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# Sweet potato storage root thioredoxin *h2* and their peptic hydrolysates exhibited angiotensin converting enzyme inhibitory activity *in vitro*

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**ABSTRACT.** Recombinant thioredoxin *h* (Trx *h*2) overproduced in *E. coli* (M15) was purified by $Ni^{2+}$-chelate affinity chromatography as previously reported (Huang et al., 2004a). The molecular mass of Trx *h*2 was ca. 14 kDa as determined by SDS (sodium dodecyl sulfate)-PAGE (polyacrylamide gel electrophoresis). Trx *h*2 had antioxidant (Huang et al., 2004b), dehydroascorbate reductase, and monodehydroascorbate reductase activities (Huang et al., 2008a). Trx *h*2 was shown by spectrophotometric methods to inhibit angiotensin converting enzyme (ACE) in a dose-dependent manner (50-200 μg/mL, with 31.9 ~ 65.9% inhibition) using *N*-[3-(2-*f*uryl) acryloyl]-Phe-*G*ly-*G*ly (FAPGG) as a substrate. A 50% inhibition ($IC_{50}$) of ACE activity required 151.8 μg/mL of Trx *h*2 and 10 nM (868 ng/mL) of Captopril. TLC data also showed Trx *h*2 as an ACE inhibitor. Trx *h*2 acted as a mixed type inhibitor against ACE using FAPGG as a substrate. When 200 μg/mL Trx *h*2 were added, $V_{max}$ and $K_m$ were, respectively, 0.010 ΔA/min and 0.125 mM; without Trx *h*2 they were 0.0096 ΔA/min and 0.495 mM. Trypsin was used for Trx *h*2 hydrolysis over different time periods. ACE inhibitory activity was found to increase from 52% to about 72% after 16 h of hydrolysis. The results suggested that the ACE inhibitory capacity of small peptides increased through trypsin hydrolysis of Trx *h*2 up to 16 h and then decreased, which may have been due to the disappearance of some active ingredients. Four peptides, namely EVPK, VVGAK, FTDVDFIK and MMEPMVK, were synthesized based on the simulated trypsin digestion of Trx *h*2 and then tested for ACE inhibitory activity. The $IC_{50}$ values of individual peptides were 1.73 ± 0.24, 1.14 ± 0.13, 0.42 ± 0.02, and 1.03 ± 0.58 mM, respectively, suggesting that FTDVDFIK might be the main active site of ACE inhibition.The results for Trx *h*2 and its hydrolysates might mean that consumption of sweet potato storage roots can aid in the control of hypertension and other diseases.

**Keywords:** Angiotensin converting enzyme (ace); Hydrolysis peptides; Sweet potato; Thioredoxin *h*2.

## INTRODUCTION

Many bioactive peptides have common structural properties that include a relatively short peptide residue length (e.g. 2-9 amino acids) and the possession of 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 (Fiat et al., 1993), antioxidant or angiotensin-converting enzyme inhibitor (Liu et al., 2007), antithrombotic (Scarborough et al., 1991), or bactericidal (Bellamy et al., 1993) functions have recently been the focus of research.

ACE (angiotensin converting enzyme, 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; Lee et al., 2006). ACE removes a dipeptide from the C-terminus of angiotensin I to form

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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 casein (Maruyama et al., 1987), α-lactoalbumin, β-lactoglobulin (Pihlanto-Leppälä et al., 1998), and mucilage (Huang et al., 2006) 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).

Thioredoxins, the ubiquitous small proteins with a redox active disulfide bridge, are important regulatory elements in a number of cellular processes (Besse and Buchanan, 1997). They all contain a unique active site, WCGPC, which is able to reduce disulfide bridges of target proteins. Initially described as hydrogen carriers in ribonucleotide reduction in *E. coli*, they were found to serve as electron donors in a variety of cellular redox reactions (Holmgren, 1985). From genome sequencing data, a significant diversity of thioredoxin genes containing five different multigenic families (f, m, x, o and h) was observed (Mestres-Ortega and Meyer, 1999; Balmer and Buchanan, 2002; Meyer et al., 2002). The ferredoxin-thioredoxin system (thioredoxins f and m) has been proven to regulate several enzymatic activities associated with photosynthetic $CO_2$ assimilation in chloroplasts. Thioredoxin x contains a transit peptide similar to those required for chloroplast and mitochondria targeting; however, its function is not clearly defined (Mestres-Ortega and Meyer, 1999). A new type of plant mitochondrial thioredoxin, Trx o, was also shown to regulate the activities of several mitochondrial proteins by disulfide bond reduction (Laloi et al., 2001).

Thioredoxin *h* is generally assumed to be cytosolic, which was supported by the absence of a transit peptide in the genes cloned for the isoforms from *Arabidopsis* (Rivera-Madrid et al., 1995), *Triticum aestivum* (Gautier et al., 1998), tobacco (Marty and Meyer, 2001), germinating wheat seeds (Serrato et al, 2001), and barley seed proteome (Kenji et al., 2003). Moreover, the existence of several forms of thioredoxin *h* detected in wheat flour (Johnson et al., 1987), and spinach leaves (Florencio et al., 1988), supports the view that higher plants possess multiple and divergent thioredoxin genes (Rivera-Madrid et al., 1995).

In our previous report, Trx *h*2 exhibited both dehydroascorbate reductase and monodehydroascorbate reductase activities (Huang et al., 2008a). Trx *h*2 also exhibited antioxidant activities against different radicals (Huang et al., 2004b). In this work we report for the first time that Trx *h*2 exhibited dose-dependent ACE inhibitory activity when Captopril was used as a positive control. Commercial bovine serum albumin (BSA), frequently found in the literature as the peptide resource of ACE inhibitors, was chosen for comparison. The $K_i$ values of Trx *h*2 against ACE were calculated. We also used trypsin to hydrolyze Trx *h*2 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), glutamate 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, peptides (GL Biochem, China), and other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

### Expression of thioredoxin *h2* in *E. coli*

Thioredoxin *h*2 (Gene Bank accession number: AY344228; Trx *h*2) was expressed in *E. coli*. The coding sequence was amplified from Trx *h*2 cDNA using an oligonucleotide (5′-GAG AGG ATC CAA TGG GAG GGG CT-3′), with a *Bam*HI site (underlined) at the putative initial Met residue, and an oligonucleotide (5′- ATT TGA AGC TTG ATT GAT GCT -3′), with a *Hind*III site at the 3′ end. The PCR fragment was subcloned in pGEM T-easy vector. The plasmid was then digested with *Bam*HI and *Hind*III and subcloned in pQE-32 expression vector (QIAexpress expression system, Qiagen). The resulting plasmid, termed pQE-Trx *h*2, was introduced into *E. coli* (M15). Cultures of the transformed *E. coli* (M15) overexpressed a protein of the expected molecular mass, which was purified by affinity chromatography in Ni-NTA columns (Qiagen) according to the method of Huang et al. (2004b).

### Protein staining of thioredoxin *h*2 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 (2-ME). Coomassie brilliant blue G-250 was used for protein staining (Huang et al., 2004).

### Protein stainings and electroblotting analysis of thioredoxin *h2* in 15% SDS–PAGE gels

Trx *h*2 were examined by protein staining in 15% SDS-PAGE (sodium dodecylsulfate-polyacrylamide gel electrophoresis) gels. Twenty microliter samples were mixed with 25 μL sample buffer containing 60 mM Tris buffer (pH 6.8), 2% SDS, 25% glycerol and 0.1% bromophenol blue, with 2-ME in a final concentration of 14.4 mM, and heated at 100°C for 5 min for protein staining. Coomassie brilliant blue G-250 was used for protein staining. The Trx *h*2 was subjected to 15% SDS-PAGE according to the

method of Huang et al. (Huang et al., 2007). After electrophoresis, gels were equilibrated in transfer buffer (25 mM Tris-HCl, pH 8.3, 150 mM glycine and 10 % (w/v) methanol). The separated proteins were transferred to an Immobilon PVDF membrane (Millipore, Bedford, MA) in transfer buffer at pH 8.3 for 1 h at 100 V. Membranes were blocked for 2 h at room temperature in 5% nonfat dry milk powder and then incubated with polyclonal antibodies as the primary antibodies against thioredoxin from *E. coli* (Sigma, USA). After incubation, membranes were washed in Tris-buffered saline with 0.05% Tween (TBST) three times, 10 min each time, and incubated with anti-rabbit horseradish peroxidase-conjugated antibody. They were then washed in TBST three times, 10 min each time, and developed using diaminobenzidine (DAB) (Sigma, USA).

### Determination of ACE inhibitory activity by spectrophotometry

The ACE inhibitory activity was measured according to the method of Lee et al. (2003) with some modifications. Four microliters (4 microunits) of commercial ACE was mixed with 50 µL of different amounts of Trx *h*2 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 over 5 min at room temperature. Deionized water was used instead of sample solution for blank experiments (ΔA control). Captopril (molecular mass 217.3 kDa) 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 Δ*A* 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 Trx *h*2 was also determined by the TLC method (Holmquist et al., 1979). The reactions between Trx *h*2 and ACE or BSA and ACE were measured according to the method of Anzenbacherova et al. (2001) with some modifications. Each 100 µL of Trx *h*2 or BSA (225 µg/mL) was premixed with 15 microunits of 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; 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 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 thioredoxin *h*2

The kinetic properties of ACE (4 mU) without or with Trx *h*2 (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 Trx *h*2) and $K_m'$ (with Trx *h*2) were calculated from Lineweaver-Burk plots, where $K_m'$ was the Michaelis constant in the presence of 200 µg/mL Trx *h*2.

### Determination of the ACE inhibitory activity of peptic hydrolysates of thioredoxin *h*2

Six mg of Trx *h*2 were dissolved in 1 mL of 0.1 M KCl buffer (pH 2.0). Then 0.1 mL of 12 mg of trypsin was added and hydrolysis was carried out 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 trypsin was heated before thioredoxin *h*2 hydrolysis for the 0 h control reaction. Each of the 60 µL Trx *h*2 hydrolysates was used for determinations of ACE inhibition using spectrophotometry.

### Statistical analysis

Means of triplicates were calculated. Student's *t* test was used for comparison between two treatments. A difference was considered to be statistically significant when $p < 0.05$.

## RESULTS and DISCUSSION

### Expression and purification of thioredoxin *h*2

To express sweet potato thioredoxin *h2* in *E. coli*, the coding sequence of Trx2 was subcloned in a pQE-32 expression vector so that sweet potato Trx *h*2 was produced with a 6x His-tag at the N-terminus. SDS-PAGE analysis of crude extracts from transformed *E. coli* (M15) showed a high level of a polypeptide with the expected molecular mass (ca. 14 kDa). This polypeptide was found as a soluble protein in the supernatant and was absent in protein extracts obtained from *E. coli* with pQE-32 vector. The expressed protein was purified from crude extracts by $Ni^{2+}$-chelate affinity chromatography, which yielded highly purified His-tagged thiredoxin *h2*. The next step of Trx *h*2 purification was the use of prepared SDS-PAGE (Figure 1). In our previous report, Trx *h*2 exhibited antioxidant, dehydroascorbate reductase and monodehydroascorbate reductase activities. The expression of Trx *h*2 with 6x His-tag protein did not affect the activity (Huang et al., 2008a; 2004b).

### Determination of ACE inhibitory activity of thioredoxin *h*2 by spectrophotometry

The purified Trx *h*2 was used for determinations of ACE inhibitory activity. Figure 2 shows the time course of the effect of different amounts of Trx *h*2 (0, 50, 100, and

200 μg/mL) on ACE activity (Δ*A* 345 nm). Compared with the ACE only (control), it was found that the higher the amount of Trx *h*2 added, the lower the ΔA 345 nm found during the 300-s reaction period. The results of Figure 2 show that purified Trx *h*2 could inhibit ACE activity in a dose-dependent manner.

### Effects of thioredoxin *h*2, BSA and captopril on ACE activity shown by spectrophotometry

We also wanted to know whether BSA exhibited ACE inhibitory activity. Figure 3A shows the effects of Trx *h*2 (0, 50, 100, and 200 μg/mL) and BSA (0, 50, 100, and 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, 1736, 3472 and 6944 ng/mL, respectively) on ACE activity. BSA was found to show less ACE inhibitory activity (less than 15% inhibition) and no dose-dependent inhibition patterns. However, Trx *h*2 exhibited dose-dependent ACE inhibitory activity (50~200 μg/mL, giving, respectively, 31.9 ~ 65.9% inhibition). We calculated the 50% inhibition ($IC_{50}$) of Trx *h*2 against ACE activity as 151.8 μg/mL, compared to 10 nM (868 ng/mL) for Captopril, which was similar to what Pihlanto-Leppälä et al. reported (7 nM). The $IC_{50}$ of yam dioscorin was 250 μg/mL (Hsu et al., 2002). Both BSA and purified Trx *h*2 were proteins, but only the purified Trx *h*2 showed specific dose-dependent ACE inhibitory activity. In the literature, the protein hydrolysates were used as sources to purify peptides as ACE inhibitors (Maruyama et al., 1987; Mullally et al., 1996). We calculated the $IC_{50}$ of Trx *h*2 against ACE activity as 151.8 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 Trx *h*2. For example, the Tyr-Pro of whey proteins were 8.1 μg/mL (Yamamoto et al., 1999), and the HHL of soybean proteins were 2.2

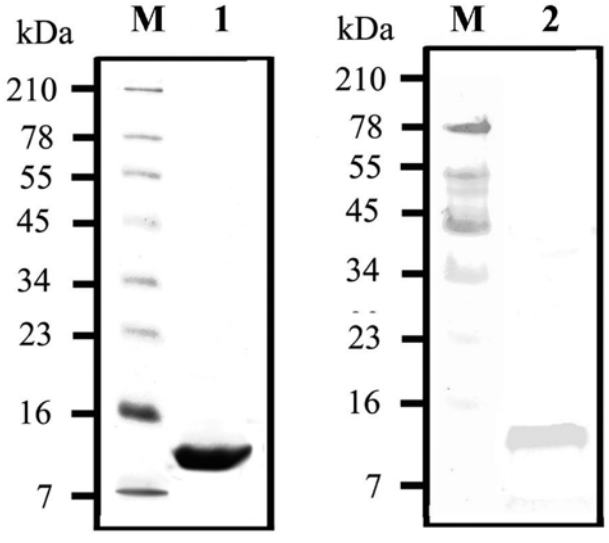


**Figure 1.** The protein staining (lane 1) and western blot analysis (lane 2) of thioredoxin *h*2. Both protein staining of Trx *h*2 with Coomassie brilliant blue R and Western blot analysis of sweet potato Trx *h*2 were performed on 15% SDS-PAGE gels. The gels were then transferred onto PVDF membranes that were probed with a 1:1000 (v/v) dilution of rabbit polyclonal antibodies raised against Trx *h*2, using goat-antirabbit horseradish peroxidase assay as the second antibody. M indicates the Seeblue™ prestained markers of SDS-PAGE. Ten micrograms of Trx *h*2 were loaded in each well.

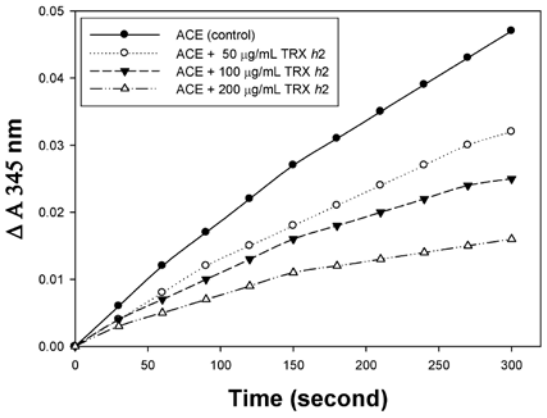


**Figure 2.** Inhibitory activity of thioredoxin *h*2 (0, 50, 100 and 200 μg/mL) of sweet potato storage root on ACE activity (ΔA 345 nm).

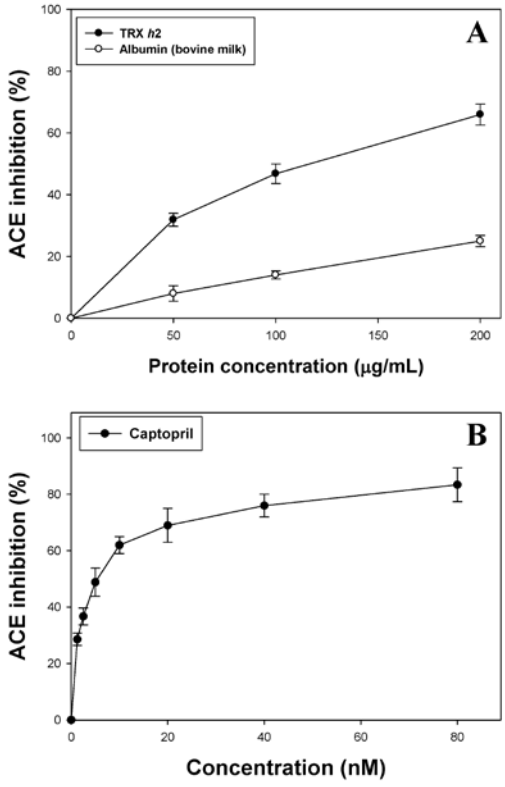


**Figure 3.** The effects of thioredoxin *h*2, albumin, and Captopril on ACE activity as determined by spectrophotometry. Thioredoxin *h*2 (0, 50, 100 and 200 μg/mL) or bovine serum albumin (0, 50, 100 and 200 μg/mL) was used. The inhibition of ACE (%) was calculated according to the equation [1-(ΔA inhibitor ÷ ΔA control)] × 100%.

μg/mL (Shin et al., 2001). On the other hand, several identified peptide fragments exhibited much higher $IC_{50}$ values than our purified Trx *h*2. Hydrolysates of whey proteins (α-lactalbumin and β-lactoglobulin), for example, had $IC_{50}$ values of 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. In our previous paper, the $IC_{50}$ of trypsin inhibitor from sweet potato was 188 μg/mL, higher than that of purified Trx *h*2 (Huang et al., 2008b).

## Determinations of ACE inhibitory activity of thioredoxin *h*2 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 qualitative results of the TLC chromatograms using silica gel 60 F254 which demonstrated the effects of 225 μg/mL of commercial BSA (lane 3) or Trx *h*2 (lane 4) on 15 microunits of ACE. Compared to the control test (lane 2), Trx *h*2 (lane 4) was found to better inhibit the ACE reaction, with less FAP production evident under UV light. However, the FAP amounts in the control test (lane 2) were similar to those found with BSA (lane 3). This demonstrated again that Trx *h*2 exhibited ACE inhibitory activity.

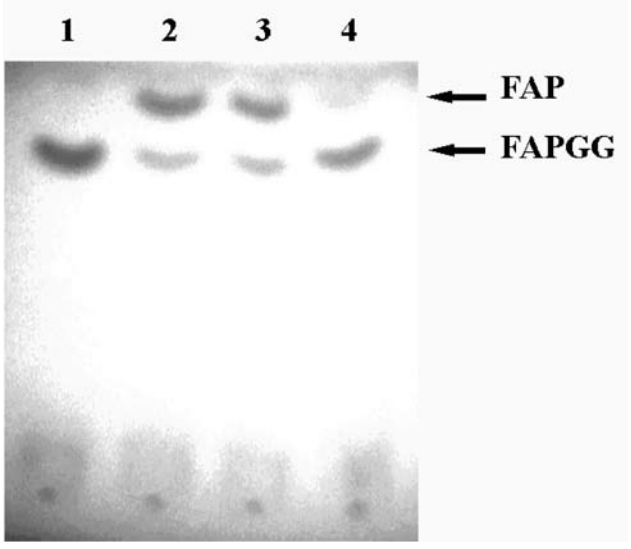


**Figure 4.** The TLC chromatograms using silica gel 60 F254 showing the effects of thioredoxin *h*2 of sweet potato storage root or bovine serum albumin on ACE activity. Lane 1, blank test (FAPGG only); lane 2, control test (ACE reacted with FAPGG to produce FAP); lane 3, control test plus 225 μg/mL bovine serum albumin; lane 4, control test plus 225 μg/mL thioredoxin *h*2. Each solution was dried under reduced pressure and redissolved with 400 μL methanol. Each 50 μL was spotted on 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 indicate the positions of both FAP and FAPGG.

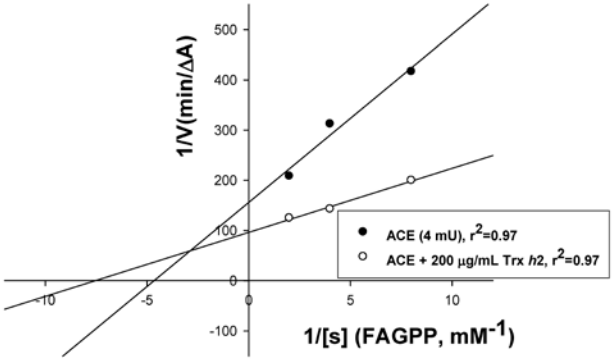


**Figure 5.** The Lineweaver-Burk plots of ACE (4 mU) without or with thioredoxin *h*2 (200 μg/mL) of sweet potato storage root using different concentrations of FAPGG (0.1 to 0.5 mM).

## Determination of the kinetic properties of ACE inhibition by thioredoxin *h*2.

The Lineweaver-Burk plots of ACE (4 mU) without or with purified Trx *h*2 (200 μg/mL) under different concentrations of FAPGG are shown in Figure 5. The results indicated that purified Trx *h*2 acted as a mixed type inhibitor against ACE using FAPGG as a substrate. When 200 μg/mL Trx *h*2 were added, $V_{max}$ and $K_m$ were, respectively, 0.010 ΔA/min and 0.125 mM while without Trx *h*2 they were 0.0096 ΔA/min and 0.495 mM. In conclusion, Trx *h*2 exhibited dose-dependent ACE inhibitory activity and acted as a mixed type inhibitor with respect to the substrate (FAPGG). A similar work calculated the $K_m$ as 0.255 mM FAPGG for ACE, and in the presence of purified dioscorin, the calculated $K_m'$ was 0.3304 mM (Hsu et al., 2002).

## Determination of the ACE inhibitory activity of peptic thioredoxin *h*2 hydrolysates

Trypsin has been frequently used for protein hydrolysis to purify potential ACE inhibitory peptides (Pihlanto-Leppälä et al., 2000). Therefore, we used it to hydrolyze Trx *h*2. Figure 6 shows the ACE inhibitory activity (Δ*A* 345 nm) of peptic Trx *h*2 hydrolysates. Figure 6 shows the ACE inhibition (percent) of peptic Trx *h*2 hydrolysates collected at different trypsin hydrolysis times. From the results, it was found that the ACE inhibitory activity increased from 52% (0 h) to about 72% (16 h). The ACE inhibitor activities of peptic Trx *h*2 hydrolysates decreased after 16 h of hydrolysis (Figure 6) suggesting that some active ingredients got lost.

Kohmura et al. (1989) synthesized some peptide fragments of human β-casein and found that the length of those peptides had an influence on ACE inhibitory activity. Namely, peptides composed of 3-10 amino acids with proline on the C-terminal were necessary as 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 and Kim (2002) studied the ACE inhibitory activity of a series of dipeptides and indicated

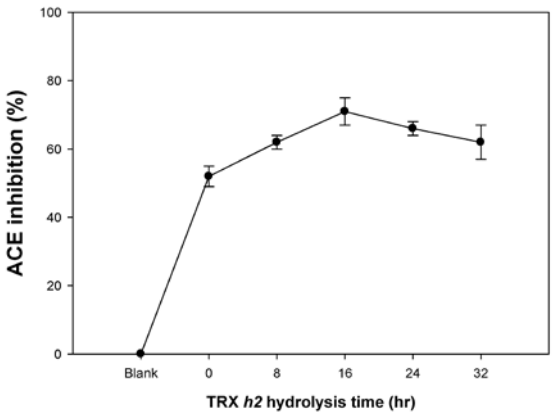


**Figure 6.** ACE inhibitor activity of peptic hydrolysates of sweet potato thioredoxin *h*2. The plot shows the ACE inhibition (%) of peptic thioredoxin *h*2 hydrolysates obtained at different trypsin hydrolysis times. The inhibition of ACE (%) was calculated according to the equation [1-(ΔA inhibitor ÷ ΔA control)] × 100%.

that tryptophan, tyrosine, proline, or phenylalanine at the *C*-terminal and branched-chain aliphatic amino acids at the *N*-terminal were required for a peptide to bind to ACE (Byun and Kim, 2002).

We measured the ACE inhibitory activity of some synthetic peptides based on Trx *h*2 gene sequences. Synthetic peptides were designed by simulating the trypsin cutting sites of sweet potato *Trx h2* gene (accession number: AY344228) products (pH >2, http://expasy.nhri.org.tw/tools/peptidecutter/). Four new inhibitory peptides (Table 1) for ACE, namely EVPK, VVGAK, FTDVDFIK, and MMEPMVK, were synthesized according to simulation. $IC_{50}$ values of individual peptides were 1.73 ± 0.24, 1.14 ± 0.13, 0.42 ± 0.02, and 1.03 ± 0.58 mM, respectively. These results demonstrated that simulated synthetic peptides from peptic Trx *h*2 hydrolysates exhibited ACE inhibitory activities. Our work suggests that (1) FTDVDFIK might represent the main active site for the ACE inhibition; (2) peptides with antihypertensive, immunomodulatory, and antioxidant activities have marked structural similarities, and these may be used as criteria for selecting or designing the multifunctional ingredients of functional foods to control cardiovascular diseases.

**Table 1.** Thioredoxin *h*2 peptides with ACE inhibitor activity.

| TRX *h*2 peptides | $IC_{50}$ |
|---|---|
| EVPK | 1.73 ± 0.24 mM |
| VVGAK | 1.14 ± 0.13 mM |
| FTDVDFIK | 0.42 ± 0.02 mM |
| MMEPMVK | 1.03 ± 0.58 mM |

Note: The sequence of Trx *h*2 had pre-pro-sequence. These sequences were retrieved from the NCBI (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov) with accession number AY344228.

In summary, Trx *h*2 exhibited dose-dependent ACE inhibitory activity. Trx *h*2 acted as a mixed type inhibitor toward ACE with an $IC_{50}$ of 151.8 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 human hypertension control through the consumption of sweet potato deserves further investigation.

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# 甘藷塊根中硫氧化還原蛋白 *h*2及其合成之胜肽具有血管收縮素轉化酶抑制活性

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在大腸桿菌 (M15) 中大量表現重組蛋白質硫氧化還原蛋白 *h2* (Trx *h*2)，利用鎳離子螯合之親和性管柱純化。Trx *h*2 經 SDS-PAGE 分析其分子量約為 14 kDa。由於 Trx *h*2 具有抗氧化活性，去氫抗壞血酸還原酶，單去氫抗壞血酸還原酶的活性。Trx *h*2 以 (*N*-(3-[2-furyl] acryloyl)-Phe-Gly-Gly) (FAPGG) 為受質，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 31.9-65.9 % 血管收縮素轉化酶活性 )。Trx *h*2 對於血管收縮素轉化酶之 50% 抑制濃度 ($IC_{50}$) 為 151.8 μg/mL，對照組 Captopril 為 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 Trx *h*2 對於血管收縮素轉化酶有抑制的效果。Trx *h*2 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 Trx *h*2 時，$V_{max}$ 和 $K_m$ 分別為 0.010 ΔA/min 和 0.125 mM；而未入 Trx *h*2 時，$V_{max}$ 和 $K_m$ 分別為 0.0096 ΔA/min 和 0.495 mM 利用胰蛋白酶以不同時間水解 Trx *h*2 時，發現反應 16 小時時其血管收縮素轉化酶活性有抑制的效果可以從 52 % (0 h) 增加到 72 % (16 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 16 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。利用電腦模擬胰蛋白酶水解 Trx *h*2 的結果，四種人工合成具有抑制血管收縮素轉化酶活性胜肽：EVPK, VVGAK, FTDVDFIK 和 MMEPMVK，測定其 $IC_{50}$ 為 1.73 ± 0.24, 1.14 ± 0.13, 0.42 ± 0.02 和 1.03 ± 0.58 mM。結果發現 FTDVDFIK 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，Trx *h*2 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：**甘藷；血管收縮素轉化酶；硫氧化還原蛋白 *h*2；水解胜肽。