Effect of Heating and Cooling on the Gelation Kinetics of 7S Globulin from Soybeans†

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The gelation process of 15% (w/v) 7S globulin was studied using dynamic viscoelastic measurements when temperature was kept at 80 °C for 30 min and lowered to 20 °C. In the presence of 1 M NaSCN, the increase in the storage modulus G' was inhibited in the heating process. With increasing concentration of NaCl, the increase in the storage modulus G' was inhibited. The gel was not formed in the presence of more than 4 M guanidine hydrochloride. When the heat-induced gel of 7S globulin was again heated from 20 to 80 °C, the storage modulus G' decreased, showing that the gel formation in the cooling process is thermally reversible. Difference infrared spectra also indicated that the band at 1618 cm^{-1} (associated with β-sheet structure) of 7S globulin gel was decreased with increasing temperature. These results suggest that hydrophobic interactions and hydrogen bonds are very important for the formation of 7S globulin gel.

Keywords: *Soybean, 7S globulin, heat-induced gel, dynamic rheological measurements, Fourier transform infrared (FT-IR)*

INTRODUCTION

It is very important to understand the mechanism and the molecular forces of the heat-induced gelation of soybean proteins for controlling gel properties. The effects of salts, reducing agents, and denaturants have been investigated to clarify the molecular forces in the heat-induced gel of soybean proteins (Shimada and Matsushita, 1981; Utsumi and Kinsella, 1985; Mori et al., 1986). Furthermore, Wang and Damodaran (1991) have studied the type and extent of conformational changes and their relation to the physical properties of globular protein gels. However, further information is needed to control gel properties of soybean proteins.

Dynamic rheological measurement is a useful method to study the gelation phenomenon, because it can be carried out at small strain without breaking the structure being formed during gelation (Clark and Ross-Murphy, 1987; Ziegler and Foegeding, 1990; Nishinari et al., 1991). It is expected that study on the gelation curves in the heating and cooling processes and the effects of salts on the gelation process may give useful information to understand the molecular forces in the heat-induced gel of soybean proteins.

We have studied the gelation process of 7S globulin because of the following reason (Nagano et al., 1992, 1994a). Soybean proteins consist of two major components, 7S and 11S globulins, with different structures and gelation properties (Saio and Watanabe, 1978; Hermansson, 1986; Morr, 1990). Although 7S globulin is one of the major components of soybean proteins, the gelation mechanism of 7S globulin was not understood well with comparison to that of 11S globulin.

It is believed that all of the hydrogen bonds, hydrophobic interactions, ionic attractions, and disulfide bonds are involved in cross-link bonding of protein gel structure (Nakai and Li-Chan, 1988). To investigate the contribution of ionic bonds and hydrophobic interactions to gelation process of 7S globulin, we have chosen to use structure-stabilizing and structure-destabilizing salts. Salts have been shown to affect the denaturation temperature and conformation of various proteins. The effects of salts on protein structure involve two mechanisms: the electrostatic shielding effects and ion-specific effects in hydrophobic interactions. The effectiveness of various salts on the stability of protein follows the Hofmeister series (von Hippel and Schleich, 1969; Damodaran, 1988). In the present study, NaSCN and NaCl have been selected as the representatives of structure destabilizers and structure stabilizers, respectively. Guanidine hydrochloride is also selected to investigate the contribution of hydrogen bonds, hydrophobic interactions, and disulfide bonds. In addition, the conformational change process of 7S globulin with increasing temperature was investigated by Fourier transform infrared analysis.

MATERIALS AND METHODS

Enrei variety soybeans were used in this study. Soybean seeds were defatted with *n*-hexane, and 7S globulin was prepared as described previously (Nagano et al., 1992). All chemicals used in this study were of reagent grade and were used without further purification.

Rheological Measurements. The storage modulus G' and the loss modulus G'' were determined by a CSL-500 rheometer (Carri-Med Ltd., Surrey, U.K.). The sample solution of 15% (w/v) 7S globulin was prepared in 35 mM potassium phosphate buffer at pH 7.6. The solution 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 kept at 80 °C beforehand. The sample solution was then subjected to shear oscillation of 1-Hz frequency and 5% strain, which is within the linear viscoelastic regime as described previously (Nagano et al., 1994a).

Fourier Transform Infrared (FT-IR) Measurements. Infrared spectra were recorded on a FT-300 (Horiba, Ltd., Kyoto, Japan) equipped with a triglycine sulfate (TGS) detector. Spectral measurements were carried out at 4-cm^{-1} resolution in the temperature range from 20 to 80 °C. Interferograms from 100 scans were averaged to obtain one spectrum. This required about 5 min. Nitrogen gas was allowed to continuously flow into

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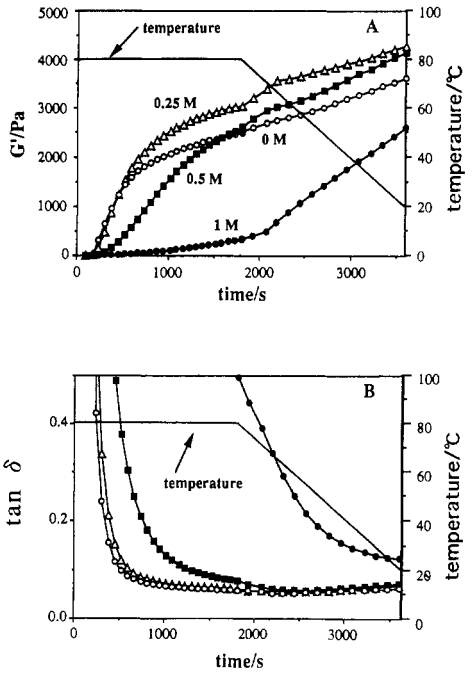

Figure 1. Gelation process of 15% (w/v) 7S globulin solution in the presence of NaSCN at various concentrations at pH 7.6. The solution was heated at 80 °C for 30 min and cooled to 20 °C at 2 °C/min. (A) Storage modulus G'; (B) mechanical loss tangent tan δ. NaSCN concentrations: (○) buffer alone; (△) 0.25 M; (■) 0.5 M; (●) 1 M.

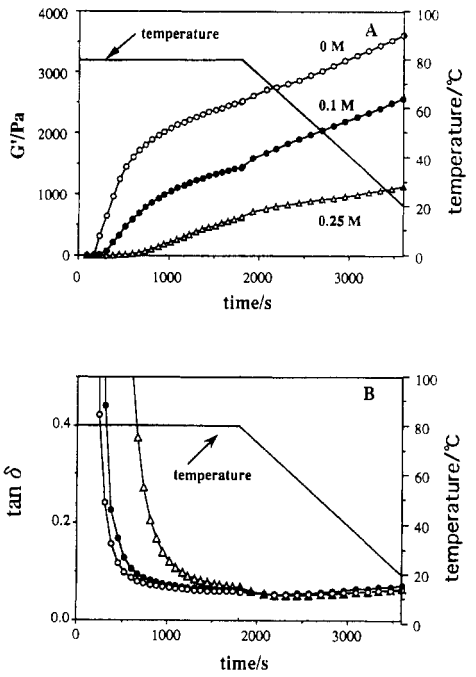

Figure 2. Gelation process of 15% (w/v) 7S globulin solution in the presence of NaCl at various concentrations at pH 7.6. The solution was heated at 80 °C for 30 min and cooled to 20 °C at 2 °C/min. (A) Storage modulus G'; (B) mechanical loss tangent tan δ. NaCl concentrations: (○) buffer alone; (●) 0.1 M; (△) 0.25 M.

the spectrophotometer during spectral measurements. 7S globulin (15% w/v) was dissolved in D_2O solution of 35 mM potassium phosphate buffer, pD 7.6. The protein solution was set between two CaF_2 disks using a 6-μm spacer. The circumferential gap between the two CaF_2 disks was sealed with glue and taped with Teflon seal to avoid evaporation of D_2O. The gel was prepared by heating at 80 °C for 30 min in an incubator (DF 62, Yamato, Tokyo, Japan) and cooled for 1 h at 20 °C. The resultant protein spectra were smoothed with a nine-point Savitzky–Golay function (Savitzky and Golay, 1964). The absorbance change at 1618 cm^{-1} was calculated as percent absorbance change compared to the spectrum of 7S globulin gel at 20 °C.

RESULTS AND DISCUSSION

Effects of Salts on the Gelation Process. Viscoelastic properties of gels are well characterized by storage modulus G′, loss modulus G'', and mechanical loss tangent tan δ (Ferry, 1980). The storage modulus G' is proportional to the energy stored in a gel per cycle, while the loss modulus G'' is proportional to the energy lost per cycle. The ratio G''/G' is called loss tangent tan δ. A perfectly elastic material will show tan $\delta = 0$, while for a purely viscous material tan δ tends to infinity. The effect of heating and cooling on the gelation process of 15% (w/v) 7S globulin was determined by dynamic viscoelastic measurements. The temperature was kept at 80 °C for 30 min and lowered to 20 °C at 2 °C/min. The storage modulus G' increased in both the heating and cooling processes (Figure 1A, curve without NaSCN). This indicates the formation of gel networks in both the heating and cooling processes. Effects of NaSCN, NaCl, and guanidine hydrochloride (GuHCl) on the gelation process of 7S globulin were then studied.

The effects of NaSCN on the gelation process of 7S globulin are also shown in Figure 1. With increasing concentration of NaSCN, the increase of the storage modulus G' was inhibited in the initial stage (<500 s) of the heating process. At the end of the heating process, however, the storage modulus G' of a gel with 0.25 M NaSCN was higher than that without NaSCN. Furthermore, the storage moduli of 7S globulin gels, which were heated at 80 °C for 30 min and cooled to 20 °C, with 0.25 and 0.5 M NaSCN were higher than that without NaSCN (Figure 1A). Salts have charge-neutralizing effects with increasing concentration; therefore, the suppression of repulsion by counterions may enhance protein–protein interactions. These results suggest that the contribution of ionic attraction for the formation of 7S globulin gel might be neglected.

In the presence of 1M NaSCN, the increase of the storage modulus G' was inhibited in the heating process (Figure 1A). The decreasing mechanical loss tangent tan δ indicates that the system tends to a more solid-like state with the formation of the gel networks. With increasing concentration of NaSCN, the decrease of tangent tan δ was inhibited in the heating process (Figure 1B). On the other hand, the concentration of NaSCN affects the rate of increase of the storage modulus G' in the cooling process. This behavior in the cooling process could not be explained well and should be explored in the near future.

Figure 2 shows the effects of NaCl on the gelation process of 7S globulin. With increasing concentration of NaCl, the increase of the storage modulus G' was inhibited in the gelation process.

Damodaran (1988) has reported the heating DSC curves of soybean protein isolate in the presence of NaSCN and NaCl at various salt concentrations. At higher salt concentrations, NaSCN decreased stability to heat denaturation, while NaCl increased the thermostability of 7S and 11S globulins. It is well-known that NaSCN destabilizes protein structure and NaCl stabilizes protein molecules (von Hippel and Schleich, 1969). The effects of salts on protein structure involve two mechanisms: the electrostatic shielding effects and ion-specific effects in hydrophobic interactions. To determine the effects of salts on the hydrophobic interactions in the protein, Damodaran and Kinsella (1981) investigated how the equilibrium between the bound and the free ligand (2-nonanone) to bovine serum albumin is altered by various salts. The

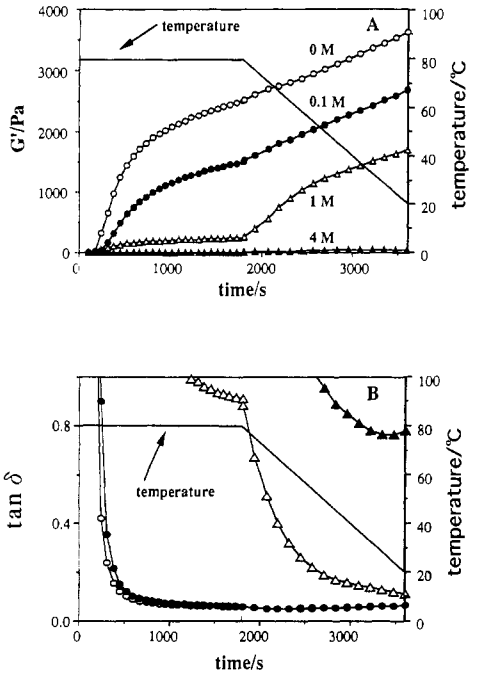

Figure 3. Gelation process of 15% (w/v) 7S globulin solution in the presence of guanidine hydrochloride (GuHCl) at various concentrations at pH 7.6. The solution was heated at 80 °C for 30 min and cooled to 20 °C at 2 °C/min. (A) Storage modulus G'; (B) mechanical loss tangent tan δ. GuHCl concentrations: (○) buffer alone; (●) 0.1 M; (△) 1 M; (▲) 4 M.

binding affinity for the ligand decreased with increasing concentration of NaSCN, while the binding affinity increased with increasing concentration of NaCl. These results suggest that NaSCN weakens and NaCl strengthens hydrophobic interactions.

The gel formation of 7S globulin was clearly inhibited in the heating process in the presence of 1 M NaSCN (Figure 1). It is considered that NaSCN suppresses intermolecular bonds through hydrophobic interactions in the presence of NaSCN because NaSCN weakens hydrophobic interactions as mentioned above. On the other hand, the increase of the storage modulus G' was inhibited with increasing concentration of NaCl (Figure 2A). This reason might be attributed to the fact that NaCl stabilizes protein molecules to heat denaturation (Damodaran, 1988). With increasing concentration of NaCl, the number of functional groups available for gel network formation is reduced because of insufficient denaturation of 7S globulin.

Guanidine hydrochloride (GuHCl) is a strong ionic denaturing agent (Tanford, 1968), which weakens hydrophobic interactions in proteins and inhibits hydrogen bonds and ionic attractions. Figure 3 shows the effects of GuHCl on the gelation process of 7S globulin. With increasing concentration of GuHCl, the increase of the storage modulus G' was inhibited in the gelation process and the gel was not formed in the presence of more than 4 M GuHCl. The decrease of tan δ was also inhibited with increasing concentrations of GuHCl. These results suggest that hydrophobic interactions and hydrogen bonds are very important for the formation of 7S globulin gel.

Gels can be formed by some proteins in an environment of denaturing agents such as GuHCl or urea, because of the formation of intermolecular disulfide bonds. The gel of bovine serum albumin (BSA) is not formed in 8 M urea plus sulfhydryl reagents which destroy SH groups, and carboxymethylated BSA is unable to form a gel in urea (Huggins et al., 1951). In the present study, the storage

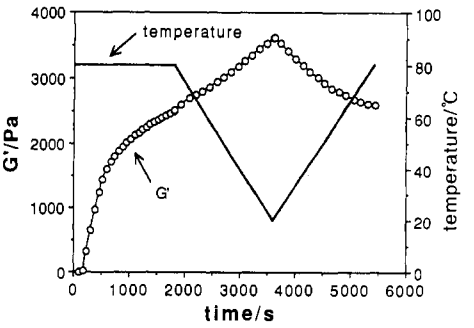

Figure 4. Gelation process of 15% (w/v) 7S globulin solution at pH 7.6. The solution was heated at 80 °C for 30 min, cooled to 20 °C at 2 °C/min, and then heated to 80 °C at 2 °C/min.

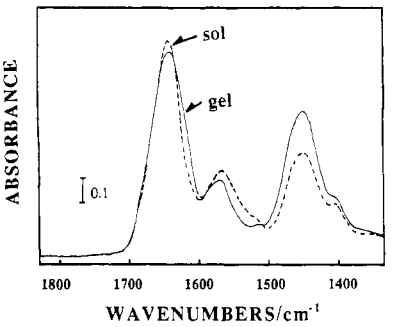

Figure 5. FT-IR spectra of sol (broken line) and gel (solid line) of 15% (w/v) 7S globulin at 20 °C in 35 mM phosphate buffer, pD 7.6. The gel was obtained by heating at 80 °C for 30 min and kept at 20 °C for 1 h.

modulus G' of 7S globulin decreased with increasing concentrations of GuHCl and could not be detected in the presence of more than 4 M GuHCl (Figure 3). Moreover, 7S globulin contains little cysteine (Coates et al., 1985). These results suggest that the contribution of intermolecular disulfide bonds for the formation of 7S globulin gel is limited.

Utsumi and Kinsella (1985) studied forces for the formation of 7S globulin gel, and concluded that hydrophobic interactions and hydrogen bonds are possible molecular forces involved in the formation of 7S globulin gel. Our results are in good agreement with their proposal.

Effect of Temperature on Gel Properties. It is well-known that hydrogen bonds are weakened with increasing temperature. To investigate the contribution of hydrogen bonds to the formation of 7S globulin gel, the storage modulus G' of the heat-induced gel was observed as a function of temperature, which was raised from 20 to 80 °C at 2 °C/min (Figure 4). With increasing temperatures from 20 to 80 °C, the storage modulus G' decreased and reached the same level as the storage modulus G' of 7S globulin solution that was heated at 80 °C for 30 min. These results suggest that the increase in the storage modulus G' of 7S globulin gel in the cooling process is mainly due to the formation of hydrogen bonds and was a thermally reversible reaction.

FT-IR Measurements. To clarify how protein molecules in gels change their secondary structure with increasing temperature, FT-IR measurements were performed. FT-IR spectra of sol and gel of 7S globulin are shown in Figure 5. The gel was obtained by heating at 80 °C for 30 min and kept at 20 °C for 1 h. Figure 6 shows the difference spectrum which was obtained by subtracting the spectrum of unheated solution from that of gel. The bands at 1680, 1645, and 1618 cm^{-1} were observed. The decrease at 1645 cm^{-1} is attributed to the denaturation of

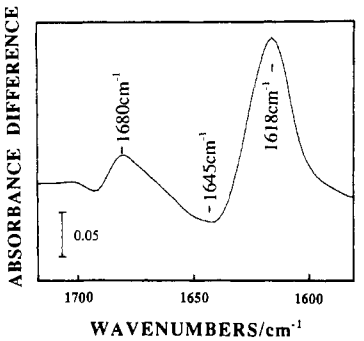

Figure 6. Difference spectrum obtained by subtracting the spectrum of sol from that of gel for 15% (w/v) 7S globulin at 20 °C in 35 mM phosphate buffer, pD 7.6.

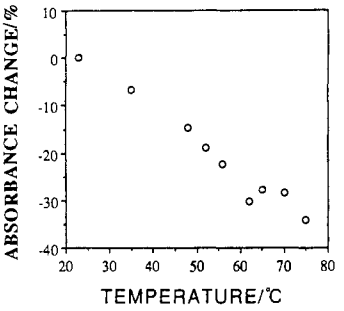

Figure 7. Absorbance change at 1618 cm^{-1} of 15% (w/v) 7S globulin gel with increasing temperature.

protein molecule. The absorbance decrease in the amide I′ region provides a good measure for estimating the degree of denaturation (Yamamoto et al., 1991; Yamamoto and Tasumi, 1991). The bands at 1680 and 1618 cm^{-1} are observed in characteristic β-sheet structure (Krimm and Banderkar, 1986; Surewicz and Mantsch, 1988; Grinberg et al., 1992).

The band at around 1618 cm^{-1} is considered to be associated with intermolecular β-sheet structure (Clark et al., 1981; Nagano et al., 1994b). Clark et al. (1981) observed the development of the band at 1620 cm^{-1} during heat-induced gelation of proteins such as bovine serum albumin, insulin, lysozyme, and α-chymotrypsin. Close comparison of this behavior with corresponding changes in small-angle X-ray scattering during heating confirms that the growth of the band at 1620 cm^{-1} correlated with the aggregation process. A band developing at 1620 cm^{-1} during protein aggregation suggests the formation of new β-sheet structures and assumes that the formation of intermolecular β-sheet structure was taking place (Clark et al., 1981; Clark and Tuffnell, 1986). Furthermore, the increases of absorption at 1618 cm^{-1} were determined by FT-IR measurements when gels were formed by heating at various temperatures for 7S and 11S globulins. A good correlation was observed between the value of the storage modulus G' and the increase of absorption at 1618 cm^{-1} (Nagano et al., 1994b).

When the temperature of the 7S globulin gel increased, the bands at both 1645 (data not shown) and 1618 cm^{-1} (Figure 7) decreased. These results indicate that denaturation of protein molecules proceeds and contents of intermolecular β-sheet structure decrease with increasing temperature. The absorbance change at 1618 cm^{-1} was calculated as percent absorbance change compared to the spectrum of 7S globulin gel at 20 °C. For example, a −10% absorbance change in Figure 7 means that the absorbance at 1618 cm^{-1} decreases by 10% from that of 7S globulin gel at 20 °C. If rheological data are taken into account, the decrease at 1618 cm^{-1} suggests that hydrogen bonds, which maintain the gel structure of 7S globulin, are weakened with increasing temperature.

Conclusion. It is considered that hydrophobic interactions and hydrogen bonds are very important in the heat-induced gel formation of 7S globulin. The gel formation in the cooling process is a thermally reversible reaction. FT-IR measurements suggest that hydrogen bonds are mainly responsible for the reversible reaction.

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