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Studies on the inhibitory effect of *Graptopetalum paraguayense* E. Walther extracts on the angiotensin converting enzyme

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Abstract

This study was aimed at evaluating the kinetic properties and capacities of water (GWE), 50% ethanolic (GE50) and 95% ethanolic (GE95) extracts from *Graptopetalum paraguayense* for the potential to inhibit angiotensin converting enzyme (ACE). The results showed that GWE, GE50 and GE95 showed potent inhibitory effects on ACE. It was found that the ACE inhibitory activities of all the tested extracts increased with the increase of their concentrations. In addition, the ACE inhibition of the tested extracts of *G. paraguayense* were significantly reduced after the addition of 1.5 mM $ZnCl_2$, suggesting the inhibitory action of the extracts may have resulted from the chelation of the ACE zinc cofactor. The inhibition kinetics, analyzed by Lineweaver–Burk plots, revealed that *G. paraguayense* extracts showed a mixed-type inhibition. A comparison of the 50% inhibition concentration (IC_{50}) and K_i values showed that the ethanolic extracts, including GE50 and GE95 exhibited the more effective ACE inhibitory activity than the water extracts of *G. paraguayense*.

Keywords: *Graptopetalum paraguayense* E. Walther; Angiotensin converting enzyme; ACE inhibitor; Mixed-type inhibition

1. Introduction

Hypertension is a multifactorial process and also a risk factor involved in many diseases, including cardiovascular diseases, renal disease and diabetics. Angiotensin-converting enzyme (ACE; peptidyldipeptide hydrolase EC 3.4.15.1) is a zinc-containing enzyme, and plays an important physiological role in regulating blood pressure. This enzyme increases blood pressure by hydrolyzing the decapeptide angiotensin I to potent vasoconstrictor, angiotensin II, which exercises a powerful vasoconstrictive action and stimulates the secretion of aldosterone, which promotes sodium and water retention in the kidneys and the consequent increase in artery pressure. Furthermore, ACE also catalyses the degradation of bradykinin, a vasodialator (Erdös, 1975; Hernaändez-Ledesma, Martiän-Aälvarez, & Pueyo, 2003; Skeggs, Kahan, & Sumway, 1956). Therefore, inhibition of ACE results in an overall anti-hypertensive effect. Many studies have been directed toward the attempted synthesis of ACE inhibitors, such as captopril, benazepril, enalapril and lisinopril, which are widely used to treat cardiovascular diseases, including essential hypertension, heart failure, coronary artery disease and kidney failure (Ondetti, Rubin, & Cushman, 1977; Patchett et al., 1980). However, these synthetic drugs are believed to cause certain side effects, such as cough, taste disturbances and skin rashes (Atkinson & Robertson, 1979). As foods and natural herbal plants are rich sources of bioactive chemicals, and are mostly free from harmful side effects, there is an increasing interest in finding natural and effective ACE inhibitors from them. In recent years, many ACE inhibitory peptides have been isolated from the hydrolysates of many food materials (Ariyoshi, 1993; Choi, Cho, Yang, Ra, & Suh, 2001; Je, Park, Kwon, & Kim, 2004; Wu & Ding, 2002; Yamamoto, 1997). The

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occurrence of ACE inhibitors in some fermented foods have also been reported (Gobbetti, Ferranti, Smacchi, & Goffredi, 2000; Je, Park, Jung, Park, & Kim, 2005; Okamato et al., 1995). In addition, some herbal extracts, such as tannins and procyanidins, which have antioxidant ability also possess ACE inhibitory activity (Actis-Goretta, Ottaviani, Keen, & Fraga, 2003; Liu et al., 2003).

Graptopetalum paraguayense E. Walther, belonging to the class of Crassvalaceae, is a plant originally cultivated in Mexico and widely distributed in tropical and sub-tropical countries. Our in vitro study had showed that all the water (GWE), 50% ethanolic (GE50), and 95% ethanolic (GE95) extracts of *G. paraguayense* E. Walther exhibited strong antioxidative activities, including α,α-diphenyl-β-picryl-hydrazyl (DPPH) radicals scavenging effects, reducing power, and lipid peroxidation inhibitory ability (Chung, Chen, Hsu, Chang, & Chou, 2005). According to an archaic Chinese prescription, *G. paraguayense* E. Walther is used for alleviating hepatic disorders, diuretic effects, relieving pains and infections; furthermore, it is considered to have a blood-pressure lowering effect. However, there is no literature available regarding to the effects of *G. paraguayense* against ACE activity. Therefore, the objectives of this study were to investigate the ACE inhibitory effect of the *G. paraguayense* extracts, including GWE, GE50 and GE95, and to characterize such inhibition from a kinetic point of view. In addition, the results were compared with those of the well-known ACE inhibitor, captopril.

2. Materials and methods

2.1. Chemicals

ACE (1 U/ml, rabbit lung), *N*-[3-(2-furyl)acryloyl]-Phe-Gly-Gly (FAPGG) and captopril were purchased from Sigma Chemicals Co. (St. Louis, MO). All other chemicals were reagent grade or purer.

2.2. Preparation of the water (GWE), 50% ethanolic (GE50) and 95% ethanolic (GE95) extracts from G. paraguayense

The *G. paraguayense* extracts, including GWE, GE50 and GE95, were prepared as follows according to the procedures described previously (Chung et al., 2005). The *G. paraguayense* E. Walther was grown in a pot. When the leaves grew to a length of 3–5 cm, the leaves were cleaned, washed, cut into small pieces, and then freeze-dried by a vacuum freeze-dryer. Each 20 g of the plant was extracted with 700 ml of distilled water at 100 °C, 50% ethanol at 85 °C, or with 95% ethanol at 75 °C for 3 h. The decoction was filtered and then dried by a vacuum freeze-dryer. The yields of dry GWE, GE50 and GE95 were 3.84%, 1.95% and 2.50%, respectively. The extracts were sealed in plastic bottles and stored at −70 °C until use.

2.3. Assay for ACE inhibitory activity

The ACE activity, using FAPGG as substrate, was performed according to Holmquist, Bunning, and Riordan (1979), with modifications by Hsu, Lin, Lee, Lin, and Hou (2002). First, twenty microliters (20 μU) of commercial ACE was mixed with 190 μl of 50 mM Tris–HCl buffer (pH 7.5) and 10 μl of different amounts of *G. paraguayense* (0.16–7.5 mg/ml) and then incubated at 30 °C for 5 min. After incubation, 1 ml of 0.5 mM FAPGG was added to the reaction mixture. The solution was immediately monitored and the linear decreased in optical density recorded at 345 nm.

Inhibition effects on the enzyme activity by test samples were represented as % of inhibition, % inhibition $= (1 - B/A) * 100$, where $A = \Delta OD_{345}$/min without test sample and $B = \Delta OD_{345}$/min with test sample. Means of triplicate readings were determined. The 50% inhibition (IC_{50}) of ACE activity was calculated as the concentrations of test samples that inhibited 50% of ACE activity under the experimental conditions.

2.4. Kinetic analysis

Different concentrations of *G. paraguayense* extracts were added to each reaction mixture according to the method of Huang, Chen, Chang, and Chou (2005). The reaction mixture consisted of four different concentrations of FAPGG (0.1–0.64 mM) as substrate and ACE (20 μunits) in 50 mM Tris–HCl buffer (pH 7.5). Four different concentrations of GWE (5.1–61.5 μg/ml), GE50 (5.1–41.0 μg/ml), GE95 (2.6–41.0 μg/ml) or captopril (0.14–3.6 ng/ml) were added to the reaction mixture. The kinetics of the ACE in the presence of the inhibitor were determined by Lineweaver–Burk plots and the dissociation constant for the binding of inhibitor to the free enzyme (K_i) was also calculated.

2.5. The effect of $ZnCl_2$ on the inhibitory activity of ACE

As ACE is a Zn^{2+}-containing enzyme, 1.5 mM $ZnCl_2$ was added to the reaction mixture according to the method of Liu et al. (2003) to assess whether the inhibitory action of the *G. paraguayense* extracts resulted from the chelation of the Zn^{2+} ions. The experimental approaches including the supplementation of the ACE activity test system with or without $ZnCl_2$ were performed.

2.6. Statistical analysis

All the assays to determine enzyme activity, the ACE inhibitory effect of *G. paraguayense* and captopril and the enzyme kinetics, were conducted at least three times with three different sample preparations. All data were expressed as mean ± SD. Analysis of variance was performed by using SPSS (SPSS Inc., USA). Duncan's new multiple range tests was used to determine the difference

of means, and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Effect of G. paraguayense extracts on the activity of ACE

In the ACE inhibition assays, ACE catalyses the degradation of the substrate, FAPGG, so that the ACE activity could be derived from the decrease in absorbance during the reaction. FAPGG is the substrate of choice since it exhibits one of the highest activities with ACE (Vermeirssen, Camp, & Verstraete, 2002). Fig. 1 shows the dose–response curve for the ACE inhibitory effect of the *G. paraguayense* extracts. It was found that all *G. paraguayense* extracts had potent inhibitory effects on ACE activity and the inhibitory activities increased with the increase of extract concentrations. The results showed GE95 and GE50 had significantly higher ACE inhibitory activity than GWE. On the basis of the half-inhibition concentration (IC_{50}) for the extracts, GE95 had the highest ACE inhibitory activity as shown by the lowest value of IC_{50}, while GWE showed the least ability (Table 1). However, the IC_{50} values for GE50 and GE95 were not significantly different. These results suggest that the solvent for the extraction process may affect ACE inhibitory activity of the *G. paraguayense*. From a comparison of the IC_{50} values, the ACE inhibitory activity of captopril, a well-known ACE inhibitor, was found to be significantly more pronounced than those of *G. paraguayense* extracts. The obtained IC_{50} value for captopril was found to be 0.64 ng/ml (approximately 2.9 nM, Table 1). The determined value is similar to the literature values, where IC_{50} values for captopril range from 2.27 nM to 0.58 μM (Carr et al., 1990; Dyck, Nováková, Schepdael, & Hoogmartens, 2003; Inada et al., 1986; Udupa & Rao, 1997). The difference in IC_{50}value for captopril resulted from the different test conditions, the different origin of the ACE enzyme, and the different substrates used in the determination of ACE inhibitory activity (Vermeirssen et al., 2002). To obtain approximately 96% ACE inhibitory activity, the concentrations needed for GWE, GE50, GE95 and captopril were 61.5 μg/ml, 41.0 μg/ml, 41.0 μg/ml, and 3.6 ng/ml, respectively. In other words, to reach a similar extent of ACE inhibitory effect, the concentration required for *G. paraguayense* extracts was significantly higher than that required for captopril. Although the ACE inhibitory abilities of the extracts were significantly less than that of captopril, it was evident that the *G. paraguayense* extracts did have potent ACE inhibitory activities.

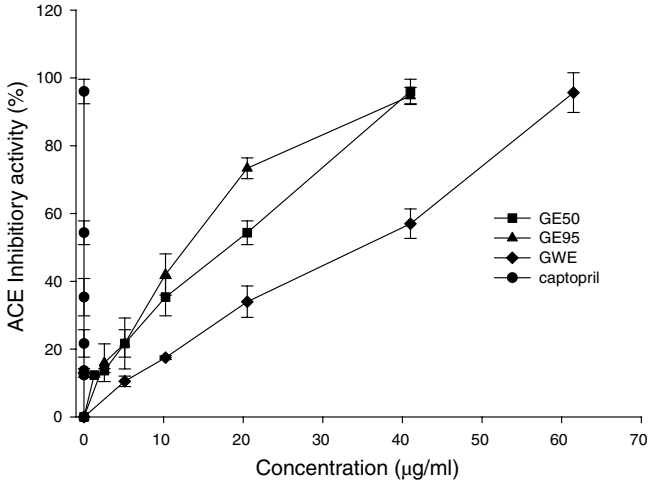

Fig. 1. ACE inhibitory activity of *G. paraguayense* extracts and captopril. Each value represents mean ± SD ($n = 3$).

Table 1
Inhibition constants of the *Graptopetalum paraguayense* extracts and captopril for angiotensin converting enzyme

Inhibitor	IC_{50}	K_i	Inhibition type
GWE	46.8 ± 2.5[a,d] μg/ml	16.2 μg/ml	Mix
GE50	19.6 ± 2.5[c] μg/ml	8.4 μg/ml	Mix
GE95	13.7 ± 2.0[c] μg/ml	9.8 μg/ml	Mix
Captopril	0. 64 ± 0.06[b] ng/ml	0.64 ng/ml	Competitive

[a] Values are given as mean ± SD ($n = 3$).
[b–d] Means in the same column followed by different letters are significantly different ($p < 0.05$).

3.2. Determination of the inhibition type of G. paraguayense extracts on ACE

The inhibition kinetics for the GWE, GE50 and GE95 were analyzed by Lineweaver–Burk plots as shown as Fig. 2. The five lines, obtained from the inhibited enzyme and from the four different concentrations of *G. paraguayense* extracts, intersected to the left of the $1/V$-axis above the $1/S$-axis (Fig. 2a–c). The results indicated that each of the three tested *G. paraguayense* extracts exhibited a mixed-type of inhibition with respect to the substrate (FAPGG). Without the *G. paraguayense* extracts, the calculated K_m was 0.275 mM FAPGG for ACE, which was close to the result (0.3 mM) of Holmquist et al. (1979). The mixed-type inhibition implies that *G. paraguayense* extracts affected the affinity of the enzyme for substrate, FAPGG, but did not bind at the active site (Webb, 1963). The kinetic and inhibition constants of *G. paraguayense* extracts were listed in Table 1. From the equilibrium constant for inhibitor binding, K_i, of the extracts, it was indicated that GE50 had the most effective binding capacity to the enzyme as evidenced by the lowest value of K_i, while GWE showed the least capacity. In other words, GE50 showed the highest ACE inhibitory ability among the extracts, followed by GE95 and GWE, in decreasing order.

Captopril, the first marketed orally active ACE inhibitor approved for treatment of human hypertension, was discussed by Cushman and Ondetti and coworkers (Cushman, Cheung, Subo, & Ondetti, 1977; Ondetti et al., 1977). The inhibition kinetics for captopril were also analyzed by

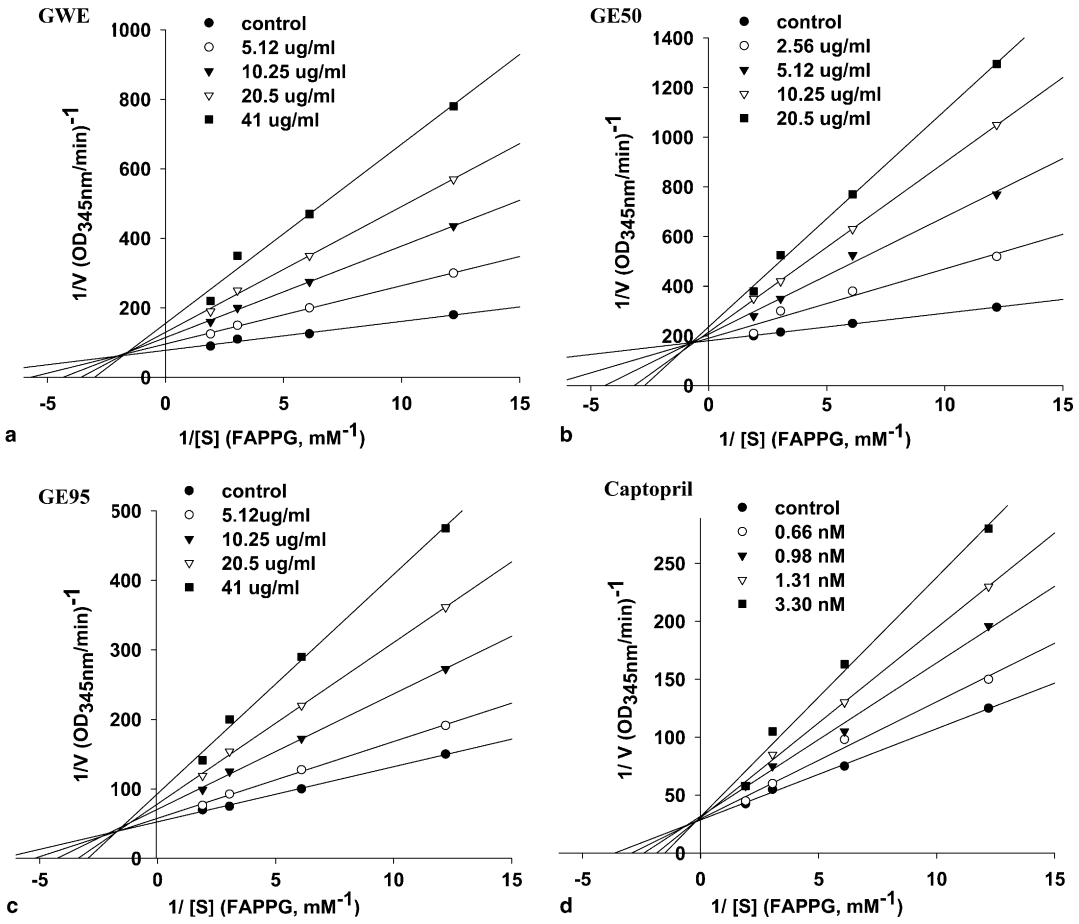

Fig. 2. Lineweaver–Burk plots for the inhibition of angiotensin-converting enzyme by *G. paraguayense* extracts and captopril. The reaction mixture contained the indicated concentrations of FAPPG (0.1, 0.2, 0.4, and 0.64 mM) as substrate and ACE (20 μU) in 50 mM Tris–HCl buffer (pH 7.5).

Lineweaver–Burk plots and it was found to be competitive (Fig. 2d). The result suggests that captopril inhibits the ACE activity by competing with the substrate for the active sites. As K_i in Table 1 indicated, captopril exerted much more effective inhibition on ACE than *G. paraguayense* extracts did, since its K_i value is in the subnanogram range (0.64 ng/ml). Because the most effective inhibitory component has not yet been isolated from *G. paraguayense* extracts, it is therefore apparent that crude *G. paraguayense* extracts do not exhibit superior activity compared to the captopril. However, safety is a primary consideration for ACE inhibitors, especially for those in anti-hypertensive agents. From the previous study in our laboratory, it appeared that *G. paraguayense* extracts were safe in genotoxicity (Chou, Chen, Yeh, & Chung, 2005) and exhibit antioxidative activities (Chung et al., 2005). It is therefore clear that the most effective ACE inhibitory component in *G. paraguayense* is worthy of further studies as a potential ACE inhibitor.

3.3. Effect of $ZnCl_2$ on the ACE inhibitory activity of the G. paraguayense extracts

The addition of 1.5 mM $ZnCl_2$ to the assay of the tested extracts-induced ACE inhibitory action showed that for GWE (added to 61.5 μg/ml), GE50 (41.0 μg/ml) and GE95 (41.0 μg/ml), this level of Zn reduced the inhibition of ACE activity significantly by 36.7% (95.7–60.6%), 32.6% (96.0–64.7%) and 39.1% (94.7–57.7%), respectively (Table 2). ACE is a zinc-metalloenzyme, and the zinc ion at the catalytic site is essential for enzyme activity. Supplementation of the ACE activity test system with $ZnCl_2$ is designed to reduce inhibition resulting from the chelation of the Zn^{2+} ion by *G. paraguayense* extracts. $ZnCl_2$ decreases the inhibitory activity of *G. paraguayense* extracts by approximately 33–39%, suggesting that the chelation of Zn^{2+} may be responsible, in a significantly part, at least,

Table 2
Effects of $ZnCl_2$ on the inhibitory activity of *Graptopetalum paraguayense* extracts on angiotensin converting enzyme

Extracts	Amount added (μg/ml)	Inhibition of activity (%) in the presence or absence of $ZnCl_2$	
		None	$ZnCl_2$ (1.5 mM)
GWE	61.5	95.7 ± 5.9[a]	60.6 ± 2.2*
GE50	41.0	96.0 ± 3.6	64.7 ± 2.2*
GE95	41.0	94.7 ± 2.5	57.7 ± 1.7*

Means in the same extract followed by asterisk are significantly different ($p < 0.05$) between two groups.
[a] Values are given as mean ± SD ($n = 3$).

for the ACE inhibitory activity of the *G. paraguayense* extracts.

4. Conclusions

In summary, this study implied a hypotensive effect of *G. paraguayense* extracts and showed a dose-dependent inhibitory effect on ACE. A study of the kinetics of inhibition of ACE shows that the *G. paraguayense* extracts are mixed-type inhibitors. The isolation and the structural elucidation of the active constituents of the extracts will provide useful leads in the development of anti-hypertensive agents. Therefore, purification of the effective ACE inhibitory component in *G. paraguayense* and investigation regarding the in vivo anti-hypertensive action await further study.

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References

Actis-Goretta, L., Ottaviani, J. I., Keen, C. L., & Fraga, C. G. (2003). Inhibition of angiotensin converting enzyme (ACE) activity by flavan-3-ols and procyanidins. *FEBS Letters, 555*, 597–600.

Ariyoshi, Y. (1993). Angiotensin-converting enzyme inhibitors derived from food proteins. *Trends in Food Science & Technology, 4*, 139–144.

Atkinson, A. B., & Robertson, J. I. S. (1979). Captopril in the treatment of clinical hypertension and cardiac failure. *Lancet Neurology, 2*, 836–839.

Carr, R. D., Higgs, L., Killingback, P. G., Nicol, A. K., O'Connor, S. E., Robson, A., et al. (1990). Pharmacological properties of FPL 63547, a novel inhibitor of angiotensin converting enzyme. *British Journal of Pharmacology, 100*, 83–89.

Choi, H. S., Cho, H. Y., Yang, H. C., Ra, K. S., & Suh, H. J. (2001). Angiotensin I-converting enzyme inhibitory from *Grifola frondosa*. *Food Research International, 34*, 177–182.

Chou, S. T., Chen, S. J., Yeh, J. Y., & Chung, Y. C. (2005). Antimutagenic potential of *Graptopetalum paraguayense* E. Walther extracts. *Food Science and Agricultural Chemistry, 43*, 349–357.

Chung, Y. C., Chen, S. J., Hsu, C. K., Chang, C. T., & Chou, S. T. (2005). Studies on the antioxidative activity of *Graptopetalum paraguayense* E. Walther. *Food Chemistry, 91*, 419–424.

Cushman, D. W., Cheung, H. S., Subo, E. F., & Ondetti, M. A. (1977). Design of potent competitive inhibitors of angiotensin converting enzyme. Carboxyalkanoyl and mercaptoalkanoyl amino acid. *Biochemistry, 16*, 5484–5491.

Dyck, S. V., Nováková, S., Schepdael, A. V., & Hoogmartens, J. (2003). Inhibition study of angiotensin converting enzyme by capillary electrophoresis after enzyme reaction at capillary inlet. *Journal of Chromatography A, 1013*, 149–156.

Erdös, E. G. (1975). Angiotensin-converting enzyme. *Circulation Research, 36*, 247–255.

Gobbetti, M., Ferranti, P., Smacchi, E., & Goffredi, F. (2000). Production of angiotensin I-converting enzyme inhibitory peptide in fermented milks started by *Lactobacillus delbruekii* subsp. *bulgaricus* SS1 and *Lactococcus lactis* subsp. *cremoris* FT5. *Applied and Environmental Microbiology, 66*, 3898–3904.

Hernaändez-Ledesma, B., Martiän-Aälvarez, P. J., & Pueyo, E. (2003). Assessment of the spectrophotometric method for determination of angiotensin-converting-enzyme activity: influence of the inhibition type. *Journal of Agricultural and Food Chemistry, 51*, 4175–4179.

Holmquist, B., Bunning, P., & Riordan, J. F. (1979). A continuous spectrophotometric assay for angiotensin converting enzyme. *Analytical Biochemistry, 95*, 540–548.

Hsu, F. L., Lin, Y. H., Lee, M. H., Lin, C. L., & Hou, W. C. (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. *Journal of Agricultural and Food Chemistry, 50*, 6109–6113.

Huang, K. F., Chen, Y. W., Chang, C. T., & Chou, S. T. (2005). Studies on the inhibitory effect of *Graptopetalum paraguayense* E. Walther extracts on mushroom tyrosinase. *Food Chemistry, 89*, 583–587.

Inada, Y., Terashita, Z., Imura, Y., Tanabe, M., Nishikawa, K., & Kikuchi, S. (1986). Inhibition of angiotensin converting enzyme by CV-3317, a non sulfhydryl compound. *The Japanese of Journal Pharmacology, 42*, 99–108.

Je, J. Y., Park, P. J., Kwon, J. Y., & Kim, S. K. (2004). A novel angiotensin I converting enzyme inhibitory peptide from Alaska Pollack (*Theragra chalcogramma*) frame protein hydrolysate. *Journal of Agricultural and Food Chemistry, 52*, 7842–7845.

Je, J. Y., Park, J. Y., Jung, P. W., Park, P. J., & Kim, S. K. (2005). Isolation of angiotensin I converting enzyme (ACE) inhibitor from fermented oyster sauce, *Crassostrea gigas*. *Food Chemistry, 90*, 809–814.

Liu, J. C., Hsu, F. L., Tsai, K. C., Chan, P., Liu, J. Y. H., Thomas, G. N., et al. (2003). Antihypertensive effects of tannins isolated from traditional Chinese herbs as non-specific inhibitors of angiotensin converting enzyme. *Life Science, 73*, 1543–1555.

Okamato, A., Hanagata, H., Matsumoto, E., Kawamura, Y., Koizumi, Y., & Yanagida, F. (1995). Angiotensin I converting enzyme inhibitory activities of various fermented foods. *Bioscience Biotechnology Biochemistry, 59*, 1147–1149.

Ondetti, M. A., Rubin, B., & Cushman, D. W. (1977). Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. *Science, 196*, 441–444.

Patchett, A. A., Harris, E., Tristram, E. W., Wyvratt, M. J., Wu, M. T., Taub, D., et al. (1980). A new class of angiotensin-converting enzyme inhibitors. *Nature, 298*, 280–283.

Skeggs, I. T., Kahan, J. E., & Sumway, N. P. (1956). The preparation and function of the angiotensin-converting enzyme. *The Journal of Experimental Medicine, 103*, 295–299.

Udupa, E. G. P., & Rao, N. M. (1997). Sheep testicular and epididymal angiotensin converting enzyme: inhibitions by captopril, lisinopril and enalapril. *Biochemistry and Molecular Biology International, 43*, 1063–1070.

Vermeirssen, V., Camp, J. V., & Verstraete, W. (2002). Optimization and validation of an angiotensin-converting enzyme inhibition assay for the screening of bioactive peptides. *Journal of Biochemical and Biophysical Methods, 51*, 75–87.

Webb, J. L. (1963). *Enzyme and metabolic inhibitors* (pp. 149–188). New York: Academic Press.

Wu, J., & Ding, X. (2002). Characterization of inhibition and stability of soy-protein-derived angiotensin I-converting enzyme inhibitory peptides. *Food Research International, 35*, 367–375.

Yamamoto, N. (1997). Antihypertensive peptides derived from food proteins. *Biopolymer, 43*, 129–134.