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Sweet potato storage root trypsin inhibitor and their peptic hydrolysates exhibited angiotensin converting enzyme inhibitory activity *in vitro*

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ABSTRACT. Trypsin inhibitor (TI), the root storage protein of sweet potato, was shown by spectrophotometric methods to inhibit angiotensin converting enzyme (ACE) in a dose-dependent manner (50-200 μg/mL, with 31.9-53.2% inhibition) using *N*-[3-(2-*f*uryl) acryloyl]-Phe-*G*ly-*G*ly (FAPGG) as a substrate. The 50% inhibition (IC_{50}) of ACE activity required 187.96 μg/mL TI compared to 10 nM (868 ng/mL) of Captopril. The use of TLC also showed TI as ACE inhibitor. TI acted as a mixed type inhibitor against ACE using FAPGG as a substrate. When 200 μg/mL TI were added, Vmax and Km were, respectively 0.013 ΔA/min and 0.715 mM while without TI they were 0.027 ΔA/min and 0.419 mM. Pepsin was used for TI hydrolysis for different times. ACE inhibitory activity was found to increase from 34% to about 83% after 24 h of hydrolysis. The results suggested that when small peptides increased by pepsin hydrolysis of the TI ACE inhibitory capacity also increased up to 24 h and then decreased, it may be due to the disappearance of some conformational requirements. Ten peptides—namely HDHM, LR, SNIP, VRL, TYCQ, GTEKC, RF, VKAGE, AH and KIEL—were synthesized based on the simulated pepsin digestion of trypsin inhibitor and then tested for ACE inhibitory activity. IC_{50} values of individual peptides were 276.2, 746.4, 228.3, 208.6, 2.3, 275.8, 392.2, 141.56, 523.5 and 849.7 μM, suggesting that TYCQ might represent the main active site for the ACE inhibition. TI and its hydrolysates might be good for control of hypertension and other diseases when people consume sweet potato tuberous roots.

Keywords: Angiotensin converting enzyme (ACE); Trypsin inhibitor; Pepsin; Sweet potato.

INTRODUCTION

Many bioactive peptides have common structural properties that include a relatively short peptide residue length (e.g. 2-9 amino acids), possessing hydrophobic amino acid residues in addition to proline, lysine or arginine groups. Bioactive peptides are among the many functional components identified in foods. These are small protein fragments that have biological effects once they are released during gastrointestinal digestion in the organism or by previous *in vitro* protein hydrolysis. Bioactive peptides with immunostimulating (Parker et al., 1984; Fiat et al., 1993), opioid (Zioudrou et al., 1979), antithrombotic (Scarborough, 1991), caseino-phosphopeptic (Maubois and Leonil, 1989), bactericidal (Bellamy et al., 1993), antioxidant or angiotensin-converting enzyme inhibitor (Ehlers and Riordan, 1989) functions have been the research focus in recent years.

ACE (peptidyldipeptide hydrolyase EC 3.4.15.1) is a glycoprotein and a dipeptide-liberating exopeptidase classically associated with the renin-angiotensin system regulating peripheral blood pressure (Mullally et al., 1996). 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. Several food-derived peptides from α-lactoalbumin, β-lactoglobulin (Pihlanto-Leppälä et al., 1998), casein (Maruyama et al., 1987), zein, mucilage (Huang et al., 2006), and azein (Yano et al., 1996) also inhibited ACE. Several antioxidant peptides (reduced glutathione and carnosine-related peptides) (Hou et al., 2003) and synthetic peptides also exhibited ACE inhibitor activities (Chen et al., 2003).

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Protease inhibitors in plants may be important in regulating and controlling endogenous proteases and in acting as protective agents against insect and/or microbial proteases (Ryan, 1973; Ryan, 1989). Sohonie and Bhandarker (Sohonie and Bhandarker, 1954) reported for the first time the presence of trypsin inhibitor (TI) in sweet potato (SP). Later, it was indicated that TI activities in SP are positively correlated with concentrations of water-soluble protein (Lin and Chen, 1980), and that they increase in response to drought (Lin, 1989). Polyamines, including cadaverine, spermidine and spermine, were bound covalently to SPTI, which might participate in regulating the growth and developmental processes of SP (Hou and Lin, 1997). TIs in SP storage roots account for about 60% of total water-soluble proteins and could be recognized as storage proteins (Lin, 1989). Matsuoka et al. (Matsuoka et al., 1990) identified sporamin as the major storage protein in SP root, accounting for 80% of the total proteins there. A dramatic decrease of the amount of sporamin to 2% of the original value was found during sprouting. Lin (Lin, 1993; Huang et al., 2007) considered sporamin as one form of TIs in SP, a finding confirmed later by Yeh et al. (1997).

In our previous report, TI exhibited both dehydroascorbate reductase and monodehydroascorbate reductase activities, and 33 kDa TI exhibited antioxidant activities against different radicals (Hou et al., 2001; Huang et al., 2007). In this work we report for the first time that TI exhibited dose-dependent ACE inhibitory activity when Captopril was used as a positive control. Commercial bovine serum albumin (BSA), which was frequently found in the literature as the peptide resource of ACE inhibitors, was chosen for comparison. The K_i values of trypsin inhibitor against ACE were calculated. We also used pepsin to hydrolyze TI for different times, and the changes of ACE inhibitory activity were determined. The IC_{50} of ACE inhibitory activities by synthetic peptides was also determined.

MATERIALS AND METHODS

Materials

Tris, electrophoretic reagents, and silica gel 60 F254 were purchased from E. Merck Inc. (Darmstadt, Germany); Captopril was purchased from Calbiochem Co. (CA, USA); 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); FAPGG, ACE (1 unit, rabbit lung); coomassie brilliant blue G-250; peptide (GL Biochem, China), and other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Plant materials

Fresh storage roots of sweet potato (*Ipomoea batatas* (L.) Lam. 'Tainong 57') were purchased from a local market.

Purification of sweet potato trypsin inhibitor

Sweet potato storage roots were washed and peeled, and then were cut into strips and extracted with distilled water. The crude extracts were loaded directly onto a trypsin Sepharose-4B affinity column. The adsorbed TI was eluted by pH changes with 0.2 M KCl (pH 2.0) according to Huang et al. (2005).

Protein staining and activity staining of trypsin inhibitor on 15% denaturing polyacrylamide gels

Samples were mixed with 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. Coomassie brilliant blue G-250 was used for protein staining (Huang et al., 2004). For sweet potato TI activity staining, the gel was stained according to the method of Hou et al. (2002).

Determination of ACE inhibitory activity by spectrophotometry

The ACE inhibitory activity was measured according to the method of Holmquist et al. (1979) with some modifications. Four microliters (4 microunits) of commercial ACE (1 unit, rabbit lung) was mixed with 50 μL of different amounts of trypsin inhibitor or BSA (50, 100, and 200 μg/mL), and then 200 μL of 0.5 mM *N*-[3-(2-*f*uryl) *acryloyl*]-*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 (ΔA inhibitor) was recorded during 5 min at room temperature. Deionized water was used instead of sample solution for blank experiments (ΔA control). Captopril (molecular mass 217.3 Da) was used as a positive control for ACE inhibitor (1.25, 2.5, 5, 10, 20, 40, and 80 nM). The ACE activity was expressed as ΔA 345 nm, and the ACE percent inhibition was calculated as follows: [1 - (ΔA inhibitor / ΔA control)] × 100. Means of triplicates were determined. The 50% inhibition (IC_{50}) of ACE activity was defined as the concentrations of samples that inhibited 50% of ACE activity under experimental conditions.

Determination of ACE inhibitory activity by TLC

The ACE inhibitory activity of TI was determined by TLC method (Holmquist et al., 1979). The reactions between TI and ACE or BSA and ACE were according to the method of Anzenbacherova et al. (2001) with some modifications. Each 100 μL of TI and BSA (225 μg/mL) was premixed with 15 microunits ACE for 1 min, and then 200 μL of 0.5 mM 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, and 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 F254. The FAPGG and FAP (ACE hydrolyzed product) were separated by TLC in 1-butanol-acetic acid-water, 4:1:1 (v/v/v) and observed under UV light.

Determination of the kinetic properties of ACE inhibition by trypsin inhibitor

The kinetic properties of ACE (4 mU) without or with purified TI (200 µg/mL) in a total volume of 250 µL were determined using different concentrations of FAPGG as substrate (0.1 mM to 0.5 mM). The Km (without TI) and Km′ (with TI) were calculated from Lineweaver-Burk plots, where Km′ was the Michaelis constant in the presence of 200 µg/mL TI.

Determination of the ACE inhibitory activity of peptic hydrolysates of trypsin inhibitor

Six mg of TI were dissolved in 1 mL of 0.1 M KCl buffer (pH 2.0). Then 0.1 mL of 12 mg of pepsin was added 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 enzyme reaction. The pepsin was heated before TI hydrolysis for the 0 h reaction. Each of the 60 µL TI hydrolysates was used for determinations of ACE inhibition by spectrophotometry.

Chromatograms of peptic hydrolysates of trypsin inhibitor on a Sephadex G-50 column

The unhydrolyzed TI and peptic TI hydrolysates at 24 h were separated by Sephadex G-50 chromatography (1 × 60 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, the absorbance of which was determined at 280 nm.

RESULTS and DISCUSSION

Extraction and purification of trypsin inhibitor from sweet potato storage root

TI was purified from sweet potato storage root according to the method of Huang et al. (2005) Figure 1 shows protein staining (lane 1 and lane 3) and activity staining (lane 2 and lane 4) of purified TI without (lane 1 and lane 2) or with (lane 3 and lane 4) 2-mercaptoethanol treatment on a 15% SDS-PAGE gel.

Determination of ACE inhibitory activity of trypsin inhibitor by spectrophotometry

The purified TI was used for determinations of ACE inhibitory activity. Figure 2 shows time course of the effect of the different amounts of TI (0, 50, 100, and 200 µg/mL) on ACE activity (Δ*A* 345 nm). Compared with ACE only (control), it was found that the higher the amount of TI added, the lower the Δ*A* 345 nm was during the 300-s reaction period. Results of Figure 2 shows that purified TI could inhibit ACE activity in a dose-dependent manner.

Effects of trypsin inhibitor, BSA, and captopril on ACE activity shown by spectrophotometry

We wanted also to know whether BSA also exhibited ACE inhibitory activity. Figure 3A shows the effects of TI (0, 50, 100, 200 µg/mL), BSA (0, 50, 100, 200 µg/mL), or Captopril (Figure 3B; 0, 1.25, 2.5, 5, 10, 20, 40 and 80 nM; corresponding to 0, 108.5, 217, 434, 868, 1,736, 3,472 and 6,844 ng/mL, respectively) on ACE activity. BSA displayed less ACE inhibitory activity (less than 15% inhibition) and without dose-dependent inhibition patterns. However, TI exhibited dose-dependent ACE inhibitory

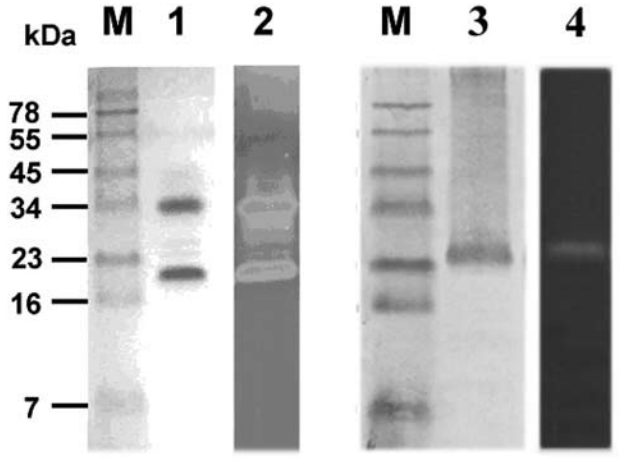

Figure 1. The protein (lane 1 and lane 3) and activity (lane 2 and lane 4) stainings of the trypsin inhibitor from sweet potato storage root on SDS-PAGE gels without (lane 1 and lane 2) or with (lane 3 and lane 4) 2-mercaptoethanol. The gel system contained 2.5 cm, 4% stacking gel and 4.5 cm, 15 % separating gel. M indicates the Seeblue™ prestained markers of SDS-PAGE. Ten micrograms of trypsin inhibitor were loaded in each well.

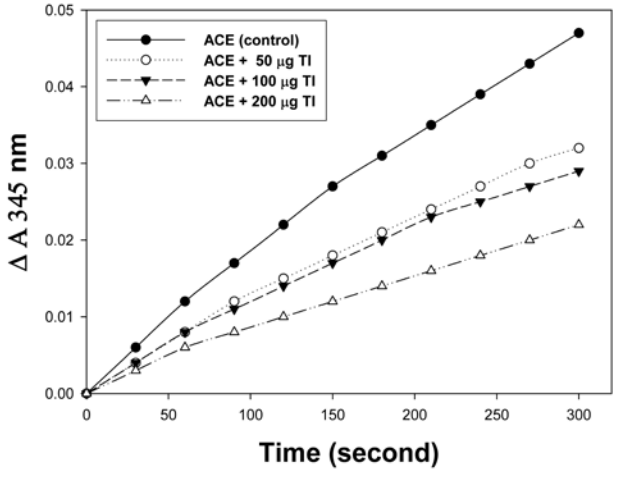

Figure 2. Inhibitory activity of different amounts of trypsin inhibitor (0, 50, 100 and 200 µg/mL) from sweet potato storage root on the ACE activity (Δ*A* 345 nm).

activity (50~200 μg/mL giving, respectively, 31.9 ~ 53.2% inhibition). From calculations, the 50% inhibition (IC_{50}) of TI against ACE activity was 187.96 μg/mL, compared to 10 nM (868 ng/mL) for Captopril, which was similar to the 7 nM reported by Pihlanto-Leppälä et al. (1998). The IC_{50} of yam dioscorin was 250 μg/mL (Hsu et al., 2002). Both BSA and purified TI are proteins, but only the purified TI showed specific dose-dependent ACE inhibitory activity. In the literature, the protein hydrolysates are used as sources to purify peptides like ACE inhibitors (Mullally et al., 1996; Maruyama et al., 1987). By our calculations, the IC_{50} of TI against ACE activity was 187.96 μg/mL, which was smaller than that of the synthetic peptide α-lactorphin (YGLF, 322.7 μg/mL). Several identified peptide fragments exhibited much lower IC_{50} values than our purified TI: for example, Tyr-Pro of whey proteins, 8.1 μg/mL (Yamamoto et al., 1999) and HHL of soybean proteins, 2.2 μg/mL (Shin et al., 2001). Conversely, several identified peptide fragments exhibited IC_{50} values much higher than our purified TI: for example, hydrolysates of whey proteins (α-lactalbumin and β-lactoglobulin) with IC_{50} values between 345-1,733 μg/mL (Pihlanto-Leppälä, et al., 2000); LAHKAL of α-lactalbumin hydrolysates, 406 μg/mL; GLDIQK of β-lactoglobulin hydrolysates, 391 μg/mL; and VAGTWY of β-lactoglobulin hydrolysates, 1,171 μg/mL.

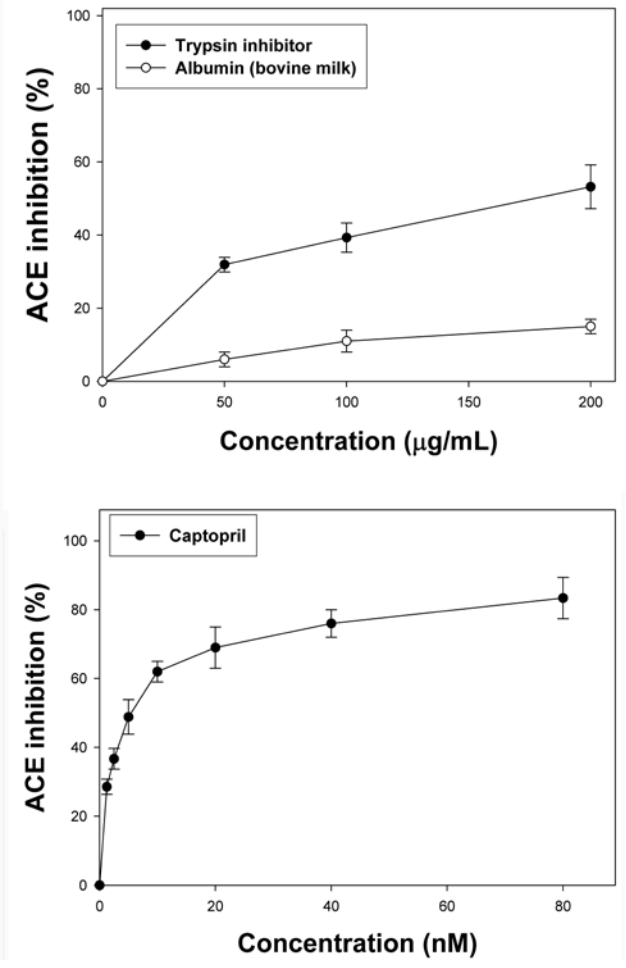

Figure 3. The effects of trypsin inhibitor, albumin, and Captopril on ACE activity determined by spectrophotometry. Trypsin inhibitor (0, 50, 100 and 200 μg/mL) or bovine serum albumin (0, 50, 100 and 200 μg/mL) was used. The inhibition of ACE (%) was calculated according to the equation [1-(ΔA inhibitor ÷ ΔA control)] × 100%.

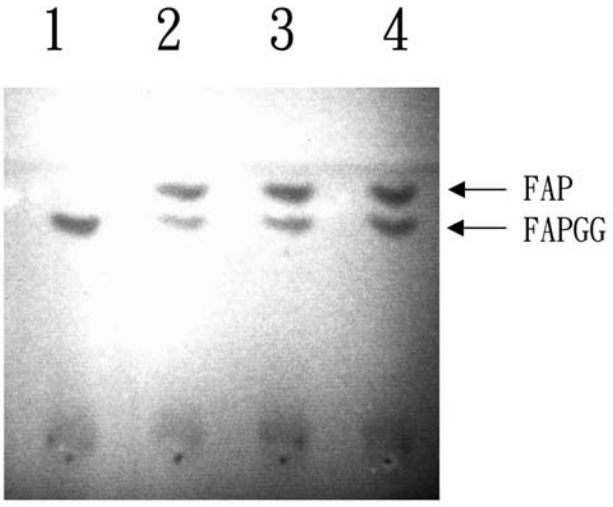

Figure 4. The TLC chromatograms of a silica gel 60 F254 showing the effects of trypsin inhibitor from sweet potato storage root or bovine serum albumin on ACE activity. Lane 1, blank test (FAPGG only); lane 2, control test (ACE reacted with FAPGG to produce FAP); lane 3, 225 μg/mL bovine serum albumin added; lane 4, 225 μg/mL trypsin inhibitor added. Each solution was dried under reduced pressure and redissolved with 400 μL methanol. Each 50 μL was spotted on a silica gel 60 F254. The FAPGG and FAP were separated by water saturated 1-butanol : acetic acid : water, 4:1:1 (V/V/V). Arrows indicated the positions of both FAP and FAPGG.

Determinations of ACE Inhibitory activity of trypsin inhibitor by TLC

The FAPGG and FAP (product of ACE catalyzed hydrolysis reaction) were separated by TLC using water-saturated 1-butanol: acetic acid: water, 4:1:1 (V/V/V) as developing solvents according to the method of Holmquist et al. (1979). Figure 4 shows the TLC chromatograms of a silica gel 60 F254 and the qualitative results of the effects of 225 μg/mL of commercial BSA (lane 3) or TI (lane 4) on 15 microunits of ACE. Compared to the control test (lane 2), TI (lane 4) was found to inhibit the ACE reaction with less FAP production observable under UV light. However, the control test (lane 2) and BSA (lane 3) produced similar amounts of FAP. These results demonstrated again that TI exhibited ACE inhibitory activity.

Determination of the kinetic properties of ACE inhibition by trypsin inhibitor

The Lineweaver-Burk plots of ACE (4 mU) without or with purified TI (200 μg/mL) under different concentrations of FAPGG are shown in Figure 5. The

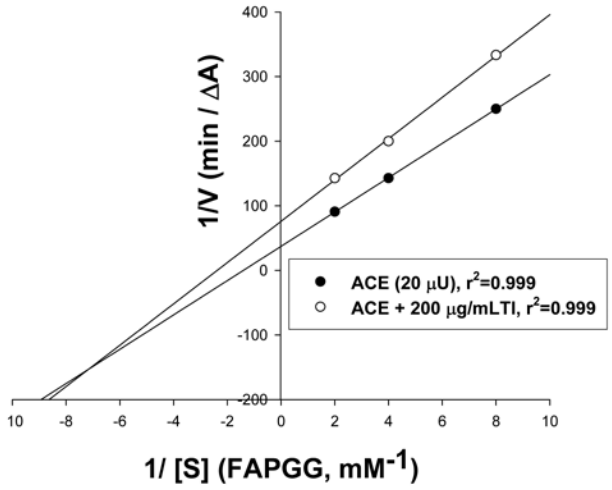

Figure 5. The Lineweaver-Burk plots of ACE (4 mU) without or with trypsin inhibitor (200 μg/mL) from sweet potato storage root using different concentrations of FAPGG (0.1 to 0.5 mM).

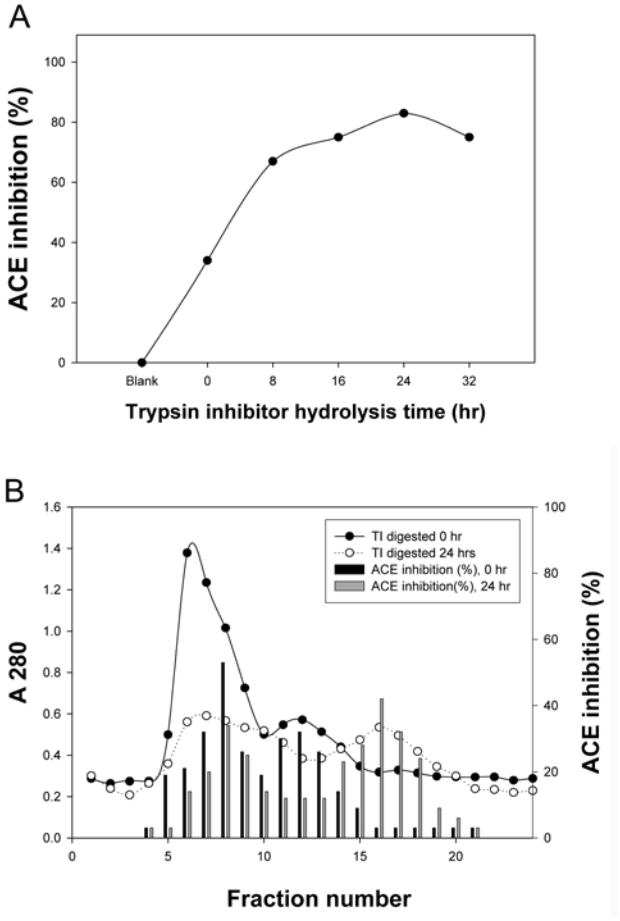

Figure 6. ACE inhibitory activity of peptic hydrolysates of sweet potato trypsin inhibitor. The plot shows the ACE inhibition (%) of peptic trypsin inhibitor hydrolysates obtained at different pepsin hydrolysis times (A). The proteins and the inhibition of ACE (%) were shown (B). The inhibition of ACE (%) was calculated according to the equation [1-(ΔA inhibitor ÷ ΔA control)] × 100%.

results indicated that purified TI acted as a mixed type inhibitor against ACE using FAPGG as a substrate. When 200 μg/mL TI were added, Vmax and Km were, respectively, 0.013 ΔA/min and 0.715 mM; while without TI they were 0.027 ΔA/min and 0.419 mM. In conclusion, TI exhibited dose-dependent ACE inhibitory activity and acted as a mixed type inhibitor with respect to the substrate (FAPGG). A similar finding was reported with the calculated Km as 0.255 mM FAPGG for ACE, and in the presence of purified dioscorin, the calculated Km´ was 0.3304 mM (Hsu et al., 2002).

Determination of the ACE inhibitory activity of peptic trypsin inhibitor hydrolysates and their peptide distributions

Pepsin is frequently used for protein hydrolysis to purify potential ACE inhibitory peptides (Pihlanto-Leppälä et al., 2000). Therefore, we used pepsin to hydrolyze TI. Figure 6 shows the ACE inhibitory activity (Δ*A* 345 nm) of peptic TI hydrolysates. Figure 6A shows the ACE inhibition (percent) of peptic TI hydrolysates collected at different pepsin hydrolysis times. The results (Figure 6A) show ACE inhibitory activity increasing from 34% (0 h) to about 83% (24 h). Figure 6B shows the chromatograms of unhydrolyzed TI and peptic TI hydrolysates (24 h) on Sephadex G-50 chromatography. It was found that smaller peptides increased with increasing pepsin hydrolytic time. The ACE inhibitor activities of peptic TI hydrolysates decreased after 36 h of hydrolysis (Figure 6A), suggesting that some proper conformational requirements got lost thereafter.

Lin (Lin, 1993) considered sporamin as one form of TI in sweet potato, a finding confirmed later by Yeh et al. (1997). So we used synthetic peptides to measure ACE inhibitor activity by the *sporamin A* and *B* gene sequences. Kohmura et al. (1989) synthesized some peptide fragments of human β-casein and found that the length of those peptides had an influence on the ACE inhibitory activity. Namely, peptides composed of 3-10 amino acids with proline on the C-terminal were necessary for ACE inhibitors (Kohmura et al., 1990). Thus the peptide Leu-Arg-Pro from food protein hydrolysates has been reported to be the most potent natural ACE inhibitor, with an IC_{50} value of 0.27 or 1.0 μM. Byun et al. (1980) studied the ACE inhibitory activity of a series of dipeptides and indicated that tryptophan, tyrosine, proline, or phenylalanine at the *C*-terminal and branched-chain aliphatic amino acid at the *N*-terminal were suitable for a peptide binding to ACE (Byun and Kim, 2002).

Synthetic peptides were designed by simulating the pepsin cutting sites of *sporamin A* (accession number: P14715) and *B* (accession number: P14716) gene products from sweet potato (pH >2, http://expasy.nhri.org.tw/tools/peptidecutter/). Ten new inhibitory peptides (Table 1) for ACE— HDHM, LR, SNIP, VRL, TYCQ, GTEKC, RF, VKAGE, AH and KIEL—were synthesized according to simulation. IC_{50} values of individual peptides were 276.2,

Table 1. Trypsin inhibitor (sporamin A and B) peptides with ACE inhibitor activity.

	IC_{50} (μM)
Sporamin A peptides	523.5
^{37}AH^{38}	523.5
^{127}KIEL^{130}	849.7
^{141}TYCQ^{144}	2.3
^{157}HDHM^{160}	276.2
Sporamin B peptides	
^{37}VRL^{39}	208.6
^{83}RF^{84}	392.2
^{121}VKAGE^{125}	141.5
^{144}GTEKC^{148}	275.8
^{167}SNIP^{170}	228.3
Sporamin A and B peptides	
^{35}RL^{36} or ^{34}RL^{35}	746.4
^{85}RF^{86} or ^{83}RF^{84}	392.2

Note: The sequences of sporamin A and B were retrieved from the NCBI (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov) with the following accession numbers: sporamin A: ABB97547 and sporamin B: P14716, neither of which includes pre-pro-sequence.

746.4, 228.3, 208.6, 2.3, 275.8, 392.2, 141.56, 523.5 and 849.7 μM, respectively. These results demonstrated that simulated synthetic peptides from peptic TI hydrolysates exhibited ACE inhibitory activities. In addition, some of these synthetic peptides also had antioxidantive activity (Huang et al., 2004, 2007). Our work suggests: (1) TYCQ might represent the main active site of the ACE inhibition, and (2) there are marked structural similarities for peptides with antihypertensive, immunomodulatory, and antioxidant activities, and these may be used as criteria for selecting or designing multifunctional ingredients of functional foods to control cardiovascular diseases.

In summary, trypsin inhibitor purified from sweet potato storage roots exhibited dose-dependent ACE inhibitory activity. TI acted as a mixed type inhibitor toward ACE with an IC_{50} of 187.96 μg/mL. Its peptic hydrolysates also showed ACE inhibitory activities. Some peptides derived from food proteins were demonstrated to have antihypertensive activities against spontaneously hypertensive rats (Fujita et al., 2000; Yoshii et al., 2001). The potential for hypertension control when people consume sweet potato deserves further investigation.

LITERATURE CITED

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

Bellamy, W., R.H. Wakabayashi, M. Takase, K. Kawase, S. Shimamura, and M. Tomita. 1993. Role of cell-binding in the antibacterial mechanism of Lactoferricin B. J. Appl. Bacteriol. **75:** 478-484.

Byun, H.G. and S.K. Kim. 2002. Structure and activity of angiotensin I converting enzyme inhibitory peptides derived from alaskan pollack skin. J. Biochem. Mol. Biol. **35:** 239-243.

Chen, T.L., Y.C. Lo, W.T. Hu, M.C. Wu, S.T. Chen, and H.M. Chang. 2003. Microencapsulation and modification of synthetic peptides of food proteins reduces the blood pressure of spontaneously hypertensive rats. J Agric. Food Chem. **51:** 1671-1675.

Ehlers, M.R. and J.F. Riordan. 1989. Angiotensin-converting enzyme new concepts converting its biological role. Biochemistry **28:** 5311-5317

Fiat, A.M., M.S. Daniele, and J. Pierre. 1993. Biological active peptides from milk proteins with emphasis on two examples concerning antithrombotic and immunomodulating activities. J. Dairy Sci. **76:** 301-310.

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

Holmquist, B., P. Bunning, and J.F. Riordan. 1979. A continuous spectrophotometric assay for angiotensin converting enzyme. Anal. Biochem. **95:** 540-548.

Hou, W.C. and Y.H. Lin. 1997. Polyamine-bound trypsin inhibitors in sweet potato (*Ipomoea batatas* [L.] Lam cv. Tainong 57) storage roots, sprouted roots and sprouts. Plant Sci. **126:** 11-19.

Hou, W.C., Y.C. Chen, H.J. Chen, Y.H. Lin, L.L. Yang, and M.H. Lee. 2001. Antioxidant activities of trypsin inhibitor, a 33 kDa root storage protein of sweet potato (*Ipomoea batatas* (L.) Lam cv. Tainong 57). J Agric. Food Chem. **49:** 2978-2981.

Hou, W.C., D.J. Huang, and Y.H. Lin. 2002. An aspartic type protease degrades trypsin inhibitors, the major root storage proteins of sweet potato (*Ipomoea batatas* (L.) Lam cv. Tainong 57). Bot. Bull. Acad. Sin. **43:** 271-276.

Hou, W.C., H.J. Chen, and Y.H. Lin. 2003. Antioxidant peptides with angiotensin converting enzyme inhibitory activities and applications for angiotensin converting enzyme purification. J. Agric. Food Chem. **51:** 1706-1709.

Hsu, F.L., Y.H. Lin, M.H. Lee, C.L. Lin, and W.C. Hou. 2002. 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. **50:** 6109-6013.

Huang, D.J. C.D. Lin, H.J. Chen, and Y.H. Lin. 2004. Antioxidant and antiproliferative activities of sweet potato (*Ipomoea batatas* [L.] Lam 'Tainong 57') constituents. Bot. Bull. Acad. Sin. **45:** 179-186.

Huang, D.J., H.J. Chen, W.C. Hou, T.E. Chen, and Y.H. Lin. 2004. *In vitro* reduction of trypsin inhibitor by purified NADPH/ Thioredoxin system from sprouts of sweet potato (*Ipomoea batatas* (L.) Lam) storage roots. Plant Sci. **166:** 435-441.

Huang, D.J., H.J. Chen, W.C. Hou, T.E. Chen, and Y.H. Lin. 2005. Isolation and characterization of cysteine proteinase cDNA from sweet potato (*Ipomoea batatas* [L.] Lam 'Tainong 57') storage roots. Plant Sci. **169:** 423-431.

Huang, D.J., W.C. Hou, H.J. Chen, and Y.H. Lin. 2006. Sweet potato (*Ipomoea batatas* [L.] Lam 'Tainong 57') storage roots mucilage exhibited angiotensin converting enzyme inhibitory activities *in vitro*. Bot. Stud. **45:** 397-402.

Huang, G.J., H. J. Chen, Y-S Chang, M-J Sheu, and Y.H. Lin. 2007. Recombinant sporamin and its synthesized peptides with antioxidant activities *in vitro*. Bot. Stud. **48:** 133-140.

Huang, G.J., M.J. Sheu, H.J. Chen, Y.S. Chang, and Y.H. Lin. 2007. Inhibition of reactive nitrogen species *in vitro* and *ex vivo* by trypsin inhibitor from sweet potato 'Tainong 57' storage roots. J. Agric. Food Chem. **55:** 6000-6006**.**

Kohmura, M., N. Nio, K. Kubo, Y. Minoshima, E. Munekata, and Y. Ariyoshi. 1989. Inhibition of angiotensin-converting enzyme by synthetic peptide fragments of human β-casein. Agric. Biol. Chem. **53:** 2107-2114.

Kohmura, M., N. Nio, and Y. Ariyoshi. 1990. Inhibition of angiotensinconverting enzyme by synthetic peptide fragments of human β-casein. Agric. Biol. Chem. **54:** 835-836.

Lin, Y.H. and H.L. Chen. 1980. Level and heat stability of trypsin inhibitor activity among sweet potato (*Ipomoea batatas* Lam.) varieties. Bot. Bull. Acad. Sin.**21:** 1-13.

Lin, Y.H. 1989. Relationship between trypsin-inhibitor activity and water-soluble protein and cumulative rainfall in sweet potato. J. Am. Soc. Hortic. Sci. **114:** 814-818.

Lin, Y.H. 1993. Trypsin inhibitors of sweet potato: review and prospect. *In* Y. I. Hsing and C. H. Chou (eds.), Recent Advances in Botany, Academia Sinica Monograph series 13, Taipei, Taiwan, pp. 179-185.

Maruyama, S., H. Mitachi, J. Awaya, M. Kurono, N. Tomizuka, and H. Suzuki. 1987. Angiotensin I-converting enzyme inhibitory activity of the C-terminal hexapeptide of as1-casein. Agric. Biol. Chem. **51:** 2557-2561.

Matsuoka, K., S. Matsumoto, T. Hattori, Y. Machida, K. Nakamura, and M. Maeshima. 1990. Vacuolar targeting and posttranslational processing of the precursor to the sweet potato tuberous root storage protein in heterologous plant cells. J. Biol. Chem. **265:** 19750-19957.

Maubois, J.L. and J. Leonil. 1989. Peptides du lait a activite biologique. Lait. **69:** 245-269.

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

Parker, F., O. Migliore-Samour, F. Floch, A. Zerial, G.H. Werner, J. Jolles, M. Casaretto, H. Zahn, and P. Jolles. 1984. Immunostimulating hexapeptide from human case in amino acid sequence, synthesis and biological properties. Eur. J. Biochem. **145:** 677-682.

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

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

Ryan, C.A. 1973. Proteolytic enzymes and their inhibitors in plants. Annu. Rev. Plant Physiol. **24:** 173-196.

Ryan, C.A. 1989. Protease inhibitor gene families: Strategies for transformation to improve plant defenses against herbivores. BioEssays **10:** 20-24.

Scarborough, R.H., J.W. Rose, M.H. Hsu, D.R. Phillips, V.A. Fried, A.M. Campbell, L. Manniaai, and I. F.Charo. 1991. Barbourin A GpIIb-IIIa specific integrin antagonist from the venom of sistrurus *M. Barbouri*. J. Biol. Chem. **266:** 9359-9360.

Shin, Z.I., R. Yu, S.A Park, D.K Chung, C.W. Ahn, H.S. Nam, K.S. Kim, and H.J. Lee. 2001. His-His-Leu, an angiotensin I converting enzyme inhibitory peptide derived from Korean soybean paste, exerts antihypertensive activity in vivo. J. Agric. Food Chem. **49:** 3004-3009.

Sohonie, K. and A.P. Bhandarker. 1954. Trypsin inhibitors in Indian foodstuffs: I. Inhibitors in vegetables. J. Sci. Ind. Res. **13B:** 500-503.

Yamamoto, N., M. Maeno, and T. Takano. 1999. Purification and characterization of an antihypertensive peptide from a yogurt-like product fermented by *Lactobacillus helveticus* CPN4. J. Dairy Sci. **82:** 1388-1393.

Yano, S., K. Suzuki, and G. Funatsu. 1996. Isolation from azein of thermolysin peptides with angiotensin I-converting enzyme inhibitory activity. Biosci. Biotechnol. Biochem. **60:** 661-663.

Yeh, K.W., J.C. Chen, M.I. Lin, Y.M. Chen, and C.Y. Lin. 1997. Functional activity of sporamin from sweet potato (*Ipomoea batatas* Lam): a tuber storage protein with trypsin inhibitory activity. Plant Mol. Biol. **33:** 565-570.

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

甘藷塊根胰蛋白酶抑制因子及其經胃蛋白酶水解的胜肽具有血管收縮素轉化酶抑制活性

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甘藷 (*Ipomoea batatas* [L.] Lam 'Tainong 57') 塊根胰蛋白酶抑制因子 (TI) 以 (*N*-(3-[2-furyl] acryloyl)-Phe-Gly-Gly) (FAPGG) 爲受質，利用分光光度計的方法分析抑制血管收縮素轉化酶 (angiotensin converting enzyme, ACE) 的能力，其效果隨劑量增加而增加 (50 到 200 μg/mL TI，分別抑制 31.9-53.2% 血管收縮素轉化酶活性)。TI 對於血管收縮素轉化酶之 50% 抑制濃度 (IC_{50}) 爲 187.96 μg/mL，對照組 captopril 爲 10 nM (868 ng/mL)。另外利用螢光 silica TLC 偵測 FAPGG 及其水解產物 FAP，結果也顯示 TI 對於血管收縮素轉化酶有抑制的效果。TI 對於血管收縮素轉化酶是屬於混合型抑制。而當加入 200 μg/mL TI 時，Vmax 和 Km 分別爲 0.013 △A/min 和 0.715 mM；而未入 TI 時，Vmax 和 Km 分別爲 0.027 △A/min 和 0.419 mM。利用胃蛋白酶以不同時間水解 TI 時，發現反應 24 小時時其血管收縮素轉化酶活性有抑制的效果可以從 34% (0 h) 增加到 83% (24 h)。由結果可知小分子的胜肽會隨著水解時間增加且血管收縮素轉化酶活性抑制也有增加，但水解時間超過 24 h 時，血管收縮素轉化酶活性抑制會降低，可能是由於一些胜肽的結構被破壞。利用電腦模擬胃蛋白酶水解 TI 的結果，十種人工合成具有抑制血管收縮素轉化酶活性胜肽：HDHM, LR, SNIP, VRL, TYCQ, GTEKC, RF, VKAGE, AH 和 KIEL，測定其 IC_{50} 爲 276.2, 746.4, 228.3, 208.6, 2.3, 275.8, 392.2, 141.56, 523.5 和 849.7 μM。結果發現 TYCQ 具有很好的抑制血管收縮素轉化酶活性。當人們食用甘藷塊根時，胰蛋白酶抑制因子及其胜肽也許對於高血壓和其他疾病的控制是有益的。

關鍵詞：血管收縮素轉化酶；胰蛋白酶抑制因子；胃蛋白酶；甘藷。