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Angiotensin I-converting enzyme inhibitory peptide from yellowfin sole (*Limanda aspera*) frame protein and its antihypertensive effect in spontaneously hypertensive rats

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Abstract

In order to utilize yellowfin sole (*Limanda aspera*) frame protein, which is normally discarded as industrial waste in the process of fish manufacture, yellowfin sole frame protein was hydrolysed by α-chymotrypsin. Yellowfin sole frame protein hydrolysates (YFPHs) were fractionated into three ranges of molecular weight (YFPH-I, 30–10 kDa; YFPH-II, 10–5 kDa; YFPH-III, below 5 kDa) using an ultrafiltration (UF) membrane bioreactor system. Angiotensin I-converting enzyme (ACE) inhibitory activity was detected on YFPH-III, and the ACE inhibitory peptide (YFP) was purified from YFPH-III using consecutive chromatographic techniques. The YFP with a molecular mass of 1.3 kDa consisted of 11 amino acids, Met-Ile-Phe-Pro-Gly-Ala-Gly-Gly-Pro-Glu-Leu, and its IC_{50} value was 28.7 μg/ml. Lineweaver–Burk plots suggest that YFP acts as a non-competitive inhibitor to inhibit ACE. Antihypertensive effects of YFP on spontaneously hypertensive rats (SHR) following oral administration was determined as the blood pressure significantly decreased after peptide ingestion.

Keywords: Yellowfin sole frame protein; Hydrolysates; ACE inhibitory peptide; Antihypertensive effect

1. Introduction

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 the 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 converts an inactive form of the decapeptide, angiotensin I, to the octapeptide angiotensin II, a potent vasoconstrictor, and inactivates bradykinin, which has a depressor action. Since the discovery of ACE inhibitors in snake venom, many studies have been attempted in the 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).

For many years, food researchers have extensively studied peptides derived from food proteins as potential nutraceuticals in relation to the development of functional foods (Ariyoshi, 1993; Yamamoto, 1997; Meisel, 1997). Recently, a new relationship between food and health has drawn considerable attention, that being

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physiological functions of some food components against certain ailments. Bioactive peptides can be released by enzymatic proteolysis of food proteins and may act as potential physiological modulators of metabolism during the intestinal digestion of the diet. The possible regulatory effects of peptides relate to nutrient uptake, immune defense, opioid and antihypertensive activities (Pihlanto-Leppälä, 2001). Hypertension is a worldwide problem of epidemic proportions, which affects 15–20% of all adults. It is the most common serious chronic health problem and 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 in controlling hypertension may be associated with the presence of an antihypertensive peptide motif. Recently, many ACE inhibitory peptides have been isolated from various food proteins such as cheese whey (Abubakar, Saito, Kitazawa, Kawai, & Itoch, 1998), casein (Maruyama et al., 1989; Maeno, Yamamoto, & Takano, 1996), zein (Yano, Suzuki, & Funatsu, 1996), corn gluten (Suh & Whang, 1999), and bovine blood plasma (Hyun & Shin, 2000). In addition, some ACE inhibitors have also been reported in some marine animals like tuna (Kohama et al., 1988) and sardines (Ukeda et al., 1992). These peptides are less potent than synthetic ones, but, they do not exhibit known side effects.

In the present study, the objective was to isolate and characterize ACE inhibitory peptides derived from an enzymatic hydrolysate of yellowfin sole frame (*Limanda aspera*) protein, which is normally discarded as industrial waste in the process of fish manufacture. Moreover, we have also investigated antihypertensive action of the purified peptide by oral administration in spontaneously hypertensive rats (SHR).

Every year, about 100 million tons of fish are harvested. However, 30% of the total catch is transformed into fishmeal (Kim, Jeon, Byun, Kim, & Lee, 1997). Over 50% of the harvest is processing waste and includes bone, skin, fins, internal organs, head, and so on (Nair & Gopakumar, 1982). In Korea, the annual yellowfin sole (*Limanda aspera*) harvest exceeds 13,828 tons and 1327 tons were processed in Korean fish plants in 2002. In particular, fish frames obtained after filleting include bones, heads and tails. The total solid mass of the frame consists of considerable amounts of protein, which can be used as potential bioactive substances.

2. Materials and methods

2.1. Materials

Fresh samples of yellowfin sole (*Limanda aspera*) frame (moisture, 72.12%; muscle, 16.16%; bone, 11.72%) was donated by Daerim Co. (Busan, Korea), and stored at −20 °C until use. ACE (EC 3.4.15.1) from rabbit lung, hippuryl-histidyl-leucine (HHL) as a substrate peptide of ACE, α-chymotrypsin (EC 3. 4. 21 1) from bovine pancreas, SP-Sephadex C-25, and Sephadex G-25 were purchased from Sigma Chemical Co. (St. Louis, MO). Ultrafiltration membrane (UF) reactor (Minitan™) system and membranes for the fractionation of yellowfin sole frame protein hydrolysates (YFPHs) based on molecular mass were purchased from Millipore Co. (Bedford, MA, USA). OHpak SB-803 HQ (8.0 × 300 mm) gel permeation chromatography column (Shoko Co., Tokyo, Japan), SP Nucleosil 100-7 C_{18} (1.0 × 250 mm) reversed phase semi-prep column (Macherey-Nagel Ltd., Middleton Cheney OX17 2PA, UK), and Zorbax SB C_{18} (4.6 × 250 mm) reversed phase analytical column (Agilent Technologies, Wilmington, DE, USA) were utilized for purification of ACE inhibitory peptide. All other reagents used in this study were reagent grade chemicals.

2.2. Preparation of chymotryptic hydrolysate

Yellowfin sole (*Limanda aspera*) frame protein was hydrolysed with α-chymotrypsin as adjusting substrate/enzyme ratio to 100:1 (w/w) at 37 °C for 8 h (pH 2.0). Yellowfin sole frame protein hydrolysate (YFPH) was subsequently boiled for 10 min to inactivate the enzyme. The resultant YFPH was fractionated through three different UF membrane bioreactor system having a range of molecular weight cut-offs (MWCO), i.e., 30, 10 and 5 kDa. YFPH-I, YFPH passed through the 30 kDa membrane but not passed through the 10 kDa membrane. YFPH-II, YFPH passed through the 10 kDa membrane but not passed through the 5 kDa membrane. YFPH-III, YFPH was passed through the 5 kDa membrane. All of YFPHs recovered by UF membrane reactor were lyophilized in a freeze-drier.

2.3. Determination of ACE inhibitory activity of fractionated YFPH

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 1.0 M HCl. The resulting hippuric acid was extracted with 0.5 ml of ethyl acetate. After centrifugation (800*g*, 15 min), 200 μl 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 read at 228 nm using an UV-spectrophotometer (Cary 1C, Varian Inc., Victoria,

Table 1
ACE inhibitory activities of YFPHs fractionated by UF membrane bioreactor system

Hydrolysates	ACE inhibitory activity (%)
YFPH-I (MW 30–10 kDa)	47.6
YFPH -II (MW 10–5 kDa)	34.5
YFPH-III (MW 5 kDa below)	68.8

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 inhibitory peptide

YFPH-III (1.0 g) with the highest activity among YFPHs (Table 1) was loaded onto a SP-Sephadex C-25 ion-exchange column (35 × 350 mm) equilibrated with 20 mM sodium acetate buffer (pH 4.0), and eluted with a linear gradient of NaCl (0–2 M) in the same buffer at a flow rate of 1.0 ml min^{-1}. The absorbance of the eluent was monitored at 215 and 280 nm. Active fractions were pooled and lyophilized, immediately. The lyophilized fraction was further purified on an OHpak SB-803 HQ (8.0 × 300 mm) gel permeation high-performance liquid chromatography column equilibrated with the same buffer. The column was eluted at a flow rate of 1.0 ml min^{-1}, and the fraction exhibiting ACE inhibitory activity was further purified using reversed phase high-performance liquid chromatography (RP-HPLC) on a SP Nucleosil 100-7 C_{18} (1.0 × 250 mm) reversed phase semi-prep column with a linear gradient of acetonitrile (0–19% in 40 min) containing 0.1% trifluoroacetic acid (TFA) at a flow rate of 2.0 ml min^{-1}. Active peak representing the ACE inhibitory activity was rechromatographed on a Zorbax SB C_{18} (4.6 × 250 mm) reversed phase analytical column with a linear gradient of acetonitrile (0–19% in 30 min) containing 0.1% TFA at a flow rate of 0.5 ml min^{-1}.

2.5. Dertermination of molecular mass and amino acid sequence

During gel permeation chromatography, a molecular mass of ACE inhibitory yellowfin sole frame peptide (YFP) purified from YFPH-III was examined using standard materials produced from Sigma Chemical Co. (cytochrome *c*, 12.4 kDa; aprotinin, 6.5 kDa; angiotensine converting enzyme I, 1.2 kDa; HHL, 0.43 kDa). Amino acid sequence of the YFP was determined by automated Edman degradation with a Perkin–Elmer 491 protein sequencer (Branchburg, NJ., USA).

2.6. Determination of the inhibition pattern on ACE

Different concentrations of ACE inhibitory peptide were added to each reaction mixture according to Bush, Herny, and Slusarchyk (1984) with some modifications. The enzyme activity was measured with different concentrations of the substrate. The kinetics of ACE in the presence of the inhibitor was determined by the Lineweaver–Burk plots.

2.7. Animals and measurement of blood pressure

Spontaneously hypertensive rats (10-week-old, male, SHR/Hos, SPF, 180–240 g BW) with tail systolic blood pressure (SBP) over 180 mmHg were obtained from Korea Research Institute of Bioscience and Biotechnology (DaeJeon, Korea). Spontaneously hypertensive rats (SHRs) were housed individually in steel cages in a room kept at 24 °C with a 12 h light–dark cycle, and fed a standard laboratory diet. Tap water was freely available. The ACE inhibitory peptide purified from YFPH was dissolved in saline at a dose of 10 mg kg^{-1} body weight (BW) and injected orally using a metal gastric zoned in SHR. The lowering efficacy of peptide on systolic blood pressure (SBP) was compared with that of captopril. Captopril was injected the same method at that of the peptide from YFPH. Control rats were administrated with the same volume of saline solution. Following oral administration of sample, SBP was measured by tail-cuff method with a Softron BP system (Softron BP-98A, Tokyo, Japan) after warming SHR in a chamber maintained at 37 °C for 10 min.

2.8. Statistical analysis

All results were expressed as means ± SEM ($n = 6$). The significance of the differences between SBPs before and after administration was analyzed using Student's *t* test.

3. Results and discussion

3.1. ACE inhibitory activity of fractionated YFPHs

Yellowfin frame protein (100 g) was hydrolized by α-chymotrypsin and α-chymotrypsin resulted in a high degree of hydrolysis (60.3% of total protein). Yellowfin frame protein hydrolysates (YFPHs) were fractionated into YFPH-I (30–10 kDa), YFPH-II (10–5 kDa), and YFPH-III (below 5 kDa). As shown in Table 1, the ACE inhibitory activities of YFPHs varied with the molecular mass distribution, and the YFPH-III with MW below 5 kDa showed the most potent ACE inhibitory activity with an IC_{50} value of 0.883 mg ml_{-1}.

As reported by Margaret, Mullally, and FitzGerald (1997), bioactive peptides can be released by enzymatic proteolysis of food proteins, so pancreatic enzymes, chymotrypsin and trypsin have been used for derivation of bioactive peptides. Among digestive enzymes, α-chymotrypsin cleaves peptide bonds involving amino acids

with bulky side chains and non-polar amino acids (Jung, Park, Byun, Moon, & Kim, 2004). Thus, natural peptides derived by α-chymotryptic hydrolysis can have C-terminus amino acids such as valine (Val), alanine (Ala), leucine (Leu), proline (Pro), tyrosine (Tyr), phenylalanine (Phe), histidine (His) and tryptophan (Trp). It is suggested that α-chymotryptic peptides may inhibit ACE with a high affinity to substrates having C-terminus amino acids such as Ala, Val, Leu, His, and Pro.

3.2. Purification of ACE inhibitory peptide

YFPH-III was dissolved in 20 mM of sodium acetate buffer (pH 4.0), and loaded onto a SP-Sephadex C-25 (35 × 350 mm) column with the linear gradient of NaCl (0–2.0 M), and fractionated into four non-adsorptive portions and five adsorptive portions (Fig. 1(a)). Fraction G eluted with 1.4–1.5 N NaCl was found to possess a strong activity with the IC_{50} of 210 μg/ml. Active fraction G was subjected to gel permeation chromatography on an OHpak SB-803 HQ (8.0 × 300 mm) gel permeation HPLC column equilibrated with the same buffer, and fractionated into four portions (Fig. 1(b)). The fractions were pooled, lyophilized, and fraction H-3 exhibited the strongest ACE inhibitory activity with an IC_{50} value of 93 μg/ml. This active fraction was further separated by RP-HPLC on a SP Nucleosil 100-7 C_{18} (1.0 × 250 mm) reversed phase semi-prep column (Macherey-Nagel Ltd., Middleton Cheney OX17 2PA, UK) using the linear gradient of acetonitrile (0–20%) containing 0.1% TFA, and the fractions were divided into two portions (Fig. 1(c)). Fraction H-3b showed the most potent ACE inhibitory activity with an IC_{50} value of 56 μg/ml. In order to obtain a purified peptide we rechromatographed on a Zorbax SB C_{18} (4.6 × 250 mm) reversed phase analytical column using a 20% acetonitrile concentration containing 0.1% TFA (Fig. 1(d)). Finally, we obtained a purified yellowfin sole frame

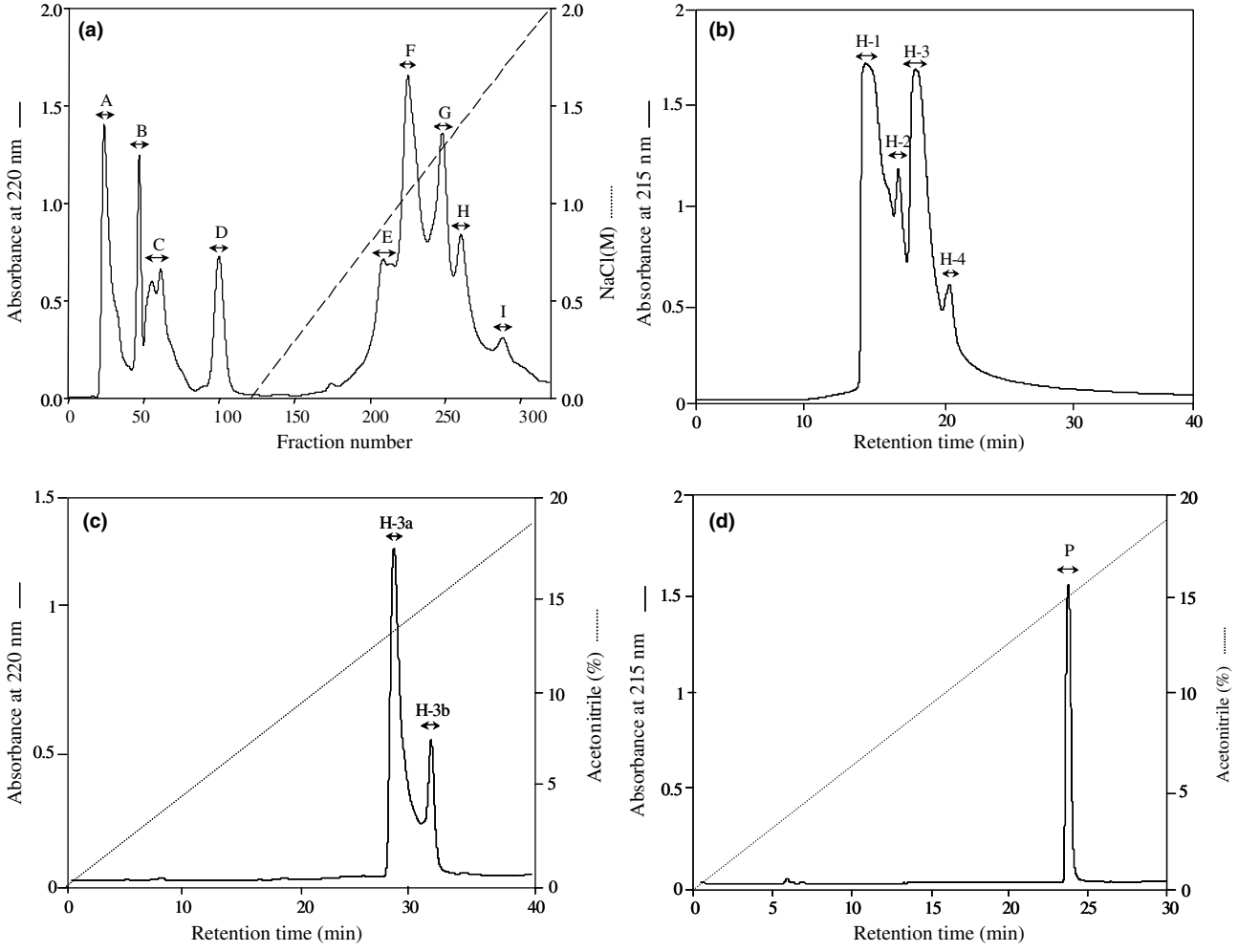

Fig. 1. Purification profiles of a novel ACE inhibitory peptide from yellowfin sole frame protein. (a) SP-Sephadex C-25 ion-exchange chromatogram of YFPH-III. Elution was performed with the linear gradient of NaCl (0–2.0 M) at a flow rate of 1.0 ml min^{-1}. (b) Elution profile of active fraction G on a gel permeation HPLC column. (c) RP-HPLC C_{18} chromatography of active fraction H-3 obtained by gel filtration. Elution was performed with the linear gradient of acetonitrile (0–20%) containing 0.1% TFA. (d) RP-HPLC C_{18} rechromatography of active fraction H-3b. The elution was carried out 20% acetonitrile containing 0.1% TFA.

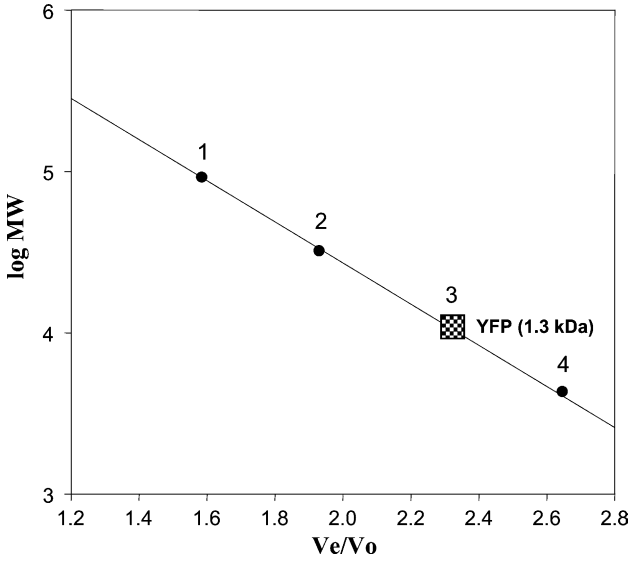

Fig. 2. Determination of molecular mass of YFP. A molecular weight of YBP was measured by gel permeation chromatography. V_e/V_o (V_e, volume of eluted protein; V_o, volume of eluted Blue dextran). ● (standard materials) 1, cytochrome *c* (12.4 kDa); 2, aprotinin (6.5 kDa); 3, angiotensine converting enzyme I (1.2 kDa); 4, HHL (0.4 kDa); ▩, YFP.

Table 2
Purification of ACE inhibitory peptide from YFPH-III

Purification step	IC_{50}[a] (mg ml^{-1})	Purification fold
YFPH-III	0.883	1.00
Cation exchange chromatography	0.210	4.21
Gel permeation chromatography	0.093	9.50
1st RP-HPLC	0.056	15.8
2nd RP-HPLC	0.029	30.4

[a] IC50 value was defined as the concentration of inhibitor required to inhibit 50% of the ACE activity.

peptide (YFP) and its IC_{50} value was measured to be 29 μg/ml. The molecular mass of YFP was estimated to be 1.2 kDa (Fig. 2). The results obtained during the purification steps are summarized in Table 2. The ACE inhibitory peptide was purified 30.8-fold from YFPH-III using five-step purification procedure.

3.3. Determination of amino acid sequence

The amino acid sequence of YFP was determined as Met-Ile-Phe-Pro-Gly-Ala-Gly-Gly-Pro-Glu-Leu, and it exhibited a hydrophobic nature since it is composed of a majority of hydrophobic amino acids. As shown in Fig. 2, the molecular mass (1.2 kDa) adequately corresponds with its sequence. It was observed during purification on the analytical column that this peptide eluted with a longer retention time confirming its hydrophobic nature (Fig. 1(a) and (b)).

As reported by Cushman, Cheung, Sabo, and Ondetti (1981), active sites of two domains of somatic ACE are structurally and functionally homologous as a dipeptidyl carboxypeptidase, and the zinc coordination geometry is critical for their hydrolytic action. However, the two catalytic sites are differentially activated by chloride ions and the physiological substrate Ang I is preferentially bound to the C-domain catalytic site. The substrate also makes a contribution to the chloride-mediated activation of the active site. Therefore, these differences indicate that, despite the higher level of primary sequence homology, structural and functional differences do exist between two active sites of C and N domains. Three subsites, S_1 (antepenultimate), S'_1 (penultimate) and S'_2 (ultimate) with unshared distinct characteristics for the binding of carboxy-terminal amino acids of substrate or inhibitor are located on two homologous active sites. For the inhibitor-enzyme binding and interaction, three main sub-sites on the active site of the enzyme with different amino acid sequence should be bound with the substrate. Binding of inhibitor or the natural substrate to the enzyme takes place predominantly via the carboxy terminal tripeptide residues. C-terminus amino acid residues of the inhibitor such as tryptophan, tyrosine, proline and phenylalanine are reported to be most favorable for the ACE inhibition. More specifically aromatic amino acids and proline for antipenultimate position (S_1) also Ala, Val, and Leu. Ile is more favorable for penultimate position (S'_1). Pro and Leu in the substrate sequence are most favorable for the ultimate position (S'_2) with regard to the affinity exerted on the enzyme (Cushman et al., 1981). This fact is further confirmed as the natural substrate Ang I also contains Leu for the ultimate position. YFP sequence appears to reflect the favorable positioning of Leu as the amino acid to bind with the ultimate position (S'_2) of the enzyme. Also suitability of Pro for the antipenultimate position of this peptide is further confirmed by the fact that Bradykinin, another natural substrate of ACE also possesses the same character in its C-terminal tripeptide sequence.

3.4. Identification of ACE inhibition pattern

The ACE inhibition pattern of the purified peptide (YFP) was estimated using Lineweaver–Burk plots, and was found to be non-competitive (Fig. 3). This means that the peptide can combine with an enzyme molecule to produce a dead-end complex, regardless of whether a substrate molecule is bound or not. Recently, many ACE inhibitory peptides have been isolated from food proteins, such as cheese whey (Abubakar et al., 1998), casein (Maeno et al., 1996; Maruyama et al., 1989), zein (Yano et al., 1996), tuna muscle (Kohama et al., 1988), sardine (Ukeda et al., 1992), corn gluten (Suh & Whang, 1999), bovine skin gelatin (Kim et al., 2001), Alaska Pollack skin gelatin (Byun & Kim, 2001), and bonito (Yokoyama, Chiba, & Yoshikawa, 1993). In addition, Phe-Phe-Val-Ala-Pro (Maruyama et al., 1989) and Ile-Pro-Pro (Kohmura et al., 1989) derived from casein exhibited ACE inhibitory activity

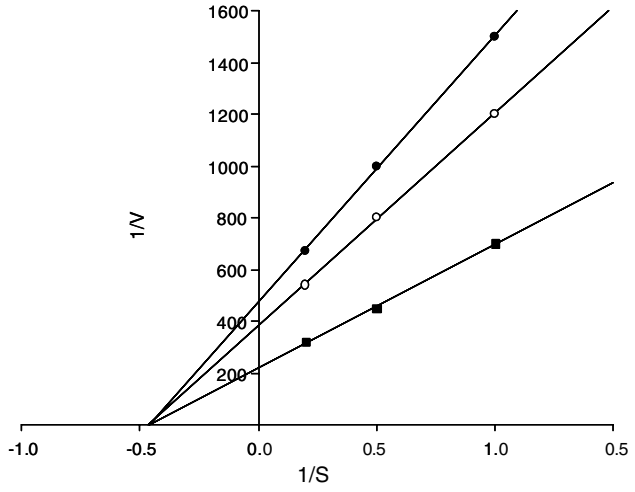

Fig. 3. Lineweaver–Burk plots on ACE inhibitory activity in the presence of the peptide. ■, Control; ○, 250 μM of YFP; ●, 500 mM of YFP.

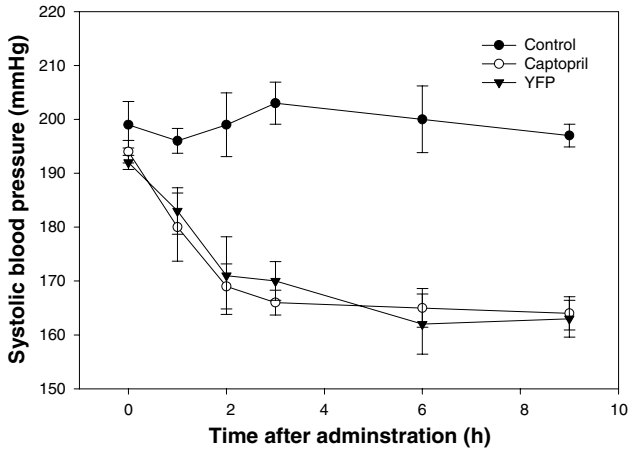

Fig. 4. Change in systolic blood pressure of SHR by administering ACE inhibitory peptide. Single oral administration was performed with the dose of 10 mg kg^{-1} body weight, and SBP was measured 0, 1, 2, 3, 6 and 9 h after the administration. Different from control at $^{*}P < 0.05$, $^{**}P < 0.01$.

(IC_{50} = 2.0 and 5.0 μM), and Leu-Gln-Pro (Miyoshi et al., 1991) derived from zein showed IC_{50} value of 9.6 μM. Ile-Val-Gly-Arg-Pro-Arg-His-Glu-Glu (IC50 = 6.2 μM), Ala-Leu-Pro-His-Ala (IC_{50} = 10 μM), Phe-Gln-Pro (IC_{50} = 12 μM), Leu-Lys-Pro-Asn-Met (IC_{50} = 17 μM), Asp-Tyr-Gly-Leu-Tyr-Pro- (IC_{50} = 62 μM), and Ile-Lys-Pro-Leu-Asn-Tyr (IC_{50} = 43 μM) were isolated from a thermolysin digest of dried bonito (Yokoyama et al., 1993).

ACE inhibitory peptide purified from YFPH-III was composed of hydrophobic amino acids at the C-terminal with IC_{50} of 22.3 μM. This IC_{50} value exhibited higher or similar activity compared to those of peptides derived from dried bonito, however, it was lower activity than those of peptides from the hydrolysate of casein and zein. Among the naturally occurring peptides with ACE inhibitory activity, the most potent and specific inhibitors were several peptides with similar structures that have been isolated from the venom of the South American pit viper *Bothrops jararaca*, and Japanese pit viper *Agkistrodon halys blomhoffi* (Ferrira, Bartet, & Greene, 1970; Ondetti et al., 1971). ACE is strongly influenced by the C-terminal tripeptide sequence of the substrate, it is suggested that peptides which contain hydrophobic amino acids at these position are potent inhibitors.

3.5. Antihypertensive activity of the purified peptide

Antihypertensive activity of the purified peptide was evaluated by measuring the change of SBP at 1, 2, 3, 6 and 9 h after oral administration of 10 mg kg^{-1} of body weight. There was no change in SBP in the controll group over the investigation period. As shown in Fig. 4, a recorded SBP reduction of 22 mmHg at 3 h after administration was observed, and the activity was maintained for 9 h. Recently, many ACE inhibitory peptides were isolated from food proteins and their antihypertensive effect tested in SHR. Fujita and Yoshikawa (1999) reported that LKPNM is a prodrug type ACE inhibitory peptide because LKPNM was found to be hydrolysed by ACE to produce LKP, which had 8-fold higher ACE inhibitory activity compared to LKPNM. After oral administration in SHR, the antihypotensive effect of LKPNM showed maximal effect at 6 h, while LKP showed maximal effect at 4 h. Leclerc, Gauthier, Bachelard, Santure, and Roy (2002) reported antihypotensive activity of casein-enriched milk fermented by *L. helveticus*. Some antihypertensive drugs are known to produce side effects such as an abnormal elevation of the blood pressure after administration. However, we could not find any side effect after sample administration. The result of this study suggests that ACE inhibitory peptide derived from yellowfin sole frame protein could be utilized to develop nutraceuticals and pharmaceuticals. In addition, it is expected that this will contribute developing interest in basic research and potential applications of bioactive peptides.

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References

Abubakar, A., Saito, T., Kitazawa, H., Kawai, Y., & Itoch, T. (1998). Structural analysis of new antihypertensive peptides derived from cheese whey protein by proteinase K digestion. *Journal of Dairy Science, 81*, 3131–3138.

Ariyoshi, Y. (1993). Angiotensin-converting enzyme inhibitors derived from food proteins. *Trends in Food Science and Technology, 4*, 139–144.

Bush, K., Herny, P. R., & Slusarchyk, D. S. (1984). Muraceins-muramyl peptides produced by Norcardia orientalis as angiotensin converting enzyme inhibitors. *Journal of Antibiotics, 37*, 330–335.

Byun, H. G., & Kim, S. K. (2001). Purification and characterization of angiotensin I converting enzyme (ACE) inhibitory peptides from alaska pollack (*Theragra chalcogramma*) skin. *Process Biochemistry, 36*, 1155–1162.

Curtiss, C., Chon, J. N., Vrobel, T., & Francious, J. A. (1978). Role of the rennin-angiotensin system in the systemic vasoconstriction of chronic congestive heart failure. *Circulation, 58*, 763–770.

Cushman, D. W., & Cheung, H. S. (1971). Spectrophotometric assay and properties of the angiotensin I-converting enzyme of rabbit lung. *Biochemical Pharmacology, 20*, 1637–1648.

Cushman, D. W., Cheung, H. S., Sabo, E. F., & Ondetti, M. A. (1981). Angiotensin converting enzyme inhibitors: Evaluation of a new class of antihypertensive drugs. In Z. P. Horovitz (Ed.), *Angiotensin converting enzyme inhibitors: Mechanism of action and clinical implications* (pp. 1–25). Urban & Schwarzenberg Baltimore-Munich.

Ferrira, S. H., Bartet, D. C., & Greene, L. J. (1970). Isolation of bradykinin-potentiating peptides from *Bothrops jararaca* venom. *Biochemistry, 9*, 2583–2593.

Fujita, H., & Yoshikawa, M. (1999). LKPNM: a prodrug-type ACE-inhibitory peptide derived from fish protein. *Immunopharmacology, 44*, 123–127.

Hyun, C. K., & Shin, H. K. (2000). Utilization of bovine blood plasma proteins for the production of angiotensin I converting enzyme inhibitory peptides. *Process Biochemistry, 36*, 65–71.

Jung, W. -K., Park, P. -J., Byun, H. -G, Moon, S. -H., & Kim, S. -K. (2004). Preparation of hoki (*Johnius belengerii*) bone oligophosphopeptide with a high affinity to calcium by carnivorous intestine crude proteinase, *Food Chemistry, 91*(2), 333–340.

Kim, S.-K., Jeon, Y.-J., Byun, H.-G., Kim, Y.-T., & Lee, C.-K. (1997). Enzymatic recovery of cod frame proteins with crude proteinase from tuna pyloric caeca. *Fisheries Science, 63*, 421–427.

Kim, S.-K., Kim, Y.-T., Byun, H.-G., Nam, K.-S., Joo, D.-S., & Shahidi, F. (2001). Isolation and characterization of antioxidative peptides from gelatin hydrolysate of Alaska Pollack skin. *Journal of Agricultural and Food Chemistry, 49*(4), 1984–1989.

Kohama, Y., Matsumoto, S., Oka, H., Teramoto, T., Okabe, M., & Mimura, T. (1988). Isolation of angiotensin-converting enzyme inhibitory from tuna muscle. *Biochemical and Biophysical Research Communications, 155*, 332–337.

Kohmura, M., Nio, N., Kudo, K., Minoshima, Y., Munekata, E., & Ariyoshi, Y. (1989). Inhibition of angiotensin-converting enzyme by synthetic peptides of human β-casein. *Agricultural and Biological Chemistry, 53*, 2107–2114.

Leclerc, P.-L., Gauthier, S. F., Bachelard, H., Santure, M., & Roy, D. (2002). Antihypertensive activity of casein-enriched milk fermented by *Lactobacillus helveticus*. *International Dairy Journal, 12*, 995–1004.

Maeno, M., Yamamoto, N., & Takano, T. (1996). Identification of an antihypertensive peptide from casein hydrolysate produced by a proteinase from *Lactobacillus helveticus* CP790. *Jounal of Dairy Science, 79*, 1316–1321.

Margaret, M., Mullally, H. M., & FitzGerald, R. J. (1997). Identification of a novel angiotensin-I-converting enzyme inhibitory peptide corresponding to a tryptic fragment of bovine β-lactoglobulin. *FEBS Letters, 402*, 99–101.

Maruyama, S., Mitachi, H., Awaya, J., Kurono, M., Tomizuka, N., & Suzuki, H. (1989). Angiotensin I-converting enzyme inhibitory activity of the C-terminal hexapeptide of α_{s1}-casein. *Agricultural and Biological Chemistry, 53*, 2107–2114.

Meisel, H. (1997). Biochemical properties of bioactive peptides derived from milk proteins: potential nutraceuticals for food and pharmaceutical applications. *Livestock Production Science, 50*, 125–138.

Miyoshi, S., Ishikawa, H., Kaneko, T., Fukui, F., Tanaka, H., & Maruyama, S. (1991). Structure and activity of angiotensin-converting enzyme inhibitors in an α-zein hydrolysate. *Agricultural and Biological Chemistry, 55*, 1313–1318.

Nair, A. L., & Gopakumar, K. (1982). Soluble protein isolate from low cost fish and fish wastes. *Fishery Technology, 19*, 101–103.

Ondetti, M. A. (1977). Design of specific inhibitors of angiotensin-converting enzyme: New class of orally active antihypertensive agents. *Science, 196*, 441–444.

Ondetti, M. A., Williams, N. J., Sabo, E. F., Pluscec, J., Weaver, E. R., & Kocy, O. (1971). Angiotensin-converting enzyme inhibitors from the venom of *Bothrops jararaca*. Isolation, elucidation of structure, and synthesis. *Biochemistry, 10*, 4033–4039.

Patchett, A. A., Harris, E., Tristram, E. W., Wyvratt, M. J., Wu, M. T., Taub, D., et al. (1980). A new class of angiotensin-converting enzyme inhibitors. *Nature, 298*, 280–283.

Pihlanto-Leppälä, A. (2001). Bioactive peptides derived from bovine whey proteins: opioid and ace-inhibitory peptides. *Trends in Food Science and Technology, 11*, 347–356.

Skeggs, L. T., Kahn, J. E., Kahn, J. E., & Shumway, N. P. (1957). The preparation and function of the angiotensin-converting enzyme. *Journal of Experimental Medicine, 103*, 295–299.

Suh, H. J., & Whang, J. H. (1999). A peptide from corn gluten hydrolysate that is inhibitory toward angiotensin I converting enzyme. *Biotechnology Letters, 21*, 1055–1058.

Ukeda, H., Matsuda, H., Osajima, K., Matsufuji, H., Matsui, T., & Osajima, Y. (1992). Peptides from peptic hydrolyzate of heated sardine meat that inhibit angiotensin I-converting enzyme. *Nippon Nogeikagaku Kaishii, 66*, 25–29.

Yamamoto, N. (1997). Antihypertensive peptides derived from food proteins. *Biopolymers, 43*, 129–134.

Yang, H. Y. T., Erdös, E. G., & Levin, Y. (1971). Characterization of a dipeptide hydrolase (Kininase II: angiotensin I converting enzyme). *Journal of Pharmacology and Experimental Therapeutics, 177*, 291–300.

Yano, S., Suzuki, K., & Funatsu, G. (1996). Isolation from α-zein of thermolysin peptides with angiotensin I-converting enzyme inhibitory activity. *Bioscience Biotechnology and Biochemistry, 60*, 661–663.

Yokoyama, K., Chiba, H., & Yoshikawa, M. (1993). Peptide inhibitors for angiotensin I converting enzyme from a thermolysin digest of dried bonito. *Bioscience Biotechnology and Biochemistry, 56*, 1541–1545.