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# Sweet potato storage root defensin and its tryptic hydrolysates exhibited angiotensin converting enzyme inhibitory activity *in vitro*

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**ABSTRACT.** Sweet potato defensin (SPD1) overproduced in *E. coli* (M15) was purified by $Ni^{2+}$-chelate affinity chromatography. The molecular mass of SPD1 is about 8,600 Da determined by SDS (sodium dodecyl sulfate)-PAGE (polyacrylamide gel electrophoresis). Our previous paper showed that SPD1 had antimicrobial, dehydroascorbate reductase and monodehydroascorbate reductase activities. The activity of SPD1 to inhibit angiotensin converting enzyme (ACE) was shown using N-[3-(2-furyl) acryloyl]-Phe-Gly-Gly (FAPGG) as substrate in a dose-dependent manner (27.56 ~ 52.58 % inhibition). The 50% inhibition ($IC_{50}$) of ACE activity required 190.47 μg/mL SPD1 while that of Captopril was 10 nM (868 ng/mL). Thin layer chromatography (TLC) also identified SPD1 as an ACE inhibitor. SPD1 acted as a mixed type inhibitor against ACE using FAPGG as a substrate. When 200 μg/mL SPD1 (10 μg) were added, Vmax and Km were 0.01 ΔA/min and 0.69 mM, respectively; without SPD, 0.03 ΔA/min and 0.42 mM. Trypsin was used for SPD1 hydrolysis and part of the reaction mixture was removed and analyzed at set times. ACE inhibitory activity increased from 52.47% to about 74.38% after 24 h hydrolysis. The results suggested that small peptides increased by trypsin hydrolysis of the SPD1 ACE inhibitory capacity also increased up to 24 h, then decreased, which may be due to the disappearance of some active ingredients. Six peptides, namely GFR, FK, IMVAEAR, GPCSR, CFCTKPC and MCESASSK, were synthesized based on the simulated trypsin digest of SPD1, then tested for ACE inhibitory activity. $IC_{50}$ values of individual peptides were 94.25 ± 0.32, 265.43 ± 1.24, 84.12 ± 0.53, 61.67 ± 0.36, 1.31 ± 0.07 and 75.93 ± 0.64 μM, respectively, suggesting that CFCTKPC might represent the main domain for the ACE inhibition. The consumption of sweet potatoes may thus help alleviate hypertension and other diseases due to their SPD1 and hydrolysate content.

**Keywords:** Angiotensin converting enzyme (ACE); Defensin; Inhibition; Sweet Potato.

## INTRODUCTION

Many bioactive peptides have common structural properties that include a relatively short peptide residue length (e.g. 2-9 amino acids) and possession of hydrophobic amino acid residues, including proline, lysine or arginine groups (Lin et al., 2006). 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. Recent research has focused on bioactive peptides with immunostimulating (Huang et al., 2010), antioxidant or angiotensin-converting enzyme (ACE) inhibitor (Liu et al., 2007), antithrombotic (Scarborough et al., 1991) and bactericidal (Bellamy et al., 1993) functions.

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ACE (peptidyldipeptide hydrolase 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 (Lin et al., 2008). Several endogenous peptides, such as enkephalins, β-endorphin, and substance P, were reported to be competitive substrates and ACE inhibitors. Numerous food-derived peptides from α-lactoalbumin, β-lactoglobulin (Pihlanto-Leppälä et al., 1998), mucilage (Huang et al., 2006) and trypsin inhibitors (TI) (Huang et al., 2007) also inhibited ACE, as did a number of antioxidant (reduced glutathione and carnosine-related peptides) (Hou et al., 2003) and synthetic peptides (Huang et al., 2006).

Plant defensins were originally termed $\gamma$ -thionins because they have a similar size (5 kDa) and the same number of disulfide bridges (four) as $\alpha$- and $\beta$-thionins. $\gamma$-Thionins however, are structurally different from $\alpha$- and $\beta$-thionins and are more similar, both in structure and in function, to insect and mammalian defensins. This class of plant peptides was thus named 'plant defensins' (Haasen and Goring, 2010). A variety of plant defensins, from both monocotyledonous and dicotyledonous plants, have been isolated and characterized (Terras et al., 1992). Plant defensin families have been known as potent growth inhibitors of a broad spectrum of fungi and bacteria, however their antimicrobial activity is quite diverse. They have been divided into two main groups (A and B), whose amino acid sequence shares only 25% similarity (Harrison and Lederberg, 1998). Plant defensins have been detected in the leaves, flowers, seeds, tubers and other parts of plants (Moreno et al., 1994). Furthermore, the expression of some defensin genes is developmentally regulated, whereas that of other genes is greatly elevated in response to external biotic and abiotic stimuli (Epple et al., 1997).

In our previous report, SPD1 exhibited antimicrobial, dehydroascorbate reductase and monodehydroascorbate reductase activities (Huang et al., 2008a). In this work, we report for the first time that SPD1 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 SPD1 against ACE were calculated. We also used trypsin to hydrolyze SPD1, analyzed it at set times, and determined the changes in ACE inhibitory activity. $IC_{50}$ of ACE inhibitory activities using synthetic peptides were also determined for comparison.

## 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).

### Defensin expression in *E. coli*

Defensin with its pre-pro-sequence (SPD1) was expressed in *E. coli*. The coding sequence was amplified from cDNA SPD1 using an oligonucleotide (5′-A GGAT CCATG GCTTC ATCTC TTCGT TC -3′) with a *Bam*HI site (underlined) at the putative initial Met residue, and an oligonucleotide (5′-GCCTT GCTAA TTCAG TCGAC CGCTG T -3′) with a *Sal*I site at the 3′ end. The PCR fragment was subcloned in a pGEM T-easy vector. The plasmid was then digested with *Bam*HI and *Sal*I and the excised fragments were subcloned in a pQE30 expression vector (QIAexpress expression system, Qiagen). The resulting plasmid, termed pQE-SPD1, was introduced into *E. coli* (M15). Cultures of the transformed *E. coli* (M15) overexpressed a protein of expected molecular mass, which was purified by affinity chromatography in Ni-nitrilotriacetic acid (NTA) columns (Qiagen), according to Huang et al. (2004).

### ACE inhibitory activity determination

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 SPD1 or BSA (50, 100, and 200 μg/mL), then 200 μL of 0.5 mM *N*-[3-(2-furyl) acryloyl]-Phe-Gly-Gly [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 after 5 min at room temperature. Deionized water was used instead of sample solution for blank experiments ($\Delta A$ control). Captopril (molecular mass 217.3 Da) was used as a positive control for the ACE inhibitor (1.25, 2.5, 5, 10, 20, 40 and 80 nM). ACE activity was expressed as $\Delta A$ 345 nm, and the ACE percent inhibition was calculated as follows: $[1 - (\Delta A \text{ inhibitor} / \Delta A \text{ control})] \times 100$. 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.

### ACE inhibitory activity determination using TLC

The ACE inhibitor activity of SPD1 was also determined using TLC (Holmquist et al., 1979). Each 100 μL of SPD1 or BSA (225 μg/mL) was premixed with 15 micro-units of ACE for 1 min, and then 200 μL of 0.5 mM FAPGG was added and allowed to react at room tempera-

ture 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 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.

### Kinetic properties of ACE inhibition determination by defensin

The kinetic properties of ACE (4 µU) without or with SPD1 (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 SPD1) and Km´ (with SPD1) were calculated from Lineweaver-Burk plots, where Km´ was the Michaelis constant in the presence of 200 µg/mL SPD1.

### ACE inhibitory activity determination by defensin trypsin hydrolysates

Six mg of SPD1 were dissolved in 1 mL of 50 mM Tris-HCl buffer (pH 7.9). Then 0.1 mL of 12 mg of trypsin was added and hydrolysis was carried out at 37°C for 8, 16, 24 and 32 h. After hydrolysis the solution was heated at 100°C for 5 min to stop enzyme reaction. The trypsin was heated before SPD1 hydrolysis for the 0 h control reaction. Each of the 60 µL SPD1 hydrolysates was used in spectrophotometry to determine ACE inhibition.

### Defensin tryptic hydrolysate chromatograms on a Sephadex G-50 column

The unhydrolyzed SPD1 and tryptic SPD1 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 570 nm.

### 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 of defensin in *E. coli*

SDS-PAGE analysis of SPD1 crude extracts from the transformed *E. coli* (M15) showed high amounts of a polypeptide with the expected molecular mass (ca. 8,600 Da). This polypeptide was found as a soluble protein in the supernatant, and was absent in protein extracts obtained from *E. coli* transformed with pQE-30 vector. The expressed protein was purified from crude extracts by $Ni^{2+}$-chelate affinity chromatography, which yielded highly purified His-tagged SPD1. Preparing SDS-PAGE (Huang et al., 2008a) was used as the next step for SPD1 purification.

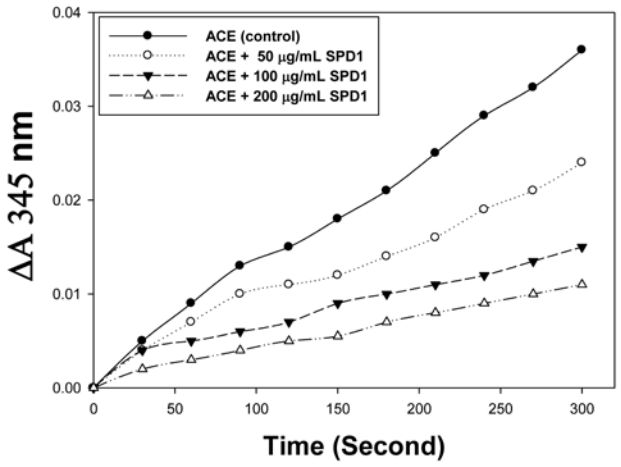


**Figure 1.** Inhibitory activity of different amounts of defensin (SPD1) (0, 50, 100 and 200 µg/mL) from sweet potato storage root on ACE activity (Δ*A* 345 nm).

### Spectrophotometric determination of ACE inhibitor activity of defensin

Purified SPD1 was used to determine ACE inhibitory activity. Figure 1 shows time progression for the effect of the different amounts of SPD1 (0, 50, 100 and 200 µg/mL) on the ACE activity (Δ*A* 345 nm). Compared with the ACE only (control), it was found that the higher the amount of SPD1 added during a 300 sec reaction period, the lower the Δ*A* 345 nm would be. Figure 1 shows that purified SPD1 could inhibit ACE activity in a dose-dependent manner.

### Effects of defensin, BSA and captopril on ACE activity shown by spectrophotometry

We were interested in learning whether BSA also exhibited ACE inhibitory activity. Figure 2A shows the effects of SPD1 (0, 50, 100, and 200 µg/mL), BSA (0, 50, 100, and 200 µg/mL) or Captopril (Figure 2B; 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 showed less ACE inhibitory activity (less than 25% inhibition) without dose-dependent inhibition patterns. However, SPD1 exhibited dose-dependent ACE inhibitory activity (50~200 µg/mL giving, respectively, 27.56 ~ 52.58% inhibition). From calculations, the 50% inhibition ($IC_{50}$) of SPD1 against ACE activity was 190.47 µg/mL compared to that of 10 nM (868 ng/mL) for Captopril, which was similar to the report (7 nM) by Pihlanto-Leppälä et al. (1998); while the $IC_{50}$ of yam dioscorin was 250 µg/mL (Hsu et al., 2002). Both BSA and purified SPD1 were proteins, but only the purified SPD1 showed specific dose-dependent ACE inhibitory activity. In the

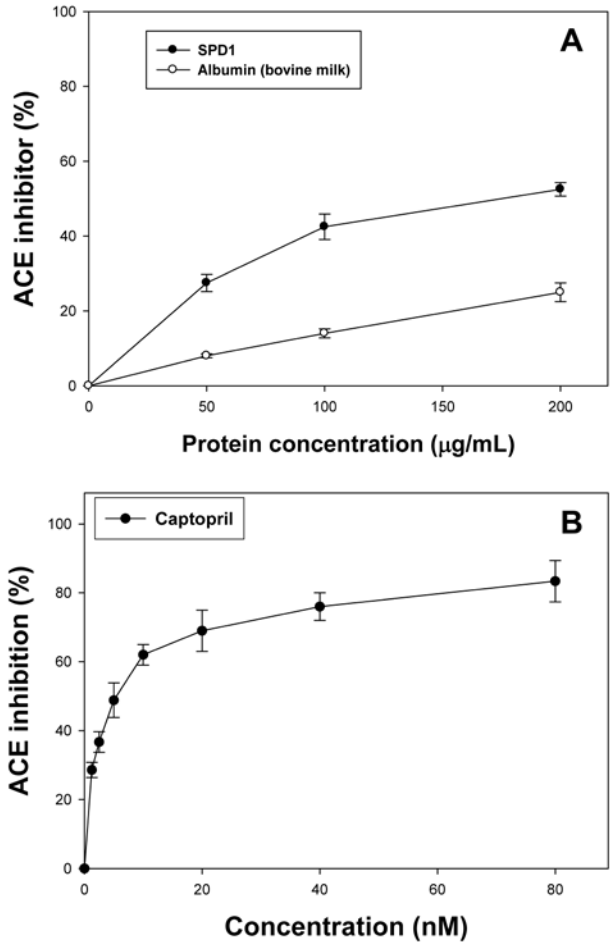


**Figure 2.** The effects of SPD1, albumin and Captopril on ACE activity determined by spectrophotometry. SPD1 (0, 50, 100 and 200 μg/mL) or bovine serum albumin (0, 50, 100 and 200 μg/mL) was used. ACE inhibition (%) was calculated according to the equation [1-(ΔA inhibitor ÷ ΔA control)] × 100%.

literature, the protein hydrolysates were used as sources to purify peptides as ACE inhibitors (Mullally et al., 1996). From calculations, the $IC_{50}$ of SPD1 against ACE activity was 190.47 μg/mL, which was smaller than the synthetic peptide α-lactorphin (YGLF, 322.7 μg/mL). Several identified peptide fragments exhibited much lower $IC_{50}$ values than our purified SPD1; 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). Alternately, several peptide fragments exhibited much higher $IC_{50}$ values than our purified SPD1; for example, hydrolysates of whey proteins (α-lactalbumin and β-lactoglobulin) were effective 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. In our previous paper (Huang et al., 2008b), the $IC_{50}$ of ACE activity required 187.96 μg/mL TI from sweet potato, which was lower than the $IC_{50}$ value of purified SPD1.

## Defensin ACE inhibitor activity determination using TLC

FAPGG and FAP (products 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 3 shows the qualitative results of TLC chromatograms of a silica gel 60 F254 about the effects of 225 μg/mL of commercial BSA (lane 3) or SPD1 (lane 4) on 15 microunits of ACE. Compared to the control test (lane 2), SPD1 (lane 4) inhibited ACE reaction showing less amounts of FAP production observed under UV light. However, similar amounts of FAP were found between the control test (lane 2) and BSA (lane 3). These results demonstrated again that SPD1 exhibited ACE inhibitor activity.

## Determination of the kinetic properties of ACE Inhibition by defensin

The Lineweaver-Burk plots of ACE (4 μU) with or without purified SPD1 (200 μg/mL) under different concentrations of FAPGG are shown in Figure 4. The results indicated that purified SPD1 acted as a mixed type inhibitor against ACE using FAPGG as a substrate. When 200 μg/mL SPD1 (10 μg) were added, Vmax and Km were, respectively, 0.01 ΔA/min and 0.69 mM; while they were 0.03 ΔA/min and 0.42 mM without SPD1. In conclusion,

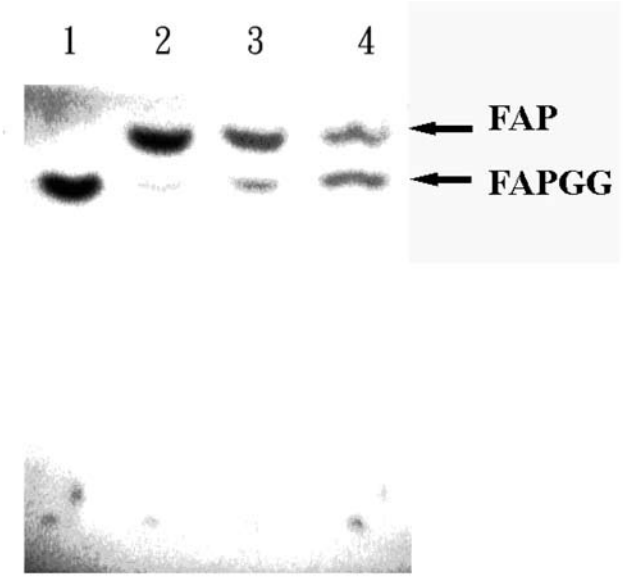


**Figure 3.** The TLC chromatograms of a silica gel 60 F254 showing the effects of defensin from sweet potato storage root or bovine serum albumin on ACE activity. Lane 1, blank test (FAPGG only); lane 2, control test (ACE reacted with FAPGG to produce FAP); lane 3, 225 μg/mL bovine serum albumin added; lane 4, 225 μg/mL SPD1 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. 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.

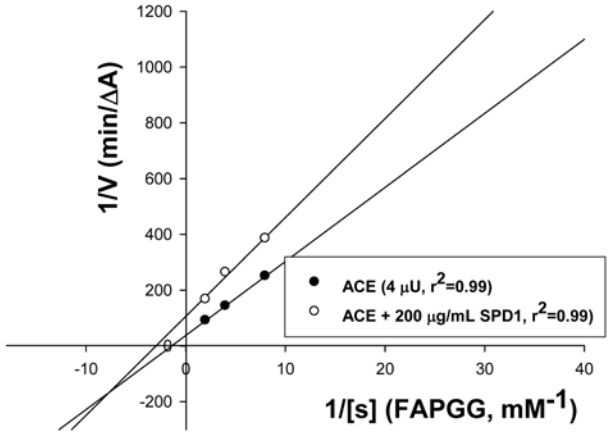


**Figure 4.** The Lineweaver-Burk plots of ACE (4 μU) without or with SPD1 (200 μg/mL) from sweet potato storage root using different concentrations of FAPGG (0.1 to 0.5 mM).

SPD1 exhibited dose-dependent ACE inhibitory activity and acted as a mixed type inhibitor with respect to the substrate (FAPGG). A similar work was reported with the calculated Km as 0.25 mM FAPGG for ACE in the presence of purified dioscorin, the calculated Km′ was 0.33 mM (Hsu et al., 2002).

The mixed-type inhibition suggests that there might be an inhibitor-binding site (or I-site) for SPD1 on the enzyme (ACE) surface in addition to the substrate-binding site (or S-site). It is noteworthy that SPD1 binds tighter to the I-site on the substrate-bound form of ACE than that on the free form. The $K_m$ values in the presence of the inhibitors are also higher than in their absence. In other words, binding of the substrate to the S-site increases the binding affinity of the inhibitor to the I-site, whereas binding of the inhibitor to the I-site decreases the binding affinity of the substrate to the S-site. The inhibitory mode of SPD1 was unique and hardly analyzed with a simple Michaelis-Menten-type interaction between the enzyme and inhibitor. The inhibitory mode of SPD1 must be examined kinetically and thermodynamically in the next phase of research.

## Determination of ACE inhibitory activity by defensin trypsin hydrolysates and their peptide distributions

Figure 5 shows the ACE inhibitory activity (Δ*A* 345 nm) of tryptic SPD1 hydrolysates. Figure 5A shows the ACE inhibition (percent) of tryptic SPD1 hydrolysates collected at set times. From the results (Figure 5A), ACE inhibitory activity increased from 52.47% (0 h) to about 74.38% (24 h). Figure 5B shows the chromatograms of unhydrolyzed SPD1 and tryptic SPD1 hydrolysates (24 h) on a Sephadex G-50 column. It was found that smaller peptides increased the longer that trypsin was hydrolyzed. The ACE inhibitor activities of tryptic SPD1 hydrolysates decreased after 24 h hydrolysis (Figure 5A), suggesting that some active ingredients got lost after 24 hours.

We used synthetic peptides (according to SPD1 gene sequence) to measure ACE inhibitor activity (Table 1). Kohmura et al. (1989) synthesized some human β-casein peptide fragments and found that their length had an influence on the ACE inhibitory activity. The necessary ACE inhibitors were namely peptides composed of 3-10 amino acids with proline on the C-terminal, and Leu-Arg-Pro, from food protein hydrolysates, is reportedly the most potent natural ACE inhibitor, with an $IC_{50}$ value of 0.27 μg/mL or 1.0 μM. Byun and Kim (2002) 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 required for a peptide to bind to ACE.

Synthetic peptides were designed by simulated trypsin cutting sites of *SPD1* gene (accession number: AY552546) products from sweet potato (http://www.expasy.org/tools/peptidecutter/). Six peptides, namely GFR, FK, IMVAEAR, GPCSR, CFCTKPC and MCESASSK, were synthesized based on the simulated trypsin digest of SPD1, then tested for ACE inhibitory activity (Table 1). $IC_{50}$ values

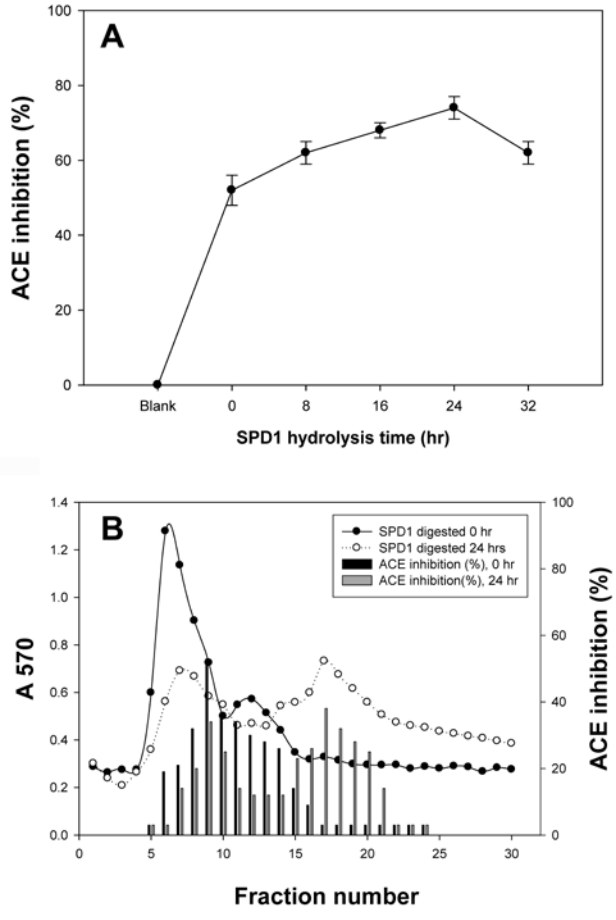


**Figure 5.** ACE inhibitor activity of tryptic hydrolysates of sweet potato SPD1. The plot shows the ACE inhibition (%) of tryptic SPD1 hydrolysates obtained at different trypsin hydrolysis time (A). The proteins and the inhibition of ACE (%) were shown (B). The inhibition of ACE (%) was calculated according to the equation [1-(ΔA inhibitor ÷ ΔA control)] × 100%.

**Table 1.** SPD1 peptides with ACE inhibition activity.

| SPD1 peptides | $IC_{50}$ (μM) |
|---|---|
| GFR | 94.25 ± 0.32 |
| FK | 265.43 ± 1.24 |
| IMVAEAR | 84.12 ± 0.53 |
| GPCSR | 61.67 ± 0.36 |
| CFCTKPC | 1.31 ± 0.07 |
| MCESASSK | 75.93 ± 0.64 |

Note: The sequence of SPD1 contains pre-pro-sequence. These sequences were retrieved from the NCBI (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov) with the following accession numbers AY552546.

of individual peptides were 94.25± 0.32, 265.43± 1.24, 84.12± 0.53, 61.67 ± 0.36, 1.31 ± 0.07 and 75.93 ± 0.64 μM, suggesting that CFCTKPC might represent the main domain for the ACE inhibition (Table 1). These results demonstrated that simulated synthetic peptides from tryptic SPD1 hydrolysates exhibited ACE inhibitory activities. Our work suggests that (1) CFCTKPC might represent the main active site for the ACE inhibition; (2) there are marked structural similarities in peptides with antihypertensive, immunomodulatory and antioxidant activities that may be used as criteria in selecting or designing multifunctional foods to control cardiovascular diseases.

In our previous paper, TI inhibited ACE activation in a dose-dependent manner. The $IC_{50}$ of ACE activity required 187.96 μg/mL TI. And TYCQ was synthesized based on the simulated pepsin digest of TI. The $IC_{50}$ values of the TYCQ peptide was 2.30 μM (Huang et al., 2008b). In this work the $IC_{50}$ values of the CFCTKPC peptide was 1.31 μM. This value was much better than TI and its hydrolysates. The CFCTKPC peptide contains seven amino acids with three cysteine residues. Thus far, the structure-activity correlations among the various ACE inhibitory peptides remain ambiguous. On the basis of some common structure patterns, it seems that the most favorable amino-terminal residues are branched amino acids such as Val and Ile and the most preferred carboxyterminus residues are among Trp, Tyr, Pro, or Phe (Zhou et al., 2010).

In summary, SPD1 exhibited dose-dependent ACE inhibitory activity. SPD1 acted as a mixed type inhibitor toward ACE with $IC_{50}$ of 1.31 ± 0.07 μM. Its peptic hydrolysates also showed ACE inhibitory activities. Some peptides derived from food proteins demonstrated antihypertensive activities against spontaneously hypertensive rats (Yoshii et al., 2001). The role of sweet potato in hypertension control deserves further investigation.

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# 甘藷塊根中防禦素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) 中大量表現甘藷塊根之重組蛋白質防禦素 (SPD1)，利用鎳離子螯合之親和性管柱純化。SPD1 經 SDS-PAGE 分析其分子量約為 8,600 Da。由以前的研究發現 SPD1 具有抗微生物活性，去氫抗壞血酸還原酶，單去氫抗壞血酸還原酶的活性。SPD1 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 SPD1，分別抑制 27.56 ~ 52.58% 血管收縮素轉化酶活性 )。SPD1 對於血管收縮素轉化酶之 50% 抑制濃度 ($IC_{50}$) 為 190.47 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 SPD1 對於血管收縮素轉化酶有抑制的效果。SPD1 對於血管收縮素轉化酶是屬於混合型抑制。利用胰蛋白腖以不同時間水解 SPD1 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 52.47 % (0 h) 增加到 74.38 % (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，血管收縮素轉化腖活性抑制會降低，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 SPD1 的結果，得到六種人工合成具有抑制血管收縮素轉化酶活性胜肽 : GFR, FK, IMVAEAR, GPCSR, CFCTKPC 和 MCESASSK，測定其 $IC_{50}$ 為 94.25± 0.32, 265.43± 1.24, 84.12 ± 0.53, 61.67 ± 0.36, 1.31 ± 0.07 和 75.93 ± 0.64 μM。結果發現 CFCTKPC 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，SPD1 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；防禦素 ; 血管收縮素轉化酶 ; 抑制作用。