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Angiotensin I-Converting Enzyme Inhibitory Peptides Derived from Wakame (*Undaria pinnatifida*) and Their Antihypertensive Effect in Spontaneously Hypertensive Rats

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Seven kinds of angiotensin I-converting enzyme (ACE) inhibitory peptides were isolated from the hydrolysates of wakame (*Undaria pinnatifida*) by Protease S "Amano" (from *Bacillus stearothermophilus*) by using three-step high-performance liquid chromatography (HPLC) on a reverse-phase column. These peptides were identified by amino acid composition analysis, sequence analysis, and liquid chromatography−mass spectrometry (LC−MS), as Val-Tyr (IC_{50} = 35.2 μM), Ile-Tyr (6.1 μM), Ala-Trp (18.8 μM), Phe-Tyr (42.3 μM), Val-Trp (3.3 μM), Ile-Trp (1.5 μM), and Leu-Trp (23.6 μM). These peptides have resistance against gastrointestinal proteases in vitro. Each peptide was determined to have an antihypertensive effect after a single oral administration in spontaneously hypertensive rats (SHR). Among them, the blood pressure significantly decreased by Val-Tyr, Ile-Tyr, Phe-Tyr, and Ile-Trp in a dose of 1 mg/kg of body weight (BW). The present study showed that antihypertensive effect in the hydrolysates of wakame by Protease S "Amano" was attributed to these peptides.

KEYWORDS: Wakame; *Undaria pinnatifida*; angiotensin I-converting enzyme; peptide; antihypertension; spontaneously hypertensive rat; LC−MS; isolation; identification; digestive resistance

INTRODUCTION

Seaweed is a popular traditional foodstuff in Japan. Among the seaweeds, wakame (*Undaria pinnatifida*) is the most widely eaten brown seaweed. In daily cooking, it is served in salads, as additives to miso soup or noodles, and as a garnish. Wakame is known to contain large quantities of soluble dietary fibers, and to be rich in various kinds of minerals (*1*). Wakame has several physiological effects: prevention of hyperlipidemia in rats (*2*), suppression of chemically induced mammary tumors in rats (*3*), and hypotensive effect in hypertensive humans (*4*).

Increased blood pressure appears to be one of the primary risk factors related to the incidence of apoplectic stroke in humans. Angiotensin I-converting enzyme (ACE) plays an important role in the renin−angiotensin system, which regulates blood pressure. Antihypertensive drugs such as captopril and enalapril are potent ACE inhibitors (*5*, *6*). Recently, several inhibitory peptides derived from food proteins have been isolated from, for example, casein (*7*), zein (*8*), sake (*9*), sour milk (*10*), sardine muscle (*11*), dried-salted fish (*12*), dried bonito (*13*), and Korean soybean (*14*).

We noted that wakame had an antihypertensive effect in hypertensive humans. We reported that wakame was hydrolyzed by various proteases, and the inhibitory activity of the hydrolysates for ACE was measured. Among the digests, Protease S "Amano" (from *Bacillus stearothermophilus*) digest showed the most potent inhibitory activity. We also reported that the hypertension in spontaneously hypertensive rats (SHR) was suppressed in both a single oral administration test and long-term feeding test with the wakame hydrolysates by Protease S "Amano" (*15*).

In this study, we report the isolation and identification of the ACE inhibitory peptides from the Protease S "Amano" digest of wakame. We have also investigated the resistance of the ACE inhibitory peptides against gastrointestinal proteases in vitro, and whether their peptides from the digest of wakame show hypotensive action by oral administration in SHR.

MATERIALS AND METHODS

Materials. The wakame hydrolysates by Protease S "Amano" were prepared as previously described (*15*). Pepsin (from porcine gastric mucosa), trypsin (from bovine pancreas), chymotrypsin (from bovine

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pancreas), ACE (from rabbit lung), hippuryl-L-histidyl-L-leucine and valyl-tyrosine were purchased from Sigma Chemical Co. (St. Louis, MO). Isoleucyl-tyrosine, isoleucyl-tryptophan, leucyl-tryptophan, and phenylalanyl-tyrosine were purchased from BACHEM AG, (Bubendorf, Switzerland). Valyl-tryptophan was purchased from Research Organics Inc., (Cleveland, OH). Alanyl-tryptophan was purchased from Tokyo Kasei Co., Ltd. (Tokyo, Japan).

Assay for ACE Inhibitory Activity. The ACE inhibitory activity was measured by the method of Yamamoto et al. (*16*) with some modification. Sample solution (35 μL) and 100 μL of hippuryl-L-histidyl-L-leucine solution (12.5 mM in a borate buffer containing 200 mM NaCl at pH 8.3) were incubated with 35 μL of 250 mU/mL of ACE at 37 °C for 1 h. The reaction was stopped by addition of 125 μL of 0.5 N HCl. The hippuric acid liberated from hippuryl-L-histidyl-L-leucine by ACE was extracted with 2.0 mL of ethyl acetate. An aliquot of ethyl acetate extract (1.5 mL) was evaporated to dryness, and the residue was dissolved in 4.25 mL of 1 M NaCl solution. The amount of hippuric acid liberated was measured spectrophotometrically at 228 nm. The inhibition was shown as equal to $[(E_c - E_s)/(E_c - E_b)] \times 100$, where E_s is absorbance with test sample added to the reaction mixture, E_c is absorbance with buffer added (instead of the test sample), and E_b is absorbance without ACE. The activity of an ACE inhibitory content was expressed as the amount needed to inhibit 50% of ACE activity (IC_{50}) under these conditions.

Purification of ACE Inhibitory Peptides. Wakame hydrolysates (5 g) were added in 250 mL of distilled water and 500 mL of 1-butanol. The mixture was then homogenized and centrifuged at 3000*g* for 5 min. The upper layer was recovered, and the lower layer was added in 60 mL of distilled water and 500 mL of 1-butanol. This procedure was repeated twice, then all the upper layers were combined, then concentrated in vacuo, and lyophilized. Thus, 850 mg of the "butanol-soluble fraction" powder was obtained.

The butanol soluble fraction powder (500 mg) in 10 mL of 0.1% trifluoroacetic acid (TFA) was applied on a reverse-phase column, μBondasphere C18 (300 × 30 mm i.d.; Waters, Milford, MA). The column was eluted in the linear-gradient mode from 0.1% TFA to acetonitrile containing 0.07% TFA (0 to 35% for 140 min) at a flow rate of 30 mL/min. The elution was monitored at 220 nm. Individual fractions (F1−F8) were lyophilized and their ACE inhibitory activities were measured. The active fractions (F4−F6) in 50 mM ammonium acetate (pH 10)/acetonitrile (99:1, v/v) were applied on a reverse-phase column XTerra RP18 (150 × 4.6 mm i.d.; Waters), and the column was eluted in the isocratic mode (10 min) followed by the gradient mode from 50 mM ammonium acetate (pH 10)/acetonitrile (99:1, v/v) to 50 mM ammonium acetate (pH 10)/acetonitrile (5:95, v/v) (0 to 20% for 40 min) at a flow rate of 1 mL/min. The elution was monitored at 220 nm. The ACE inhibitory activities of the individual peaks were measured. The fractions of each active peak (A−F) were collected and concentrated; then these peaks were purified by either of the two kinds of reverse-phase columns. Among the six active peaks, two peaks were applied on a reverse-phase column, ODP50-4D (150 × 4.6 mm i.d.; Showadenko, Tokyo, Japan), and the column was eluted in the linear gradient mode from 50 mM ammonium acetate (pH 10)/acetonitrile (99:1, v/v) to 50 mM ammonium acetate (pH 10)/acetonitrile (5:95, v/v) (0 to 20% for 30 min) at a flow rate of 0.5 mL/min. Among the six active peaks, the other four peaks were applied on an XTerra RP18 (150 × 4.6 mm i.d.) and the column was eluted in the linear gradient mode from 0.1% TFA/0.07% TFA in acetonitrile (99:1, v/v) to 0.1% TFA/0.07% TFA in acetonitrile (5:95, v/v) (0 to 30% for 40 min) at a flow rate of 1 mL/min. The elution was monitored at 220 nm.

Analysis of Peptides. The amino acid composition of each peptide was analyzed by the methods of Li et al. (17) with a high-performance liquid chromatography (HPLC) system (JASCO HPLC800 series, JASCO Co. Ltd., Tokyo, Japan) after hydrolysis in 6 N HCl for 24 h at 110 °C. The amino acid sequence of each peptide was analyzed by automated Edman degradation using a PPSQ-10 protein sequencer (Shimadzu Co. Ltd., Kyoto, Japan).

Analysis of Wakame Hydrolysates. The quantitative determination of the peptide contents in the wakame hydrolysates was carried out by liquid chromatography−mass spectrometry (LC−MS). The LC−MS system consisted of a model 2695 chromatography manager HPLC pump/autosampler (Waters), and a ZQ 4000 mass detector (Waters). Both systems were controlled by MassLynx software (Waters). Chromatography was performed at 35 °C using an Xterra MS C18 column (150 × 2.1 mm i.d., 3.5 μm particle; Waters), and elution was made in the linear gradient mode from 0.05% TFA to acetonitrile containing 0.05% TFA (3 to 20% for 40 min) at a flow rate of 0.2 mL/min. The following settings were used: cone voltage at +30 V, capillary voltage at 3.00 kV, desolvation temperature at 300 °C, source block temperature at 100 °C, desolvation gas flow at 350 L/h, and cone gas flow at 50 L/h.

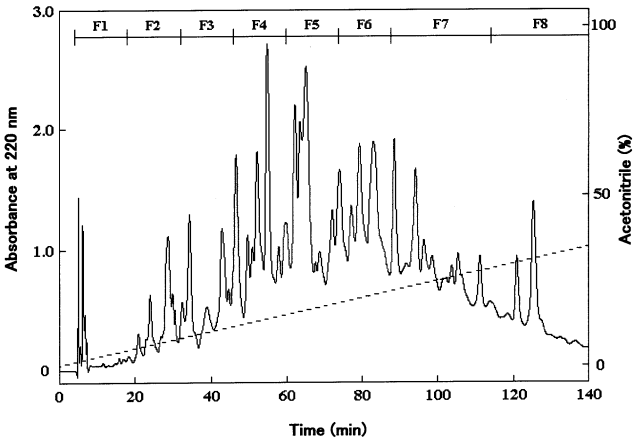

Figure 1. Separation of the butanol-soluble fraction on μBondasphere C18 column. Each fraction (F1–F8) was collected individually. The elution was done as described under Materials and Methods. Fractions F1, F2, F3, F4, F5, F6, F7, and F8 had IC_{50} values of 1119.4, 462.2, 234.9, 39.4, 41.2, 31.0, 46.1, and 110.1 μg/mL, respectively. Solid line is the absorbance at 220 nm. Dotted line is the percentage of acetonitrile in the eluting solution.

Digestion Test. The digestive stability was assayed by the modified method of Seki et al. (*18*). Each synthesized peptide (1.5 mg) was incubated in 2.5 mL of pepsin solution (0.12 mg, pH 2.0) for 4 h at 37 °C. On successive digestion by trypsin and chymotrypsin after pepsin treatment, the pepsin solution was heated for 10 min in a boiling-water bath, and the pH was adjusted to 8.0. Then, trypsin and chymotrypsin were added (0.075 mg each) to the solution, followed by incubation for 4 h at 37 °C. The reaction was stopped by boiling for 10 min. The contents of the peptides were analyzed by HPLC and their ACE inhibitory activities were measured.

Animals and Measurement of Blood Pressure. Male SHR, 18 weeks old, were purchased from Funabashi Nojyo Co., Ltd. (Chiba, Japan). The SHR were housed individually in steel cages in a room kept at 24 °C with a 12-h light−dark cycle (lights on 8:00−20:00), and fed a standard laboratory diet (Labo MR Stock; Nihon Nosan Kogyo Co., Ltd., Kanagawa, Japan). Tap water was freely available. Systolic blood pressure (SBP) was measured by the tail-cuff method with a programmed electro-sphygmomanometer (model UR-5000; Ueda Co., Ltd., Tokyo, Japan) after warming the rat in a chamber maintained at 40 °C for 8 min. At least five readings were recorded, the maximum and minimum values were discarded, and average SBP was calculated from the remaining three values.

Oral Administration. The SHR having average SBPs higher than 180 mmHg were used. Samples were dissolved in 1 mL of distilled water and orally administered to SHR by intubation. Control rats were given the same volume of distilled water. Systolic blood pressure was measured before and 3, 6, 9, and 24 h after the administration.

Statistical Analysis. The results were expressed as means and standard errors (SE). The significance of the differences in SBPs before and after administration was analyzed using Student's *t* test. Statistical analyses were performed using the computer program SPSS (SPSS Inc., Chicago, IL).

RESULTS

Isolation of ACE Inhibitors. The ACE inhibitory activity (IC_{50}) was 99.5 μg/mL for the hydrolysate of wakame by

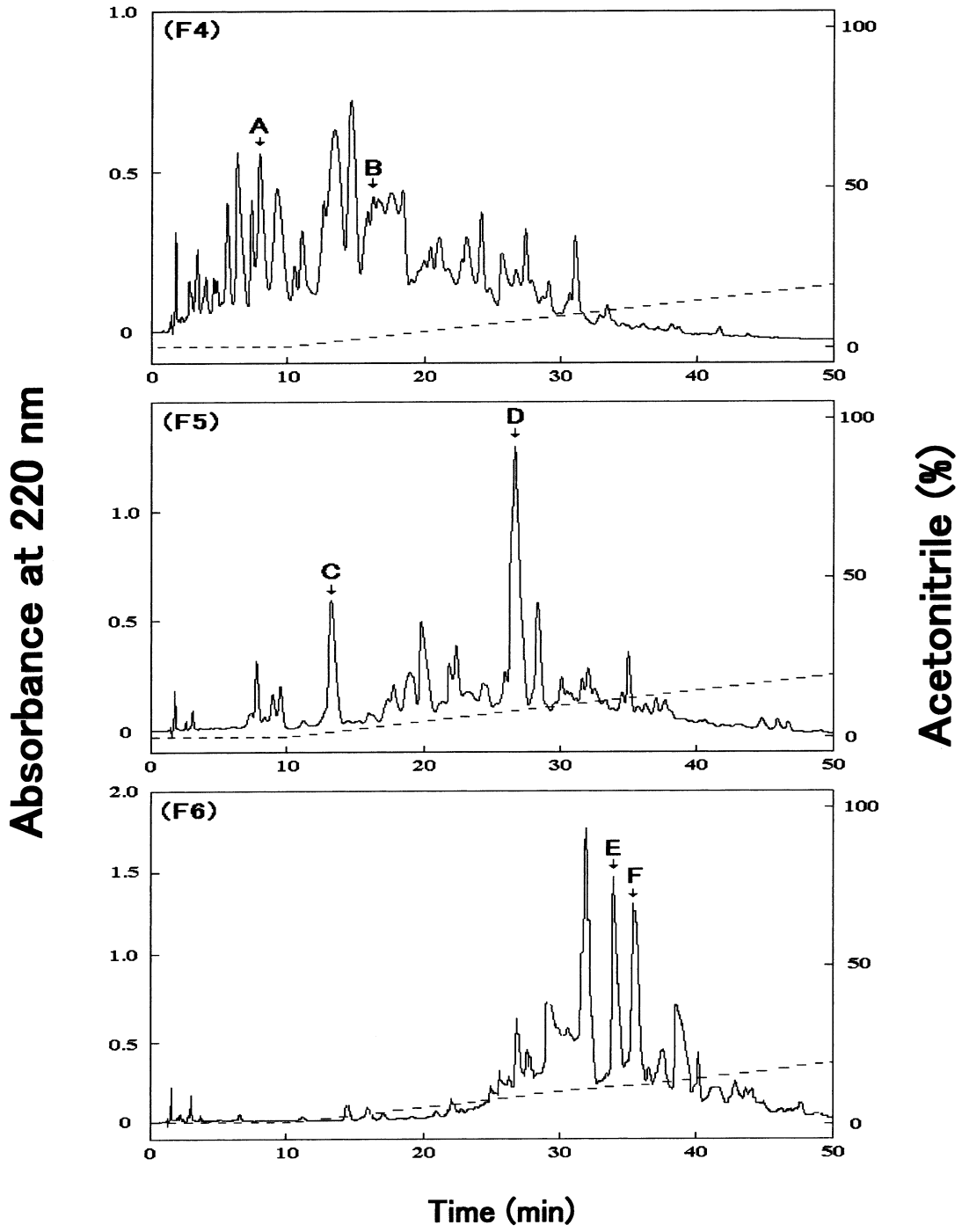

Figure 2. Separation of active fraction (F4–F6) on Xterra RP18 column. Arrows indicate active peaks (A–F). The elution was done as described under Materials and Methods. Solid line is the absorbance at 220 nm. Dotted line is the percentage of acetonitrile in the eluting solution.

Protease S "Amano". After water−butanol partition, it was 50.7 μg/mL for the butanol-soluble fraction and 159.3 μg/mL for the aqueous fraction, demonstrating a stronger ACE inhibitory activity in the butanol-soluble fraction. The butanol-soluble fraction was separated into eight fractions (F1−F8) by reverse-phase HPLC (μBondasphere C18 column). The stronger potent ACE inhibitory activities were recognized in fractions F4−F6 (**Figure 1**). These were further fractionated by subjection to the second reverse-phase HPLC (Xterra RP18 column) (**Figure 2**). The fraction of each peak obtained was measured for the ACE inhibitory activity. The peaks (A, B, C, D, E, and F) exhibiting potent activities were purified on the third reverse-phase HPLC (ODP50-4D or Xterra RP18 column) to isolate seven peptides (**Figure 3**). These peptides were identified by structural analysis, using amino acid analysis method and amino acid sequencer, to be Val-Tyr, Ile-Tyr, Ala-Trp, Phe-Tyr, Val-Trp, Ile-Trp, and Leu-Trp, respectively (**Table 1**). The retention time of HPLC and mass spectrometry of each peptide were confirmed to be completely identical with those of its corresponding synthetic peptide. As a typical example, **Figure 4** shows the chart of Phe-Tyr. Similar results were obtained with the other peptides. In addition, calibration curves were prepared by LC−MS using the corresponding synthetic peptides to examine the content of each peptide in the hydrolysate of wakame. **Table 1** shows the peptide contents in the hydrolysate of wakame. The values of IC_{50} are also shown in **Table 1**.

Digestion Stability. The seven ACE inhibitory peptides obtained from the hydrolysate of wakame were treated by

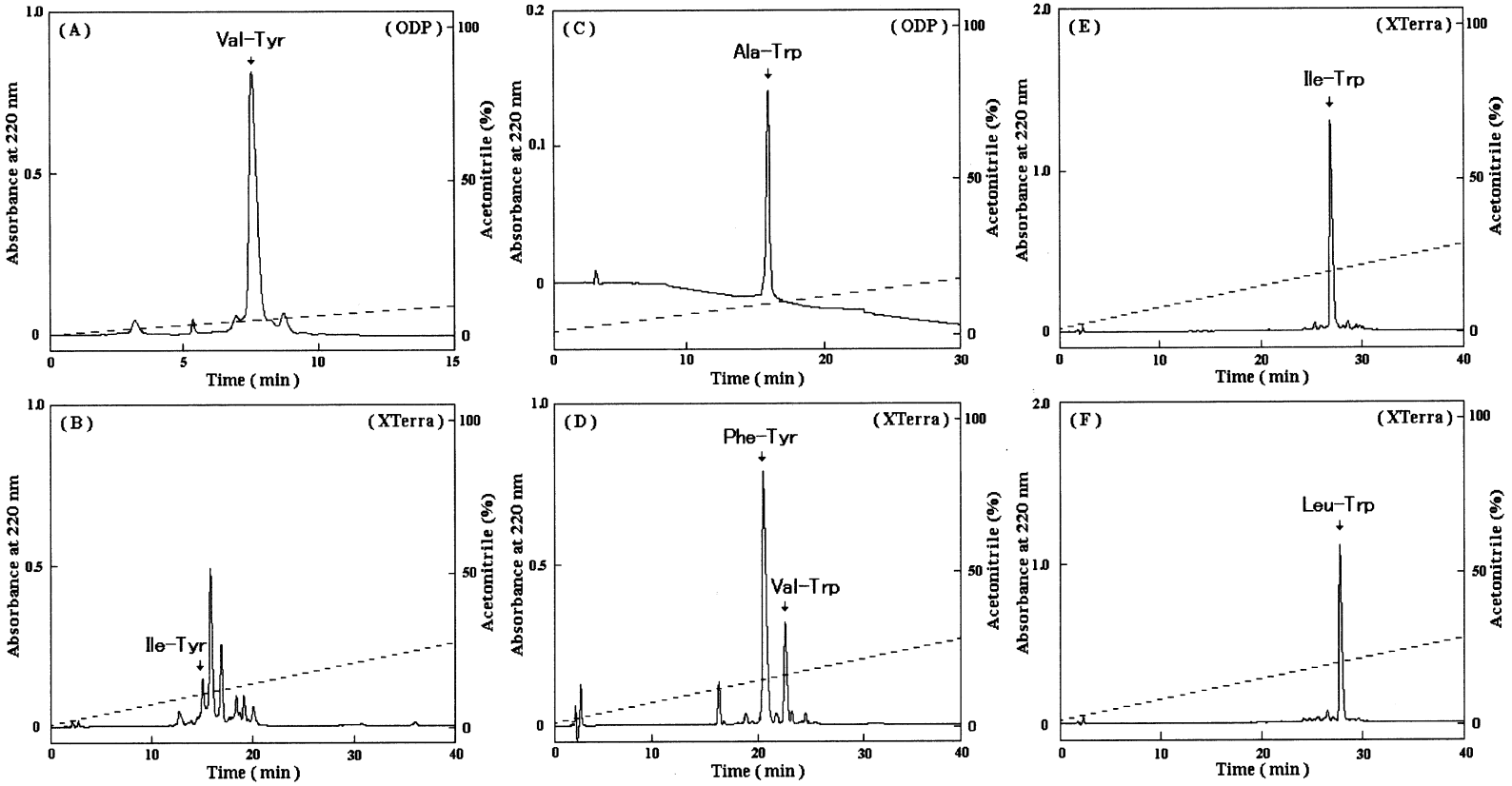

Figure 3. Purification of active peaks (A–F) on ODP50-4D column (ODP) or Xterra RP18 column (Xterra). Arrows indicate purified peptides. The elution was done as described under Materials and Methods. Solid line is the absorbance at 220 nm. Dotted line is the percentage of acetonitrile in the eluting solution.

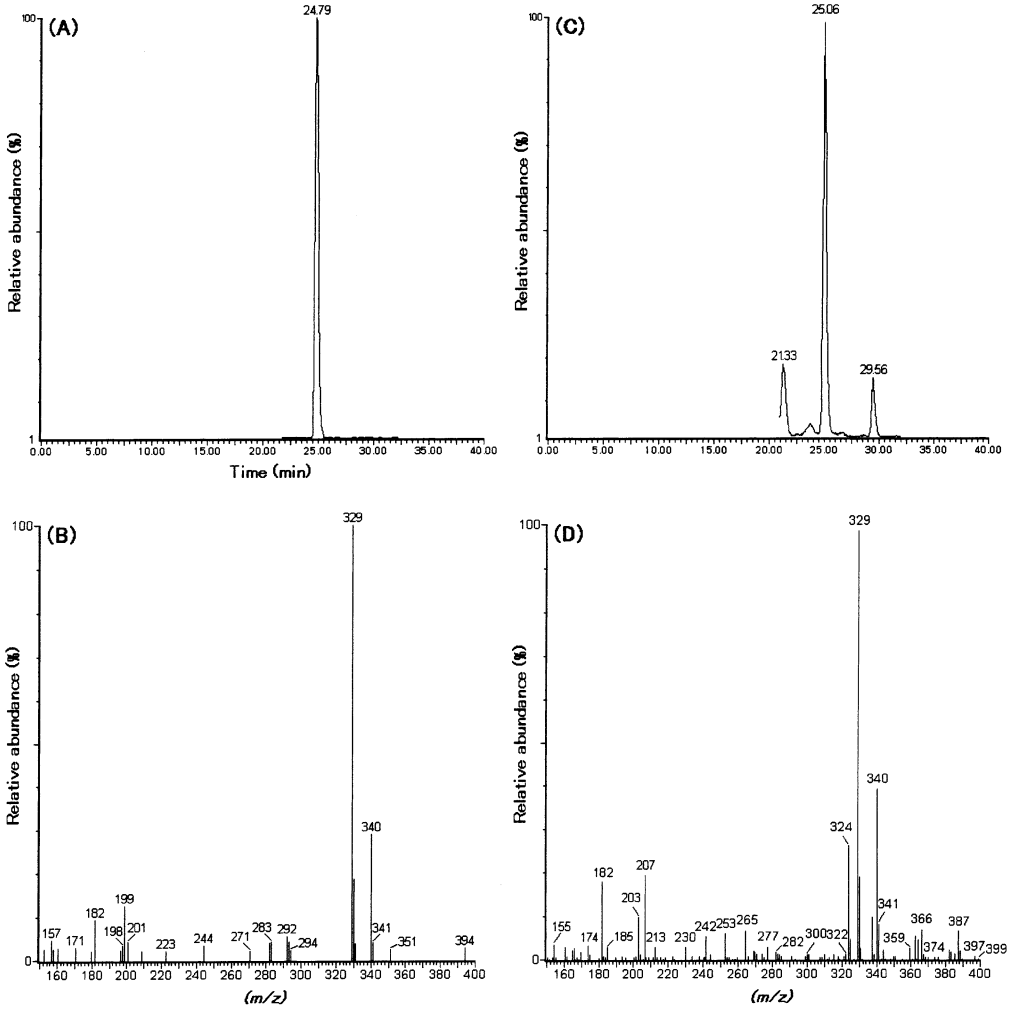

Figure 4. Mass spectrum of the phenylalanyl-tyrosine: (A, B) corresponding synthetic phenylalanyl-tyrosine; (C, D) phenylalanyl-tyrosine in the hydrolysate of wakame. Chromatograms (A, C) and mass spectra (B, D) were obtained by LC–MS.

Table 1. Analytical Data for Dipeptides Isolated from Wakame and ACE Inhibitory Activity of Synthetic Dipeptides

peptide	amino acid ratio in acid hydrolysate	IC50 (μM)	$[M + H]^+$	content (%)[a]
Val-Tyr	Val 1.00, Tyr 1.15	35.2	281	0.09
Ile-Tyr	Ile 1.00, Tyr 0.85	6.1	295	0.02
Ala-Trp	Ala 1.00, Trp 1.20	18.8	329	0.05
Phe-Tyr	Phe 1.00, Tyr 1.25	42.3	276	0.09
Val-Trp	Val 1.00, Trp 1.28	3.3	304	0.01
Ile-Trp	Ile 1.00, Trp 1.01	1.5	318	0.02
Leu-Trp	Leu 1.00, Trp 1.28	23.6	318	0.02

[a] Content in the hydrolysate of wakame by Protease S "Amano".

digestive proteases (pepsin, trypsin, and chymotrypsin) to examine digestive resistance against the gastrointestinal proteases. The digestive resistance was examined by the change in the ACE inhibitory activity between the peptides added with gastrointestinal proteases and those without, as well as the change in peptide content by HPLC analysis. As a result, all the peptides received neither decomposition by the gastrointestinal proteases nor reduction of the ACE inhibitory activities.

Antihypertensive Effect of a Single Oral Administration. **Table 2** shows the changes of SBP after single oral administration of each peptide in SHR. A statistically significant antihypertensive effect was recognized with four of the seven peptides at the dose of 1 mg/kg of body weight (BW). With the four peptides (Val-Tyr, Ile-Tyr, Phe-Tyr, and Ile-Trp) showing hypotensive effect at 1 mg/kg of BW, single doses of 0.1 and 10 mg/kg of BW were given to SHR. Although the dose-dependence was not obvious, the antihypertensive effect was observed in the test groups. The effect of the 1 mg/kg of BW dose was almost equivalent to that of captopril, which was administered as positive control.

DISCUSSION

It was previously (*15*) shown that the hydrolysate of wakame by Protease S "Amano" had an ACE inhibitory activity, and that oral administration of the hydrolysate of wakame to SHR showed antihypertensive effect. This report addresses our study conducted to identify the hypotensive ingredients in the hydrolysate of wakame.

First, we tried to identify the main ACE inhibitory ingredients in the hydrolysate of wakame. Cheung et al. (*19*) reported that dipeptides having hydrophobic amino acids such as Val and Ile at the amino terminus have higher ACE inhibitory activities. There are also many peptides having hydrophobic amino acids among the various peptides having ACE inhibitory activities obtained from food proteins (*8*, *10*). To attempt the partition of the hydrophobic peptides and the hydrophilic peptides, we applied water−butanol partition to the hydrolysate of wakame to measure the ACE inhibitory activity of each fraction. As expected, the butanol-soluble fraction had more potent ACE

Table 2. Effect of Oral Administration of Various Peptides on Systolic Blood Pressure in SHR

		systolic blood pressure (mmHg)[b]			
			time after administration (h)		
sample	dose (mg/kg)	before administration	3	6	9
control		211.4 ± 8.4	218.3 ± 6.0	209.6 ± 4.8	210.1 ± 4.9
Val-Tyr	0.1	241.9 ± 3.7	239.9 ± 5.2	237.4 ± 2.1	231.6 ± 3.9
	1	228.2 ± 3.4	220.4 ± 6.2	220.0 ± 7.6	206.7 ± 9.5*
	10	216.6 ± 3.6	214.3 ± 1.5	216.6 ± 4.1	198.9 ± 4.3*
Ile-Tyr	0.1	230.7 ± 4.7	218.9 ± 5.4**	224.7 ± 5.2*	222.1 ± 5.0*
	1	205.6 ± 5.2	201.7 ± 5.8	193.4 ± 3.6	184.3 ± 4.5*
	10	227.1 ± 8.0	213.9 ± 5.9*	212.8 ± 3.4	208.3 ± 5.2*
Phe-Tyr	0.1	236.3 ± 8.6	225.4 ± 6.4	219.8 ± 7.6	210.2 ± 9.6*
	1	208.7 ± 4.4	204.4 ± 3.9	198.4 ± 5.0	193.0 ± 5.1**
	10	242.7 ± 8.5	224.8 ± 4.3	221.3 ± 3.7*	221.1 ± 6.6
Ile-Trp	0.1	231.7 ± 5.1	228.9 ± 6.4	222.6 ± 2.2	217.1 ± 4.5*
	1	213.3 ± 3.4	212.0 ± 3.5	209.2 ± 2.5	199.5 ± 5.9*
	10	224.9 ± 3.3	220.7 ± 5.7	221.3 ± 5.5	223.4 ± 5.7
Ala-Trp	1	231.7 ± 6.7	226.9 ± 4.7	230.6 ± 6.8	230.4 ± 2.2
Leu-Trp	1	210.4 ± 5.1	209.3 ± 4.9	209.8 ± 2.7	199.8 ± 3.6
Val-Trp	1	193.3 ± 7.5	188.1 ± 3.9	186.1 ± 6.2	182.0 ± 4.8
Captopril[a]	1	238.7 ± 6.9	222.2 ± 3.9*	221.1 ± 4.0*	224.9 ± 4.1*

[a] Captopril as a positive control. [b] Systolic blood pressure is shown as mean ± SE, $n = 6$. * Significantly different from the before administration at $p < 0.05$ by paired t test. ** Significantly different from the before administration at $p < 0.01$ by paired t test.

inhibitory activity, suggesting that the ACE inhibitory ingredients were concentrated in this fraction. We were successful in isolating seven dipeptides by three steps of HPLC of the butanol soluble fraction. All seven of the isolated peptides were recovered, more than 90%, in butanol phase when treated by water−butanol partition. And all seven isolated peptides have an aliphatic hydrophobic amino acid at the N-terminus (except Phe) with an aromatic residue at the C-terminus (**Table 1**). It was expected that the method of water−butanol partition would concentrate hydrophobic peptides with potent ACE inhibitory activity. Because we examined the active peptide of the hydrolysate of wakame for the ACE inhibitory activity and the quantity of peptide as search point, it was suggested that all seven isolated peptides were the main active peptides of the hydrolysate of wakame. These peptides were already reported as having ACE inhibitory activities as obtained from naturally occurring substances. Val-Tyr was previously isolated from sake (*9*), sardine (*20*), and whey protein (*21*), etc. Similarly, Ile-Tyr was isolated from dried bonito (*13*) and wheat germ (*22*), Ala-Trp was isolated from human serum albumin (*23*), Phe-Tyr was isolated from α-zein (*24*) and garlic (*25*), Val-Trp was obtained from sake lees (*9*) and fish sauce (*26*), Ile-Trp was isolated from fish sauce (*26*) and dried bonito (*27*), and Leu-Trp was isolated from ovalbumin (*27*). Val-Tyr, Ile-Tyr, Ala-Trp, Val-Trp, and Ile-Trp were confirmed to have ACE inhibitory activities by Cheung et al. (*19*), consistent with the results of our present study.

Quantitative determination of the seven peptides was carried out by LC−MS. Conventionally, for qualitative and quantitative determinations of the objective peptides from a peptide mixture it was necessary to apply HPLC procedures repeatedly to be successful in complete isolation of each individual peptide. On the other hand, as it is now possible by LC−MS to identify the attribution of each peak by the molecular weight and fragmentation information obtained from MS about each of the peaks of the nonisolated peptides, a simple and accurate quantitative determination is feasible if the objective peptide is a known compound. In addition, even when the amino acid sequence is unknown, the molecular weight and fragmentation information obtained from MS is also helpful for the subsequent sequence analysis. As for the ACE inhibitory peptides in the hydrolysate of wakame, the analysis by LC−MS can determine their primary structures by using corresponding synthetic peptides so that it has become possible to determine several peptides all at once. Thus, it is considered that LC−MS is a very useful analytical apparatus for determination of specific peptides from a peptide mixture.

We examined the digestive resistance of seven ACE inhibitory peptides against the gastrointestinal proteases using the synthetic peptides. The seven kinds of peptides obtained from the hydrolysate of wakame had resistance to digestion against the gastrointestinal proteases. It was known that small peptides such as dipeptides and tripeptides are absorbed in their intact form from the intestinal tract (*28*−*31*). It has also been reported that the tripeptides having ACE inhibitory activities are absorbed in their intact form (*32*). These suggest that the peptides having ACE inhibitory activities, which we isolated from the hydrolysate of wakame, could be absorbed in their intact form from the intestinal tract, and showed antihypertensive effect.

The inhibition mode of each peptide against ACE was examined by performing Lineweaver−Burk plots. As a result, Val-Tyr and Val-Trp showed a competitive inhibition, Ile-Tyr and Leu-Trp showed a noncompetitive inhibition, and the other three peptides (Ala-Trp, Phe-Tyr, Ile-Trp) showed an uncompetitive inhibition (data not shown). The inhibition mode of the peptides obtained from wakame was various. The inhibition mode against ACE appears unaffected by the carboxyl terminal amino acid residue (Tyr, Trp). When the amino-terminal amino acid was valine, the inhibition mode of the dipeptides seemed to display a competitive inhibition. However, because there are few cases in this study, further investigations are necessary concerning to relationship between the inhibition mode and the structure of dipeptides.

Single oral administration test in SHR was first performed at the dose level of 1 mg/kg of BW for all seven peptides. As a result, four of the seven peptides showed statistically significant antihypertensive effect. Regarding the four peptides which showed significant blood-pressure-lowering effect, single oral administration tests were performed at the dose levels of 0.1 and 10 mg/kg of BW. The SBP significantly decreased at 0.1 mg/kg of BW for Ile-Tyr, Phe-Tyr, and Ile-Trp, and at 10 mg/kg of BW for Val-Tyr, Ile-Tyr, and Phe-Tyr.

The single oral administration of Val-Tyr in SHR has been reported in detail by Saito et al. (*33*) and Seki et al. (*34*). The results of the present study were well in accord with those by Seki et al. Results of Ile-Tyr and Ile-Trp in SHR were reported by Yoshikawa et al. (*27*). According to their report, the dose of 60 mg/kg of BW produced a blood-pressure-lowering effect of −19 mmHg and −22 mmHg, respectively. In the present study, statistically significant reductions of −21 mmHg for Ile-Tyr and −14 mmHg for Ile-Trp were observed at a lower oral dose (1 mg/kg of BW). Results of Phe-Tyr in SHR have been reported by Suetsuna (*25*). The SBP significantly decreased by about −30 mmHg at the dose of 200 mg/kg of BW. In this study, significant reductions of −21 mmHg at 10 mg/kg of BW, −16 mmHg at 1 mg/kg of BW, and −26 mmHg even at 0.1 mg/kg of BW were observed. The peptides of Ile-Tyr, Ile-Trp, and Phe-Tyr showed significantly antihypertensive effect in SHR at lower amounts of these peptides. It was shown that these peptides have more powerful hypotensive activity in the previously reported literature.

The present study revealed the four peptides having ACE inhibitory activity from the hydrolysate of wakame, and showed that antihypertensive effect in the hydrolysates of wakame by Protease S "Amano" was attributed to these peptides. Our next focus will be an evaluation of the blood-pressure-lowering effect of the hydrolysate of wakame in clinical trials in humans.

ABBREVIATIONS USED

ACE, angiotensin I-converting enzyme; SHR, spontaneously hypertensive rats; TFA, trifluoroacetic acid; HPLC, high-performance liquid chromatography; LC−MS, liquid chromatography−mass spectrometry; SBP, systolic blood pressure; SE, standard error; BW, body weight.

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