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Antioxidant Peptides with Angiotensin Converting Enzyme Inhibitory Activities and Applications for Angiotensin Converting Enzyme Purification

WEN-CHI HOU,*,† HSIEN-JUNG CHEN,§ AND YAW-HUEI LIN*,#

Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei 110, Taiwan, Republic of China; Department of Horticulture, Chinese Culture University, Taipei 111, Taiwan; and Institute of Botany, Academia Sinica, Nankang, Taipei 115, Taiwan, Republic of China

Five commercial peptides, namely, reduced glutathione (GSH), oxidized glutathione (GSSG), carnosine, homocarnosine, and anserine, were used to test angiotensin converting enzyme inhibitory (ACEI) activities using *N*-[3-(2-furyl)acryloyl]-Phe-Gly-Gly (FAPGG) as a substrate. All of these peptides showed dose-dependent ACEI activities. Using 50% inhibition (IC_{50}) of captopril as 0.00781 μM for the reference, the IC_{50} values of GSH, carnosine, homocarnosine, and anserine were determined to be 32.4 μM, 5.216 mM, 6.147 mM, and 6.967 mM, respectively. GSH or carnosine showed mixed noncompetitive inhibition against ACE. When 0.0164 mM GSH or 0.4098 mM carnosine was added, the apparent inhibition constant (K_i) was 49.7 μM or 3.899 mM, respectively. Commercial glutathione-Sepharose 4 fast flow, GSH-coupled CNBr-activated and GSH-coupled EAH-activated Sepharose gels were used for ACE purification. Commercial ACE could be adsorbed only by EAH-coupled GSH gels and eluted off the gels by increasing salt concentrations. These EAH-coupled GSH gels might be developed as affinity aids for ACE purification.

KEYWORDS: Angiotensin converting enzyme (ACE); glutathione; *N*−[3-(2-furyl)acryloyl]-Phe-Gly-Gly (FAPGG); peptide; EAH-activated gel

INTRODUCTION

Several risk factors are associated with stroke, including age, gender, elevated cholesterol, smoking, alcohol, excessive weight, race, family history, and hypertension (*1*). Although some of these risk factors cannot be modified, one factor that can be controlled and has the greatest impact on the etiology of stroke is high blood pressure (*2*). Hypertension is considered to be the central factor in stroke, with ∼33% of deaths due to stroke attributed to untreated high blood pressure (*1*). There are several classes of pharmacological agents that have been used in the treatment of hypertension (*1*); one class of antihypertensive drugs known as angiotensin I converting enzyme (ACE) inhibitors (i.e., peptidase inhibitors) has a low incidence of adverse side effects and are the preferred class of antihypertensive agents for the treatment of patients with concurrent secondary diseases (*3*).

ACE (peptidyldipeptide hydrolyase EC 3.4.15.1) is a dipeptide-liberating exopeptidase, which has been classically associated with the renin−angiotensin system regulating peripheral blood pressure (*4*). ACE removes a dipeptide from the C terminus of angiotensin I to form angiotensin II, a very hypertensive compound. Several endogenous peptides such as enkephalins, β-endorphin, and substance P were reported to be competitive substrates and inhibitors of ACE (*4*). Several food-derived peptides can inhibit ACE (*5*), which include α-lactalbumin and β-lactoglobulin (*6*−*8*), casein (*9*−*11*), zein (*12*, *13*), gelatin (*14*), and yam dioscorin (*15*), all of which were hydrolyzed by pepsin, trypsin, or chymotrypsin.

Reduced glutathione (GSH) is a tripeptide that plays many roles in protective mechanisms and critical physiological functions in cells (*16*−*18*). GSH is widely distributed in cells including in the cytosol (1−10 mM; *16*, *19*), mitochondria (5−11 mM; *19*), nucleus (1−10 mM; *19*), and extracellular compartments (10−800 μM; *19*). Carnosine is a dipeptide (β-alanyl-L-histidine) that is often found in long-lived mammalian tissues at relatively high concentrations (up to 20 mM; *20*). Carnosine has antioxidant activities (*21*) and can delay aging in cultured cells (*22*). Some carnosine-related aminoacyl-histidine dipeptides, such as homocarnosine (γ-aminobutyric-histidine) and anserine (β-alanyl-1-methyl-histidine), were also found in the mammalian nerve system in high amounts (*23*). In this work we used five peptides, namely, GSH, oxidized glutathione (GSSG), carnosine, homocarnosine, and anserine to test ACE inhibitory activities using *N*-[3-(2-furyl)acryloyl]-Phe-Gly-Gly (FAPGG) as a substrate and captopril as a positive

* Address correspondence to Prof. Hou at the Graduate Institute of Pharmacognosy, Taipei Medical University, No. 250, Wu-Hsing St., Taipei 110, Taiwan [fax 886 (2) 2378-0134; e-mail wchou@tmu.edu.tw] or to Prof. Lin at the Institute of Botany, Academia Sinica, Nankang, Taipei 115, Taiwan, ROC [fax 886(2) 2782-7954; e-mail boyhlin@ccvax.sinica.edu.tw].
† Taipei Medical University.
§ Chinese Culture University.
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control. K_i values of GSH and carnosine against ACE were also calculated. We also reported that commercial ACE could be adsorbed by EAH-coupled GSH gels and eluted by increasing salt concentrations. Because of the high cost of commercial ACE (such as rabbit lung and porcine kidney sources), the use of natural peptide inhibitors (e.g., GSH) to prepare affinity aids for the purification of ACE from different sources and then to search for inhibitors from plant sources seems reasonable and highly wanted.

MATERIALS AND METHODS

Materials. Captopril was purchased from Calbiochem Co. (La Jolla, CA); CNBr-activated Sepharose 4B, EAH-activated Sepharose 4B, and glutathione-Sepharose 4 fast flow were purchased from Pharmacia Biotech AB (Uppsala, Sweden). FAPGG, ACE (I unit, rabbit lung), GSH, GSSG, carnosine, anserine, Coomassie brilliant blue R-250, *N*-hydroxysuccinimide, and *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals and reagents were from Sigma Chemical Co.

Determination of ACE Inhibitory Activity by Spectrophotometry. The ACE inhibitory activity was measured according to the method of Holmquist et al. (*24*) with some modifications. Twenty microliters (20 μU) of commercial ACE (1 U/mL, rabbit lung, Sigma Chemical Co.) was mixed with 200 μL of different amounts of peptides [dissolved in 50 mM Tris-HCl buffer (pH 7.5) containing 0.3 M NaCl, for GSH, 0.0041−0.0656 mM; for aminoacyl-histidine dipeptides, 0.2049−8.196 mM; for GSSG, 0.2049−1.639 mM], and then 1 mL of 5×10^{-4} M FAPGG [dissolved in 50 mM Tris-HCl buffer (pH 7.5) containing 0.3 M NaCl] was added. The decreased absorbance at 345 nm ($\rho A_{\text{inhibitor}}$) was recorded during 5 min at room temperature. Deionized water was used instead of sample solution for blank experiments (ρA_{blank}). Captopril (MW = 217.3 Da) was used as a positive control for ACE inhibitor at 0.00189, 0.00377, 0.00566, 0.00754, and 0.0188 μM. The ACE activity was expressed as $\rho A_{345\text{nm}}$ and the ACE inhibition (percent) was calculated as follows: $[1 - (\rho A_{\text{inhibitor}} \div \rho A_{\text{control}})] \times 100\%$. Means of triplicates were determined. The 50% inhibition (IC_{50}) of ACE activity was calculated as the concentrations of samples that inhibited 50% of ACE activity under these conditions.

Determination of the Kinetic Properties of ACE Inhibition by Antioxidant Peptides. The kinetic properties of ACE (20 μU) without or with GSH (0.0164 mM) or carnosine (0.4098 mM) were determined using different concentrations of FAPGG as substrate [(1−5 $\times$ 10^{-4} M]. The K_m (without antioxidant peptides) was calculated from Lineweaver−Burk plots, and the K_i (with GSH or carnosine) was calculated using the equation $K_i = [I]/(K_m'/K_m) - 1$, where [I] is the concentration of GSH or carnosine added and K_m' is the Michaelis constant in the presence of inhibitor at concentration [I].

GSH Was Coupled onto CNBr-Activated Sepharose 4B or EAH-Activated Sepharose 4B. GSH was coupled onto CNBr-activated Sepharose 4B or EAH-activated Sepharose 4B, and each was used as an affinity aid for ACE purifications. The coupling procedure was according to the manufacturer's guidelines. The brief coupling procedure of GSH onto CNBr-activated Sepharose 4B is described below. Powders of CNBr-activated Sepharose 4B (4 g) were activated with 2 mM HCl, 1000 mL for 15 min, and filtered, and then 300 mg of GSH in 100 mM $NaHCO_3$ buffer (adjusted to pH 8.3) was added and gently shaken at room temperature for 2 h. After filtration through a sintered glass filter (porosity G3), the coupled resins were blocked with 0.2 M glycine (pH 8.0) for another 2 h. For EAH-activated Sepharose 4B, *N*-hydroxysuccinimide was used to extend the spacer arms. Then 160 mg of GSH and 0.92 g of *N*-hydroxysuccinimide in 24 mL of distilled water were added into the gels, which were then washed successively with 800 mL of 0.5 M NaCl and 200 mL of distilled water while 0.92 g of *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride in 8 mL of distilled water was added drop by drop into the gels. The coupling reaction was performed at room temperature for 20 h with gentle shaking. During the first hour of the coupling reaction, the pH of coupling should be kept at 4.5−6.0. For affinity purifications, 1 unit of commercial ACE (1 mL) was loaded onto each affinity column (1.0 $\times$ 10 cm), including CNBr-activated GSH-Sepharose 4B gels, EAH-GSH Sepharose 4B gels, and commercial glutathione-Sepharose 4 fast flow. The column was first washed with 25 mM Tris-HCl buffer (pH 7.5) for 20 fractions (buffer A), then eluted with the same buffer containing 0.3 M NaCl (buffer B) for 20 fractions, and finally eluted with the same buffer containing 1.0 M NaCl (buffer C) for another 20 fractions. Flow rate was 40 mL/h, and each fraction contained 4 mL. Each fraction was used for ACE activity determinations and expressed as $\rho A_{345\text{nm}}/200$ μL.

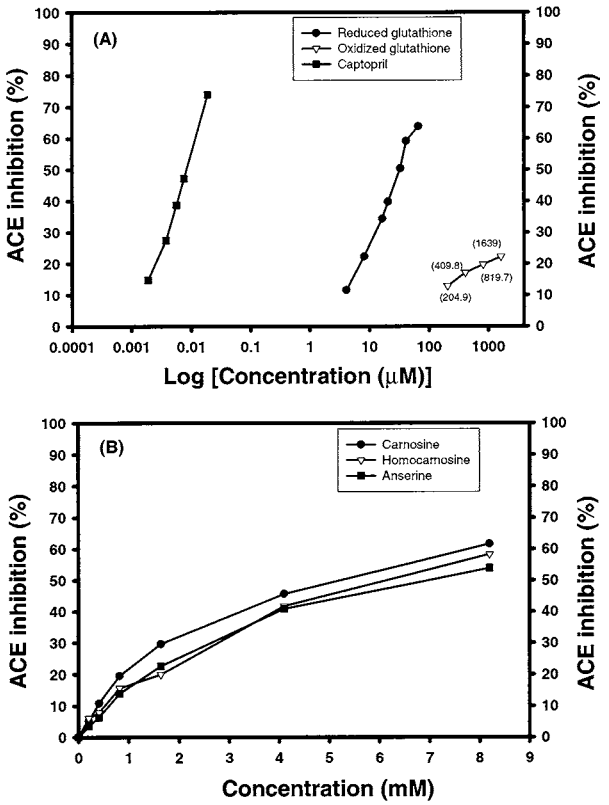

Figure 1. Effects of peptides (A) GSH, 0.0041–0.0656 mM, and GSSG, 0.2049, 0.4098, 0.8197, and 1.639 mM, and (B) aminoacyl-histidine dipeptides (carnosine, homocarnosine, and anserine), 0.2049–8.196 mM, and captopril (0.00189, 0.00377, 0.00566, 0.00754, and 0.0188 μM) on ACE activity by spectrophotometry. ACE inhibition (%) was calculated according to the equation $[1 - (\rho A_{\text{inhibitor}} \div \rho A_{\text{control}})] \times 100\%$.

RESULTS AND DISCUSSION

Determination of ACE Inhibitory Activity of Antioxidant Peptides by Spectrophotometry. Several food-derived peptides can inhibit ACE (*5*), including α-lactalbumin and β-lactoglobulin (*6*−*8*), casein (*9*−*11*), zein (*12*, *13*), gelatin (*14*), and yam dioscorin (*15*), all of which were hydrolyzed by proteases. Several food-derived peptides were also reported to have antihypertensive activity using spontaneously hypertensive rats (SHR) as model systems (*25*−*28*). However, no available data were found between the antioxidant peptides and antihypertensive activity. It was reasonable to postulate that some antioxidant peptides present in cells might have effects on ACE. GSH, GSSG, and aminoacyl-histidine related peptides (carnosine, homocarnosine, and anserine) with high amounts in cells (*19*, *20*, *23*) were chosen for ACEI activities. The results are shown in **Figure 1** using captopril as a positive control. It was found that all of these peptides showed ACEI activities in a dose-dependent manner in vitro. **Figure 1A** shows that the 50%

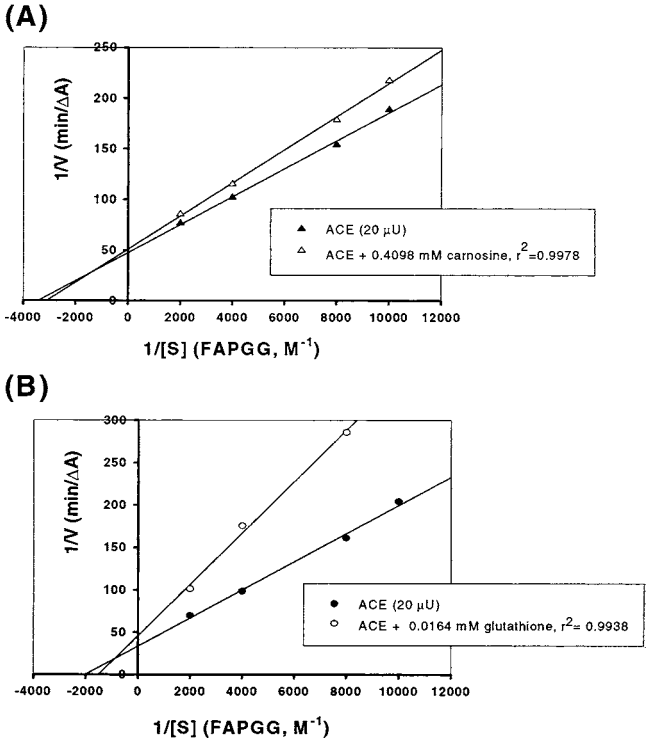

Figure 2. Lineweaver–Burk plots of ACE (20 μU) without or with (A) 0.4098 mM carnosine or (B) 0.0164 mM GSH in different concentrations of FAPGG [(1–5) $\times$ 10^{-4} M].

inhibition (IC_{50}) of GSH against ACE was 32.4 μM versus 0.00781 μM for captopril, which was similar to the value (0.007 μM) reported by Pihlanto-Leppälä et al. (*6*). The oxidized form of GSSG showed less ACEI activity and was about $^1/_{100}$ that of GSH at 20% ACE inhibitory activity (**Figure 1A**). The IC_{50} values of carnosine, homocarnosine, and anserine against ACE were 5.216 mM, 6.147 mM, and 6.967 mM, respectively (**Figure 1B**), which were $^1/_{161}$, $^1/_{190}$, and $^1/_{215}$, respectively, that of GSH.

The IC_{50} of GSH was 32.4 μM, which was much lower than those of the synthetic peptides of β-lactorphin (YLLF, 171.8 μM), α-lactorphin (YGLF, 733.3 μM), and β-lactotensin (HIRL, 1153 μM) (*4*). Several identified peptide fragments (*7*) of α-lactalbumin hydrolysates (such as VGINYWLAHK, 327 μM, and WLAHK, 77 μM) and β-lactoglobulin hydrolysates (such as LAMA, 556 μM, and LDAQSAPLR, 635 μM) also exhibited much higher IC_{50} values than GSH. IVGRPR, isolated from bonito hydrolysates (*26*), has an IC_{50} of 300 μM and showed antihypertensive activity following intravenous and oral administration in SHR. Although GSH plays many roles in protective mechanisms and critical physiological functions in cells (*16*−*18*), its ACE inhibitory activity in vitro has never been reported before. The antihypertensive activity of GSH in the SHR model needs further investigations.

Determination of the Kinetic Properties of ACE Inhibition by GSH or Carnosine. Lineweaver−Burk plots of ACE (20 μU) without or with (A) 0.4098 mM carnosine or (B) 0.0164 mM GSH in different concentrations of FAPGG [(1−5 $\times$ 10^{-4} M] are shown in **Figure 2**. The results indicated that carnosine or GSH acted as a mixed noncompetitive inhibitor with respect to the substrate FAPGG. Without the antioxidant peptides, the calculated K_m was 2.92 $\times$ 10^{-4} M FAPGG for ACE, which was close to the result (3 $\times$ 10^{-4} M) of Holmquist et al. (*24*). In the presence of 0.4098 mM carnosine, the calculated K_m' was 3.53 $\times$ 10^{-4} M, and in the presence of 0.0164 mM GSH, the calculated K_m' was 6.61 $\times$ 10^{-4} M. From the equation $K_i = [I]/(K_m'/K_m) - 1$, the K_i values were 3.899 mM and 49.7 μM FAPGG, respectively, for carnosine and GSH.

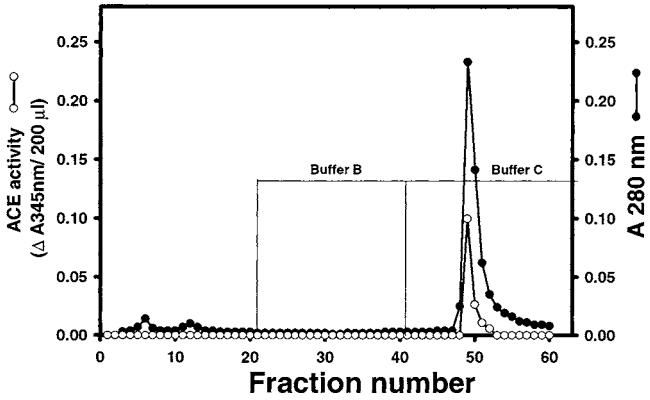

Figure 3. Chromatograms of ACE activity on EAH-GSH column (1 $\times$ 10 cm). The column was first washed with 25 mM Tris-HCl buffer (pH 7.5) for 20 fractions (buffer A), then eluted with the same buffer containing 0.3 M NaCl (buffer B) for 20 fractions, and finally eluted with the same buffer containing 1.0 M NaCl (buffer C) for another 20 fractions. Flow rate was 40 mL/h, and each fraction contained 4 mL. Each fraction was used for ACE activity determinations and expressed as ρA_{345nm}/200 μL.

Chromatograms of ACE Activity on EAH-GSH Column. The use of affinity aids, such as *N*-[1(*S*)-carboxy-5-aminopentyl]-phenylalanylglycine-coupled agarose (*29*), lisinopril-Sepharose (*30*, *31*), and captopril-Sepharose (*32*−*34*), was reported for ACE purification. All of the ligands used for ACE purification were shown to have ACEI activities. Therefore, the use of GSH as an affinity aid for ACE purification was tested. First, the commercial glutathione-Sepharose 4 fast flow (epoxy-activated) gel was chosen for commercial ACE purifications. However, the ACE did not bind this gel and appeared in flow through (data not shown). Second, the GSH-coupled CNBr-activated Sepharose 6B was prepared according to the manufacturer's guidelines. However, ACE did not bind this gel either and also appeared in flowthrough (data not shown). Third, the GSH-coupled EAH-activated Sepharose 6B was prepared according to the manufacturer's guidelines, and *N*-hydroxysuccinimide was used as spacer arms. The chromatogram is shown in **Figure 3**. After washings with 25 mM Tris-HCl buffer (pH 7.5) and the same buffer containing 0.3 M NaCl (buffer B), all of the ACE activities were eluted with 25 mM Tris-HCl buffer (pH 7.5) containing 1.0 M NaCl (buffer C). Epoxy-activated gel was coupled to groups containing $-NH_2$, $-OH$, or $-SH$; CNBr-activated gel was coupled to groups containing $-NH_2$. The EAH-activated gel was coupled to groups containing $-COOH$. The extended *N*-hydroxysuccinimide EAH gel was coupled to groups containing $-NH_2$. Reasoning from present results, the ability of EAH-coupled GSH gels to adsorb commercial ACE may be due to the SH group in GSH. However, the free amino group in GSH and steric factor may be also involved in ACE binding. This EAH-coupled GSH gel might be developed as an affinity aid for ACE purification.

In conclusion, cells are known to contain high concentrations of antioxidant peptides such as GSH, carnosine, and its related peptides. In addition to their well-known antioxidant activities, these peptides also have ACE inhibitory activities in vitro. The antihypertensive activity of GSH in SHR model systems needs further investigations. This EAH-coupled GSH gel might be developed as an affinity aid for ACE purification.

LITERATURE CITED

(1) Mark, K. S.; Davis, T. P. Stroke: development, prevention and treatment with peptidase inhibitors. *Peptides* **2000**, *21*, 1965−1973.

(2) Dunbabin, D. Cost-effective intervention in stroke. *Pharmacoeconomics* **1992**, *2*, 468−499.

(3) Fotherby, M. D.; Panayiotou, B. Antihypertensive therapy in the prevention of stroke: what, when, and for whom? *Drugs* **1999**, *58*, 663−674.

(4) Mullally, M. M.; Meisel, H.; FitzGerald, R. J. Synthetic peptides corresponding to α-lactalbumin and β-lactoglobulin sequences with angiotensin-I-converting enzyme inhibitory activity. *Biol. Chem.* **1996**, *377*, 259−260.

(5) Ariyoshi, Y. Angiotensin-converting enzyme inhibitors derived from food proteins. *Trends Food Sci. Technol.* **1993**, *4*, 139−144.

(6) Pihlanto-Leppälä, A.; Rokka, T.; Korhonen, H. Angiotensin I converting enzyme inhibitory peptides derived from bovine milk proteins. *Int. Dairy J.* **1998**, *8*, 325−331.

(7) Pihlanto-Leppälä, A.; Koskinen, P.; Piilola, K.; Tupasela, T.; Korhonen, H. Angiotensin I-converting enzyme inhibitory properties of whey protein digest: concentration and characterization of active peptides. *J. Dairy Res.* **2000**, *67*, 53−64.

(8) Pihlanto-Leppälä, A. Bioactive peptides derived from bovine whey proteins: opioid and ace-inhibitory peptides. *Trends Food Sci. Technol.* **2001**, *11*, 347−356.

(9) Maruyama, S.; Mitachi, H.; Awaya, J.; Kurono, M.; Tomizuka, N.; Suzuki, H. Angiotensin I-converting enzyme inhibitory activity of the C-terminal hexapeptide of α_{s1}-casein. *Agric. Biol. Chem.* **1987**, *51*, 2557−2561.

(10) Kohmura, M.; Nio, N.; Kubo, K.; Minoshima, Y.; Munekata, E.; Ariyoshi, Y. Inhibition of angiotensin-converting enzyme by synthetic peptides of human β-casein. *Agric. Biol. Chem.* **1989**, *53*, 2107−2114.

(11) Maeno, M.; Yamamoto, N.; Takano, T. Identification of an antihypertensivepeptide from casein hydrolysate produced by a proteinase from *Lactobacillus helveticus* CP790. *J. Dairy Sci.* **1996**, *79*, 1316−1321.

(12) Miyoshi, S.; Ishikawa, H.; Kaneko, T.; Fukui, F.; Tanaka, H.; Maruyama, S. Structures and activity of angiotensin-converting enzyme inhibitors in an α-zein hydrolysate. *Agric. Biol. Chem.* **1991**, *55*, 1313−1318.

(13) Yano, S.; Suzuki, K.; Funatsu, G. Isolation from α-zein of thermolysin peptides with angiotensin I-converting enzyme inhibitory activity. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 661−663.

(14) Chen, T. L.; Ken, K. S.; Chang, H. M. Study on the preparation of angiotensin-converting enzyme inhibitors (ACEI) from hydrolysates of gelatin. *J. Chin. Agric. Chem. Soc.* **1999**, *37*, 546−553.

(15) Hsu, F. L.; Lin, Y. H.; Lee, M. H.; Lin, C. L.; Hou, W. C. Both dioscorin, the tuber storage protein of yam (*Dioscorea alata* cv. Tainong No. 1), and its peptic hydrolysates exhibited angiotensin converting enzyme inhibitory activities. *J. Agric. Food Chem.* **2002**, *50*, 6109−6113.

(16) Smith, C. V.; Jones, D. P.; Guenthner, T. M.; Lash, L. H.; Lauterburg, B. H. Compartmentation of glutathione: implications for the study of toxicity and disease. *Toxicol. Appl. Pharmacol.* **1996**, *140*, 1−12.

(17) Noctor, G.; Foyer, C. H. Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1998**, *49*, 247−279.

(18) Sies, H. Glutathione and its role in cellular functions. *Free Radical Biol. Med.* **1999**, *27*, 916−921.

(19) Schafer, F. Q.; Buettner, G. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radical Biol. Med.* **2001**, *30*, 1191−1212.

(20) Brownson, C.; Hipkiss, A. R. Carnosine reacts with a glycated protein. *Free Radical Biol. Med.* **2000**, *28*, 1564−1570.

(21) Kohen, R.; Yamamoto, Y.; Cundy, K. C.; Ames, B. N. Antioxidant activity of carnosine, homocarnosine and anserine present in muscle and brain. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *95*, 2175−2179.

(22) McFarland, G. A.; Holliday, R. Retardation of the senescence of cultured human diploid fibroblasts by carnosine. *Exp. Cell Res.* **1994**, *212*, 167−175.

(23) Peretto, P.; Luzzati, F.; Bonfanti, L.; Marchis, S. D.; Fasolo, A. Aminoacyl-histidine dipeptides in the glial cells of the adult rabbit forebrain. *Peptides* **2000**, *21*, 1717−1724.

(24) Holmquist, B.; Bunning, P.; Riordan, J. F. A continuous spectrophotometric assay for angiotensin converting enzyme. *Anal. Biochem.* **1979**, *95*, 540−548.

(25) Matsui, T.; Li, C. H.; Tanaka, T.; Maki, T.; Osajima, Y.; Matsumoto, K. Depressor effect of wheat germ hydrolysate and its novel angiotensin I-converting enzyme inhibitory peptide, Ile-Val-Tyr, and the metabolism in rat and human plasma. *Biol. Pharm. Bull.* **2000**, *23*, 427−431.

(26) Fujita, H.; Yokoyama, K.; Yoshikawa, M. Classification and antihypertensive activity of angiotensin I-converting enzyme inhibitory peptides derived from food proteins. *J. Food Sci.* **2000**, *65*, 564−569.

(27) Yoshii, H.; Tachi, N.; Ohba, R.; Sakamura, O.; Takeyama, H.; Itani, T. Antihypertensive effect of ACE inhibitory oligopeptides from chicken egg yolks. *Comp. Biochem. Physiol. C* **2001**, *128*, 27−33.

(28) Matsui, T.; Tamaya, K.; Seki, E.; Osajima, K.; Matsumoto, K.; Kawasaki, T. Absorption of Val-Tyr with in vitro angiotensin I-converting enzyme inhibitory activity into the circulating blood system of mild hypertensive subjects. *Biol. Pharm. Bull.* **2002**, *25*, 1228−1230.

(29) Pantoliano, M. W.; Holmquist, B.; Riordan, J. F. Affinity chromatographic purification of angiotensin converting enzyme. *Biochemistry* **1984**, *23*, 1037−1042.

(30) Bull, H. G.; Thornberry, N. A.; Cordes, E. H. Purification of angiotensin-converting enzyme from rabbit lung and human plasma by affinity chromatography. *J. Biol. Chem.* **1985**, *260*, 2963−2972.

(31) Lanzillo, J. J.; Stevens, J.; Dasarathy, Y.; Yotsumoto, H.; Fanburg, B. L. Angiotensin-converting enzyme from human tissues. Physicochemical, catalytic, and immunological properties. *J. Biol. Chem.* **1985**, *260*, 14938−14944.

(32) Taira, H.; Mizutani, S.; Narita, O.; Tomoda, Y. Angiotensin I-converting enzyme in human placenta. *Placenta* **1985**, *6*, 543−549.

(33) Ryan, J. W. Angiotensin-converting enzyme, dipeptidyl carboxypeptidase I, and its inhibitors. *Methods Enzymol.* **1988**, *163*, 194−209.

(34) Polanco, M. J.; Agapito, M. T.; Recio, J. M. Inhibition and affinity chromatography of chicken lung angiotensin I-converting enzyme with captopril. *Comp. Biochem. Physiol. B: Comp. Biochem.* **1992**, *102*, 527−533.

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