Dynamic Viscoelastic Study on the Gelation Properties of β-Conglycinin-Rich and Glycinin-Rich Soybean Protein Isolates

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Preheated soybean protein isolates (SPIs) were prepared from new soybean lines that have extremely different compositions of β-conglycinin and glycinin by a typical method for manufacturing commercial SPI. The gelation properties of these SPIs were investigated by dynamic viscoelastic measurements. The storage modulus G' of 12% SPI was measured at pH 7.0 when the temperature was increased from 30 to 80 °C and then lowered to 20 °C at 2 °C/min. Gelation properties of the preheated SPI were different from those of the non-pre-heated SPI. In the absence of NaCl, an increase in the storage modulus G' was observed for preheated β-conglycinin-rich SPI and preheated control SPI, but not for preheated glycinin-rich SPI. On the other hand, in the presence of 2.5% NaCl, an increase in the storage modulus G' was observed for all preheated SPIs in the order β-conglycinin-rich SPI > control SPI > glycinin-rich SPI. These results suggest that β-conglycinin plays an important role in the heat-induced gel formation of commercial SPI.

Keywords: *β-Conglycinin; glycinin; gelation; dynamic viscoelastic measurements*

INTRODUCTION

Soybean seeds contain between 35 and 46% protein on a dry weight basis at maturity. This protein has a high nutritional value and a wide variety of uses. Therefore, soybean protein is commonly used as food ingredients, such as quality improvers, meat extenders, and so on. Soybean protein consists of two major components, β-conglycinin and glycinin, which together constitute ∼70% of the total seed storage protein at maturity and are believed to reflect the functional properties of soybean protein (Nielsen, 1985). It is also well known that β-conglycinin and glycinin have different structures and functional properties (Saio and Watanabe, 1978; Hermansson, 1986; Kilara and Sharkasi, 1986; Nagano et al., 1994).

The molecular weights of the two major soy proteins are generally considered to be ∼180 000 (β-conglycinin) and 320 000 (glycinin; Nielsen, 1985). β-Conglycinin is a trimeric glycoprotein of various combinations of three subunits, α′, α and β, which have molecular weights of 69 000, 68 000, and 42 000, respectively (Thanh and Shibasaki, 1977). On the other hand, glycinin is a hexamer composed of various combinations of five different subunits (G1−G5) with molecular weights of 54 000−64 000 (Nielsen et al., 1989). Each subunit has the generalized structure A-SS-B, where A represents an acidic polypeptide with a molecular weight of 34 000−44 000 and B is a basic polypeptide of with a molecular weight of ∼20 000. The A and B polypeptides are linked by a single disulfide (SS) bond (Staswick et al., 1984).

Van Kleef (1986) characterized the effect of pH and ionic strength on the storage modulus G' of soybean protein isolate (SPI) and glycinin gels. The gels were prepared in boiling water for 45 min. The curve of the storage modulus G' as a function of pH for SPI showed a remarkable resemblance to that for glycinin, and the storage modulus G' for glycinin was greater than that for SPI at comparable pH and ionic strength. Therefore, Van Kleef (1986) considered that the mechanical properties of SPI gels were mainly determined by glycinin. Nakamura et al. (1986) also observed that glycinin formed a harder gel than β-conglycinin when heated at 100 °C, pH 7.6, and ionic strength of 0.5. On the other hand, Shimada and Matsushita (1980) recognized that β-conglycinin formed a harder gel than glycinin when heated at 80 °C for 30 min in water at pH 7.5. Utsumi and Kinsella (1985) also reported that the hardness of gels decreased in the order β-conglycinin ≫ SPI > glycinin, when heated at 80 °C for 30 min in 30 mM Tris-HCl buffer at pH 8.0. These findings indicate that glycinin plays an important role in the formation of gels prepared by heating SPI at 100 °C. On the other hand, β-conglycinin plays a significant role in gel formation at 80 °C.

Commercial SPI is mainly used as a food ingredient in meat products such as sausage and ham. In these systems, the ability to form gels below 80 °C is a very important functional property of SPI because a high temperature affects the texture of meat products. Under these conditions, it is believed that β-conglycinin must play an important role in gel formation by SPI. However, Hermansson (1979) suggested that the gel properties of commercial SPI were different from those of native SPI. These differences in gel properties may be attributed to the fact that commercial SPI is denatured, as shown by differential scanning calorimetry measurements.

Through breeding trials to manipulate variant alleles, new soybean lines have been developed that have extremely different compositions of β-conglycinin and glycinin (Kitamura, 1993). In the present study, SPIs were prepared from these soybean seeds by a typical method for manufacturing commercial SPI. The gela-

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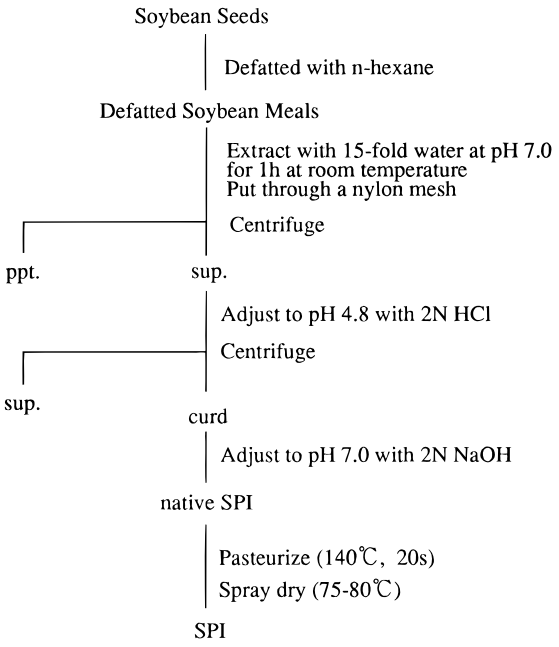

Figure 1. Schematic diagram for the preparation of SPI and preheated SPI.

tion properties of SPIs were then determined by dynamic viscoelastic measurements at temperatures <80 °C.

MATERIALS AND METHODS

Materials. Two kinds of new soybean seeds, glycinin-low and β-conglycinin-low lines, were used in this study. They were produced by Dr. K. Kitamura, National Agricultural Research Center, Tsukuba (Kitamura, 1993). Glycinin-low lines and β-conglycinin-low lines and IOM (Indiana, Ohio, Michigan) soybeans were raised in 1993. Coomassie Brilliant Blue R-250 was purchased from Fluka Chemie, AG (Buchs, Switzerland). All other chemicals used in this study were of reagent grade and used without further purification.

Preparation of Soybean Protein Isolate (SPI) and Preheated SPI. The procedure for preparing SPI and preheated SPI is shown in Figure 1. The following procedures were performed at room temperature. Defatted soybean meal was prepared from ground soybean seeds by solvent extraction with *n*-hexane. Defatted soybean meal was then extracted with 15-fold water at pH 7.0 and centrifuged to remove the insoluble material. The pH of the extract was adjusted to pH 4.8 with 2 N HCl, and the insoluble fraction was collected by centrifugation. The obtained precipitate was adjusted to pH 7.0 with 2 N NaOH, yielding SPI. Some of this SPI was lyophilized and the remainder was used to prepare preheated SPI. The solution of SPI was pasteurized at 140 °C for 20 s with steam and spray-dried at 75−80 °C to yield preheated SPI.

Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) was conducted with 12% polyacrylamide gel according to the method of Laemmli (1970). The gel was stained with Coomassie Brilliant Blue R-250. The staining intensity of the bands on SDS-PAGE was measured by densitometry with a flying-spot scanner (CS-9000, Shimadzu Corporation, Kyoto, Japan).

Dynamic Viscoelastic Measurements. The storage modulus G', the loss modulus G'', and the mechanical loss tangent tan δ were determined with a CLS-500 rheometer (Carri-Med Ltd., Surrey, U.K.). The sample solution of 12% (w/w) SPI in water at pH 7.0 was placed between parallel plates (diameter, 40 mm), and the gap between the two plates was set to 1 mm. The lower plate had been previously maintained at 30 °C. The sample solution was then subjected to shear oscillation of 1 Hz frequency and 0.025 strain. This experimental condition of shear oscillation was considered to be within the linear viscoelastic regime. The temperature was increased from 30 to 80 °C and then lowered to 20 °C at 2 °C/min. All measurements were done in duplicate at least.

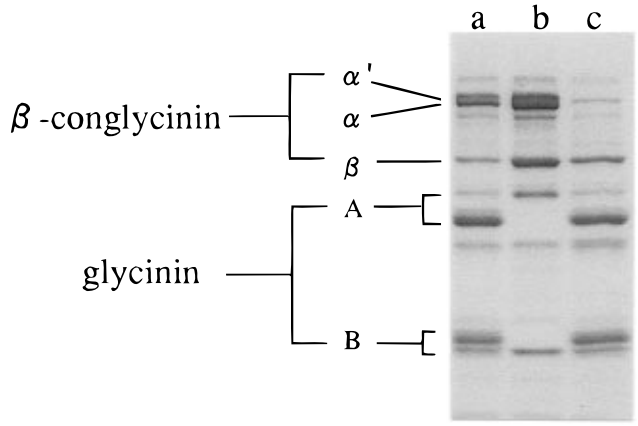

Figure 2. SDS-PAGE of SPIs obtained from three kinds of soybean seeds: (a) IOM soybeans; (b) glycinin-low lines; (c), β-conglycinin-low lines.

Table 1. Proportion of β-Conglycinin and Glycinin in SPIs Obtained from Glycinin-Low Lines, β-Conglycinin-Low Lines, and IOM Soybeans[a]

soybean seed	β-conglycinin, %	glycinin, %	total, %
glycinin-low lines	56	20	76
IOM	31	46	77
β-conglycinin-low lines	16	62	78

[a] Protein proportion was determined by SDS-PAGE with densitometry.

RESULTS AND DISCUSSION

Composition of β-Conglycinin and Glycinin of SPI. In this study we used two new soybean seeds, glycinin-low lines and β-conglycinin-low lines, with modified seed protein compositions of β-conglycinin and glycinin. The glycinin-low lines only have the G5 subunit of glycinin, whereas the β-conglycinin-low lines are characterized by the absence of an α′ subunit and low levels of both the α and β subunits of β-conglycinin. Despite their extremely different composition of β-conglycinin and glycinin, there is no reduction in total protein contents. The glycinin-low lines contain a high amount of β-conglycinin, and the β-conglycinin-low lines have a high amount of glycinin (Kitamura, 1993). IOM soybeans are usually used as materials for producing commercial SPI. Therefore, IOM soybeans were used to prepare control SPI.

The SDS-PAGE patterns of SPI obtained from IOM soybeans and the glycinin-low lines and β-conglycinin-low lines are shown in Figure 2. The staining intensity of the bands was measured by densitometry and the results in Table 1 indicate the percent areas of β-conglycinin and glycinin. The glycinin-low lines and β-conglycinin-low lines contain considerably different amounts of β-conglycinin and glycinin than IOM soybeans. In addition, the total area of β-conglycinin and glycinin is similar in the SPIs obtained from the glycinin-low lines and β-conglycinin-low lines and IOM soybeans. These results indicate that these three kinds of SPIs are suitable for studying the role of β-conglycinin and glycinin in the gel-forming abilities of SPI.

Dynamic Viscoelastic Properties of SPI. β-Conglycinin-rich and glycinin-rich SPIs are prepared from

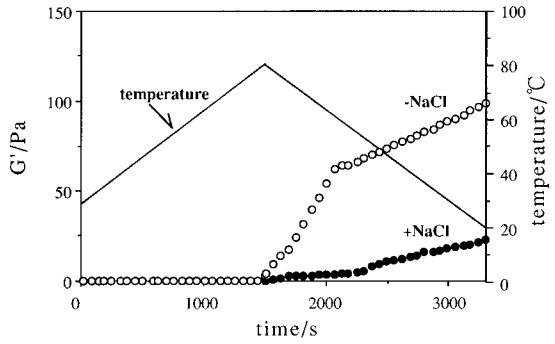

Figure 3. Gelation of 12% (w/w) β-conglycinin-rich SPI at pH 7.0 in the absence (○) and presence (●) of 2.5% NaCl. The temperature was increased from 30 to 80 °C and lowered to 20 °C at 2 °C/min.

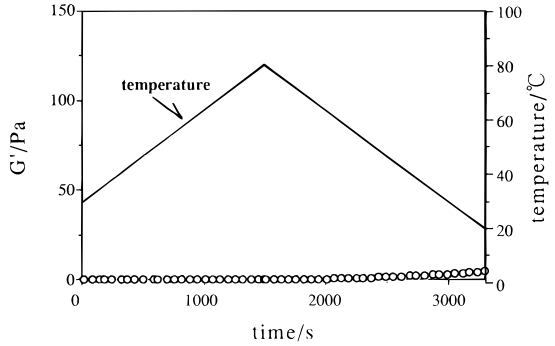

Figure 4. Gelation of 12% (w/w) SPI obtained from IOM soybeans at pH 7.0 in the absence of NaCl. The temperature was increased from 30 to 80 °C and lowered to 20 °C at 2 °C/min.

new soybean seeds, glycinin-low lines, and β-conglycinin-low lines, respectively. Control SPI was obtained from IOM soybean seeds. The effects of heating and cooling on the gelation process of 12% (w/w) SPIs in water at pH 7.0 were determined by dynamic viscoelastic measurements (Figures 3 and 4). The temperature was increased from 30 to 80 °C and then lowered to 20 °C at 2 °C/min. An increase in the storage modulus G' of β-conglycinin-rich SPI was observed with cooling. In the presence of 2.5% NaCl, the increase in the storage modulus G' of β-conglycinin-rich SPI was lower than that in the absence of NaCl (Figure 3). These results suggest that the number of functional groups available for gel network formation is reduced with the addition of NaCl because of insufficient denaturation of β-conglycinin. Bikbov et al. (1983) and Damodaran (1988) have reported the heating differential scanning calorimetry curves of SPI in the presence of NaCl at various salt concentrations. Their curves showed that the denaturation temperatures of β-conglycinin and glycinin shifted to higher temperatures with an increasing concentration of NaCl.

On the other hand, the storage moduli of control and glycinin-rich SPIs increased only slightly with both heating and cooling (Figure 4; data for glycinin-rich SPI are not shown). These results may be attributed to the fact that the denaturation temperature of glycinin is >80 °C. It is believed that denaturation is a prerequisite for the formation of SPI gel. Utsumi and Kinsella (1985) reported that gel hardness was in the order β-conglycinin ≫ SPI > glycinin, when heated at 80 °C for 30 min in 30 mM Tris-HCl buffer at pH 8.0. Theresults in the present study do not contradict their results, although a slight increase in the storage modulus G' of control and glycinin-rich SPIs was observed.

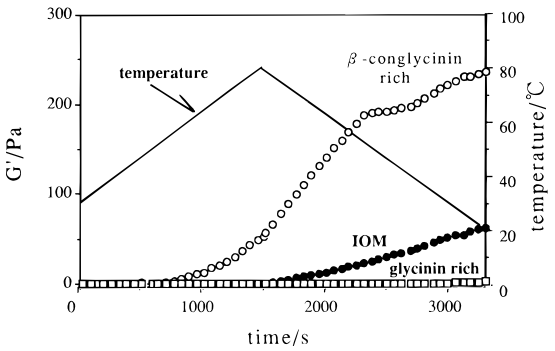

Figure 5. Gelation of 12% (w/w) preheated SPI at pH 7.0 in the absence of NaCl. The temperature was increased from 30 to 80 °C and lowered to 20 °C at 2 °C/min. (○) β-Conglycinin-rich; (●) IOM; (□) glycinin-rich.

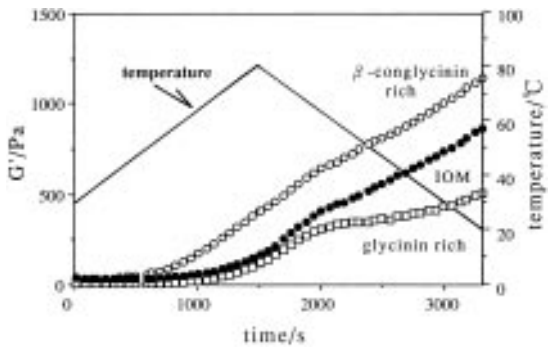

Figure 6. Gelation of 12% (w/w) preheated SPI at pH 7.0 in the presence of 2.5% NaCl. The temperature was increased from 30 to 80 °C and lowered to 20 °C at 2 °C/min. (○) β-Conglycinin-rich; (●) IOM; (□) glycinin-rich.

This might be attributable to the short duration of heating at 80 °C in this study.

Dynamic Viscoelastic Properties of Preheated SPI. Preheated SPIs were prepared from three kinds of soybean seeds, glycinin-low and β-conglycinin-low lines and IOM soybeans, by a typical method for manufacturing commercial SPI (i.e., a solution of SPI was pasteurized at 140 °C for 20 s and spray-dried; Figure 1). Because of the preheating during processing, the gelation properties of SPI should be changed. Therefore, gelation properties of preheated SPIs were studied by dynamic viscoelastic measurements.

The effects of heating and cooling on the gelation of 12% (w/w) preheated SPI were determined by dynamic viscoelastic measurements under the same conditions of heating and cooling used to study SPIs. In the absence of NaCl, the storage modulus G' of preheated β-conglycinin-rich SPI increased with both heating and cooling. In addition, the storage modulus G' of preheated SPI obtained from IOM soybeans increased with cooling, but that of preheated glycinin-rich SPI did not increase in either process (Figure 5). Clearly, the gel-forming ability of β-conglycinin-rich SPI was improved by preheating, but that of glycinin-rich SPI was not in the absence of NaCl. These results suggest that preheating has different effects on the gel properties of β-conglycinin and glycinin.

In the presence of NaCl, the storage modulus G' of all of the preheated SPIs increased with both heating and cooling (Figure 6). These results show that the gel-forming abilities of both β-conglycinin-rich and glycinin-rich SPIs were improved by preheating in the presence of 2.5% NaCl. The increase in the storage modulus G' of preheated SPI was enhanced with the addition of

Table 2. Dynamic Viscoelastic Properties of SPI Gels in the Presence of 2.5% NaCl after Cooling[a]

SPI	G', Pa	G'', Pa	tan δ
β-conglycinin-rich	1075 ± 95	110 ± 7	0.103 ± 0.015
IOM	755 ± 79	88 ± 15	0.116 ± 0.006
glycinin-rich	493 ± 14	157 ± 13	0.318 ± 0.076

[a] Mean value ± standard deviation of three replications.

NaCl (Figures 5 and 6), whereas the increase in the storage modulus G' of SPI was inhibited by adding NaCl (Figure 3). This phenomenon may be due to conformational changes in protein molecules caused by preheating. This notion should be explored in a future study.

It is well known that preheating improves the gel-forming ability of globular proteins such as glycinin (Nakamura et al., 1986), SPI (Furukawa and Ohta, 1981), and egg white (Kato et al., 1990a). Nakamura et al. (1985) postulated that gel-formation by glycinin occurred in two steps; that is, the association of glycinin molecules to form strands (stage 1), followed by interaction of the strands to form a gel networks (stage 2). A heating temperature of 100 °C was necessary only for stage 1; the temperature could be lowered to 80 °C for stage 2.

Furukawa and Ohta (1981) reported that the formation of aggregates was promoted by increasing the preheating temperature, and that gel hardness of SPI correlated with the increase in the degree of aggregates. On the other hand, Kato et al. (1990a) found that the gel strength of egg white increased with an increase in the duration of preheating at 80 °C in the dry state. They also determined the molecular weights of heated egg white and found that the molecular weight distribution curve shifted toward lower molecular weight and became sharp with an increase in the duration of preheating (Kato et al., 1990b). These results suggest that it is necessary not only to form a aggregate but also to aggregate with a certain molecular distribution to improve the gel-forming ability.

The storage modulus G' was increased in the order preheated β-conglycinin-rich SPI > preheated IOM SPI > preheated glycinin-rich SPI in the presence of 2.5% NaCl (Figure 6). The values of G', G'', and tan δ at 20 °C after cooling are shown in Table 2. A perfectly elastic material will show tan $\delta = 0$, and tan δ approaches infinity for a purely viscous material. The results of Figure 6 and Table 2 suggest that β-conglycinin plays an important role in gel formation by SPI.

The results of this study suggest that preheating has different effects on the gel properties of β-conglycinin and glycinin, and that β-conglycinin plays an important role in the gel properties of commercial SPI (Figures 5 and 6). Arrese et al. (1991), Wagner et al. (1992), and Petruccelli and Añón (1994) reported that the degree of denaturation varied among different commercial SPIs and seemed to affect functional properties such as the gel-forming ability, viscosity, and water imbibing capacity. Therefore, further investigations are needed concerning the relationship between the process used to manufacture commercial SPI and the individual gel properties of β-conglycinin and glycinin.

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