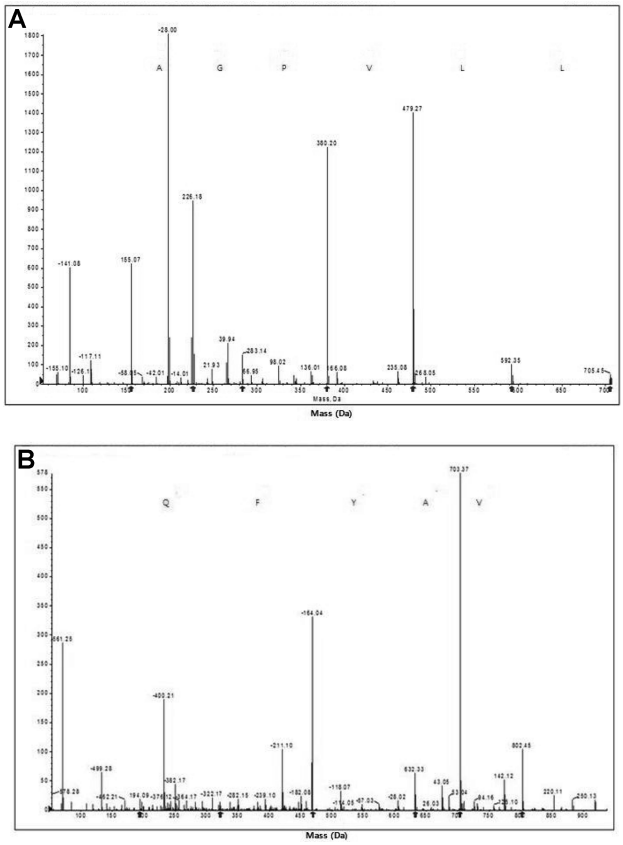

Fig. 2. Identification of the molecular mass and amino acid sequence of the purified ACE inhibitory peptides using LC-MS/MS.
(**A**) Ala-Gly-Pro-Val-Leu-Leu; (**B**) Gln-Phe-Tyr-Ala-Val).

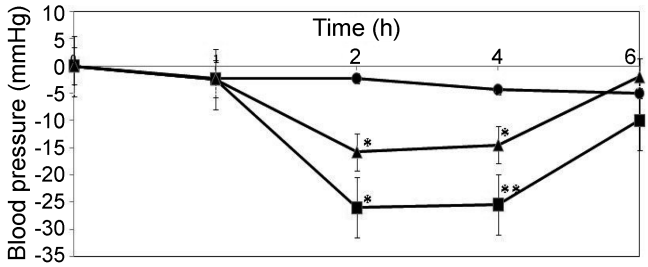

Fig. 3. Changes in systolic blood pressure of spontaneously hypertensive rat by administering partially purified ACE inhibitor from the traditional rice wine.

A single oral administration was performed with a dose of 500 mg/kg body weight, and SBP was measured 0, 2, 4, and 6 h after administration. Different from control at *P < 0.05, **P < 0.01. ●, Saline solution; ■, commercial captopril; ▲, partial purified ACE inhibitor.

are novel ACE inhibitors, which have not previously been found in any free peptides or protein hydrolysates.

Molecular Mass of the Purified ACE Inhibitors

The molecular masses of the purified ACE inhibitors were estimated to be 468.7 Da (F1-1A-1) and 357.7 Da (F1-1A-2), respectively, by LC-MS/MS analyses (Fig. 2). These molecular masses were smaller than those of other ACE inhibitory peptides [7, 11]. Therefore, it would be very useful as an antihypertensive agent because it is easy to absorb in the small intestine.

Antihypertensive Action of the ACE Inhibitor

The antihypertensive action of the ACE inhibitor was investigated using SHRs. As shown in Fig. 3, the average blood pressure of the SHRs in the ACE inhibitor group was approximately 160 mmHg just before administration of the ACE inhibitor. Two hours after the administration the ACE inhibitor at a dosage of 500 mg/kg for the rats, the blood pressure measured decreased to 145 mmHg; the average blood pressure then increased slightly. These results suggests that the purified ACE inhibitor exhibits a clear antihypertensive effect in SHRs at a dosage of 500 mg/kg.

We concluded that the Korean traditional rice wine in this study is an antihypertensive food, even though the ACE inhibitory activity was lower than those of the commercial antihypertensive drug captopril and other purified ACE inhibitors from *S. cerevisiae* [7] and *T. giganteum* [11].

Acknowledgment

This study was performed as a 2007 Cooperative Project of the Korea Food Research Institute, supported by the Korea Institute of Planning and Evaluation for Technology of Food, Agriculture, Forestry (ex-ARPC), Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

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