Effects of Angiotensin I-Converting Enzyme Inhibitory Substances Derived from Indonesian Dried-salted fish on Blood Pressure of Rats

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Indonesian dried-salted fish (DSF) was produced from skipjack tuna by soaking the flesh in 15% NaCl (DSF I) or 25% NaCl (DSF II). The DSFs were then hydrolyzed by trypsin, chymotrypsin, Pronase E, and pepsin. Angiotensin I-converting enzyme (ACE) inhibitory activity was measured. The pepsin digest showed the highest inhibitory activity (IC50: 0.63 mg protein/ml). DSF II hydrolysate had higher inhibitory activity than that in DSF I. A three-month storage period of DSF gave higher ACE-inhibitory activity than that of 6 months. An oral administration of pepsin hydrolysate significantly decreased the blood pressure of rats. From the purification steps, at least 4 inhibitor peptides were found. The amino acid sequences of the peptides were Val-Ala-Trp-Lys-Leu, Trp-Ser-Lys-Val-Val-Leu, Ser-Lys-Val-Pro-Pro, and Cys-Trp-Leu-Pro-Val-Tyr, with an IC50 value of 31.97, 156.28, 74.22, and 22.20 μM, respectively.

Proteins from natural resources are important for supplying nutrients and energy, and these roles are defined as a primary function of protein.11 Protein also has many potential tertiary functions relating to physiological regulation.23 A number of functional peptides derived from milk and soybean have already been identified. The peptides promote calcium absorption,3 reduce blood pressure,4,6 and regulate cholesterol in serum.5

There is great interest in the preparation and isolation of peptides from natural resources involved in blood pressure regulation. These peptides have been known as potent inhibitors of angiotensin I-converting enzymes. Angiotensin I-converting enzyme (ACE; peptidyldepeptidase hydrolyase, EC 3.4.15.1), belongs to the class of zinc proteases that need zinc and chloride for their activation.6

This enzyme plays an important physiological role in regulating blood pressure by converting an inactive form of decapetide, angiotensin I, to a potent vasoconstrictor, octapeptide angiotensin II (by cleaving the dipetide at the C-terminal of angiotensin I), and inactivating bradykinin, which has depressor action.7–10

Some specific inhibitors of ACE, such as captopril and enalapril, have been proved to be useful as antihypertensive drugs. ACE-inhibitory peptides derived from food proteins may prevent hypertensive diseases. For this purpose, ACE-inhibitory peptides have been isolated from casein,11–12 zein,13–15 gelatin,21 soy sauce,16 soybean, corn, wheat,17 and other food proteins. Much research has also been done using fish muscle or fish products, such as sardine muscle,16–20 tuna muscle,21–22 and bonito.23–25

Since the ACE-inhibitory activities from Indonesian traditional fish products had never been reported, the objectives of this work were to study the ACE-inhibitory activity in Indonesian dried-salted fish (DSF) and to test its effect lowering the blood pressure of rats.

Materials and Methods

Materials. Pepsin (porcine stomach mucosa), trypsin (bovine pancreas, type I), chymotrypsin (bovine pancreas, type VII), and Pronase E (type XIV from Streptomyces griseus) were purchased from Sigma Chemical Co. Hippuryl-histidyl-leucine (HHL) was obtained from the Peptide Institute Inc., and angiotensin I-converting enzyme (ACE) was from Wako Chemical Ltd.

Fresh (non-frozen) Skipjack tuna (Katsuwonus pelamis), mean weight about 4 kg and mean length about 60 cm, was purchased from Tsukiji Fish Market, Tokyo. The fish was iced during transportation to the laboratory and then processed as described below.

Preparation of samples. DSF products were prepared as described previously.24 The fish were split, gutted, washed with tap water, and filleted. Fillets were cut into 60–70 g slices, and divided into 3 groups. The control group (unprocessed fish) was freeze-dried. The other groups were immersed for 24 h in 15% (DSF I) or 25% pure NaCl solution (w/v) (DSF II) with a fish-to-brine ratio of 1:2, v:v. After salting, the fish was removed and placed on drying trays and dried in an artificial drier at 45°C for 40 h.

The DSF I and DSF II were divided into 3 groups. The first group was immediately freeze-dried to represent 0 month of storage period. The other groups were scaled with minimal headspace in loose-fitting polyethylene bags and stored at 28°C for 3 and 6 months. This storage condition was chosen to model the typical storage time seen in Indonesia.

The desalting was done by soaking the DSF in tap water for 12 h. The product was then freeze-dried and used as a sample. Defatting was done using chloroform and methanol (2:1),27 and then drying at 45°C to evaporate the chloroform.

Fish protein hydrolysates were prepared by the method of Yokoyama et al.28 with a little modification. Two-g freeze-dried samples were suspended in 100 ml of distilled water, homogenized for 2 min, and boiled at 98°C for 15 min. After cooling, the homogenate was digested by various proteases (trypsin, chymotrypsin, Pronase E, and pepsin). The digestion

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Abbreviations: DSF, dried-salted fish; ACE, angiotensin I-converting enzyme; IC50, the concentration of an ACE inhibitor required to inhibit 50% of the ACE activity.
was done in the presence of 880 μg enzyme/ml homogenate at 37°C for 3 to 30 h. Before pepsin digestion, the homogenate was adjusted to pH 2 with hydrochloric acid. Digestions by other proteases were done at pH 7.5. The digests were boiled for 15 min to inactivate the enzymes. The pepsin digest was adjusted to pH 7 with sodium hydroxide before boiling. After centrifugation at 10,000 rpm for 15 min, the supernatant was filtered and analyzed.

The hydrolysate was lyophilized to obtain a powder product. The powder was used for further purification steps and to test its effects on lowering blood pressure in rats.

**Chemical analysis.** Protein contents of fresh fish and DSF were measured by the Kjeldahl procedure, while the protein content of hydrolysates was measured by the Lowry method. Total fat was measured by extraction with n-hexane, and methanol (2:1) as described by Folch et al. Moisture was measured by oven drying at 105°C to constant weight, ash by heating at 600°C, and salt by titration against 0.1 M silver nitrate.

**In vitro assays of ACE-inhibitory activity.** Inhibitory activity of ACE was analyzed by spectrophotometric measurement of the rate of formation of hippuric acid from hippuryl-l-histidyl-l-leucine (HHL), by the method of Cheung and Cushman. The concentration of ACE inhibitors (hydrolysates) reducing 50% of ACE activity was defined as the IC₅₀ value.

**Purification of ACE-inhibitory substance.** Purification of the ACE-inhibitory substance was done with column chromatography as described by Maruyama and Suzuki, with a little modification. About 0.35 g of freeze-dried powder (obtained from pepsin hydrolysate), was diluted in 5 ml of distilled water and then put on a Sephadex G-25 column (2.5 × 80 cm) and eluted with distilled water. The active fraction was collected, concentrated to 5 ml, put on a second column (1.5 × 55 cm) packed with SP-Sephadex C-25 and eluted with a linear gradient using 200 ml each of distilled water and 0.5 M ammonium formate (pH 7.0). The most active fraction was put on a third column (2.5 × 65 cm) packed with Sephadex G-25, and eluted with distilled water.

The active substances obtained from the last column were further purified by high pressure liquid chromatography (HPLC). The substances were injected into a Tosoha TSK gel ODS-80 Ts column (6.0 × 150.0 mm). The column was developed at a flow rate of 2.0 ml/min by a linear gradient of acetonitrile (0-50%/30 min) containing 0.1% trifluoroacetic acid. The active fractions were collected and concentrated with a centrifugal concentrator and then were purified in the same column, and eluted with 15-25% acetonitrile (1.5 ml/min). Each chromatogram was monitored by the absorbance at 210 nm of ultraviolet light.

Amino acid analysis of each ACE-inhibitory substance was done with a Tosoha HPLC on a Tosoha TSK Octadex-2PW column (4.6 × 150 mm) after hydrolysis with 6 N HCl at 130°C for 3 h in a vacuum. Amino acid sequencing of peptides was done with a Shimadzu PPSQ-10 protein-peptide sequencer.

**In vivo assays of ACE-inhibitory activity.** Six-week-old male Wistar rats (Tokyo Experimental Animal Co., Ltd.) weighing approximately 72-75 g, were used. The rats were fed a commercial non-purified diet (type MF, Oriental Yeast Co.) for 3 days to help them acclimate to their new environment. After 3 days, the rats were weighed and housed in individual metabolism cages with wire bottoms in a room maintained at 22 ± 3°C, relative humidity of 50 ± 10% and 12 h light-dark cycle beginning at 7:00 a.m. The rats were divided into 5 groups of 6 rats each according to the source of dietary protein. Lactic casein, defatted DSF, desalted DSF, untreated DSF, and untreated DSF + ACE-inhibitory substances were the sole protein sources in the diet. The chemical composition of these samples is shown in Table I. The powder of pepsin hydrolysate was used as a source of ACE-inhibitory substances. As much as 5 g of powder per kg body weight per day was fed orally for 16 days. Before giving it to the rats (at around 11:00 a.m.), the powder was diluted with 1 ml of 0.9% NaCl solution and then orally administered.

**Diets** were prepared as recommended by the Association of Official Analytical Chemists (AOAC) (Table II), containing 10% of protein. The rats were fed a restricted diet, only 10 g per day per rat (control pair fed) for 16 days. The main purpose of this treatment was to obtain a maximum growth rate of rat during the feeding periods. Because of the almost constant body weight, it becomes easier to observe some effects of diets without any concern with body weight gain. This treatment was done

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>NaCl (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated DSF</td>
<td>3.8</td>
<td>22.7</td>
<td>22.8</td>
<td>12.7</td>
<td>57.0</td>
</tr>
<tr>
<td>Desalted DSF</td>
<td>2.6</td>
<td>7.3</td>
<td>7.6</td>
<td>16.8</td>
<td>60.8</td>
</tr>
<tr>
<td>Defatted DSF</td>
<td>4.3</td>
<td>25.4</td>
<td>24.4</td>
<td>1.3</td>
<td>65.8</td>
</tr>
</tbody>
</table>

* Freeze dried sample.

DSFs were produced by immersing the flesh of fish in 25% NaCl solution for 12 h and then drying at 45°C for 40 h.

**Results and Discussion**

**Chemical composition of samples**

The effects of desalting or defating treatment on the chemical composition of samples are presented in Table I. Desalting treatment significantly decreased the NaCl (from 22.8 to 7.6%) and ash, but slightly increased the fat and protein contents of DSF. Defating treatment significantly decreased the fat content of DSF from 12.7 to 1.3% (dry basis) and consequently increased the protein content from 57.0 to 65.8% (dry basis). We hoped that the variation in values of NaCl, fat, and protein in the samples would be meaningful in the relation with their ACE-inhibitory activity and their effects on the blood pressure of rats.

**ACE-inhibitory activity**

Our preliminary experiment indicated that the ultraviolet absorption spectrum test of hydrolysates gave a maximum absorbance at around 280 nm wavelength. This suggested that the hydrolysates consisted of peptides. Three hours of proteolysis time produced hydrolysates with higher

Table I. Chemical Composition of Dried-salted Fish with Various Treatments for Rat Bioassay

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
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<td>65.8</td>
</tr>
</tbody>
</table>

* Freeze dried sample.

DSFs were produced by immersing the flesh of fish in 25% NaCl solution for 12 h and then drying at 45°C for 40 h.

Table II. Composition of Diet (% of Weight) for Rats Experiment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>NaCl (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated DSF</td>
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<td>22.7</td>
<td>22.8</td>
<td>12.7</td>
<td>57.0</td>
</tr>
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<td>7.6</td>
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<td>24.4</td>
<td>1.3</td>
<td>65.8</td>
</tr>
</tbody>
</table>

* Based on AOAC (1990) to give 10% of protein level.

**Blood pressure.** Mean blood pressure of rats was measured by the tail cuff method, using a programmable electrophysgmonanometer apparatus (Type PS-100, Riken Kaitatsu Co.). The rats were warmed for 10 min in a 40°C thermostated box before measuring their blood pressure. To increase the reliability of blood pressure measurements, the rats were familiarized with the blood pressure apparatus before measurement. At least five readings were recorded and averaged for each rats.
ACE-inhibitory activities than 6, 9, and 15 h did, and markedly decreased at 24 and 30 h of proteolysis. As compared with trypsin, chymotrypsin, and Pronase E, pepsin hydrolysates was the most potent as inhibitor. All the data are not shown.

Effects of salt content (DSF I and II) and the storage periods (0, 3, and 6 months) on their IC_{50} values are shown in Table III. It was observed that DSF II had a tendency to give a higher ACE-inhibitory activity than DSF I. This might be associated with the physical characteristics of protein, where 25% NaCl treatment caused more denaturation than 15% NaCl treatment did, consequently the enzyme digests the protein easily to produce some active substances.

It was also observed (Table III) that DSF after 3 months of storage had a tendency to produce a hydrolysate with a higher ACE-inhibitory activity than DSFs stored for 0 and 6 months. In many cases, storage time also enhanced protein denaturation and consequently increased the digestibility. However, longer storage time (6 months in this case) allowed the protein to be denatured more intensively. As a result, protein would be digested more easily and produce many short peptides or free amino acids with a very low or no inhibitory activity.

The digestion of DSF by pepsin for 3 h gave an IC_{50} value of 0.63 mg protein/ml hydrolysate. A similar value has been reported by Ukeda et al.\textsuperscript{19,20} for sardine peptic hydrolysate (IC_{50} value; 0.62 mg/ml). Agreeing with our results, they also reported that pepsin was the best as compared with trypsin, chymotrypsin, and Denazyme AP.

However, Yokoyama et al.\textsuperscript{23} found that a thermolysin digest obtained from hydrolyzation of katsuobushi (a Japanese traditional seasoning made from skipjack muscle) showed a higher inhibitory activity (IC_{50} value; 29 μg protein/ml) than pepsin, trypsin, or chymotrypsin. And Matsui et al.\textsuperscript{11} found an IC_{50} value of 0.26 mg/ml in a sardine muscle digest obtained by bacterial alkaline protease hydrolysis for 17 h at 50°C and pH 9.0.

Although the previous researchers had indicated that thermolysin and alkaline protease were more effective in yielding peptide inhibitors than pepsin was, our laboratory tended to use pepsin. Since alkaline protease and thermolysin are enzymes isolated from bacteria, the peptides produced may not be yielded in vivo. As a consequence, if the peptides are to be used medicinally, some antigen-antibody reaction (food allergy) or digestive resistance to proteases may arise.\textsuperscript{11} So apparently it is much better to use pepsin, which is naturally produced by the internal systems of our bodies.

Table IV shows the effects of desalting or defatting treatment before pepsin digestion on IC_{50} values of fresh fish and DSFs. Desalting or defatting treatment gave higher ACE-inhibitory activity than the untreated one. The proteolysis reaction in treated samples is likely to be faster or more specific than in untreated samples, which may be caused by the increase of the ratio of substrate to enzyme. Defatting of freeze-dried DSF in this study reduced the fat content from 12.7 to 1.3%, and subsequently increased the protein content from 57.0 to 65.8% (dry basis).

Defatting treatment of DSF II produced the best potent inhibitor with an IC_{50} value of 0.30 mg protein/ml of ACE-inhibitory activity (IC_{50} value) of defatted sardine meal hydrolysate by alkaline protease treatment was 0.18 mg/ml, which was different from our result. The different was likely to be associated with the different of fish species, enzyme, and sample preparation procedure.

Compared to defatted-DSF, the desalted one seems had a smaller effect on increasing of ACE-inhibitory activity. It was also observed from Table III that salt content of samples seem had no effect on ACE-inhibitory activity, as the IC_{50} values of fresh fish and DSFs were similar, even though their NaCl contents were very different. The NaCl content in fresh, DSF I and II were 1.2, 18.7, and 22.8%, respectively. These results appear to support the notion that salting and drying of fishes affect least the ACE-inhibitory activities originally present in fresh fish.

| Table III. IC_{50} Values of Fresh Fish and DSF Hydrolyzed by Pepsin* |
|-------------------------|------|
| Treatments             | IC_{50} (mg protein/ml) |
| Fresh fish             | 0.75 |
| DSF I: 0 months        | 0.81 |
| DSF I: 3 months        | 0.76 |
| DSF I: 6 months        | 0.85 |
| DSF II: 0 months       | 0.82 |
| DSF II: 3 months       | 0.63 |
| DSF II: 6 months       | 0.76 |

* Digestion was conducted at 37°C for 3 h.

* DSF I: Dried-salted fish produced by 15% NaCl solution.

* DSF II: Dried-salted fish produced by 25% NaCl solution.

DSF products were stored at 28°C for 0, 3, and 6 months.

<table>
<thead>
<tr>
<th>Table IV. Effects of Desalting or Defatting Treatment on IC_{50} Values of Fresh Fish and DSF Hydrolysates</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC_{50} (mg protein/ml)</td>
</tr>
<tr>
<td>Fresh fish</td>
</tr>
<tr>
<td>Untreated</td>
</tr>
<tr>
<td>Dried-salted fish</td>
</tr>
<tr>
<td>DSF I</td>
</tr>
<tr>
<td>Untreated</td>
</tr>
<tr>
<td>Defatted</td>
</tr>
<tr>
<td>DSF II</td>
</tr>
<tr>
<td>Untreated</td>
</tr>
<tr>
<td>Defatted</td>
</tr>
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</table>

1. Hypotensive Effect of ACE-Inhibitory Substances

Fig. 1. Sephadex G-25 Column Chromatogram of Pepsin Hydrolysate and Its ACE-Inhibitory Activity (Absorbance at 280 nm, E\textsubscript{280}, % of ACE-Inhibitory Activity).

The experimental details are described in Materials and Methods.
Isolation of ACE-Inhibitors

Isolation of ACE-inhibitors from DSF II stored for 3 months was done as described in Materials and Methods. Figure 1 shows the Sephadex G-25 column chromatogram of the pepsin hydrolysate. From four peaks yielded (identified as peak I, II, III, and IV), peak III showed the highest ACE-inhibitory activity. Those active fractions were then collected, concentrated, and put on the second column (SP-Sephadex C-25). The active fractions obtained (figure not shown) were further purified by a third column. Two peaks were observed (figure not shown), but only the second peak had a high activity as an ACE-inhibitor.

The active peak fractions obtained from the third column were then purified by HPLC. About ninety peaks were detected by using the ODS column (Fig. 2), but only 3 peaks (A, B, and C) had a good ACE-inhibitory activity. The active peaks of A, B, and C were further purified on the same column. Peak A was eluted with 20% acetonitrile (1.5 ml/min), while peaks B and C were eluted with 25% acetonitrile (1.5 ml/min). A1 and B1 were the active peptides of A and B fractions, while C1 and C2 were the active peptides obtained from the C fraction (figure not shown).

Amino acid sequences

The amino acids sequences and the IC50 values of the active inhibitors are shown in Table V. It was observed that the inhibitor C2 (Cys-Trp-Leu-Pro-Val-Tyr) had the highest activity (IC50 value: 22.20 μM), then inhibitor A (IC50 value: 31.97 μM).

Inhibitors of ACE that were first obtained from the venom of snakes such as Bothrops jararaca and Agkistrodon halys blomhoffii have many proline residues. Fragments of food proteins, such as casein, zein, gelatin, fish and others, also inhibit ACE and each of these inhibitors has at least one proline residue. In this study only inhibitors C1 and C2 had proline residues, but all inhibitors contained valine, tryptophan, and leucine residues.

Cheung et al.38 and Kawakami and Kayahara39 concluded that the COOH-terminal amino acid of peptides made the most important contribution to binding of these substrates with the active site of angiotensin-converting enzyme (ACE). Further more they suggested that the most favorable COOH-terminal amino acids were the aromatic acids (tryptophan, tyrosine, and phenylalanine), and the imino acid (proline). Our results indicated that the inhibitor C2, Cys-Trp-Leu-Pro-Val-Tyr, had the highest inhibitory activity (IC50 value: 22.20 μM). Cheung et al.38 also found that the dipeptide Val-Tyr had an IC50 value of 22.0 μM. The similarity of these results is easy to understand, since the ACE-inhibitory activity of peptides is mostly dependent on the COOH-terminal dipeptide residues.38

In the case of inhibitor C1 (Ser-Lys-Val-Pro-Pro), although the peptide was ended by a proline residue, its ACE-inhibitory activity was not very good (74.22 μM). According to Cheung et al.38, among the NH2-terminal amino acids of dipeptides, the branched-chain aliphatic amino acids (valine and isoleucine) were the most effective for increasing peptide binding to ACE, and proline was an unfavorable NH2-terminal amino acid. It was observed by Cushman et al.40 that replacement of the terminal sequence Pro-Pro of SQ 20,881 (tetroptide), Glu-Trp-Pro-Arg-Pro-Gln-Ile-ile-Pro-Pro, by the sequence Ala-Pro, increased the affinity of this nonapeptide inhibitor for ACE.

Hypotensive activity of crude peptide

A number of previous researchers proved that although the ACE-inhibitory activities of food-derived peptides were weaker than those of commercial drugs, some of them have been shown to be effective in lowering blood pressure in spontaneously hypertensive rats (SHR) after oral or intraperitoneal administrations.12–14,16,18,23

The blood pressure of rats fed casein and DSF with various treatments is shown in Table VI. It was observed that untreated-DSF and defatted-DSF groups had the highest blood pressure (180 mmHg). This blood pressure correlated with the NaCl content in the diets (Table VI). The NaCl content in untreated-DSF, defatted-DSF, and desalted-DSF diets were 3.99, 3.70, and 1.26%, respectively.

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Structure</th>
<th>IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Val-Ala-Trp-Lys-Leu</td>
<td>31.97</td>
</tr>
<tr>
<td>B</td>
<td>Trp-Ser-Lys-Val-Leu</td>
<td>156.28</td>
</tr>
<tr>
<td>C1</td>
<td>Ser-Lys-Val-Pro-Pro</td>
<td>74.22</td>
</tr>
<tr>
<td>C2</td>
<td>Cys-Trp-Leu-Pro-Val-Tyr</td>
<td>22.20</td>
</tr>
</tbody>
</table>

Table VI. NaCl Content in the Diets and Mean Blood Pressure of Rats after Feeding for 16 Days

<table>
<thead>
<tr>
<th>Treatments</th>
<th>NaCl content in diets* (g/100 g)</th>
<th>Mean blood pressureb (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>0.50</td>
<td>123.35 ± 7.64</td>
</tr>
<tr>
<td>Desalted DSF</td>
<td>1.26</td>
<td>126.28 ± 5.66</td>
</tr>
<tr>
<td>Defatted DSF</td>
<td>3.70</td>
<td>180.00 ± 1.79</td>
</tr>
<tr>
<td>Untreated DSF</td>
<td>3.99</td>
<td>179.12 ± 2.35</td>
</tr>
<tr>
<td>Untreated DSF +</td>
<td>3.99</td>
<td>115.22 ± 4.67</td>
</tr>
</tbody>
</table>

* Diets contain 10% protein and were fed 10 g/day.

b Values are means of 6 replicates ± SD.

Different following letters represent a significant difference (p <0.01).
It is well known that ingestion of excess NaCl causes hypertension.

The association between dietary salt (NaCl) and blood pressure has been assumed to be related primarily to the sodium content of salt. A number of investigators, however, noted that the hypertensive effect of sodium chloride is dependent on the concomitant presence of both chloride and sodium ions. Greger and Tseng conclude that dietary chloride has more a rapid and greater effect on blood pressure than dietary sodium.

The addition of ACE-inhibitory substances (pepsin hydrolysate) that were orally administered to rats fed untreated DSF significantly decreased the blood pressure (−65 mmHg) compared with the rats fed untreated DSF without ACE-inhibitory substances. This showed that the ACE-inhibitory substances derived from Indonesian dried-salted fish has a potent effect on reducing the blood pressure of rats fed a diet high in NaCl. No significant difference in blood pressure was observed between rats fed casein and desalted DSF, and also between casein and untreated DSF + ACE-inhibitory substances groups. These results also indicate that the hypotensive effect of ACE-inhibitory substances obtained from DSF was stronger than the hypertensive effect of NaCl in the DSF diet.

Since the results of this study were obtained by using pepsin hydrolysate that had not been purified, the main cause of decreasing of the blood pressure is still not fully understood. The decreased may be caused by the peptides that were isolated and purified from peak III of Fig. 1, or by other compounds produced during the pepsin digestion (see peaks I, II, and IV, in Fig. 1). Our preliminary experiment only indicated that peak III had the highest ACE-inhibitory activity among the peaks. Further studies concerning peaks I, II, and IV need to be done to clarify the status of these peaks (peptides or other substances).

To study the effects of pure peptides (isolated from peak III of Fig. 1) in lowering the blood pressure of spontaneously hypertensive rats (SHR), further experiments using peptide A (Val-Ala-Trp-Lys-Leu, IC₅₀ value: 31.97 μM) and peptide C2 (Cys-Trp-Leu-Pro-Val-Tyr, IC₅₀ value: 22.20 μM) are now being done in our laboratory.

Based on these results, it can be concluded that DSF products contain potent ACE-inhibitory substances. These substances had a significant effect on lowering the blood pressure of salt-induced hypertension rats. The traditional desalting method practiced by Indonesian people on DSF products is very useful in reducing the NaCl content and this lead to prevention of the hypertension after consuming the products. It is proposed that protein intake can be increased by use of water-soaked DSF with less concern about producing hypertension.

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References