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Note

Preparation of Angiotensin I-Converting Enzyme Inhibiting Peptides from Soybean Protein by Enzymatic Hydrolysis

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Soybean protein isolate was hydrolyzed using two proteases (Protease M and Orientase 90N), and the inhibitory activity of angiotensin I-converting enzyme (ACE) and bitterness of the hydrolysates were investigated. The ACE inhibitory activity of the hydrolysates increased with increasing hydrolysis time. Hydrolysates obtained using Protease M for 4 to 10 h and Orientase 90N for 6 to 10 h showed a high ACE inhibitory activity, and the bitterness was negligible. The ACE inhibitory peptides were shown to be oligopeptides composed of 2–5 amino acid residues. These peptides might be useful for therapeutic applications based on the consumption of an anti-hypertensive food.

Keywords: soybean protein, peptides, angiotensin I-converting enzyme, bitterness

Hypertension is one of the major risk factors for arteriosclerosis, stroke, myocardial infraction, and end-stage renal disease. Angiotensin I-converting enzyme (ACE, EC [3.4.15.1]) plays a key role in the renin-angiotensin system which regulates the blood pressure and the blood cyclic system, and ACE is known to increase the blood pressure (Maruyama, 1996). Therefore, ACE inhibitory peptides were considered to be useful for preventing hypertension. Dietary ingestion of a moderate amount of ACE inhibitors derived from food proteins is thought to play an important role in the prophylaxis and therapy. The ACE inhibitory peptides derived from katsuo-bushi (dried bonito) have been commercialized as an anti-hypertensive food in Japan (Hasegawa, 1996).

Soybean protein is an economical and high quality vegetable protein. Recently, it has been reported that soybean protein hydrolysates contained ACE inhibitory peptides, which were resistant to gastrointestinal protease *in vitro* and could exert an ACE inhibitory activity in vascular tissues *in vivo* (Yu *et al*., 1996). However, the ACE inhibitory activities of the hydrolysates derived from soy protein already reported were not high enough for practical use (Sang *et al*., 2000). Furthermore, most of the hydrolysates had a bitter taste, which limited their utilization on an industrial scale.

In this study, we investigated the conditions of soy protein hydrolysis using two selective commercial proteases for the purpose of industrialization of the ACE inhibitory peptides. We intended to produce peptides not only with a high ACE inhibitory activity but also with a bland taste.

Materials and Methods

Materials Soybean protein isolate (SPI) was obtained from Jiling Buer Protein Co., Ltd. (Jiling province, China). Protease M and Orientase 90N were obtained from Amano Enzyme Inc. (Nagoya, Japan) and HBI Enzyme Inc. (Osaka, Japan), respectively, and were derived from selective strains of *Aspergillus oryzae* and *Bacillus subtilis,* respectively. Hippury-L-histidyl-L-leucine (Hip-His-Leu, HHL), angiotensin I-converting enzyme (ACE; from rabbit lung), and *o*-phthaldialdehyde (OPA) were purchased from Sigma Chemical Co. (St. Louis, MO).

Protein hydrolysis SPI was hydrolyzed using Protease M as follows: SPI was dissolved in distilled water (5.0%, w/w) and pH was adjusted to 4.5 with 1 M HCl, enzyme (200 U/g protein) was added and the mixture was kept at 40°C. Hydrolysis using Orientase 90N was carried out as follows: SPI was dissolved in distilled water (5.0%, w/w) and pH was adjusted to 7.5 with 1 M NaOH, enzyme (enzyme/protein=1/1000) was added and the mixture was kept at 40°C.

After the hydrolysis, samples were heated at 80°C for 30 min in a water bath to inactivate the proteases. Then the samples were adjusted to pH 7.0, centrifuged (3000×*g*, 20 min, 4°C) and the supernatants were filtered (ϕ25 μm).

Measurement of degree of hydrolysis The degree of hydrolysis (DH) for the soy protein hydrolysate was determined using the trinitrobenzenesulfonic acid (TNBS) method described by Jense (1979). A 1.5 mM L-leucine solution was used as the standard. DH was defined as the percentage ratio of the number of peptide bonds broken (h) to the total number of bonds per unit weight (h_{tot}), and calculated as follows:

$$DH\ \% = h/h_{tot} \times 100.$$

The total number of peptide bonds (h_{tot}) in SPI was assumed to be 7.8 meq./g (Adler-Nissen, 1986).

Determination of ACE inhibitory activity The ACE inhibitory activity was determined according to the method of Horie (1999) as follows: a 0.05 ml aliquot of a sample was mixed with 0.1 ml of 0.025 U/ml ACE and 0.1 ml of solution (4.7 mM HHL

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and 600 mM NaCl in 400 mM pH 8.5 phosphate buffer). The mixture was incubated in a water bath at 37°C for 30 min, then 1.5 ml of 0.3 M NaOH was added to terminate the reaction. A 0.1 ml OPA solution (2% OPA in methanol) was added to the solution and after 10 min incubation at room temperature, 0.2 ml of 3 M HCl was added. The fluorescence intensity was measured with a RF5300PC-fluorescence spectrophotometer (Shimadzu, Japan) under the following conditions: excitation, 340 nm; emission, 455 nm; slit width, 5 nm. The ACE activity was calculated from the following equation and expressed as ACE%:

$$ACE\% = (b-c)/a \times 100,$$

where a is the fluorescence intensity of the standard solution (ACE and water), b is the fluorescence intensity of ACE and the sample solution, and c is the fluorescence intensity of the blank solution (without ACE).

Sensory evaluation of bitterness The bitterness of the hydrolysate solution (5% w/w) was evaluated immediately after the hydrolysis and compared with a standard quinine sulfate solution (Minagawa *et al*., 1989). Quinine sulfate solutions ranging from 10^{-8} M to 10^{-4} M were used as standards. The degree of bitterness was evaluated according to the following criteria: 8×10^{-7} M quinine sulfate solution, not bitter (score 0); 3×10^{-6} M, slightly bitter (score 1); 7×10^{-6} M, distinctly bitter (score 2); 2×10^{-5} M, very bitter (score 3) and 1×10^{-4} M, extremely bitter (score 4). A 5-semi-trained-member taste panel was selected to perform several discrimination tests on the 5 tastes (sweet, sour, salty, bitter, and the taste of monosodium glutamate). The degree of bitterness and the threshold value of the bitter peptides were averaged after four examinations.

Results and Discussion

DH of hydrolysate The DH values of the soybean protein hydrolysates obtained using the two proteases are shown in Fig. 1. The DH of the hydrolysates obtained using Protease M increased from 22.1% at 0.5 h to 60.5% at 10 h, and that using Orientase 90N increased from 29.0% at 0.5 h to 53.3% at 10 h.

The proximate range of number of amino acid residues of the hydrolysates (at 10 h) was estimated using the correlation between DH and the average length of the peptide chains given by Jense (1979). The number of amino acid residues of the peptides was estimated between 2 and 5.

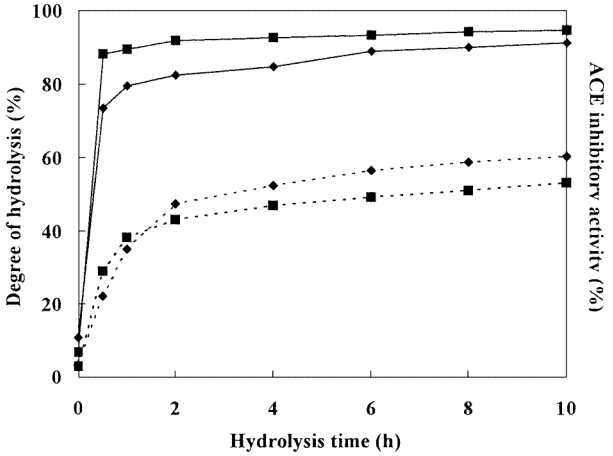

Fig. 1. Changes in the degree of hydrolysis (%) and the ACE inhibitory activity (%) of hydrolysates. ◆: Protease M, ■: Orientase 90N, ---- degree of hydrolysis, — ACE inhibitory activity.

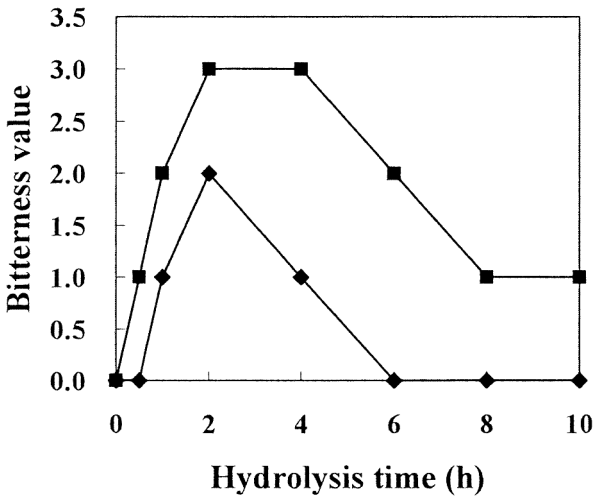

Fig. 2. Sensory evaluation of bitterness of hydrolysates. ◆: Protease M, ■: Orientase 90N.

Effect of hydrolysis time on ACE inhibitory activity The effects of the hydrolysis time on the ACE inhibitory activity of the hydrolysates are also shown in Fig. 1. All of the hydrolysates obtained using the two proteases showed some ACE inhibitory activity. The soybean protein itself displayed the activity to some extent, but the inhibitory activity increased with the hydrolysis. The hydrolysates treated for 10 h and exhibiting the highest ACE inhibitory activity of up to 91.3% for Protease M and 94.7% for Orientase 90N, indicated that both proteases could give rise to highly active ACE inhibitory peptides. Although the ACE inhibitory activity cannot be directly compared with data under different measuring conditions, the hydrolysates in this study showed a higher activity than other hydrolysates already reported. (Wu & Ding, 2001; Sang *et al*., 2000).

Oshima *et al*. (1979) reported that ACE inhibitory peptides derived from food protein mainly consisted of low-molecular-weight peptides with a MW below 1500 Da. Wu and Ding (2001) reported that the soybean ACE inhibitory peptides belonged to oligopeptides with 2–8 amino acid residues. The results of DH of the hydrolysate in this study indicated that the hydrolysates had a suitable molecular weight for such a property.

Bitterness of the peptides The degree of bitterness of the hydrolysates is shown in Fig. 2. Those obtained using Orientase 90N for 2 to 4 h displayed a bitterness, while most displayed very little or no bitterness.

When we use peptides as food materials, the bitter taste is a major problem. Some of the debittering procedures, which involved further hydrolysis with exopeptidases, resulted in a loss of bio-functionality of the peptides. In this study, the hydrolysates obtained using Protease M from 4 to 10 h, with little bitterness and a high bio-functional activity, could be utilized for industrial purposes, along with those obtained using Orientase 90N from 6 to 10 h.

We could not determine, however, the effect of these ACE inhibitory peptides on hypertension *in vivo*, because the relationship between the ACE inhibitory activity *in vitro* and the blood-pressure-suppression effects *in vivo* has not been elucidated. Although further studies must be conducted before the use of

bio-active compounds from soybean protein, the results obtained in this study may contribute to the development of a novel functional food as well as to therapeutic application.

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