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Both Dioscorin, the Tuber Storage Protein of Yam (*Dioscorea alata* cv. Tainong No. 1), and Its Peptic Hydrolysates Exhibited Angiotensin Converting Enzyme Inhibitory Activities

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Dioscorin, the tuber storage protein of yam (*Dioscorea alata* cv. Tainong No. 1), was purified to homogeneity by DE-52 ion-exchange chromatography. This purified dioscorin was shown by spectrophotometric methods to inhibit angiotensin converting enzyme (ACE) in a dose-dependent manner (12.5−750 μg, respectively, 20.83−62.5% inhibitions) using *N*-[3-(2-*f*uryl)*a*cryloyl]-*P*he-*G*ly-*G*ly (FAPGG) as substrates. The 50% inhibition (IC_{50}) of ACE activity was 6.404 μM dioscorin (250 μg corresponding to 7.81 nmol) compared to that of 0.00781 μM (0.0095 nmol) for captopril. The commercial bovine serum albumin and casein (bovine milk) showed less ACE inhibitory activity. The use of qualitative TLC also showed dioscorin as ACE inhibitors. Dioscorin showed mixed noncompetitive inhibitions against ACE; when 31.25 μg of dioscorin (0.8 μM) was added, the apparent inhibition constant (K_i) was 2.738 μM. Pepsin was used for dioscorin hydrolysis at 37 °C for different times. It was found that the ACE inhibitory activity was increased from 51.32% to about 75% during 32 h hydrolysis. The smaller peptides were increased with increasing pepsin hydrolytic times. Dioscorin and its hydrolysates might be a potential for hypertension control when people consume yam tuber.

KEYWORDS: Angiotensin converting enzyme (ACE); dioscorin; *N*-[3-(2-furyl)acryloyl]-Phe-Gly-Gly (FAPGG); mixed-type inhibition; pepsin; yam

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 etiology of stroke is high blood pressure (*2*). Hypertension is considered to be the central factor in stroke with approximately 33% of deaths due to stroke attributed to untreated high blood pressure (*1*). There are several classes of pharmacological agents which have been used in the treatment of hypertension (*1*); one class of antihypertensive drugs known as angiotensin I converting enzyme (ACE) inhibitors (i.e., a peptidase inhibitor) is associated with a low rate of adverse side effects and is the preferred class of antihypertensive agents when treating 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, which removes a dipeptide from the C-terminus of angiotensin I to form angiotensin II, is 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 inhibited ACE (*5*), which were hydrolyzed by pepsin, trypsin, or chymotrypsin, including α-lactalbumin and β-lactoglobulin (*4*, *6*−*8*), casein (*9*−*11*), zein (*12*, *13*), and gelatin (*14*, *15*). Fermented milk was also reported to exhibit ACE inhibitory activity (*16*).

Dioscorin, the storage protein of yam tuber, accounted for about 90% of extractable water-soluble proteins from different yam species (*Dioscorea batatas*, *Dioscorea alata*, *Dioscorea pseudojaponica*) estimated by the immunostaining method (*17*), and all of them exhibited carbonic anhydrase and trypsin inhibitor activities (*17*, *18*). We also proved that dioscorin exhibited both dehydroascorbate reductase and monodehydroascorbate reductase activities and might respond to environmental stresses (*19*). In our recent report, the 32 kDa dioscorin from yam (*Dioscorea batatas* Decne) exhibited

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antioxidant activities against different radicals (*20*). The dried slices of yam tuber were frequently used as Chinese medicinal plants, and the fresh tuber was also a staple food in West Africa, Southern Asia, and the Caribbean. In this work we report for the first time that 32 kDa dioscorin, the major storage protein of yam (*D. alata* cv. Tainong No. 1) tuber, exhibited dose-dependent ACE inhibitory activities when captopril was used as a positive control. Commercial proteins of bovine serum albumin (BSA), casein (bovine milk), and gelatin (bovine skin) were chosen for comparisons, which were frequently found in the literature as the peptide resources of ACE inhibitors. The K_i values of dioscorin against ACE were also calculated. We also used pepsin for dioscorin hydrolysis at different times, and the changes of ACE inhibitory activities were determined.

MATERIALS AND METHODS

Materials. Tris, gelatin (bovine skin), electrophoretic reagents, and silica gel 60 F_{254} were purchased from E. Merck Inc. (Darmstadt, Germany). Captopril was purchased from Calbiochem (CA). Sephadex G-50 (F) was purchased from Pharmacia Biotech AB (Uppsala, Sweden). Seeblue prestained markers for SDS−PAGE including myosin (250 kDa), BSA (98 kDa), glutamic dehydrogenase (64 kDa), alcohol dehydrogenase (50 kDa), carbonic anhydrase (36 kDa), myoglobin (30 kDa), and lysozyme (16 kDa) were from Invitrogen (Groningen, The Netherlands). Pepsin (3460 units/mg of solid, P-6887), FAPGG, ACE (1 unit, rabbit lung), casein (bovine milk), BSA (A-2153, fraction V), and Coomassie brilliant blue R-250 were purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals and reagents were from Sigma Chemical Co. (St. Louis, MO).

Dioscorin Purified from Yam Tuber. Fresh yam (*D. alata* cv. Tainong No. 1) tubers were purchased from a wholesaler for immediate dioscorin extraction. After being washed and peeled, the tubers were cut into strips for dioscorin extraction and purification according to the method of Hou et al. (*17*−*20*). Samples were homogenized with 4 volumes (w/v) of 50 mM Tris-HCl buffer (pH 8.3). After centrifugation at 12500*g* for 30 min, the supernatants were saved and loaded directly onto a DE-52 ion-exchange column. After being washed with 3 column volumes of 50 mM Tris-HCl buffer (pH 8.3), the adsorbed dioscorins were eluted batchwise with the same washing buffer containing 150 mM NaCl. The eluted fraction was collected and concentrated with Ultrafree-4 (molecular mass cutoff is 5 kDa; Millipore Co., Bedford, MA). The concentrated dioscorin solution was dialyzed against deionized water overnight and lyophilized for further use.

Protein Staining on SDS−PAGE Gels. The 80 μL samples were mixed with 20 μL of sample buffer, namely, 60 mM Tris-HCl buffer (pH 6.8) containing 2% SDS, 25% glycerol, and 0.1% bromophenol blue with or without 2-mercaptoethanol, heated at boiling water temperature for 5 min, and cooled to ambient temperature, followed by electrophoresis according to the method of Laemmli (*21*). Coomassie brilliant blue R-250 was used for protein staining (*22*).

Determination of ACE Inhibitory Activity by Spectrophotometry. The ACE inhibitory activity was measured according to the method of Holmquist et al. (*23*) with some modifications. Twenty microliters (20 microunits) of commercial ACE (1 unit/mL, rabbit lung; Sigma Chemical Co.) was mixed with 200 μL of different amounts of dioscorin (12.5, 31.25, 125, 250, 375, and 750 μg corresponding to 0.32, 0.8, 3.202, 6.404, 9.61, and 19.21 μM, respectively), commercial casein (125, 250, and 375 μg corresponding to 4.34, 8.68, and 13.02 μM, respectively), and BSA (125, 250, 375, and 750 μg corresponding to 1.55, 3.10, 4.66, and 9.31 μM, respectively), and then 1 mL of 5×10^{-4} M *N*-[3-(2-*f*uryl)*a*cryloyl]-*P*he-*G*ly-*G*ly [FAPGG, dissolved in 50 mM Tris-HCl buffer (pH 7.5) containing 0.3 M NaCl] was added. The decreased absorbance at 345 nm ($\Delta A_{inhibitor}$) was recorded during 5 min at room temperature. Deionized water was used instead of sample solution for blank experiments (ΔA_{blank}). Captopril (molecular mass 217.3 Da) was used as a positive control for ACE inhibitors (0.00075, 0.00189, 0.00377, 0.00566, 0.00754, 0.0188, and 0.0754 μM). The ACE activity was expressed as ΔA 345 nm, and the ACE inhibition (percent) was calculated as follows: $[1 - (\Delta A_{inhibitor} \div \Delta A_{control})] \times 100$. 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 ACE Inhibitory Activity by TLC. The ACE inhibitory activity of dioscorin was determined by the TLC method (*23*). The reactions between dioscorins and ACE or BSA and ACE were according to the methods of Anzenbacherova et al. (*24*) with some modifications. Each 100 μL of dioscorin and BSA (250 μg) was premixed with 15 microunits ACE for 1 min, and then 200 μL of 5×10^{-4} M FAPGG was added and allowed to react at room temperature for 10 min. Then 800 μL of methanol was added to stop the reaction. The blank experiment contained FAPGG only; in the control experiment, ACE reacted with FAPGG under the same conditions. Each was dried under reduced pressure and redissolved with 400 μL of methanol, and 50 μL was spotted on a silica gel 60 F_{254} by the CAMAG Linomat IV spray-on technique (CAMAG, Switzerland). The FAPGG and FAP (ACE hydrolyzed products) were separated by TLC in butanol−acetic acid−water, 4:1:1 (v/v/v), and observed under UV light (*19*).

Determination of the Kinetic Properties of ACE Inhibition by Dioscorin. The kinetic properties of ACE (20 microunits) without or with dioscorin (31.25 μg corresponding to 0.8 μM) were determined using different concentrations of FAPGG as substrate ($1 \times 10^{-3} - 1 \times 10^{-4}$ M). The K_m (without dioscorin) was calculated from Lineweaver−Burk plots, and the K_i (with dioscorin) was calculated using the equation $K_i = [I]/(K_m'/K_m) - 1$, where [I] is the concentration of dioscorin added and K_m' is the Michaelis constant in the presence of inhibitor at concentration [I].

Determination of the ACE Inhibitory Activity of Dioscorin Hydrolysates by Pepsin. The 7 mg of dioscorin was dissolved in 1 mL of 0.1 M KCl buffer (pH 2.0). Then 0.1 mL of 14 mg of pepsin was added for hydrolysis at 37 °C for 8, 12, 24, and 32 h. After hydrolysis, 0.5 mL of 0.5 M Tris-HCl buffer (pH 8.3) was added, and the solution was heated at 100 °C for 5 min to stop hydrolysis. The pepsin was heated before dioscorin hydrolysis for the 0 h reaction. Each of the 60 μL dioscorin hydrolysates (about 6.724 μM) was used for determinations of ACE inhibitions by spectrophotometry.

Chromatograms of Dioscorin Peptic Hydrolysates on a Sephadex G-50 Column. The unhydrolyzed dioscorin and peptic dioscorin hydrolysates of 8 and 32 h were lyophilized and separated by Sephadex G-50 chromatography (1 × 75 cm). The column was eluted with 20 mM Tris-HCl buffer (pH 7.9). The flow rate was 30 mL/h, and each fraction contained 2 mL. Each fraction was determined at the absorbance of 230 nm.

RESULTS AND DISCUSSION

Determination of ACE Inhibitory Activity of Dioscorin by Spectrophotometry. Dioscorin was purified from yam (*D. alata* cv. Tainong No. 1) tubers according to the method of Hou et al. (*17*−*20*). The inset of **Figure 1** shows protein stainings of purified dioscorin without (lane 1) and with (lane 2) 2-mercaptoethanol treatments on a 12.5% SDS−PAGE gel. Two bands (lane 1) without 2-mercaptoethanol treatments and a single band (lane 2) with a molecular mass of 32 kDa were found after 2-mercaptoethanol treatments which were the same as those of Hou et al. (*17*, *18*). The purified dioscorin was used for determinations of ACE inhibitory activities. **Figure 1** shows the effect of the different amounts of dioscorin (12.5, 31.25, 125, 250, 375, and 750 μg) on the ACE activity (ΔA 345 nm). Each lane (second regression) shows the positive correlation between ΔA 345 nm and reaction time. Compared with the ACE only (control), it was found that the higher the amount of dioscorin added, the lower the ΔA 345 nm found during 5 min reaction. This meant that dioscorin could inhibit ACE activity.

Effects of Dioscorin, BSA, Casein, and Captopril on ACE Activity by Spectrophotometry. From **Figure 1**, it was found that dioscorin exhibited ACE inhibitory activity. It was interesting to know if commercial proteins of BSA and casein (bovine milk) also exhibited the same ACE inhibitory activity. **Figure**

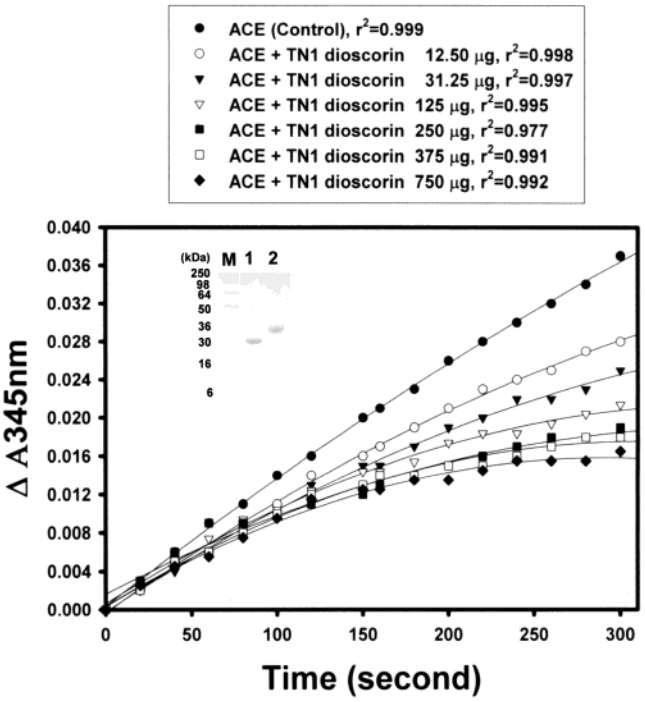

Figure 1. Different amounts of dioscorin (12.5, 31.25, 125, 250, 375, and 750 μg) on the ACE activity (ΔA 345 nm). Each of the second regressions was plotted between the ΔA 345 nm and reaction time. The inset shows protein stainings of purified dioscorin without (lane 1) and with (lane 2) 2-mercaptoethanol treatments on a 12.5% SDS–PAGE gel. M indicates the Seeblue prestained markers of SDS–PAGE. 5 μg of protein was loaded in each well.

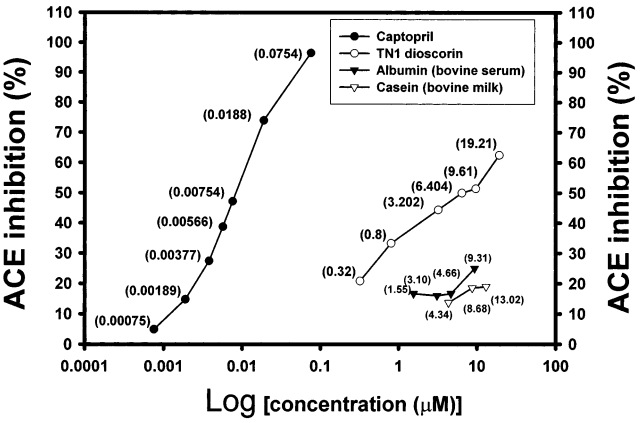

Figure 2. Effects of dioscorin (0.32, 0.8, 3.202, 6.404, 9.61, and 19.21 μM), casein (4.34, 8.68, and 13.02 μM), bovine serum albumin (1.55, 3.10, 4.66, and 9.31 μM), and captopril (0.00075, 0.00189, 0.00377, 0.00566, 0.00754, 0.0188, and 0.0754 μM) on ACE activity by spectrophotometry. The ACE inhibition (%) was calculated according to the equation $[1 - (\Delta A_{inhibitor} \div \Delta A_{control})] \times 100$.

2 shows the effect of dioscorin (0.32, 0.8, 3.202, 6.404, 9.61, 19.21 μM), casein (4.34, 8.68, 13.02 μM, respectively), bovine serum albumin (1.55, 3.10, 4.66, 9.31 μM), and captopril (0.00075, 0.00189, 0.00377, 0.00566, 0.00754, 0.0188, and 0.0754 μM) on ACE activity. It was found that BSA and casein (bovine milk) showed less ACE inhibitory activity (less than 20% inhibition) and without dose-dependent inhibition patterns. However, dioscorin exhibited dose-dependent ACE inhibitory activities (0.32−19.21 μM, respectively, 20.83−62.5% inhibitions). From calculations, the 50% inhibition (IC_{50}) of ACE activity was 6.404 μM dioscorin (7.81 nmol) compared to that

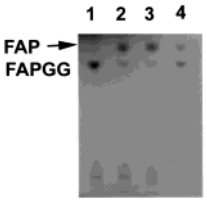

Figure 3. TLC chromatograms of a silica gel 60 F_{254} on the effects of 250 μg of BSA (lane 3) or dioscorin (lane 4) on 15 microunits of ACE. Lanes: 1, blank test (FAPGG only); 2, control test (ACE hydrolyzed FAPGG to produce FAP). Each solution was dried under reduced pressure and redissolved with 400 μL of methanol. Each 50 μL was spotted on a silica gel 60 F_{254} by the CAMAG Linomat IV spray-on technique (CAMAG, Switzerland). The FAPGG and FAP were separated by butanol–acetic acid–water, 4:1:1 (v/v/v).

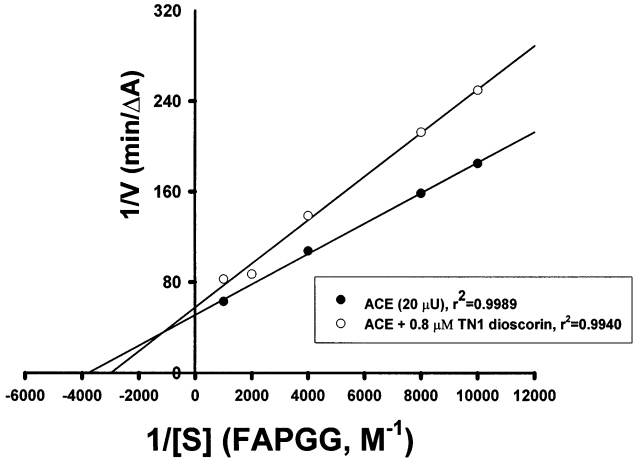

Figure 4. Lineweaver–Burk plots of ACE (20 microunits) without or with dioscorin (31.25 μg corresponding to 0.8 μM) in different concentrations of FAPGG (1×10^{-3} –1×10^{-4} M).

of 0.00781 μM (0.0095 nmol) for captopril, which was similar to the reports (0.007 μM) of Pihlanto-Leppälä et al. (*6*).

The IC_{50} of dioscorin was 6.404 μM, which was much less than that of the synthetic peptides 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 that of dioscorin. However, dioscorin showed IC_{50} values similar to those of the identified peptides of bovine skin gelatin hydrolysates (GPL, 2.55 μM, and GPV, 4.67 μM) (*15*) or ovalbumin peptic hydrolysates (FGRCVSP, 6.2 μM) (*25*).

Determinations of ACE Inhibitory Activity of Dioscorin by TLC. Figure 3 shows the qualitative results of TLC chromatograms of a silica gel 60 F_{254} on the effects of 250 μg of BSA (lane 3) or dioscorin (lane 4) on 15 microunits of ACE. Compared to the control test (lane 2), it was found that dioscorin (lane 4) inhibited ACE hydrolysis for less amounts of FAP productions under UV light observations. However, similar FAP productions were found between the control test (lane 2) and BSA (lane 3). This result demonstrated again that dioscorin exhibited ACE inhibitory activities.

Determination of the Kinetic Properties of ACE Inhibition by Dioscorin. Figure 4 shows the Lineweaver−Burk plots of ACE (20 microunits) without or with dioscorin (31.25 μg corresponding 0.8 μM) in different concentrations of FAPGG. The results indicated that dioscorin acted as mixed noncompeti-

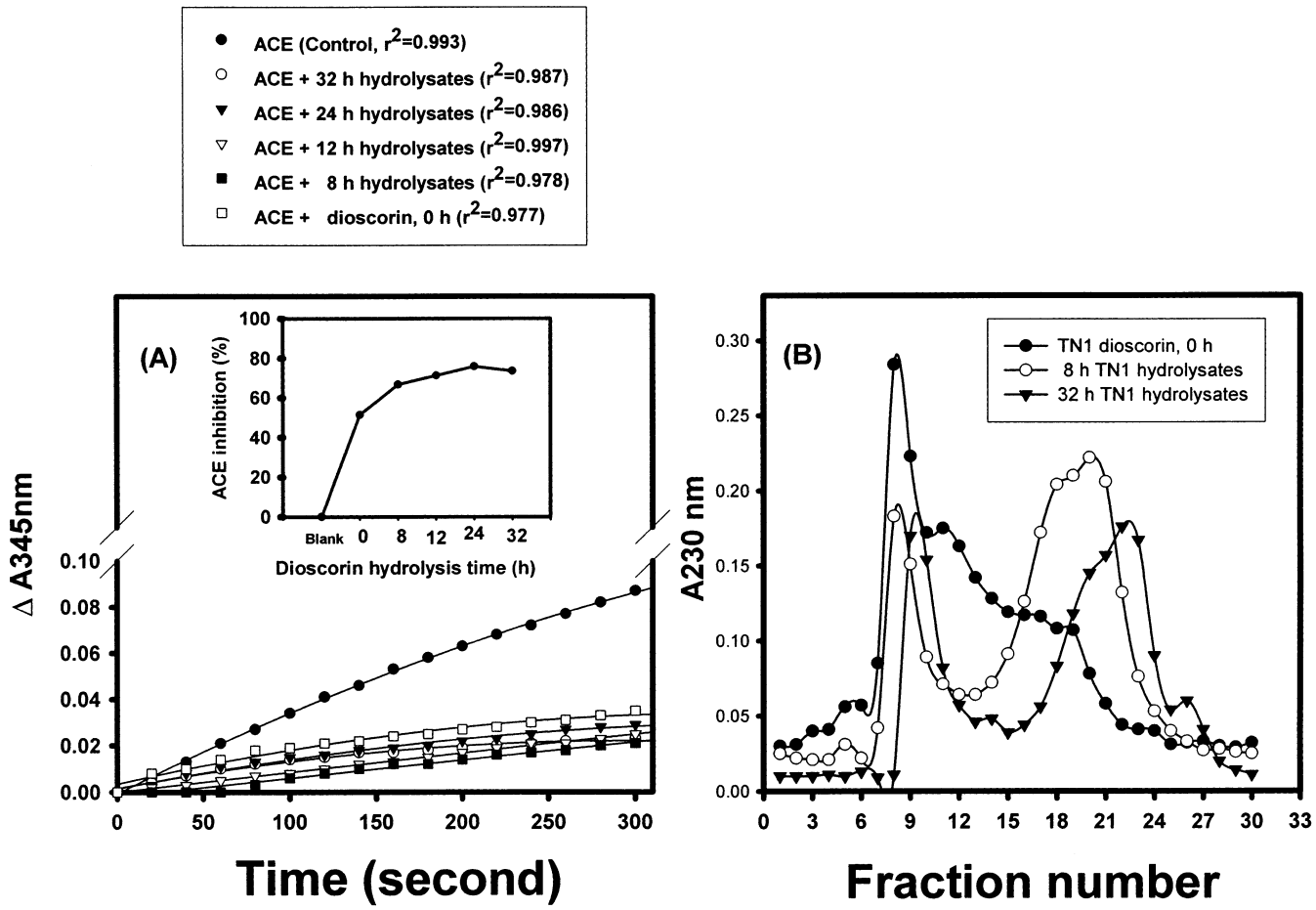

Figure 5. ACE inhibitory activity of dioscorin peptic hydrolysates. Each of the second regressions was plotted between the ΔA 345 nm and reaction time. The inset shows the ACE inhibition (%) of dioscorin hydrolysates at different pepsin hydrolysis time. The ACE inhibition (%) was calculated according to the equation $[1 - (\Delta A_{inhibitor} \div \Delta A_{control})] \times 100$.

tive inhibitors with respect to the substrates (FAPGG). Without dioscorin, the calculated K_m was 2.546×10^{-4} M FAPGG for ACE, which was closest to the result (3×10^{-4} M) of Holmquist et al. (*23*). In the presence of dioscorin (0.8 μM), the calculated K_m' was 3.29×10^{-4} M. From the equation $K_i = [I]/(K_m'/K_m) - 1$, the calculated K_i was 2.738 μM for FAPGG.

Determination of the ACE Inhibitory Activity of Dioscorin Hydrolysates by Pepsin and Their Peptide Distributions. The pepsin was frequently used for protein hydrolysis to purify the potency of ACE inhibitory peptides (*6*−*8*, *25*). Therefore, pepsin was chosen for dioscorin hydrolysis. **Figure 5** shows the ACE inhibitory activity (ΔA 345 nm) of dioscorin peptic hydrolysates. **Figure 5A** (inset) shows the ACE inhibition (percent) of dioscorin hydrolysates at different pepsin hydrolysis times. From the results of **Figure 5**, it was found that the ACE inhibitory activity was increased from 51.32% (0 h) to about 75% (32 h). **Figure 5B** shows the chromatograms of unhydrolyzed dioscorin and peptic dioscorin hydrolysates of 8 and 32 h on Sephadex G-50 chromatography. It was found that the smaller peptides were increased with increasing pepsin hydrolytic time. The purifications of potential peptides of ACE inhibitors needed further investigations.

In conclusion, the tuber storage protein of yam, dioscorin, exhibited dose-dependent ACE inhibitory activities. The IC_{50} of dioscorin was 6.404 μM and acted as mixed noncompetitive inhibitors toward ACE. Its peptic hydrolysates also showed ACE inhibitory activities. Some peptides derived from food proteins were demonstrated to have antihypertensive activities against spontaneously hypertensive rats (*25*, *26*). It might be a potential for hypertension control when people consume yam tuber and needs further investigations.

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 a_{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 antihypertensive peptide 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) Kim, S. K.; Byun, H. G.; Park, P. J.; Shahidi, F. Angiotensin I converting enzyme inhibitory peptides purified from bovine skin gelatin hydrolysate. *J. Agric. Food Chem.* **2001**, *49*, 2992−2997.

(16) Nakamura, Y.; Yamamoto, N.; Sakai, K.; Okubo, A.; Yamazaki, S.; Takano, T. Purification and characterization of angiotensin I-converting enzyme inhibitors from sour milk. *J. Dairy Sci.* **1995**, *78*, 777−783.

(17) Hou, W. C.; Chen, H. J.; Lin, Y. H. Dioscorins from different *Dioscorea* species all exhibit both carbonic anhydrase and trypsin inhibitor activities. *Bot. Bull. Acad. Sin.* **2000**, *41*, 191−196.

(18) Hou, W. C.; Liu, J. S.; Chen, H. J.; Chen, T. E.; Chang, C. F.; Lin, Y. H. Dioscorin, the major tuber storage protein of yam (*Dioscorea batatas* Decne), with carbonic anhydrase and trypsin inhibitor activities. *J. Agric. Food Chem*. **1999**, *47*, 2168−2172.

(19) Hou, W. C.; Chen, H. J.; Lin, Y. H. Dioscorin, the major tuber storage protein of yam (*Dioscorea batatas* Decne), with dehydroascorbate reductase and monodehydroascorbate reductase activities. *Plant Sci.* **1999**, *149*, 151−156.

(20) Hou, W. C.; Lee, M. H.; Chen, H. J.; Liang, W. L.; Han, C. H.; Liu, Y. W.; Lin, Y. H. Antioxidant activities of dioscorin, the storage protein of yam (*Dioscorea batatas* Decne). *J. Agric. Food Chem.* **2001**, *49*, 4956−4960.

(21) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.

(22) Neuhoff, V.; Stamm, R.; Eibl, H. Clear background and highly sensitive protein staining with Coomassie blue dyes in polyacrylamide gels: a systematic analysis. *Electrophoresis* **1985**, *6*, 427−448.

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

(24) Anzenbacherova, E.; Anzenbacher, P.; Macek, K.; Kvetina, J. Determination of enzyme (angiotensin convertase) inhibitors based on enzymatic reaction followed by HPLC. *J. Pharm. Biomed. Anal.* **2001**, *24*, 1151−1156.

(25) 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.

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

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