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ARTICLE

Isolation of an Antihypertensive Peptide from Alcalase Digest of *Spirulina platensis*

Jun Lu,[§,†,‡] Di-Feng Ren,[§,†,‡] You-Lin Xue,[†] Yoriko Sawano,[†] Takuya Miyakawa,[†] and Masaru Tanokura*[,†]

[†]Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan, and [‡]College of Biological Science and Technology, Beijing Forestry University, Hai-Dian District, Beijing 100083, People's Republic of China. [§] These two authors contributed equally to this work.

An angiotensin I-converting enzyme (ACE) inhibitory peptide Ile-Gln-Pro with an IC_{50} value of 5.77 ± 0.09 μM was purified from the alcalase digests of *Spirulina platensis* by gel filtration chromatography and two steps of reverse-phase high-performance liquid chromatography (RP-HPLC). The peptide was synthesized and showed resistance to in vitro digestion by gastrointestinal proteases. Kinetics studies indicated that the peptide was a noncompetitive inhibitor and that the K_i value was 7.61 ± 0.16 μM. Oral administration of Ile-Gln-Pro at a dosage of 10 mg/kg showed significant decreases of the weighted systolic blood pressure (SBP) and diastolic blood pressure (DBP) in spontaneously hypertensive rats (SHR) at 4, 6, and 8 h after treatment. The results showed that the ACE inhibitory peptide from *Spirulina platensis* may have potential for use in the prevention and treatment of hypertension.

KEYWORDS: ***Spirulina platensis*; angiotensin I-converting enzyme; ACE inhibitory peptide; antihypertension**

INTRODUCTION

Angiotensin I-converting enzyme (ACE), a key enzyme component of the renin−angiotensin system (RAS), plays a critical physiological role in the regulation of blood pressure and body fluid homeostasis (*1*, *2*). In the RAS, ACE cleaves a dipeptide from the C-terminal of angiotensin I to form angiotensin II, which regulates blood pressure as well as sodium and water reabsorption directly by stimulating its high-affinity type 1 receptor (*3*). Blockade of the RAS with ACE inhibitors has been an effective pharmacotherapy for a number of diseases, including hypertension, heart failure, ventricular remodeling, and diabetes (*3*).

Since the discovery of captopril (*4*), various synthetic ACE inhibitors have been used extensively in the treatment of essential hypertension and heart failure, including captopril, zofenopril, enalapril, ramipril, lisinopril, and benazepril (*5*). However, these synthetic inhibitors inevitably have some adverse side effects, such as coughing, taste disturbances, and skin rashes (*6*). In recent years, nutraceuticals and functional foods have attracted considerable interest as potential alternatives for the treatment of hypertension (*7*). More attention has been paid to the peptides from hydrolysates or enzymatic digests of food proteins due to their improved bioactivities and low side-effect profiles. Many ACE inhibitory peptides have been isolated from various food materials, such as casein (*8*), corn gluten meal (*9*), garlic (*5*), gelatin (*10*), mushroom (*11*), natto (*12*), porcine hemoglobin (*13*), potato (*14*), sake (*15*), soybean (*16*), tofuyo (*17*), and tuna (*18*, *19*).

Spirulina, a blue-green filamentous alga which has been commercially produced for more than 10 years as a human food supplement, is gaining more attention for the treatment of various diseases because it contains high-quality proteins, carotenoids, vitamin E, phycocyanine, and other nutritional components (*20*). Experimental studies have demonstrated that *Spirulina* exerts beneficial antivirus (*21*), anticancer (*22*), fatty-liver prevention (*23*), and immune-strengthening effects (*24*). Although enzymatic hydrolysates of *Spirulina* show ACE inhibitory activity (*25*), limited knowledge is available regarding their ACE inhibitory activity or antihypertensive effects. Taking into account that as much 55% to 77% of the dry weight of *Spirulina* consists of proteins, and *Spirulina* contains all of the amino acids essential for human health (*26*), it would be of interest to isolate and develop bioactive peptides with high-level ACE inhibitory properties from *Spirulina*.

In the present study, we isolated, purified, and characterized an ACE inhibitory peptide from hydrolysates and enzymatic digests of *Spirulina platensis* by alcalase. The inhibition pattern of the peptide on ACE was examined, and the stability was evaluated under simulated gastrointestinal conditions. In addition, the *in vivo* antihypertensive effect of the purified peptide was investigated in spontaneously hypertensive rats (SHR).

MATERIALS AND METHODS

Materials and Chemicals. The spray-dried powder of *Spirulina platensis* used in this study was obtained from Inner Mongolia Reiuve Biotech Co., Ltd. (Eerduosi, China) and kindly supplied by DIC Lifetec

*To whom correspondence should be addressed. Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. Tel: +81-3-5841-5165. Fax: +81-3-5841-8023. E-mail: amtanok@mail.ecc.u-tokyo.ac.jp.

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Co., Ltd. (Tokyo, Japan). Alcalase (2.4 L) from *Bacillus licheniformis* (P4860), a product of Novozymes Corp., was purchased from Sigma-Aldrich (St. Louis, MO). ACE powder extracted from rabbit lungs and the lyophilized powder of bovine serum albumin were also obtained from Sigma-Aldrich. Abz-FRK(Dnp)P-OH was from BIOMOL International L.P. (Plymouth Meeting, PA). All other reagents, unless otherwise specified, were of analytical or guaranteed reagent grade.

Preparation of Water Extracts and Enzymatic Hydrolysates of *Spirulina platensis*. Three enzymes, alcalase, papain, and thermolysin, were preliminarily tested, and alcalase was chosen because alcalase-treated hydrolysate showed the highest ACE inhibitory activity among the tested hydrolysates. Spray-dried powder of *Spirulina platensis* (20 g) suspended in 160 mL of water was repeatedly frozen with liquid N_2, thawed (5 freeze−thaw cycles in total), and then sonicated for 3 min. The cell suspension was then incubated at 30 °C for 12 h and centrifuged at 6,000*g* for 30 min to obtain water extracts of *S. platensis*.

Enzymatic hydrolysates of *S. platensis* were prepared as described above for the suspension, freeze−thawing, and sonication procedures. After sonication, the cell suspension was immediately centrifuged and lyophilized. The protein content was measured with Protein Assay (Bio-Rad Laboratories, Hercules, CA) using bovine serum as a standard. The lyophilized powder was dissolved in water at pH 8.5 at 2% (w/w) protein concentration and digested by 2.4 L of alcalase (~2.4 units/mL) with the ratio of enzyme to substrate protein being 0.04% (v/v) at 50 °C until the enzymatic digestion was completed (10 h). After the reaction was stopped by adjusting the pH to 4.0 with HCl, we cooled the mixture on ice and then centrifuged at 6,000*g* for 10 min.

Each of the water extracts and enzymatic hydrolysates was ultrafiltrated using Vivaspin 10,000, 5,000, and 3,000 molecular weight cutoff (MWCO) membranes (Sartorius Co., Goettingen, Germany). Each fraction was collected, and its ACE inhibitory activity was measured. The active fractions were lyophilized and stored at 4 °C for purification.

Purification of ACE Inhibitory Peptides. The lyophilized active fractions from enzymatic hydrolysis were dissolved in 10 mM Tris-HCl (pH 7.0). The solution was applied onto a Superdex Peptide HR 10/30 gel filtration column furnished with an ÄKTApurifier System (GE Healthcare, Buckinghamshire, England) and eluted with a solution containing 10 mM Tris-HCl (pH 7.0) and 150 mM NaCl at a flow rate of 0.6 mL/min. The eluted fractions were detected at 220 nm, and the ACE inhibitory activities were determined. The active fractions were collected and lyophilized immediately, and the dissolved solution was applied to preparative reverse-phase high-performance liquid chromatography (RP-HPLC) on a COSMOSIL MS-II C18 column (4.6 mm × 250 mm; Nacalai Tesque Co., Kyoto, Japan) at a flow rate of 1.5 mL/min. The column was equilibrated with 0.1% trifluoroacetic acid (TFA) in 1% (v/v) acetonitrile (ACN)/99% (v/v) water solution, and elution was carried out with a linear gradient of 1−45% (v/v) ACN in 0.1% (v/v) TFA for 12 column volumes (CVs). The active fractions were collected and lyophilized immediately, and were successively rechromatographed under the same RP-HPLC conditions except for the gradient (12−20% (v/v) ACN in 0.1% (v/v) TFA) for 15 CVs.

Assay of ACE Inhibitory Activity. The ACE inhibitory activity was assayed using a fluorescence resonance energy transfer (FRET) substrate, Abz-FRK(Dnp)P-OH, by a method described previously (*27*) with some modifications. Briefly, a 2 mL cuvette containing a mixture of 0.1 M Tris-HCl (pH 7.0), 50 mM NaCl, 10 μM $ZnCl_2$, 0.001 units of ACE, and 100 μL of the inhibitory peptide solution was preincubated in the thermostatted cell compartment of a fluorometer (RF-5300-PC; Shimadzu Corporation, Kyoto, Japan; λ_{ex} = 320 nm and λ_{em} = 420 nm) under agitation with a magnetic stirrer bar for 5 min at 37 °C. The reaction was initiated by adding 15 μL of the FRET substrate at a final concentration of 1.8 μM. The initial velocities (v_0) were calculated from the increase in fluorescence for 2 min. The extent of inhibition was calculated as follows:

$$\text{ACE inhibitory activity (\%)} = [1 - (v_0 \text{ with inhibitory peptide})/(v_0 \text{ without inhibitory peptide})] \times 100$$

The IC_{50} value was defined as the concentration of ACE inhibitory peptide required to inhibit 50% of the ACE activity under the above assay conditions and was determined by a regression analysis of ACE inhibition (%) versus peptide concentration.

The K_i value and inhibition mode of synthesized ACE inhibitory peptides were determined by Lineweaver−Burk plot and Dixon plot from experiments conducted at four inhibitor concentrations (3, 7, 11, and 15 μM) and at three substrate concentrations (1.05, 1.67, and 4 μM).

Mass Spectrometry and Amino Acid Sequence Analysis. The molecular masses of the purified ACE inhibitory peptides were determined using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (AXIMA CFR plus; Shimadzu). Prepared samples were analyzed in the linear positive ion mode following the common protocol of the dried-droplet technique mixing equal volumes (1 μL) of sample and matrix solution (α-cyano-4-hydroxycinnamic acid, 5 mg/mL in a mixture of ACN and 0.1% v/v TFA, 50:50 v/v). Sample measurements were performed in triplicate.

The amino acid sequence was determined by the Edman method, using PPSQ-31A/33A Automated Protein/Peptide Sequencer (Shimadzu) according to the manufacturer's instructions. Fifteen microliters of the unknown samples (more than 50 pmol) were added to a polyvinylidene difluoride membrane and were dried for the analysis of amino acid sequence.

Peptide Synthesis. The ACE inhibitory peptides were chemically synthesized by the Thermo Fisher Scientific Greiner Bio-One Co., Ltd. (Tokyo, Japan). The purity of the synthesized peptide was verified by analytical HPLC coupled with MALDI-TOF MS to be higher than 95%.

Stability of the ACE Inhibitory Peptide. The stability of the synthesized ACE inhibitory peptides against gastrointestinal proteases, pepsin, chymotrypsin, and trypsin was assessed in vitro according to a previously described method (*17*) with some modifications. Briefly, 0.2 mL of the 1.5 mM peptide solution was treated with 0.2 mL of 0.05% (w/v) protease solution containing 0.1 M KCl−HCl (pH 2.0) for pepsin or 0.1 M K_3PO_4 (pH 8.0) for chymotrypsin and trypsin for 6 h at 37 °C, and the reaction was stopped by adding 1 M NaOH or 6 M HCl to pH 7.0. The pepsin digest was also subjected to successive digestion by chymotrypsin and trypsin. The pepsin digest was evaporated with a vacuum centrifuge and redissolved in 0.2 mL of water. The solution was then treated with 0.025% (w/v) chymotrypsin as well as 0.025% (w/v) trypsin, and the reaction was stopped under the same conditions as described above.

The stopped reaction mixtures were ultrafiltrated using 10,000 MWCO membranes, and the filtrates were applied to an HPLC system (Hydrosphere C18 column, 4.6 mm ×150 mm; YMC Co., Ltd., Kyoto, Japan) at a flow rate of 1 mL/min. The column was equilibrated with 0.1% TFA in 1% ACN/water solution, and a linear gradient was carried out with 0% to 90% ACN in 0.1% TFA for 30 min.

Antihypertensive Effect in SHR. Fifteen 8-week-old male SHR, purchased from Charles River Laboratories (Kanagawa, Japan), were acclimated for one week at a temperature of 24 °C with a 12 h light−dark cycle, and fed with tap water and a common laboratory diet ad libitum. During this period, the rats became accustomed to the blood-pressure-measuring procedure: the systolic blood pressure (SBP), diastolic blood pressure (DBP), and heartbeat rate (HR) of the rats were measured by the tail cuff method everyday, and before measurement, the rats were kept at 30 °C for 10 min to make the pulsations of the tail artery detectable. The care and treatment of the rats was in accordance with The Ethical Guide for the Care and Use of Laboratory Animals of Chiba University, and the animal experiments were approved by Ethics Committee for Animal Experiments of Chiba University.

On the eighth day, the rats were assigned to 3 groups, a negative control, positive control, and treatment group, according to the balanced average blood pressure of each group. A dose of the synthesized ACE inhibitory peptide, 10 mg/kg per rat dissolved in 0.9% saline, was orally administered to the treatment group by gastric intubation. The positive control group was administered captopril at a dosage of 10 mg/kg per rat in saline, while the negative control group was administered saline only. The SBP, DBP, and HR of the rats were measured by the tail cuff method as described above before administration and at 2 h, 4 h, 6 hand 8 h after administration. Five measurements were taken, and then the maximum and minimum measurements were discarded, and the three remaining values were averaged. Data were assessed by analyses of group differences and were considered statistically significant at $p < 0.05$ by Tukey's test.

RESULTS

ACE Inhibitory Activities of Alcalase Hydrolysates and Water Extracts of *Spirulina platensis*. The ACE inhibitory activities of

Table 1. ACE Inhibitory Activities of Extracts of *Spirulina platensis*

pretreatment	molecular weight	IC_{50} (mg/mL)	yield (%)[a]
none	0−3,000	0.30 ± 0.02	9.3
	3,000−5,000	0.34 ± 0.01	18.0
	5,000−10,000	0.52 ± 0.05	7.8
alcalase	0−3,000	0.23 ± 0.02	15.0
	3,000−5,000	0.31 ± 0.02	30.8
	5,000−10,000	0.33 ± 0.02	21.9

[a] The yield was the protein recovery rate of which the protein concentration was measured with $A_{220\ nm}$.

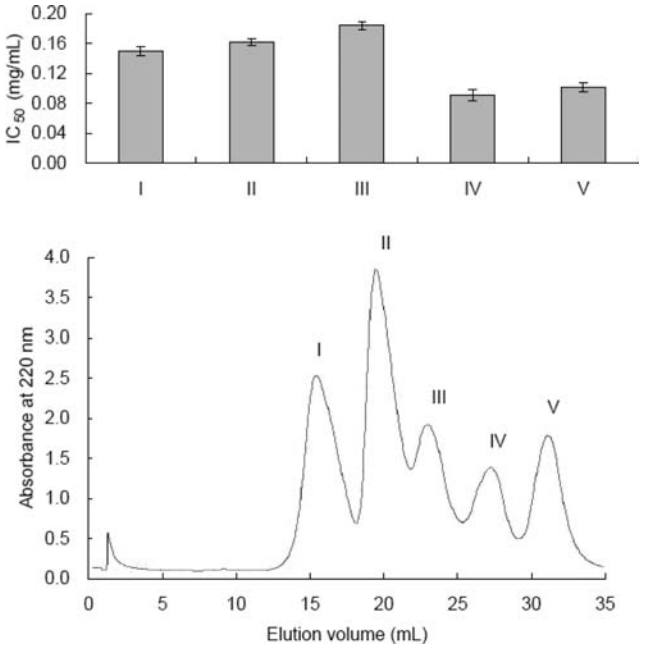

Figure 1. Gel filtration chromatogram of the alcalase hydrolysates on a Superdex Peptide HR 10/30 column (lower panel) and ACE inhibitory activity of each fraction (upper panel).

alcalase hydrolysates and the water extracts from *Spirulina platensis* are summarized in **Table 1**. The enzymatic hydrolysates had higher ACE inhibitory activity than the water extracts in each MW range. The digests by alcalase with a MW range of 0 to 3,000 showed the most potent inhibitory activity of 0.23 ± 0.02 mg/mL with a yield of 15.0%. Therefore, this fraction was chosen for the production of ACE inhibitor for the following procedures.

Purification of ACE Inhibitory Peptides. The digests by alcalase with a MW of 0 to 3,000 were further purified by using sequential chromatographic methods. First, gel filtration chromatography on Superdex Peptide HR 10/30 was used to fractionate the digests, and the fractions corresponding to major absorbance peaks were collected to evaluate ACE inhibitory activity. There were five major peaks (I−V) with an absorbance at 220 nm, among which fraction IV showed the most potent ACE inhibitory activity of 0.091 ± 0.007 mg/mL, with a yield of 1.34% (**Figure 1** and **Table 2**).

Fraction IV was then subjected to reverse-phase HPLC on a COSMOSIL MS-II C18 column, and the most active fraction (B) was found to have an inhibitory activity of 0.0039 ± 0.0003 mg/mL and a yield of 0.22% (**Figure 2** and **Table 2**). Fraction B was again chromatographed under the same RP-HPLC conditions except for the gradient, and finally, one single peak was eluted with an inhibitory activity of 0.0021 ± 0.0001 mg/mL and a yield of 0.18% (**Figure 3** and **Table 2**).

The IC_{50} values of the most active fractions in different stages were also measured to reflect the efficiency of the purification

Table 2. Purification Efficiencies of the ACE Inhibitory Peptide from *Spirulina platensis* at Different Purification Steps

purification step	IC_{50} (mg/mL)	yield (%)[a]
alcalase digests	0.47 ± 0.04	100
ultrafiltration	0.23 ± 0.02	15.0
superdex peptide HR 10/30	0.091 ± 0.007	1.34
first RP-HPLC	0.0039 ± 0.0003	0.22
second RP-HPLC	0.0021 ± 0.0001[b]	0.18

[a] The yield was the protein recovery rate of which the protein concentration was measured at $A_{220\ nm}$. [b] Equivalent to 5.77 ± 0.09 μM with respect to Ile-Gln-Pro.

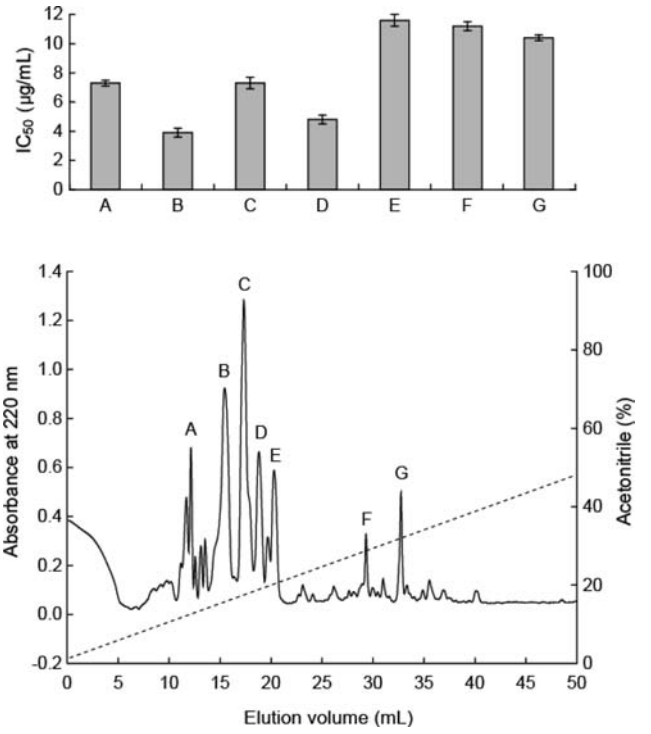

Figure 2. First reverse-phase HPLC profile on a COSMOSIL MS-II C18 column (lower panel) and ACE inhibitory activity of each fraction (upper panel).

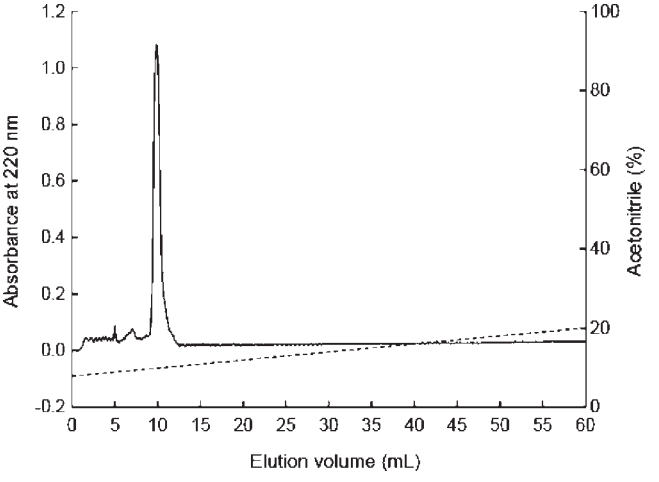

Figure 3. Second reverse-phase HPLC profile on a COSMOSIL MS-II C18 column.

procedures, and as shown in **Table 2**, the IC_{50} value was improved about 224-fold from 0.47 ± 0.04 mg/mL of the alcalase digest to 0.0021 ± 0.0001 mg/mL (equivalent to 5.77 ± 0.09 μM) of the purified peptide.

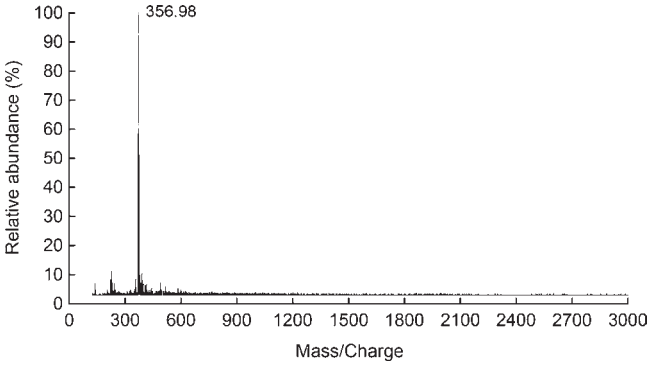

Figure 4. Mass spectrum of the purified ACE inhibitory peptide from *Spirulina platensis*.

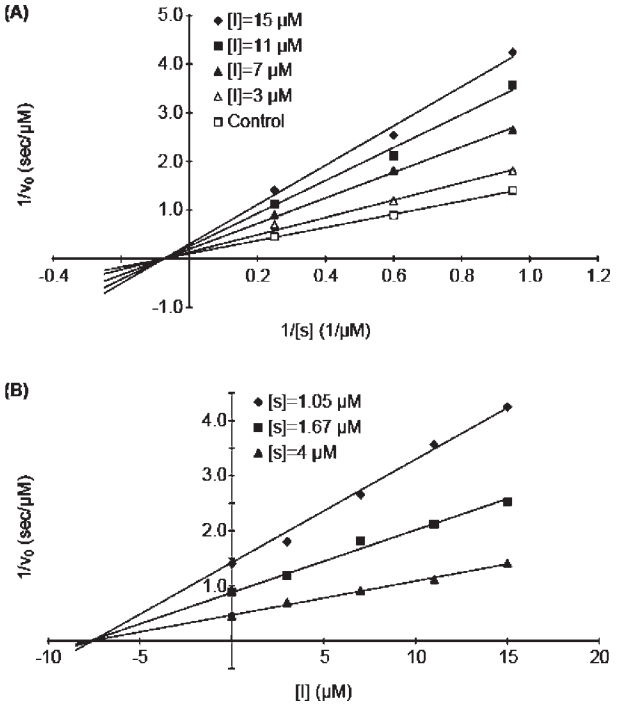

Figure 5. Inhibitory kinetics of Ile-Gln-Pro on the ACE activity using Abz-FRK(Dnp)P-OH as a substrate. (**A**) Lineweaver−Burk plot of the reciprocal velocity ($1/v$) against 1/[s] in the presence of Ile-Gln-Pro at concentrations of 0 μM (□), 3 μM (Δ), 7 μM (▲), 10 μM (■), and 15 μM (♦). (**B**) Dixon plot of $1/v$ against the concentration of Ile-Gln-Pro, [I], at substrate concentrations of 1.05 μM (♦), 1.67 μM (■), and 4 μM (▲).

Molecular Weight and Amino Acid Sequence of the ACE Inhibitor. The molecular mass of the ACE inhibitory peptide, purified from enzymatic digests of *Spirulina platensis* of alcalase, was estimated to be 356.98 Da according to MALDI-TOF-MS analysis (**Figure 4**). The peptide sequence was found to be Ile-Gln-Pro by an amino acid sequence analysis.

Determination of the Inhibition Pattern of the ACE Inhibitory Peptide. The inhibition mechanism of the ACE inhibitory peptide, Ile-Gln-Pro, was examined by kinetic studies. As shown in **Figure 5**, the Lineweaver−Burk plot with an intersection on the 1/[s] axis indicated that the peptide was a noncompetitive inhibitor, and the Dixon plot with an intersection on the [I] axis also showed the characteristics of noncompetitive inhibition. The K_i value was determined to be 7.61 ± 0.16 μM.

Determination of Stability of the ACE Inhibitory Peptide. When Ile-Gln-Pro was treated with pepsin, chymotrypsin, and trypsin separately or successively, the ACE inhibitory activity only decreased slightly (**Table 3**). The digests by these gastrointestinal proteases were also analyzed by reverse phase-HPLC, and little difference was found for the retention time and peak area before and after treatments with gastrointestinal proteases (data not shown). These results suggested that if the ACE inhibitory peptide was orally administered, it would be kept stable and resistant to digestion in the gastrointestinal tracts.

Table 3. Stability of Ile-Gln-Pro from *Spirulina platensis* against the Digestion of Gastrointestinal Proteases

digestion	IC_{50} (μM)
none	5.77 ± 0.09
pepsin	5.81 ± 0.19
chymotrypsin	5.79 ± 0.05
trypsin	5.84 ± 0.06
pepsin → chymotrypsin and trypsin	5.91 ± 0.12

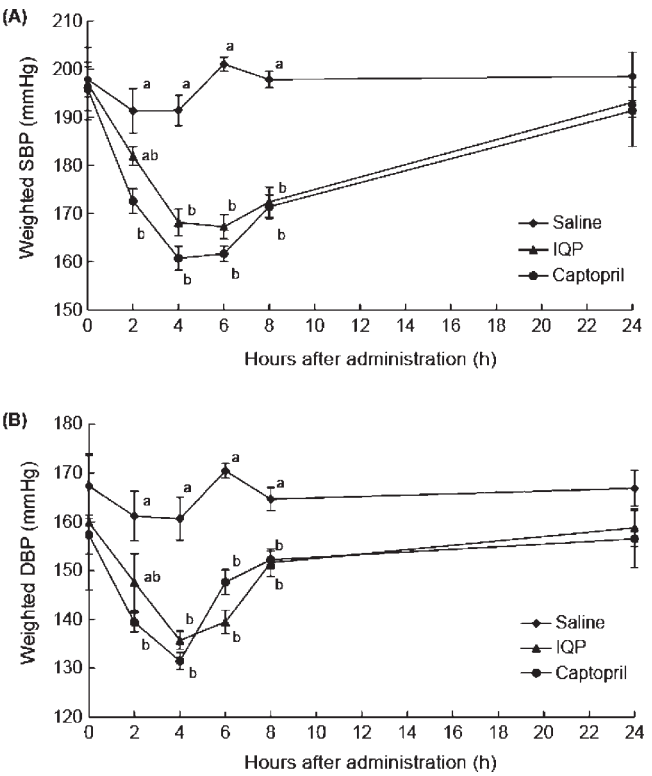

Figure 6. In vivo antihypertensive effect of the ACE inhibitory peptide Ile-Gln-Pro from *Spirulina platensis* in SHR. (**A**) Changes of the weighted SBP for 24 h after administration. (**B**) Changes of the weighted DBP for 24 h after administration. Weighted SBP or DBP = SBP or DBP/HR·AveHR, where AveHR was the average heartbeat rates of all the rats at the respective time point. Each value was expressed as the mean ± SEM. A value of $p < 0.05$ was considered to be statistically significant by one-way ANOVA followed by posthoc Scheffe's test. Values at the same time point with dissimilar lowercase letters were significantly different.

Antihypertensive Effect of the Purified ACE Inhibitory Peptide. The antihypertensive effect of Ile-Gln-Pro was evaluated by measuring the changes in the weighted SBP and DBP of SHR during a 24 h observation period following a single gastric intubation (10 mg/kg per rat). In the negative control group, no significant changes were found in the weighted SBP and DBP during the 24 h after administration. However, administration of Ile-Gln-Pro caused a significant decrease of the weighted SBP and DBP at 4, 6, and 8 h after administration, while captopril (10 mg/kg) lowered both the weighted SBP and DBP significantly at 2, 4, 6, and 8 h after administration (**Figure 6**). The results indicated that

the ACE inhibitory peptide Ile-Gln-Pro provided a clear antihypertensive effect in SHR at a dosage of 10 mg/kg per rat.

DISCUSSION

In recent years, there has been increasing awareness that certain naturally occurring compounds in plants and other bioresources confer protection against human diseases while avoiding the side effects associated with pharmaceuticals (*28*, *29*). To obtain the active antihypertensive ACE inhibitory peptide from *Spirulina*, in the present study we screened the water extracts and enzymatic hydrolysates by alcalase from *Spirulina platensis* and found that the enzymatic digests obtained using alcalase with an MW ranging from 0 to 3,000 had the most potent ACE inhibitory activity. The ACE inhibitory peptide was purified using gel filtration chromatography and two successive reverse-phase HPLCs, and the peptide sequence was identified as Ile-Gln-Pro.

Since ACE cleaves a dipeptide from the C-terminal region of angiotensin I to form angiotensin II, which is the major inducer of hypertension (*3*), the C-terminal of the inhibitory peptides is important for binding to the ACE enzyme and for their inhibitory activity (*5*, *30*). Studies have shown that peptides with hydrophobic residues, such as tryptophan, tyrosine, or phenylalanine, at the carboxy-terminal show the highest ACE inhibitory activities and that peptides having proline at the C-terminal are especially effective (*5*, *31*). Cheung et al. have also reported that peptides with branched-chain aliphatic amino acids at their amino-terminals exhibited ACE inhibitor activity (*31*). The ACE inhibitory peptide Ile-Gln-Pro purified in this study was a peptide first reported from *Spirulina platensis*. Its IC_{50} value of 5.77 ± 0.09 μM was relatively low compared to those of other ACE inhibitors derived from various sources at concentrations ranging from 3.74 μM to 12 mM (*5*, *8*, *11*, *13*−*17*, *32*, *33*), possibly because the peptide had amino acid residues at both the carboxy- and amino-terminals, and thus had an ideal structure for ACE inhibition.

Similar to many other reports in which the sequences of ACE inhibitors were generally in the range of tripeptides to oligopeptides, the purified ACE inhibitory peptide from *Spirulina platensis* was a tripeptide, and its molecular weight was very small, facilitating absorption in the intestine (*11*, *34*). Moreover, The ACE inhibitory peptide kept its inhibitory activity after incubation with various gastrointestinal proteases, suggesting that it would not be readily degraded in the gastrointestinal tracts (*16*). Although most of the reported ACE inhibitors exhibit competitive inhibition, some noncompetitive ACE inhibitors have been found in food resources such as natto (*12*), tuna (*18*), sake (*15*), and tofuyo (*17*), and the inhibition of Ile-Gln-Pro in this study was also noncompetitive according to both the Lineweaver−Burk plot and the Dixon plot. Some of the previously studied inhibitors, such as captopril, enalaprilat, and ramiprilat, are competitive according to the Lineweaver−Burk plot (*31*) but exhibit a noncompetitive mode on the Dixon plot. The reason for this phenomenon is suggested to be the slow-tight binding of these inhibitors at the ACE active sites (*17*, *31*). However, the details of the inhibition mechanism of noncompetitive ACE inhibitors, including Ile-Gln-Pro, remain to be clarified in the future.

In the present in vivo studies, a single gastric intubation administration of Ile-Gln-Pro resulted in significant decreases of both the weighted SBP and DBP in SHR from 4 to 8 h after administration, while captopril achieved significant decreases of the weighted SBP and DBP from 2 to 8 h after administration. In addition, for Ile-Gln-Pro the most potent decrease in the weighted SBP occurred at 6 h (167.3 ± 2.5 mmHg) after administration, while for captopril, the lowest point of the weighted SBP appeared at 4 h (160.7 ± 2.5 mmHg) after administration. These results showed that the antihypertensive effect of Ile-Gln-Pro was a little weaker and slower than that of captopril. However, this ACE inhibitory peptide from *Spirulina platensis* may have potential for use in the prevention and treatment of hypertension.

ABBREVIATIONS USED

ACE, angiotensin I-converting enzyme; ACN, acetonitrile; CV, column volumes; DBP, diastolic blood pressure; FRET, fluorescence resonance energy transfer; HR, heartbeat rate; MALDI-TOF MS, matrix assisted laser desorption/ionization time-of-flight mass spectrometry; MWCO, molecular weight cutoff; RAS, renin−angiotensin system; RP-HPLC, reverse-phase high-performance liquid chromatography; SBP, systolic blood pressure; SHR, spontaneously hypertensive rats; TFA, trifluoroacetic acid.

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