Isolation of Glycinin and β-Conglycinin Fractions from a Soy Protein by Utilizing Selective Proteolysis

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Summary

We verified that the glycinin and β-conglycinin fractions were selectively isolated from a soy protein by hydrolysis by pepsin and papain with optimal pH and temperature in relatively large scale. A method for the preparation of glycinin and β-conglycinin fractions from soy protein isolate, which was hydrolyzed by pepsin or papain, was carried out. The yields of SPI (undigested), pepsin treated fraction (β-conglycinin fraction) and papain treated fraction (glycinin fraction) from 100 g of defatted soybean were 29.6, 18.0 and 18.9 g, respectively.

SDS-PAGE of the hydrolysates from soy protein isolate by pepsin or papain showed that the band corresponding to glycinin disappeared with hydrolysis by pepsin at pH 2.0, and the band corresponding to β-conglycinin disappeared with hydrolysis by papain at 80 ℃. The optimum temperature of papain was 80 ℃, and pepsin had maximal activity at pH 2.0. The selective proteolysis is due to the specific denaturation of glycinin and β-conglycinin under different pH and temperature conditions, and not by the substrate specificity of proteases.

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Key words : β-conglycinin, glycinin, isolation, proteolysis, soy protein

Soy protein has functional properties, such as emulsification, water solubility, moisture holding, and foaming (Sugano, 1999). In addition, Soy protein contains significant amounts of all the essential amino acids (Sugano, 1999). Moreover, lowering of cholesterol in plasma (Sugano, 1999; Aoyama *et al*., 2001), the prevention of aging (Sugano, 1999), cardiac disease (Sugano, 1999) and obesity (Velasquez *et al*., 2007) by soy protein intake, have been reported. As just described, soy protein have various functional properties and high nutritional value. For that reason, soy protein is extensively used for traditional Japanese foods and processed foods.

Soy protein consists of two major components, glycinin and β-conglycinin, which account for approximately 70 % of the proteins in soybean seed. β-conglycinin comprises of three subunits, α' (76kDa), α (72 kDa) and β (53 kDa) (Shuttuck-Eidens and Beachy 1985). Glycinin comprises of two subunits, acidic (37-42 kDa) and basic (20 kDa) (Staswick *et al*. 1981). It is also well known that these components have different structures and show considerable differences in their functional properties such as gelation and emulsification. For that reason, many workers have investigated glycinin and β-conglycinin respectively to elucidate the gel properties of soy protein.

By the way, Angiotensin Ⅰ-converting enzyme (ACE), a dipeptidyl carboxypeptidase, catalyzes the conversion of Angiotensin Ⅰ to the potent vasoconstrictor Angiotensin Ⅱ and plays an important physiological role in regulating blood pressure. Inhibitors of ACE derived from food proteins are utilized for food for specified health use (Matsui *et al*., 1993; Nakamura *et al*., 1995). We have attempted to produce ACE inhibitory peptides from enzymatic hydrolysates of soy protein. To identify ACE inhibitory peptides from soy protein hydrolysates, it is necessary to isolate glycinin and β-conglycinin from soy protein. Many studies have demonstrated that glycinin and β-conglycinin fractions from soy protein separated based on their different solubility in pH, temperature, and salt concentration (Wolf *et al*., 1962; Koshiyama, 1965; Thanh *et al*., 1975; Nagano *et al*., 1992). Wu *et al*. (2000) reported that simplified fractionation for glycinin and β-conglycinin was attained in a pilot-scale by using pH adjustment and ultrafiltration membrane separation.

On the other hand, Tsumura *et al*. (2004) demonstrated a method for the selective proteolysis of glycinin or β-conglycinin using native soy protein isolate. In this report, a method for preparing hydrolysates enriched glycinin and β-conglycinin by selective proteolysis of soy protein was verified.

MATERIALS AND METHODS

Materials

Soybean (*Glycine max* [L] Merr. var. Enrei) seeds were used in this study. Pepsin (EC 3.4.23.1, 4,550 units/mg) and papain (EC 3.4.22.2, 22.7 units/mg) were purchased from Sigma-Aldrich Co. (St. Louis, Mo., USA). All other chemicals were reagent grade.

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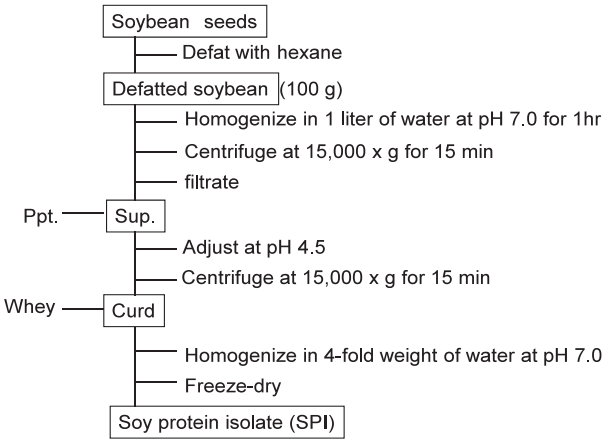

Fig. 1. Flow diagram for the preparation of soy protein isolate (SPI).

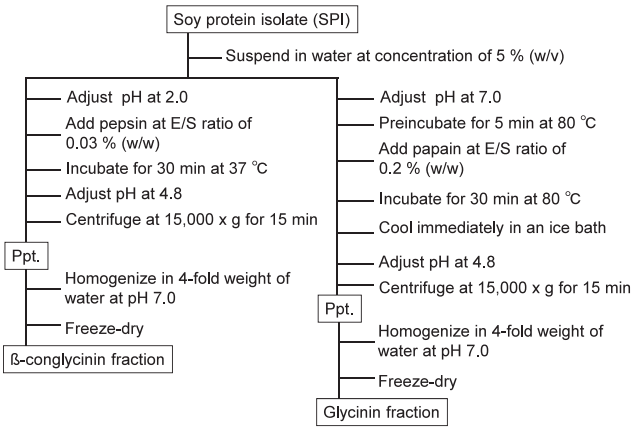

Fig. 2. Flow diagram for the preparation of glycinin and β-conglycinin fractions from SPI

Preparation of soy protein isolate

Flow diagram for the preparation of soy protein isolate is shown in **Fig. 1**. All preparation procedures were performed at room temperature. Soybean seeds were crushed and defatted with hexane. Defatted soybean flakes (100 g) were homogenized in a 1 liter of distilled water, and the pH value of the suspension was adjusted at 7.0 with 10 M NaOH. This suspension was stirred for 1 h, centrifuged (15,000 x g for 15 min) and filtered to remove insoluble material. The resulting supernatant was adjusted at pH 4.5 with 10 M HCl. After centrifugation at 15,000 x g for 15 min, the precipitate was homogenized in a 4-fold weight of distilled water and neutralized at pH 7.0 with 10 M NaOH, freeze-dried, and powdered. It will be referred as SPI (soy protein isolate) hereafter.

Proteolysis of SPI

SPI was suspended in distilled water to a final concentration of 5 % (w/v) and adjusted at pH 2.0 with 10 M HCl. Pepsin was added to the suspension at the enzyme/SPI ratio of 0.03 % (w/w), and then incubated at 37℃ for 30 min. After the prescribed incubation times, the reaction solution was adjusted at pH 4.8 with 10 M NaOH. After centrifugation at 15,000 x g for 15 min, the precipitate was suspended in distilled water, and neutralized at pH 7.0 with 10 M NaOH, freeze-dried, and powdered. Another SPI portion was suspended in distilled water to a final concentration of 5 % (w/v) and adjusted at pH 7.0 with 10 M NaOH. The suspension was preincubated at 80 ℃ for 5 min. Papain was added to the suspension at enzyme/SPI ratio of 0.2 % (w/w), and then incubated at 80 ℃ for 30 min. After the prescribed incubation times, the reaction solution was immediately cooled in an ice bath until room temperature, and then adjusted at pH 4.8 with 10 M HCl. After centrifugation at 15,000 x g for 15 min, the precipitate was suspended in distilled water, and neutralized at pH 7.0 with 10 M NaOH, freeze-dried, and powdered.

Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli (1970), using 10% polyacrylamide gel. Protein was stained with Coomassie Brilliant Blue R-250.

RESULTS AND DISCUSSION

Selective proteolysis of the glycinin and β-conglycinin fraction in SPI

Tsumura *et al.* (2004) demonstrated that the glycinin and β-conglycinin fractions in a soy protein were able to be selectively hydrolyzed by pepsin and papain with controlled pH and temperature. Based on the results, we attempted to verify that the glycinin and β-conglycinin in soy protein were selectively hydrolyzed and the hydrolysates enriched with glycinin or β-conglycinin was obtained respectively.

A method for the preparation of glycinin and β-conglycinin fractions from SPI is shown in **Fig. 2**. SPI was hydrolyzed by pepsin and precipitated. By the precipitation step, the small peptides and amino acids produced during hydrolysis were removed. Another SPI portion was hydrolyzed by papain and precipitated. The yields of SPI (undigested), pepsin treated fraction (β-conglycinin fraction) and papain treated fraction (glycinin fraction) from 100 g of defatted soybean were 29.6, 18.0 and 18.9 g, respectively.

SDS-PAGE of the hydrolysates from soy protein isolate by pepsine and papain are shown in **Fig. 3**. The band corresponding to glycinin disappeared with hydrolysis by pepsin at pH 2.0, although the band corresponding to β-conglycinin was not affected. This result indicates that the precipitate mainly contained β-conglycinin fraction. On the other hand, the band corresponding to β-conglycinin disappeared with hydrolysis by papain at 80 ℃ , although the band corresponding to glycinin was not affected. This result indicates that the precipitate mainly contained glycinin fraction.

Thermal profile of differential scanning calorimetry (DSC) measurements and denaturation are closely related. The thermal denaturation studies of soy protein by DSC have been reported (Hermansson, 1978; Bikbov *et al.*, 1983;

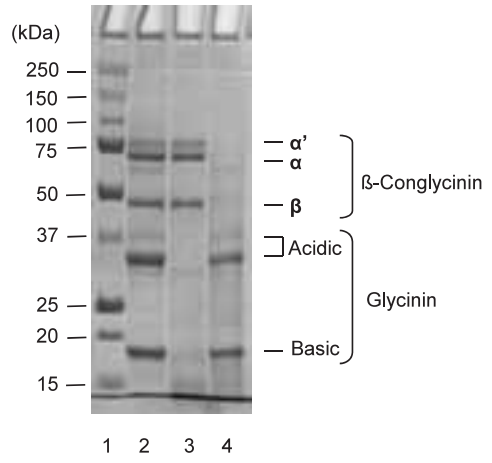

Fig. 3. SDS-PAGE of the hydrolysates from SPI by pepsine and papain.
Lane 1: molecular mass standards; lane 2: native SPI; lane 3: pepsine treated SPI; lane 4: papain treated SPI

Damodaran, 1988; Kitabatake *et al*., 1990). Tsumura *et al*. (2004) investigated the thermal stability of glycinin and β-conglycinin at various pH value by a DSC analysis. They reported that glycinin was preferentially denatured in the acidic pH range, and it was more thermally stable than β-conglycinin in the neutral pH range. The optimum temperature of papain was 80 ℃, and pepsin had maximal activity at pH 2.0. As the result, selective proteolysis would occur. Nielsen *et al*. (1988) demonstrated that native and heated legume storage proteins were digested with various protease including pepsin and papain. They concluded that all proteins were readily hydrolyzed on heating in spite of the type of proteases. Therefore, it was thought that the selective proteolysis was caused by the substrate denaturation, not by the substrate specificity of protease.

We have confirmed a method for selective proteolysis of glycinin or β-conglycinin. The selective proteolysis and precipitation could produce hydrolysates enriched glycinin or β-conglycinin fraction.

REFERENCES

Aoyama, T., M. Kohno, T. Saito, K. Fukui, K. Takamatsu, T. Yamamoto, Y. Hashimoto, M. Hirotsuka and M. Kito. 2001. Reduction by phytate-reduced soybean β-conglycinin of plasma triglyceride level of young and adult rats. *Biosci. Biotech. Biochem*. **65**: 1071-1075

Bikbov, T. M., V. Y. Grinberg, A. N. Danilenko, T. S. Chaika, I. A. Vaintraub and V. B. Tolstoguzov. 1983. Studies on gelation of soybean globulin solutions. *Colloid Polym. Sci*. **261**: 346-358.

Damodaran, S. 1988. Refolding of therally unfolded soy proteins during the cooling regime of the gelation process. *J. Agric. Food Chem*. **36**: 262-269.

Hermansson, A. M. 1978. Physico-chemical aspects of soy proteins structure formation. *J. Texture Stud*. **9**: 33-58

Kitabatake, N., M. Tahara, E. Doi. 1990. Thremal denaturation of soybean protein at low water contents. *Agric. Biol. Chem*. **54**: 2205-2212.

Koshiyama, I. 1965. Purification of the 7S component of soybean proteins. *Agr. Biol. Chem*., **29**: 885-887.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680-685.

Matsui, T., H. Matsufuji, E. Seki, K. Osajima, M. Nakashima and Y. Osajima. 1993. Inhibition of angiotensin 1-converting enzyme by Bacillus licheniformis alkaline protease hydrolyzates derived from sardine muscle. *Biosci. Biotech. Biochem*., **57**: 922-925.

Nagano, T., M. Hirotsuka, H. Mori, K. Kohyama, and K. Nishinari. 1992. Dynamic viscoelastic study on the gelation of 7S globulin from soybean. *J. Agric. Food Chem*., **40**: 941-944.

Nakamura, Y., N. Yamamoto, K. Sakai and T. Takano. 1995. Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotensin 1-converting enzyme. *J. Dairy Sci*., **78**: 1253-1257.

Nielsen, S. S., S. S. Deshpande, M. A. Hermodson and M. P. Scott. 1988. Comparative digestibility of legume storage proteins. *J. Agric. Food Chem*., **36**: 896-902.

Shuttuck-Eidens, D. M., and Beachy R. M. 1985. Degradation of β-conglycinin in early stage of soybean embryogenesis. *Plant Physiol*. **78**: 895-898

Staswick, P. E., M. A. Hermodson and N. C. Nielsen. 1981. Identification of the acidic and basic subunit complexes of glycinin. *J. Biol. Chem*., **256**: 8752-8755

Sugano, M. 1999. Daizu seihin ni motomerareru kinousei (in Japanese). pp. 1-16. In: Sugano, M. and H. Sho (eds.) *Soybean protein : Its production, processing and physiological functions*. Kenpakusha, Tokyo.

Thanh, V. H., K. Okubo and K. Shivasaki. 1975. Isolation and characterization of the multiple 7S globlins of soybean proteins. *Plant Phisiol*., **56**: 19-22.

Tsumura, K., T. Saito, W. Kufimiya, and K. Inouye. 2004. Selective proteolysis of the glycinin and β-conglycinin fractions in a soy protein isolate by pepsin and papain with controlled pH and tenmperature. *J. Food Sci*., **69**: 363-367.

Velasquez, M. T. and S. M. Bhathena. 2007. Role of dietary soy protein in obesity. *Int. J. Med. Sci*., **4**: 72-82.

Wolf, W. J., G. E. Babcock and K. Smith. 1962. Purification and stability studies of the 11S component of soybean proteins. *Arch. Biochem. Biophys*., **99**: 265-274.

Wu, S., P. A. Murphy, L. A. Johnson, M. A. Reuber and A. R. Fratzke. 2000. Simplified process for soybean glycinin and β-conglycinin fractionation. *J. Agric. Food Chem*., **48**: 2702-2708.

選択的タンパク質分解によるダイズタンパク質グリシニンとβ-コングリシニンの分離

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要　約

ダイズ種子タンパク質の主要な構成成分であるグリシニンとβ-コングリシニンをそれぞれ選択的に酵素分解することにより、グリシニンあるいは、β-コングリシニンを多く含む画分が得られることを実証した。脱脂したダイズ種子タンパク質溶液を pH 2.0に調整し、ペプシンを添加して、37℃、30分間インキュベートしたところ、グリシニンが優先的に分解され、β-コングリシニンが豊富な画分が得られることが、電気泳動像から明らかとなった。また、ダイズ種子タンパク質溶液を pH 7.0に調整し、パパインを添加して80℃、30分間インキュベートするとβ-コングリシニンを豊富に含む画分が得られた。結果的に脱脂ダイズ100g からβ-コングリシニン画分18.0g、グリシニン画分18.9g を得た。

示差走査熱量分析から、グリシニンは、pH 3.5以下の酸性域でβ-コングリシニンよりも変性しやすく、また、中性域ではβ-コングリシニンよりも熱に安定であると報告されている。酵素として用いたペプシンの至適 pH は2.0で、パパインの至適温度は80℃であることから、基質の変性条件と酵素の至適反応条件が一致する。グリシニンとβ-コングリシニンの選択的な酵素分解は、酵素の基質特異性ではなく、基質の変性条件の違いで酵素分解の優先度に差が出るために起こると考えられた。

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