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Novel angiotensin I-converting enzyme inhibitory peptides isolated from Alcalase hydrolysate of mung bean protein

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Abstract: Mung bean protein isolates were hydrolyzed for 2 h by Alcalase. The generated hydrolysate showed angiotensin I-converting enzyme (ACE) inhibitory activity with the IC_{50} value of 0.64 mg protein/ml. Three kinds of novel ACE inhibitory peptides were isolated from the hydrolysate by Sephadex G-15 and reverse-phase high performance liquid chromatography (RP-HPLC). These peptides were identified by amino acid composition analysis and matrix assisted-laser desorption/ionization time-of-flight tandem mass spectrometry (MALDI-TOF MS/MS), as Lys-Asp-Tyr-Arg-Leu, Val-Thr-Pro-Ala-Leu-Arg and Lys-Leu-Pro-Ala-Gly-Thr-Leu-Phe with the IC_{50} values of 26.5 μM, 82.4 μM and 13.4 μM, respectively. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: angiotensin I-converting enzyme peptides; mung bean protein; antihypertensive effect; spontaneously hypertensive rats; Alcalase

INTRODUCTION

Angiotensin I-converting enzyme (ACE, dipeptidyl carboxpeptidase, EC 3.4.15.1) plays an important role in the regulation of blood pressure as well as cardiovascular function. ACE converts the inactive decapeptide angiotensin I into the potent vasoconstricting octapeptide angiotensin II, and also inactivates vasodilator, bradykinin [1]. Thus, inhibition of ACE results in a decrease in blood pressure. Many potent synthetic ACE inhibitors such as captopril, enalapril, lisinopril and ramipril have been widely used in the clinical treatment of hypertension and heart failure in humans. However, synthetic ACE inhibitors can have side effects including cough, taste disturbances and skin rashes [2]. Therefore, the search for diet-related preventive measures for hypertension is obviously of interest within the scope of functional foods. ACE inhibitory peptides derived from food proteins are suitable candidates for such products. Oshima *et al.* first reported ACE inhibitory peptides produced from food protein by digestive proteases [3]. Afterward, many ACE inhibitory peptides have been discovered from enzymatic hydrolysates of different food proteins. These peptides with *in vitro* ACE inhibitory activities have been well demonstrated having *in vivo* inhibitory properties on ACE and antihypertensive effects without side effects in SHR and hypertensive humans [4].

Abbreviations: DH, degree of hydrolysis; SBP, systolic blood pressure; SHR, spontaneously hypertensive rats.

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Mung bean is a popular food in China due to its detoxifying, anti-inflammatory, antitumorogenic, cholesterol-lowering and diuretic properties. Mung bean is an excellent source of protein with the protein content ranging from 20 to 33% in addition to the bean being almost free from flatulence-causing factors [5]. In our previous studies, we found that mung bean protein hydrolysates obtained with Alcalase exhibited *in vitro* ACE inhibitory activity [6,7] and exerted antihypertensive effect in SHR [8]. The objective of this study was to isolate and identify peptides responsible for ACE inhibitory activity from mung bean protein hydrolysate produced by Alcalase.

MATERIALS AND METHODS

Materials

Mung beans (*Phaseolus radiatus* L.) were purchased from a local supermarket. The seeds were ground and defatted with hexane for 24 h in a shaker. ACE (from rabbit lung; 3.4 U/mg of protein), pepsin (1 : 60 000, 3400 U/mg of solid, from porcine stomach mucosa), hippuryl-L-histidyl-L-leucine, captopril and a-cyano-4-hydroxycinnamic acid were purchased from Sigma (St Louis, MO, USA). Alcalase 2.4 L (liquid, 2.4 AU/g) was kindly provided by Novo Nordisk (Bagsvaerd, Denmark). Alcalase 2.4 L is a food-grade endoprotease from *Bacillus licheniformis* and the main enzyme component is the serine protease subtilisin A (EC3.4.21.62).

Preparation of Mung Bean Protein Isolates

Protein isolates were prepared from defatted mung bean flour by extraction with alkaline water (pH 9.0, 1 : 10 w/v

flour : water ratio) for 30 min at room temperature (~25 °C). The suspension thus obtained was centrifuged at 2000 × *g* for 20 min. The supernatants were combined and the pH adjusted to 4.6 with 2 N HCl. The protein precipitate formed was separated by centrifugation at 2000 × *g* for 20 min. The precipitate was washed twice with distilled water and then lyophilized.

Preparation of Protein Hydrolysates

Mung bean protein hydrolysate was prepared as described by our previous study [7]. Briefly, Alcalase was added to the mung bean protein isolates solution (4%, w/v) at 20 µl/g protein (enzyme/bean protein). Hydrolysis was performed using the pH-stat method [9] for 2 h at 55 °C and pH 8.0. The enzymatic hydrolysis was terminated by heating for 10 min in a boiling water bath. The protein hydrolysate was centrifuged at 10 000 × *g* for 20 min, and the resulting supernatant was filtrated through the hollow fiber ultrafiltration membrane (Tianjin Motian Membrane Engineering and Technology Co. Ltd., Tianjin, China) with a molecular weight (MW) cut-off 6000. The filtrates were then lyophilized. The peptide content was measured by UV absorbance difference at 215 and 225 nm [10].

Assay for ACE Inhibitory Activity

ACE inhibitory activity was measured by our previously described method [6]. The IC_{50} value was defined as the concentration of peptide in mg protein/ml required to inhibit 50% of the ACE activity under the assayed conditions, and was determined by regression analysis of ACE inhibition (%) *versus* log (peptide concentration, mg/ml).

Purification of ACE Inhibitory Peptides

The lyophilized filtrate (0.15 g/ml) from MW cut-off 6000 ultrafiltration membrane was loaded onto a Sephadex G-15 column (1.8 × 60 cm) equilibrated with 20 mM sodium acetate–acetic acid buffer solution (pH 4.0) and eluted with the same buffer solution at a flow rate of 0.4 ml/min. The elution was monitored at 220 nm. The fractions were collected from at least six chromatographic runs and lyophilized. The ACE inhibitory activity of each fraction peak was measured. The highest ACE inhibitory fraction peak from Sephadex G-15 column was dissolved in 0.1% trifluoroacetic acid (TFA) (5 mg/ml) and further separated by reverse-phase high performance liquid chromatography (RP-HPLC) with Sephasil Peptide C18 12 µm ST 4.6/250 column (4.6 × 250 mm, Amersham Pharmacia Biotech, Sweden). The injection volume was 100 µl. The separation was performed with a linear gradient from solvent A (0.1% TFA in distilled water) to 100% of solvent B (60% acetonitrile containing 0.1% TFA) over 60 min at a flow rate of 1 ml/min. The elution was monitored at 220 nm. The individual peaks were collected from at least ten separate RP-HPLC runs, pooled and lyophilized. Each peak was determined for the ACE inhibitory activity. The strong active peaks were further applied onto the Sephasil Peptide C18 5 µm ST 4.6/250 column (4.6 × 250 mm, Amersham) and eluted using a linear gradient from 10 to 80% of solvent B over 40 min.

Amino Acid Sequence Analysis and Mass Spectrometry

The amino acid composition of purified peptide showing ACE inhibitory activity was determined by precolumn derivatization with *O*-phthalaldehyde on the automatic amino acid analyzer (Agilent HP1100, Agilent Co., USA) after hydrolysis for 24 h in 6 N HCl at 110 °C under vacuum. The sequences of the ACE inhibitory peptides were analyzed by matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry (MALDI-TOF MS/MS). The purified peptide was dissolved in distilled water containing 0.1% TFA. The solution of 50% acetonitrile (containing 5 mg/ml a-cyano-4-hydroxycinnamic acid and 0.1% TFA) was used as matrix solution. One microliter of matrix solution was mixed with 1 µl of sample solution and 1 µl of this mixture solution was then spotted on a stainless steel MALDI target with 192 wells (Applied Biosystems, Framingham, MA, USA). After the mixture solution dried, the samples were then subjected to MALDI-TOF MS/MS analysis. All the mass spectra and tandem mass spectra were acquired using manual method by the ABI 4700 TOF-TOF Proteomics Analyzer instrument (Applied Biosystems). The UV Nd : YAG laser was operated at a 200 Hz repetition rate with wavelength of 355 nm. The accelerated voltage and detector voltage were operated at 20 kV and 1 kV, respectively. The samples were measured in the reflectron and positive ionization mode. Myoglobin digested by trypsin was used to calibrate the mass instrument with internal calibration mode. The spectra were analyzed by 4700 Explorer™ software (Applied Biosystems). The sequences of these peptides were obtained by *de novo* Explorer and were proved by Data Explorer with the Ion Fragmentation Calculator function.

RESULTS AND DISCUSSION

Purification of ACE Inhibitory Peptide

Mung bean protein isolates were hydrolyzed with Alcalase and the generated hydrolysates were subjected to assay for ACE inhibitory activity. The unhydrolyzed mung bean protein isolates showed no inhibitory activity on ACE. ACE inhibitory activities were generated from the mung bean proteins after enzymatic hydrolysis. The hydrolysate showed the highest ACE inhibitory percentage at 2 h incubation with the IC_{50} value being 0.64 mg protein/ml, and further hydrolysis resulted in hydrolysates with decreased inhibition on ACE (data not shown). This hydrolysate also showed antihypertensive effect in SHR [8]. To identify the ACE inhibitory peptides contained in the hydrolysate, which had demonstrated ACE inhibitory activity *in vitro* and antihypertensive activity *in vivo*, the hydrolysate was initially separated using Sephadex G-15 gel filtration chromatography. As shown in Figure 1, the hydrolysate was fractionated into nine individual fractions (fraction A-I). The ACE inhibitory activity of each fraction is shown in Table 1. The ACE inhibitory activity was widely observed in all fractions, suggesting that many ACE inhibitory peptides with various ranges of MW were included in the

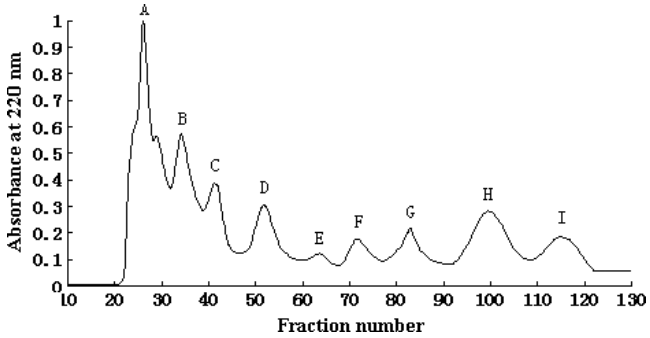

Figure 1 Gel filtration chromatography profile of hydrolysates on Sephadex G-15 column (1.8 × 60 cm). Separation was performed at a flow rate of 0.4 ml/min with 20 mM sodium acetate–acetic acid buffer solution (pH 4.0) and collected at a fraction volume of 2 ml.

Table 1 ACE inhibitory activity of peptide fractions obtained on Sephadex G-15 column chromatography[a]

Fraction	ACE inhibitory activity (%)	Fraction	ACE inhibitory activity (%)
A	5.36 ± 0.67	F	22.80 ± 1.45
B	13.66 ± 0.53	G	46.28 ± 0.96
C	52.66 ± 1.21	H	25.34 ± 1.01
D	56.19 ± 2.36	I	8.69 ± 1.34
E	32.63 ± 1.68	—	—

[a] The concentration of peptide fraction in the inhibition assay was 0.5 mg/ml.

hydrolysate. However, fraction D exhibited the highest activity, followed by fraction C. The fraction D was further purified on a Sephasil Peptide C18 by RP-HPLC. The fraction D was separated into 18 major different fractions (Figure 2(a)). Among the 18 fractions, fraction F7, F13 and F14 showed strong ACE inhibitory activities with inhibition percentages higher than 50% at a protein concentration of 100 μg/ml (Figure 2(b)). The three active fractions were again applied into the RP-HPLC column and eluted using a shallower acetonitrile gradient. The elution profiles of fraction F7, F13, and F14 are shown in Figure 2(c). After the second RP-HPLC, a single pure peptide was isolated from fraction F7 and designated as F7-1; fraction F13 was separated into two peaks (F13-1 and F13-2), among which peak F13-2 had the highest ACE inhibitory activity; fraction F14 was separated into three peaks (F14-1, F14-2 and F14-3), among which peak F14-2 showed the highest ACE inhibitory activity. Peptides F7-1, F13-2 and F14-2 were then subjected to MALDI-TOF MS analysis to detect the purity and to obtain the MWs of these peptides.

Identification of ACE Inhibitory Peptide

MALDI-TOF MS has rapidly developed as one of the most effective analytical tools for the determination and characterization of biopolymers such as peptides, proteins, oligosaccharides and nucleotides. MALDI-TOF MS is remarkably tolerant toward contaminants such as salts and buffers, requires sample loads in the subpicomolar range and features a mass accuracy (and precision) of 0.1–0.01% [11]. MALDI-TOF MS is also an effective technique for the detection of purity of peptides and proteins [12]. MALDI-TOF MS of the Peptides F7-1, F13-2 and F14-2 displayed a single positively charged ion ($[M + H]^+$) at 694.45, 656.40 and 846.68, respectively, which confirmed the purity of these peptides. These precursor ions were then subjected to TOF-TOF tandem MS analysis. Figure 3 shows the MS/MS spectrum of the three identified ACE inhibitory peptides. Structural information of these peptides was summarized in Table 2. As can be seen from Figure 3(b) and Figure 3(c), intense fragment ions were observed at m/z 439.33 of peptide Val-Thr-Pro-Ala-Leu-Arg and at m/z 605.44 of peptide Lys-Leu-Pro-Ala-Gly-Thr-Leu-Phe, both correspond to the *y*-type ion adjacent to the amino acid proline. This amino acid is associated with very abundant *y*-type ions which are often easily identifiable because these ions are over-presented in the spectrum [13]. The presence of arginine at the

Table 2 Structural characterization of ACE peptides from mung bean protein hydrolysates

Fraction	Amino acid composition (relative molar ratio)	Sequence	Calculated mass[a]	Observed mass	IC_{50} (μM)
F7-1	Lys 1.00, Asp 1.03, Tyr 0.97, Arg 0.99, Leu 1.00	Lys-Asp-Tyr-Arg-Leu	693.8	693.35	26.5
F13-2	Val 1.00, Thr 1.04, Pro 1.03, Ala 0.96, Leu 1.00, Arg 0.98	Val-Thr-Pro-Ala-Leu-Arg	655.8	655.40	82.4
F14-2	Lys 0.98, Leu 2.12, Pro 0.97, Ala1.00, Gly 1.04, Thr 1.05, Phe1.16	Lys-Leu-Pro-Ala-Gly-Thr-Leu-Phe	846.0	845.68	13.4

[a] Monoisotopic mass values.

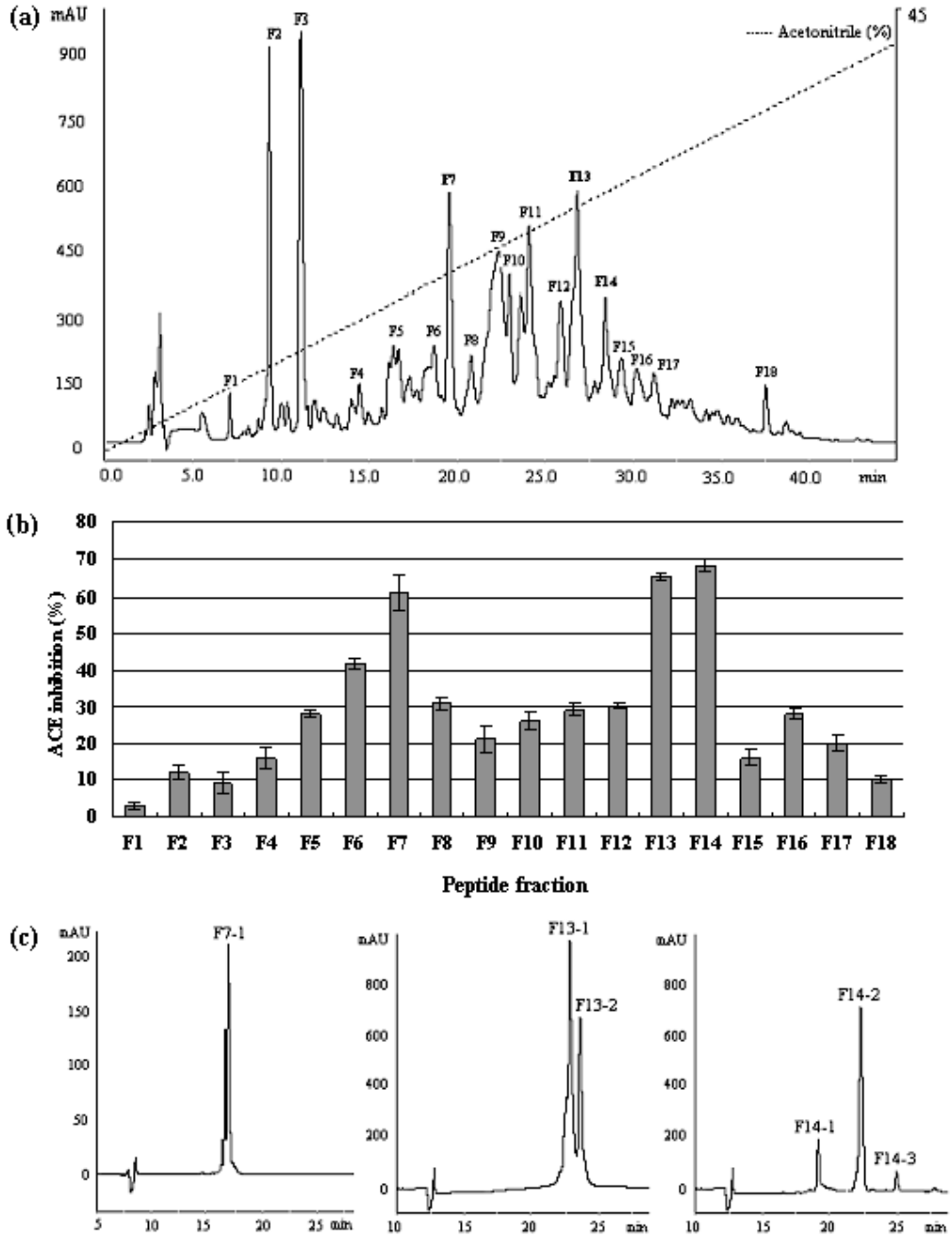

Figure 2 (a) RP-HPLC chromatogram of fraction D from Sephadex G-15; (b) ACE inhibitory activity of peptide fractions from RP-HPLC of fraction D; (c) RP-HPLC chromatograms of fractions of F7, F13 and F14 from the first RP-HPLC.

C-terminal of peptide Val-Thr-Pro-Ala-Leu-Arg favored the appearance of *y*-type fragment ions (Figure 3(b)). In the case of peptide Lys-Leu-Pro-Ala-Gly-Thr-Leu-Phe, however, the fragment ion at *m/z* 605.44 that corresponds to the y6-ion is the most noticeable ion in the spectrum (Figure 3(c)). This fragment was produced by cleavage at Leu-Pro bond and it had been previously found that abundant Xxx-Pro relative bond cleavage ratios were observed when Xxx was Val, His, Asp, Ile and Leu [14]. Internal fragment ions PAL at *m/z* 282.21 of peptide Val-Thr-Pro-Ala-Leu-Arg and PAGT at 327.21 of peptide Lys-Leu-Pro-Ala-Gly-Thr-Leu-Phe were also abundant, corresponding to peptides with proline close to the *N*- or *C*-terminus. Figure 3(a) shows the MS/MS spectrum of the singly charged precursor ion at *m/z* 694.35 corresponding to peptide Lys-Asp-Tyr-Arg-Leu. This fragmentation spectrum contained a major ion at 451.34, which was identified as a *y*-type fragment ion resulting from the cleavage *C*-terminal to aspartic acid. This unusual fragmentation pattern had also been previously shown by other authors, who found that peptide ions containing arginine and aspartic or glutamic acid residues result in selective cleavages *C*-terminal to the acidic residues

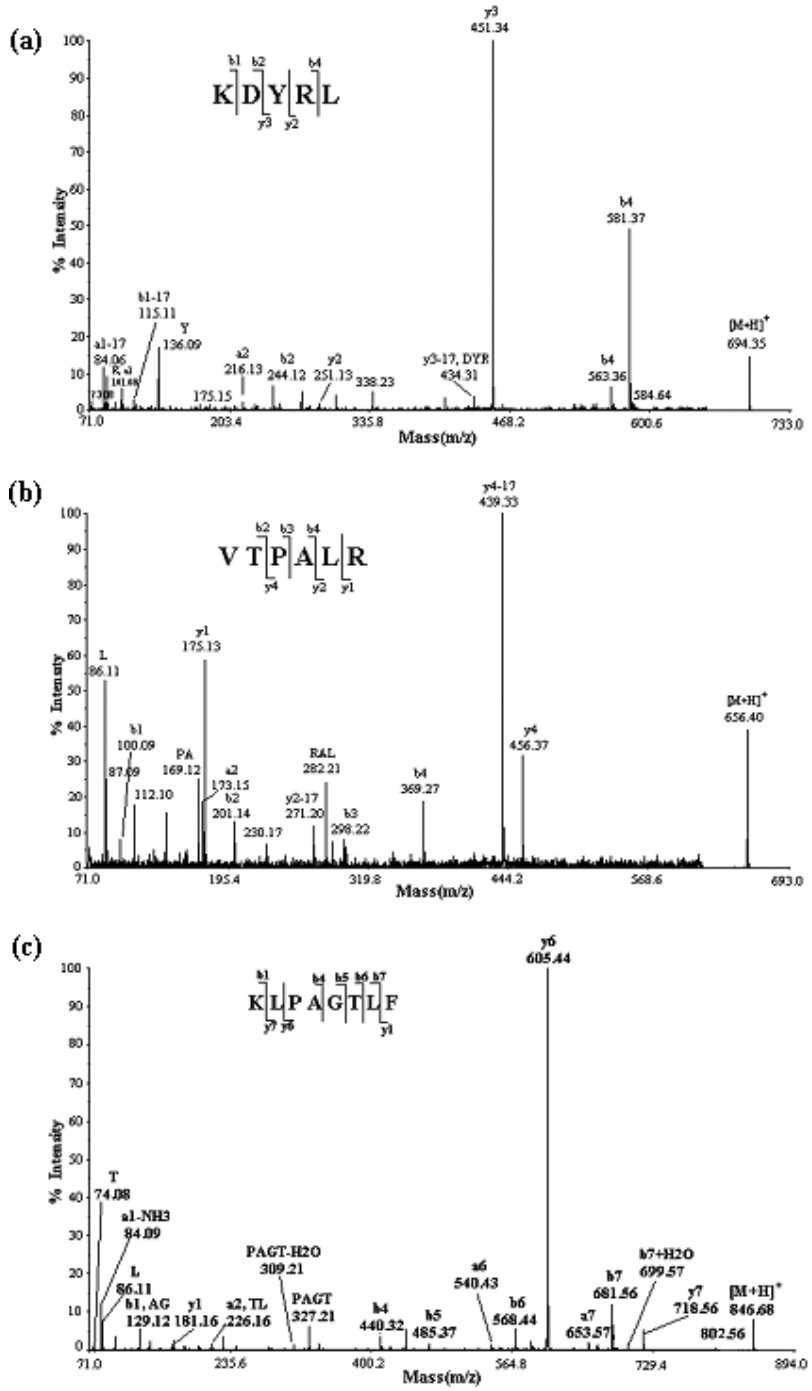

Figure 3 MS/MS spectrum of ion (a) m/z 694.35; (b) m/z 656.40; (c) m/z 846.68.

[15,16]. In all cases, some characteristic ions that correspond to decarboxyled- or deamidoed-amino acids were also observed in the MS/MS spectrum. These ions are characterized as m/z = MW of amino acid-45 or MW-17, which contributes to the confirmation of amino acid composition of identified peptides. The assignment of the *y*- and *b*-type fragment ions, together with fragment ions corresponding to losses of water and ammonium from the precursor ions or their fragments, and results of amino acid composition analysis of peptides allowed unambiguous identification of these three ACE inhibitory peptides. To the best of our knowledge, the three ACE inhibitory peptides derived from mung bean protein in this study has not been described before in any other source of protein.

Structure-activity correlations among different peptide inhibitors of ACE indicate that competitive binding to ACE is strongly influenced by the *C*-terminal tripeptide sequence of the inhibitors. ACE appears to prefer substrate or competitive inhibitors containing

hydrophobic (aromatic or branched-side chains) amino acid residues at each of the three *C*-terminal positions. Among the most favorable *C*-terminal amino acids are aromatic amino acids, as well as the imino acids proline, because ACE binds only weak peptides that have terminal dicarboxylic amino acids, aliphatic (Ile, Ala, Leu, and Met) residues in the ultimate position also contribute to binding to ACE [17]. Among the identified peptides, Lys-Asp-Tyr-Arg-Leu contained Leu as *C*-terminal amino acid, although Lys-Leu-Pro-Ala-Gly-Thr-Leu-Phe had the amino acid Phe as *C*-terminal amino acid. Meanwhile, peptide Lys-Leu-Pro-Ala-Gly-Thr-Leu-Phe shares the *C*-terminal dipeptide with α-lactalbumin-derived Tyr-Gly-Leu-Phe and β-lactoglobulin-derived Tyr-Leu-Leu-Phe, both were found to have *in vitro* ACE inhibitory activity [18] and antihypertensive activity in SHR [19]. Structure-activity data also suggest that the positive charge on the guanidine or ε-amino group of *C*-terminal arginine and lysine side chains, respectively, contribute substantially to the ACE inhibitory potency of several peptides [20]. Rohrbach *et al.* [21] showed by using *N*-blocked synthetic peptides that in terms of binding specificity to ACE, Pro as *C*-terminal amino acid and a positively charged amino acid in the penultimate position had a positive influence for peptide-enzyme binding. In this study, peptide Val-Thr-Pro-Ala-Leu-Arg possessed *C*-terminal arginine, which may contribute to the inhibitory activity of this peptide. Meanwhile, this peptide contained a high proportion of hydrophobic amino acids at *N*-terminal, which may contribute to the binding affinity of the peptide to ACE. With respect to peptide Lys-Asp-Tyr-Arg-Leu, this peptide contained arginine in the penultimate position, which was in agreement with Rohrbach *et al.* [21].

CONCLUSION

Three kinds of novel ACE inhibitory peptides were isolated from the Alcalase hydrolysate of mung bean protein isolates and their amino acid sequences were identified to be Lys-Asp-Tyr-Arg-Leu, Val-Thr-Pro-Ala-Leu-Arg and Lys-Leu-Pro-Ala-Gly-Thr-Leu-Phe. Mung bean protein is a good protein source of ACE inhibitory peptides when hydrolyzed with the protease Alcalase.

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REFERENCES

1. Erdös EG. Angiotensin I-converting enzyme. *Circ. Res.* 1975; **36**: 247–255.
2. Messerli FH. Combinations in the treatment of hypertension: ACE inhibitors and calcium antagonists. *Am. J. Hypertens.* 1999; **12**: 86S–90S.
3. Oshima G, Shimabukuro H, Nagasawa K. Peptide inhibitors of angiotensin I-converting enzyme in digests of gelatin by bacterial collagenase. *Biochim. Biophys. Acta* 1979; **566**: 128–137.
4. Li G-H, Le G-W, Shi Y-H, Shrestha S. Angiotensin I-converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. *Nutr. Res.* 2004; **27**: 469–486.
5. Adsuale RN, Kadam SS, Salunkhe DK. Chemistry and technology of green gram (Vigna radiata [L.] Wilczek). *Crit. Rev. Food Sci. Nutr.* 1986; **25**: 73–105.
6. Li G-H, Liu H, Shi Y-H, Le G-W. Direct spectrophotometric measurement of angiotensin I-converting enzyme inhibitory activity for screening bioactive peptides. *J. Pharm. Biomed. Anal.* 2005; **37**: 219–224.
7. Li G-H, Le G-W, Liu H, Shi Y-H. Mung-bean protein hydrolysates obtained with Alcalase exhibit angiotensin I-converting enzyme inhibitory activity. *Food Sci. Technol. Int.* 2005; **11**: 281–287.
8. Li G-H, Shi Y-H, Liu H, Le G-W. Antihypertensinve effect of alcalase generated mung bean protein hydrolysates in spontaneously hypertensive rats. *Eur. Food Res. Technol.* 2006; **222**: 733–736.
9. Adler-Nissen J. *Enzymic Hydrolysis of Food Proteins*. Elsevier Applied Science Publishers: London, 1986; 9–24.
10. Murphy JB, Kies MW. Note on spectrophotometric determination of proteins in dilute solutions. *Biochim. Biophys. Acta* 1960; **45**: 382–384.
11. Kaufmann R. Matrix-assisted laser desorption ionization (MALDI) mass spectrometry: a novel analytical tool in molecular biology and biotechnology. *J. Biotechnol.* 1995; **41**: 155–175.
12. Li YL, Jin SW. Application of mass spectrometry in protein and peptide chemistry. *Org. Chem.* 1996; **16**: 209–217. (in Chinese).
13. Papayannopoulus AI. The interpretation of collision-induced dissociation tandem mass spectra of peptide. *Mass Spectrom. Rev.* 1995; **14**: 49–73.
14. Gomez-Ruiz JA, Ramos M, Recio I. Identification and formation of angiotensin-converting enzyme-inhibitory peptides in Manchego cheese by high-performance liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* 2004; **1054**: 269–277.
15. Tsaprailis G, Nair H, Somogyi A, Wysocki VH, Zhong W, Futrell JH, Summerfield SG, Gaskell SJ. Influence of secondary structure on the fragmentation of protonated peptides. *J. Am. Chem. Soc.* 1999; **121**: 5142–5154.
16. Tsaprailis G, Somogyi A, Nikolaev EN, Wysocki VH. Refining the model for selective cleavage at acidic residues in arginine-containing protonated peptides. *Int. J. Mass Spectrom. Ion Physics.* 2000; **195/196**: 467–479.
17. Cheung HS, Wang FL, Ondetti MA, Sabo EF, Cushman DW. Binding of peptide substrate and inhibition of angiotensin-converting enzyme: importance of the COOH-terminal dipeptides sequence. *J. Biol. Chem.* 1980; **255**: 401–407.
18. Mullally MM, Meisel H, FitzGerald RJ. Synthetic peptides corresponding to α-lactalbumin and β-lactoglobulin sequences with angiotensin I-converting enzyme inhibitory activity. *Biol. Chem. Hoppe-Seyler* 1996; **377**: 259–260.
19. Sipola M, Finckenberg P, Vapaatalo H, Pihlanto-Leppälä A, Korhonen H, Korpela R, Nurminen M-L. α-Lactorphin and β-lactorphin improve arterial function in spontaneously hypertensive rats. *Life Sci.* 2002; **71**: 1245–1253.
20. Meisel H. Casokinins as bioactive peptides in the primary structure of casein. In *Food Protein – Structure and Functionality*, Schwenke KD, Mothes R (eds). VCH: Weinheim, New York, 1993; 67–75.
21. Rohrbach MS, Williams EB, Rolstad RA. Purification and substrate specificity of bovine angiotensin converting enzyme. *J. Biol. Chem.* 1981; **256**: 225–230.