Antihypertensive Effect of an Enzymatic Hydrolysate of Chicken Essence Residues

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Received September 17, 2001; Accepted February 18, 2002

Enzymatic hydrolysates with antihypertensive activity were obtained from the leftover residues of a chicken essence factory. Among many proteases tested, Alcalase was found to be the best to produce high angiotensin I-converting enzyme (ACE) inhibitory activity in vitro. The in vivo experiment revealed that a 3% supplement of the Alcalase-treated hydrolysate of chicken essence residues added to the control diet fed to spontaneously hypertensive rat (SHR) lowered the systolic blood pressure (SBP) of the rats after 16 weeks of treatment and at 1 week post-treatment. Repression of ACE activity of the aorta was suggested to be the reason for the antihypertensive effect of the Alcalase-treated hydrolysate of chicken essence residues.

Keywords: protein hydrolysate, peptide, chicken, angiotensin-converting enzyme, spontaneously hypertensive rat, blood pressure

The angiotensin I-converting enzyme (ACE) is important in the renin-angiotensin system (RAS) of the human body. This enzyme catalyzes the cleavage of the C-terminal dipeptide from inactive angiotensin I (DRVYIHPFHL) to become active angiotensin II (DRVYIHPF) (Skeggs et al., 1956), and inhibits the activity of the vasodilator bradykinin (Erdos, 1975). Consequently, this concerted action endows ACE with a crucial role in controlling blood pressure (Okamoto et al., 1995).

Many peptides with various inhibitory activity against ACE were isolated and identified from enzymatically hydrolyzed food proteins or fermented foods. These include peptides from α-zein (Miyoshi et al., 1991; Yano et al., 1996), bovine casein (Maruyama et al., 1985; Maruyama & Suzuki, 1982), chicken muscle (Fujita et al., 2000), dried bonito (Yokoyama et al., 1992), pepsin hydrolyzed Indonesian dried-salted fish (tuna) (Astawan et al., 1995), sour milk (Nakamura et al., 1995), sake and sake lees (Saito et al., 1994a, b), and soy sauce (Kinoshita et al., 1993). An overview of ACE inhibitory peptides derived from food proteins was well reviewed in two papers of Ariyoshi (1993) and Yamamoto (1997).

The ACE activity in several organs, such as the abdominal aorta, brain, lung and kidney, is thought to be responsible for the hypertension of spontaneously hypertensive rats (SHR) with treatments of pharmaceuticals like captopril and enalapril (Chevillard et al., 1988; Cohen et al., 1983; Unger et al., 1981). Persistent antihypertensive effect of ACE inhibitors might be related to sustained ACE inhibition in aorta and kidney (Unger et al., 1985). However, little is known about the action in vivo of ACE inhibitory peptides in the enzymatic hydrolysate of food proteins after oral administration. It was recently reported that the systolic blood pressure (SBP) and ACE activity in the aorta were significantly lower after long-term feeding of sour milk containing diet in SHR rats than in rats fed a control diet (Nakamura et al., 1996). The major ACE inhibitory peptides, Ile-Pro-Pro and Val-Pro-Pro, were further detected in the abdominal aorta of SHR rats after oral administration of sour milk (Masuda et al., 1996).

Chicken essence is a popular commercial product in several Pacific/Asia countries. The traditional method of producing chicken essence is based on steam cooking of the raw materials in a pressurized cooker for several hours, then the liquid fraction collected is further processed to remove oil and fine particles. This method produces a large amount of proteinaceous residues. Recently, enzymatic hydrolysis of the raw materials was applied in the process; still, the residues contain undigested proteins that can be further processed.

We report here the preparation of hydrolysates with different proteases from chicken essence residues of a local factory. The one with the highest ACE inhibitory activity in vitro was used as a diet supplement for SHR rats to determine its ACE inhibitory activity in vivo.

Materials and Methods

Materials The precipitated residues from hydrolyzed chicken meat were obtained from a local food factory. Alcalase (2.4L), Esperase and Flavourzyme were obtained from the Novo Nordisk Co. ( Bagsvaerd, Denmark). Bromelain is a product of HM Co. (Bangkok, Thailand).

An ACE diagnostic kit, containing Reagent (N-[3-(2-furyl)acryloyl]-L-phenylalanlyglycylglycine, FAPGG, ACE’s substrate) and Calibrator (porcine ACE in human serum) was purchased from Sigma Co. (St. Louis, MO). The UV-visible spectrophotometer used in this study is Model UV-160A of Shimadzu Co. (Tokyo) and a programmable sphygmomanometer (Model BP-98A; Softron Co., Tokyo) was used to measure the systolic blood pressure (SBP) of SHR rats.

Methods

Proximate analysis of the chicken residues Proximate analysis (ash, crude fat, crude fiber, crude protein, moisture) of the chicken residues was conducted according to the AOAC method (AOAC, 1995).

Preparation of the chicken residue hydrolysates Two parts of water was added to one part chicken residue and thoroughly mixed. To 500 g of the chicken residue suspension in a 1 l screw-capped laboratory bottle (Merk Co., Darmstadt, Germany), 1%
The IC50 of each sample was the protein concentration that resulted in a 50% inhibition of the ACE activity. The activity of calibrator was provided by Sigma and varied slightly among different lots, but was generally around 45 U/ml. A 0.05 ml aliquot of calibrator was then added and incubated in a 37˚C water bath for 5 min. A 0.05 ml aliquot of calibrator was then added and incubated for another 10 min. Absorbances at 340 nm were measured at exactly 5 min (initial A's) and 10 min (final A's) after the addition of the calibrator. The ACE activity was expressed as:

\[ \text{ACE activity} = \frac{\text{Initial A's} - \text{Final A's (sample)}}{\text{Initial A's} - \text{Final A's (blank)}} \times \text{activity of calibrator in 37°C.} \]

The activity of calibrator was provided by Sigma and varied slightly among different lots, but was generally around 45 U/ml. Five different replicated concentrations of each sample solution were measured by micro-biuret method (Itzhaki & Gill, 1964). Measurement of degree of hydrolysis (DH) was based on the following equation:

\[ \text{DH} = \frac{\text{Formol Nitrogen}}{\text{Total Nitrogen}} \times 100\%; \]

whereas the determinations of FN and TN were based on the AOAC (1995).

Determination of ACE inhibitory activity The Sigma method (1994) was used to assay the ACE inhibitory activity. Each bottle of the reagent (FAPGG) and calibrator (porcine ACE in human serum) from the Sigma kit was mixed well with 10 ml and 0.5 ml of deionized distilled water, respectively. A 0.05 ml aliquot of deionized distilled water or sample solution was added to 1 ml reagent in a cuvette and incubated in a 37˚C water bath for 5 min. A 0.05 ml aliquot of calibrator was then added and incubated for another 10 min. Absorbances at 340 nm were measured at exactly 5 min (initial A's) and 10 min (final A's) after the addition of the calibrator. The ACE activity was expressed as:

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whereas the determinations of FN and TN were based on the AOAC (1995).

**Table 1. Proximate analysis of the chicken residues.**

<table>
<thead>
<tr>
<th>Item</th>
<th>Content (g/kg)</th>
<th>Wet basis</th>
<th>Dry basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>6.8</td>
<td>22.9</td>
<td></td>
</tr>
<tr>
<td>Crude carbohydrate</td>
<td>40.3</td>
<td>136.0</td>
<td></td>
</tr>
<tr>
<td>Crude fat</td>
<td>69.7</td>
<td>235.2</td>
<td></td>
</tr>
<tr>
<td>Crude fiber</td>
<td>42.3</td>
<td>142.7</td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>137.3</td>
<td>463.2</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>703.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. IC50 values for ACE inhibition and the degrees of hydrolysis (DH) of various chicken residue hydrolysates.**

<table>
<thead>
<tr>
<th>Protease</th>
<th>IC50 (x10^2 mg protein/ml)</th>
<th>Protein concentration (mg/ml)</th>
<th>DH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>142.4</td>
<td>10.77</td>
<td>20.47</td>
</tr>
<tr>
<td>Alcalase</td>
<td>27.3</td>
<td>21.13</td>
<td>16.83</td>
</tr>
<tr>
<td>Bromelain</td>
<td>31.3</td>
<td>21.75</td>
<td>16.53</td>
</tr>
<tr>
<td>Esperase</td>
<td>37.2</td>
<td>23.67</td>
<td>13.66</td>
</tr>
<tr>
<td>Flavourzyme</td>
<td>46.3</td>
<td>14.55</td>
<td>38.14</td>
</tr>
</tbody>
</table>

**Results and Discussion**

**Proximate analysis of the chicken residues** The results of proximate analysis of the chicken residues are shown in Table 1. The crude protein content was 13.73% on a wet basis, and 46.32% on a dry basis.

**ACE inhibitory activities of hydrolysates of chicken essence residues prepared with various proteases** The properties, including IC50, protein concentration and DH, of various enzymatic hydrolysates of chicken essence residues and commercial peptide products are shown in Table 2. These parameters revealed the two facets of proteases: to solubilize proteins from the residues and to hydrolyze the proteins into small peptides and amino acids. It seemed that Esperase showed the highest ability to solubilize more protein materials, but its ability to hydrolyze protein was relatively low. Flavourzyme, on the other hand, did not show high ability to solubilize protein from the residues, but its ability to hydrolyze the solubilized protein was relatively high. Compared to Esperase, Alcalase and Bromelain showed slightly lower...
activities in solubilizing protein residues and slightly higher activities in hydrolyzing solubilized protein.

The Alcalase-treated hydrolysate showed the highest ACE inhibitory activity, i.e. the lowest IC₅₀, as determined by the Sigma method (Table 2). Flavourzyme-treated hydrolysate, on the other hand, showed the lowest ACE inhibitory activity, i.e., the highest IC₅₀ while the hydrolysates prepared with bromelain and Esperase showed IC₅₀ values between those of Alcalase and Flavourzyme treatments. The control sample, which was not treated with any protease, showed the highest IC₅₀ values, indicating the lowest ACE inhibitory effect.

The method modified from that of Cushman & Cheung (1971) is widely used for in vitro assay of the activity of ACE. The theory behind the method is treating a chromogenic substrate, hippuryl-histidyl-leucine (HHL), with rabbit lung ACE, and the amount of released hippuric acid is in proportion to the absorbance at 228 nm. However, the protocol of the method is complex and time consuming. Another argument has to do with the physiological implication in human since the ACE was from rabbit lung. Harjanne (1984) described a y=3.574x−6.06 relationship, where y is the value obtained by the Sigma method and x is the value obtained by the HHL method.

**SHR feeding experiment** The growth curves of the SHR are shown in Fig. 1a. Test groups showed similar growth rate as that of the control group. The feed consumption of the control, 1% and 3% groups was 20.1±1.5, 20.3±1.7 and 19.6±1.3 g/day, respectively. The results suggested that the consumption of the Alcalase-treated hydrolysate of chicken essence residues did not interfere with the SHR appetite nor their growth. Changes in SBP are shown in Fig. 1b. Both test groups show lower SBP than that of the control group after 6 weeks of the experiment (14-weeks of age), the difference between control and 3% groups became significant at 23-weeks of age and until the end of test feeding experiment (24-weeks of age). SBP of control group was 194±7 mmHg, the 1% group was 176±11 mmHg and the 3% group was 168±7 mmHg at 24-weeks of age. So a 18 mmHg and a 26 mmHg (p<0.05) decrease in the SBP was observed for the 1% and 3% groups, respectively. A further 1-week feeding with the control diet for rats of all groups did not show much difference on SBP to that observed at 24-weeks of age. They were 189±9 mmHg for the control group, 173±7 mmHg for the 1% group, and 168±9 mmHg for the 3% group, respectively, at 25th-week of age. A 16 mmHg and a 21 mmHg (p<0.05) decrease in the SBP was thus observed for the 1% group and 3% group, respectively, at the 25-weeks of age. Although less of a lowering effect on SBP was observed after the test diet was replaced with the control diet, the 3% group still showed a more significant lowering than the control group. Similar results were observed by Nakamura et al. (1996) with sour milk supplement to the diet of SHR. Two and one-half percent sour milk in the diet started at 7 weeks of age caused a significant lowering (19 mmHg) on SBP of the SHR at 23-weeks, with no effect on body weight. The sour milk had a sustained effect even 48 h after substitution of the diet for the control diet.

The ACE activities of various organs are shown in Table 3. Those of the 1% group did not show significant differences from those of the control group at 24-weeks of age (the end of 16 weeks of treatment) or at 25-weeks of age (1 week post-treatment). At 24-weeks, ACE activity of the aorta was more significantly lowered in the 3% group than in the control group. The feed consumption of the control, 1% and 3% groups was 20.1±1.5, 20.3±1.7 and 19.6±1.3 g/day, respectively. The results suggested that the consumption of the Alcalase-treated hydrolysate of chicken essence residues did not interfere with the SHR appetite nor their growth. Changes in SBP are shown in Fig. 1b. Both test groups show lower SBP than that of the control group after 6 weeks of the experiment (14-weeks of age), the difference between control and 3% groups became significant at 23-weeks of age and until the end of test feeding experiment (24-weeks of age). SBP of control group was 194±7 mmHg, the 1% group was 176±11 mmHg and the 3% group was 168±7 mmHg at 24-weeks of age. So a 18 mmHg and a 26 mmHg (p<0.05) decrease in the SBP was observed for the 1% group and 3% group, respectively. A further 1-week feeding with the control diet for rats of all groups did not show much difference on SBP to that observed at 24-weeks of age. They were 189±9 mmHg for the control group, 173±7 mmHg for the 1% group, and 168±9 mmHg for the 3% group, respectively, at 25th-week of age. A 16 mmHg and a 21 mmHg (p<0.05) decrease in the SBP was thus observed for the 1% group and 3% group, respectively, at the 25-weeks of age. Although less of a lowering effect on SBP was observed after the test diet was replaced with the control diet, the 3% group still showed a more significant lowering than the control group. Similar results were observed by Nakamura et al. (1996) with sour milk supplement to the diet of SHR. Two and one-half percent sour milk in the diet started at 7 weeks of age caused a significant lowering (19 mmHg) on SBP of the SHR at 23-weeks, with no effect on body weight. The sour milk had a sustained effect even 48 h after substitution of the diet for the control diet.

The ACE activities of various organs are shown in Table 3. Those of the 1% group did not show significant differences from those of the control group at 24-weeks of age (the end of 16 weeks of treatment) or at 25-weeks of age (1 week post-treatment). At 24-weeks, ACE activity of the aorta was more significantly lowered in the 3% group than in the control group. The

![Figure 1](image.png)

**Fig. 1.** (a) Effect on body weight of chronic treatment of the Alcalase-treated hydrolysate of chicken essence residues. (b) Effect on systolic blood pressure of chronic treatment of the Alcalase-treated hydrolysate of chicken essence residues for 16 weeks and its withdrawal for an additional 1 week.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma activity (mU/mg protein)</th>
<th>Aorta activity (mU/mg protein)</th>
<th>Kidney activity (mU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-weeks of age</td>
<td>87±26</td>
<td>124±28</td>
<td>5.1±1.3</td>
</tr>
<tr>
<td>1%</td>
<td>74±11 (84.9)</td>
<td>98±25 (79.0)</td>
<td>3.9±1.5 (76.5)</td>
</tr>
<tr>
<td>3%</td>
<td>91±21 (104.6)</td>
<td>58±20 (48.6)</td>
<td>4.2±0.6 (82.4)</td>
</tr>
<tr>
<td><strong>25-weeks of age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>88±20</td>
<td>106±31</td>
<td>5.1±2.0</td>
</tr>
<tr>
<td>1%</td>
<td>82±19 (93.2)</td>
<td>94±30 (88.7)</td>
<td>3.7±1.7 (72.5)</td>
</tr>
<tr>
<td>3%</td>
<td>94±26 (106.8)</td>
<td>76±28 (71.7)</td>
<td>4.0±1.0 (78.4)</td>
</tr>
</tbody>
</table>

Reported are mean±95% confidence, n=4.

Figures in parentheses represent the percentage of respective control values.

* p<0.05 treated vs. control.
ACE activities of plasma and kidney in the 3% group did not differ from those in the control group. The antihypertensive effect of the Alcalase-treated hydrolysate of chicken essence residues towards SHR at 24-weeks of age may largely be associated with inhibition of the ACE in the aorta for the 3% group. After the rats in this group were fed the control diet for one more week, the ACE activities of plasma and kidney remained the same. The ACE activity of aorta recovered from 58 mU/mg protein at 24-weeks of age to 76 mU/mg protein, or from 46.8% to 71.7% of respective control values. The result may explain the weaker lowering effect on SBP of SHR rats after the test diet was replaced with the control diet. It also suggests that the ACE activities of different organs recover from the inhibition of the Alcalase-treated hydrolysate of chicken essence residues at different rates. The delayed recovery of aorta ACE activity post-treatment may be the reason for the persistent antihypertensive effect of the Alcalase-treated hydrolysate of chicken essence residues. Unger et al. (1985) reported that ACE inhibition within peripheral tissues, such as the vascular wall or the kidney, may be equally important for the chronic antihypertensive actions of ACE inhibitors. Nakamura et al. (1996) reported that the ACE activity of aorta may be important in expression of antihypertensive activity of the sour milk in SHR rats. The ACE inhibitory tripeptides, Ile-Pro-Pro and Val-Pro-Pro, were further detected in the abdominal aorta after oral administration of the sour milk to SHR rats (Masuda et al., 1996). Results in this paper also suggest the importance of the aorta ACE activity under the repression of the Alcalase-treated hydrolysate of chicken essence residues, probably also due to accumulation of the inhibitory peptides in the aorta. However, further questions are raised by this assumption. Isolation and identification of the ACE inhibitory peptides of the Alcalase-treated hydrolysate of chicken essence residues are currently being performed. The mechanisms for ACE inhibition and metabolism of the peptides in vivo are also yet to be clarified.

Acknowledgment Financial support for this study from the Ministry of Economic Affairs, Taiwan, the Republic of China, is highly appreciated (88-EC-2-A-17-237).

References
Sigma. (1994). Angiotensin converting enzyme. Procedure 305-UV, Sigma Diagnostics, St. Louis, MO, USA.