THE STUDY OF ANTIHYPERTENSIVE PEPTIDE DERIVED FROM CHICKEN LEG BONE PROTEIN

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Introduction

Hypertension is a worldwide trend and is one of the major factors indicated for stroke, arteriosclerosis and heart failure (Brian and Rosario, 2005). Angiotension I converting enzyme, ACE (dipeptidyl carboxypeptidase, EC 3.4.15.1) is a zinc metal peptidase associated with rennin-angiotensin system, a main mechanism of blood pressure regulation. Therefore, ACE inhibition is an important target to develop medicines for pharmacologic treatment of hypertension (Li *et al.,* 2004). Peptides from enzymatic hydrolysates, including ACE inhibitory, have been investigated for many years and have shown their biological activities. ACE inhibitory peptides derived from various sources of food proteins, released after hydrolysis or fermentation processing have been studied. In Taiwan, people prefer to consume deboned chicken meat products, especially parts of the chicken legs. As a result, more and more chicken leg bones are produced during processing. The purposes of this study are to develop ACE inhibitor derived from a hydrolysate of chicken leg bone protein and to orally administrate it in spontaneously hypertensive rats (SHR) to investigate its antihypertensive effects.

Materials and Methods

Sample preparation. According to the method of Cheng *et al.* (2008), chicken leg bones (broiler) were obtained from a meat processing factory, Tai-Chung, Taiwan. Chicken leg bone was ground with water and heated at 100 ºC for 5min, then digested for 12 h by Alcalase (Sigma, USA) at pH 8.0 and 50°C. The enzymatic hydrolysis was stopped every 2 h by boiling for 10 min and the hydrolysates collected. After incubation, the hydrolysates were centrifuged at 10,000×*g* for 10 min, filtered, lyophilized and stored at -80°C.

Assay of ACE inhibitory activity. The ACE inhibitory activity of hydrolysate was determined according to Cushman and Cheung (1971) with slight modifications. ACE (Sigma) prepared with 300 mM sodium tetraborate buffer (8 mU/ 50 µL), 30µL of hydrolysate and 50 µL of 5 mM hippuryl-L-histidyl-L-leucine(HHL) was incubated at 37°C for 30 min. The reaction was terminated by adding 380 µL of 1.0 N HCl. The resulting hippuric acid was extracted with 1.5 mL of ethyl acetate and dissolved in 1.0 mL of distilled water. The absorbance was read at 228nm and inhibitory activity was calculated by using the absorbance of hippuric acid liberated from HHL by ACE. The IC_{50} value was defined as the concentration of hydrolysates (mg/mL) required to inhibit 50% of ACE activity.

Antihypertensive activity of hydrolysates. Twenty four seven-week-old male SHRs were raised in an air-conditioned room (25°C) for 1 week, then randomly divided into 3 groups and orally administrated with hydrolysate (50mg/kg bw), Captopril (1.5mg/kg bw), or water. Tail systolic blood pressure was measured by tail-cuff method using indirect blood pressure meter (BP-98-A, Softron, Japan).

Results and Discussion

ACE Inhibitory activity of hydrolysates. Figure 1 represents ACE inhibitory activity of hydrolysates derived from enzymatic hydrolysis of chicken leg bone protein by Alcalase. Hydrolysates showed significantly higher ACE inhibitory activity ($P<0.01$) after hydrolysis and a dramatic increase in initial incubation (0-2 h), followed by decreased activity. At 4 and 8 h hydrolysis resulted in higher inhibitory activity (84.33 % and 85.68%) compared with other incubation times. Moreover, even though a higher inhibitory activity was found at 8 h incubation, hydrolysate at 4 h hydrolysis had lower IC_{50} value (0.545 mg/mL). Thus, four hour incubation hydrolysate (A4) with better ACE inhibitory activity after lyophilization was selected as a potent ACE inhibitor and test on SHR.

Antihypertensive activity of hydrolysates. The immediately antihypertensive activities of hydrolysates are shown in Figure 1. The systolic blood pressure (SBP) in the control group remained at 200 mmHg, while a reduction of 30 mmHg was observed at 2 h with the clinical drug, Captopril, which is a fast-acting and potent ACE inhibitor. A4 (50 mg/kg bw) only reduced the SBP by about 10 mmHg at 2 h after oral administration. However, a maximal reduction activity of about 26 mmHg was found at 4 h and was maintained for 8 h after oral administration. Fujita *et al.* (2000) have classified ACE inhibitory peptides derived from various sources of food proteins into three groups: (1) inhibitor type; (2) substrate type; (3) prodrug-type inhibitor according to the onset of antihypertensive activity. A4, as an enzymatic hydrolysate without purification and showing long-lasting antihypertensive activities, seemed to be peptides within it belonging to both the inhibitor type and prodrug type. Figure 2 shows changes in SBP of SHR after oral administration with A4 for 8 weeks. It was observed that all SBP of SHR increased according to age. The control group showed increased SBP, from 185 mmHg to 223 mmHg, but the SBP of Catopril and A4 were below 200 mmHg. In contrast, A4 exhibited a reduction effect as significant as that of Captopril, for control of long-term SBP in SHR. These findings suggest that A4 not only

shows strongly ACE inhibitory activity *in vitro*, but also works to reduce SBP of SHR with both immediate and long-term effects.

Table 1. ACE inhibitory activity of hydrolysates derived from hydrolysis of chicken leg bone protein by Alcalase

Time (h)	Inhibition of ACE[1] (%)	IC_{50} (mg/mL)
0	21.13 ± 2.10[e2]	
2	72.03 ± 8.42[bc]	
4	84.33 ± 1.74[a]	0.545
6	75.33 ± 8.24[b]	
8	85.68 ± 2.00[a]	0.612
10	79.14 ± 7.39[b]	
12	61.39 ± 6.27[d]	

[1] Values are means ± standard deviation of four replicate analyses.
[2] Means with different superscript letters in the column are significant (P<0.05).

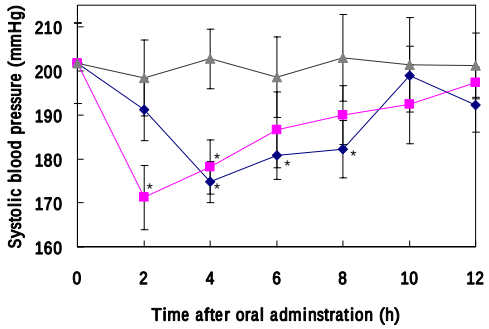

Figure 1. Changes in immediate systolic blood pressure of SHR by orally administering (50 mg/kg bw) with four hours incubation hydrolysate (A4). Captopril (1.5 mg/kg bw) was used as the positive control. Control was orally administered with deionized water. (◆,A4; ■,Captopril; ▲, Control). *significantly different from control (P<0.05). n=8.

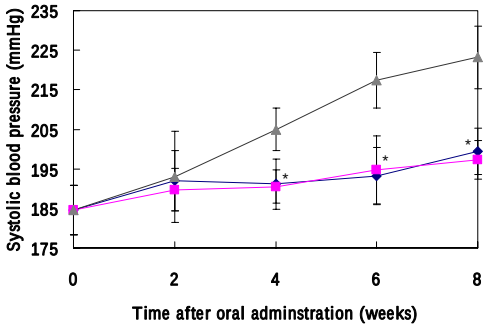

Figure 2. Changes in long-term systolic blood pressure of SHR by orally administering (50 mg/kg/bw day) for 8 weeks with A4. Captopril (1.5 mg/kg/bw day) was used as the positive control. Control was orally administered with deionized water. (◆,A4; ■,Captopril; ▲, Control).
*significantly different from control (P<0.05). n=8.

Conclusions

Chicken leg bone proteins hydrolyzed with Alcalase for 4 h showed potent ACE inhibitory activity. After oral administration in SHRs, A4 exhibited better antihypertensive activity in regards to both immediate and long-term effects as the clinical drug, Catopril. It is noted that A4 has potential to be applied and developed as ingredient of functional food for the treatment of hypertension.

References

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