Dynamic Viscoelastic Study on the Gelation of 7S Globulin from Soybeans

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7S globulin was isolated according to a new method from soybeans. Its physicochemical properties were studied by differential scanning calorimetry (DSC) and by dynamic viscoelastic measurements. Heating DSC curves for 7S globulin showed only one endothermic peak. Extrapolation of the incipient temperature (T_i) and the endothermic peak temperature (T_p) observed at slow heating rates in DSC curves to 0 °C/min led to the result T_{i0} = 64 °C and T_{p0} = 70 °C. The gelation process of 7S globulin solution was examined by dynamic rheological measurements at isothermal conditions. The storage modulus G' did not rise at temperatures below T_{i0} (64 °C) even after 120 min. The gelation time became shorter and the rate constant of gelation increased with increasing heating temperature. The storage modulus G' of 7S globulin gels heated for 120 min as a function of heating temperature increased up to T_{p0} (70 °C) and then decreased. These results indicated that the rheological behavior of 7S globulin gel was closely related to DSC curves and strongly influenced by the heating temperature.

INTRODUCTION

Soybean proteins are extensively used for processed foods and traditional Japanese foods because of their functional properties and high nutritional value. Gel-forming ability is one of the most important functions of soybean proteins, and it is worthwhile to understand their gelation properties. Soybean proteins consist of two major components, 7S and 11S globulins (Osborne, 1924; Wolf et al., 1961), which have different structures and gel properties (Nielsen, 1985; Saio and Watanabe, 1978; Morr, 1990). Individual investigation of 7S and 11S globulins is necessary to elucidate the gel properties of soybean proteins. Although 7S globulin is one of the major components of soybean proteins, the literature is scanty concerning heat-induced gels of 7S globulin (Hashizume et al., 1975; Utsumi and Kinsella, 1985; Nakamura et al., 1986) with comparison to those of 11S globulin.

Dynamic rheological measurement is a useful method to study the gelation phenomenon, because it can be carried out at small strain within the linear viscoelastic regime and gelation curves can be monitored as a function of time (Nishinari et al., 1991; Clark and Ross-Murphy, 1987). Although thermal denaturation studies of 7S globulin by differntial scanning calorimetry (DSC) have been reported, most of the observed heating DSC curves showed two endothermic peaks, one of which originated from 11S globulin (Hermansson, 1978; Bikbov et al., 1983; Damodaran, 1988; Kitabatake et al., 1990).

In the present work, the relationship between gelation properties and heating DSC curves for 7S globulin was studied. This is the first study of gel properties of pure 7S globulin which shows only one endothermic peak in heating DSC curves as far as we are aware.

MATERIALS AND METHODS

The variety of soybean seeds used in this study was Enrei. Sodium bisulfite (SO_2 contents 60–69%) was purchased from Kishida Chemical Co., Ltd. All other chemicals used in this study were of reagent grade and were used without further purification.

Isolation of 7S Globulin. The isolation protocol for 7S and 11S globulins is shown in Figure 1. Soybean seeds were defatted with *n*-hexane. The protein was then extracted by making a slurry with 15-fold volumes of water adjusted to pH 7.5 with 2 N NaOH. This slurry was put through a nylon mesh (180-mesh size), and the filtrate was collected and then centrifuged (9000*g* × 30 min). Dry sodium bisulfite (SBS) was then added to the supernatant (0.98 g of SBS/L), the pH was adjusted to 6.4 with 2 N HCl, and the mixture was kept in ice bath overnight.

The following preparation procedure was performed at 4 °C. After removal of the insoluble fraction, which turned out to be the 11S globulin fraction, by centrifugation (6500*g* × 20 min), the salt concentration was then adjusted to 0.25 M by the addition of solid NaCl. The pH of the supernatant was then adjusted to pH 5.0 with 2 N HCl. After 1 h, the insoluble fraction was removed by centrifugation (9000*g* × 30 min). The supernatant was diluted 2-fold with ice-cold water, adjusted to pH 4.8 with 2 N HCl, and then centrifuged (6500*g* × 20 min). The obtained precipitate, the 7S globulin fraction, was washed twice with distilled water, adjusted to pH 7.5 with 2 N NaOH, and then lyophilized. Protein content of this lyophilized powder was 92% by the Kjeldahl method (N × 6.25).

Differential Scanning Calorimetry (DSC) Measurements. The thermal denaturation of 7S globulin was studied by DSC measurements using a DSC 120 (Seiko Instruments Inc.). An approximately 45-mg portion of 10% (w/w) 7S globulin solution in 35 mM potassium phosphate buffer, pH 7.6, was weighed into a silver pan and sealed. Distilled water was used as the reference material. Heating rates ranged from 0.5 to 2 °C/min.

Rheological Measurements. The storage modulus G' and the mechanical loss tangent tan δ of 7S globulin were determined by a Rheograph Sol (Toyo Seiki Seisakusho Ltd.). Sample (1.5 mL) [10% (w/w) 7S globulin solution in 35 mM potassium phosphate buffer, pH 7.6] was introduced to the cell, which was kept at a constant temperature beforehand. The sample solution was then subjected to shear oscillations of 1-Hz frequency and 25-μm amplitude (Kaibara and Fukada, 1976). The measurements performed ranged from 62 to 80 °C for 120 min.

Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to the method of Laemmli (1970). The separating gel gradient was between 8% and 16% acrylamide.

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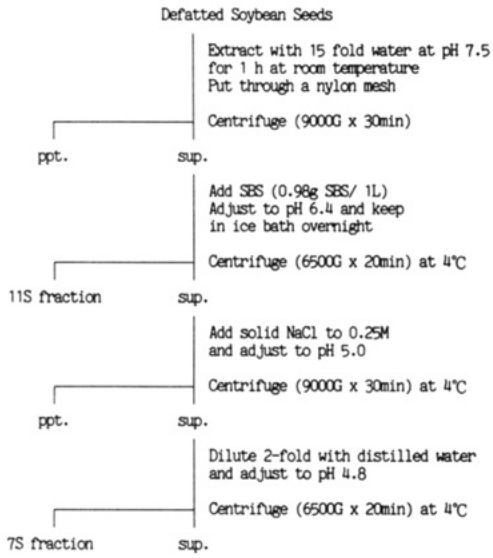

Figure 1. Schematic diagram for the isolation of 7S and 11S globulins.

RESULTS

Isolation of 7S Globulin. Suchkov et al. (1990) reported a noteworthy method to isolate 7S and 11S globulins from broad beans and peas based on the differences of solubility of 7S and 11S globulins in various NaCl concentrations at 5 °C. However, it was not possible to isolate 7S and 11S globulins properly in the case of soybeans by their method.

It was concluded that the conditions of pH 5.0 and 0.25 M NaCl concentration were best to isolate 7S globulin from soybeans at 4 °C. The presence of 2-mercaptoethanol (2-ME) has an important effect on the isolation of 7S and 11S globulins in the isoelectric precipitation step as reported previously (Nash et al., 1974; Iwabuchi and Yamauchi, 1987). It was confirmed that SBS could be used instead of 2-ME. The reducing agent was required to give a good purity of 11S globulin and a high yield of 7S globulin. The amount of 7S globulin which was obtained in the presence of SBS was 1.5-fold that without SBS.

SBS was used because the water content of the precipitated 11S globulin fraction was lower. Therefore, it was easier to separate 11S globulin by centrifugation, especially when large amounts of 11S globulin are required. The protein contents of the 11S globulin fraction were 19% in the presence of 2-ME and 41% in the presence of SBS determined according to the Kjeldahl method (N × 6.25) when the precipitate was obtained by centrifugation at 6500*g* for 20 min.

By this method, 5 g of 11S globulin and 3 g of 7S globulin were obtained from 50 g of defatted soybean seeds. Figure 2 shows the SDS–PAGE pattern of 7S and 11S globulin fractions. The purity of each fraction obtained by our method was more than 90% measured by densitometry.

DSC Measurements. A typical DSC heating curve for a 10% 7S globulin solution is shown in Figure 3. Only one endothermic peak during the heating DSC measurement of 7S globulin was observed. The incipient temperature (T_i) and the endothermic peak temperature (T_p) were determined at different scanning rates as shown in Figure 4. T_i and T_p observed at different heating rates were extrapolated to 0 °C/min. As the result, T_{i0} was 64 °C and T_{p0} was 70 °C.

Dynamic Rheological Measurements. The storage modulus G' and the loss modulus G'' of 7S globulin gels formed by heating at 80 °C for 120 min were measured as a function of strain amplitude by using the same oscillatory frequency (1 Hz). In the amplitude range 25–100 μm, both G' and G'' were constant. This result indicated that the shear oscillation of 1-Hz frequency and 25-μm amplitude were within the linear viscoelastic regime.

The gel formation curves of 7S globulin were studied at constant temperatures from 62 to 80 °C for 120 min. Figure 5 shows experimental G' values as a function of time at

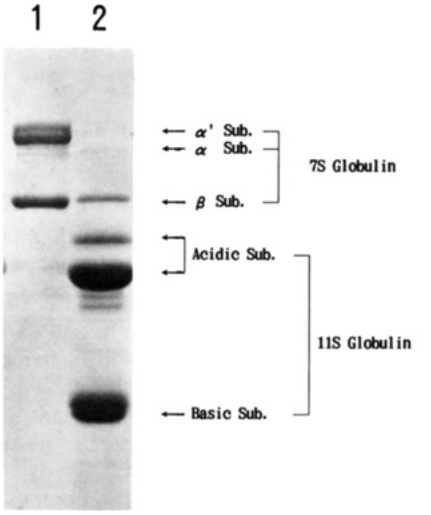

Figure 2. SDS–polyacrylamide gel electrophoresis of 7S and 11S globulin fractions. (Lane 1) 7S globulin fraction; (lane 2) 11S globulin fraction.

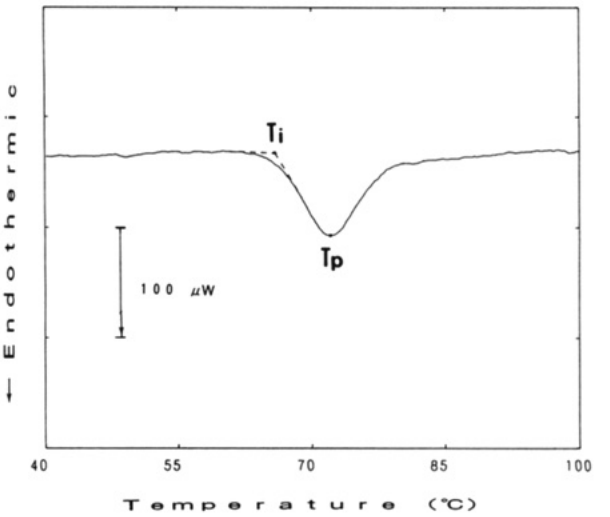

Figure 3. Typical heating DSC curve of 7S globulin solution (10% w/w) in 35 mM potassium phosphate buffer, pH 7.6. Heating rate, 1 °C/min, T_i, the incipient temperature; T_p, the endothermic peak temperature.

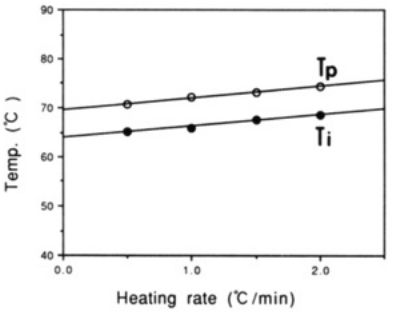

Figure 4. Incipient temperature (T_i) and endothermic peak temperature (T_p) of 7S globulin solution as a function of the scanning rate. (●) T_i; (○) T_p.

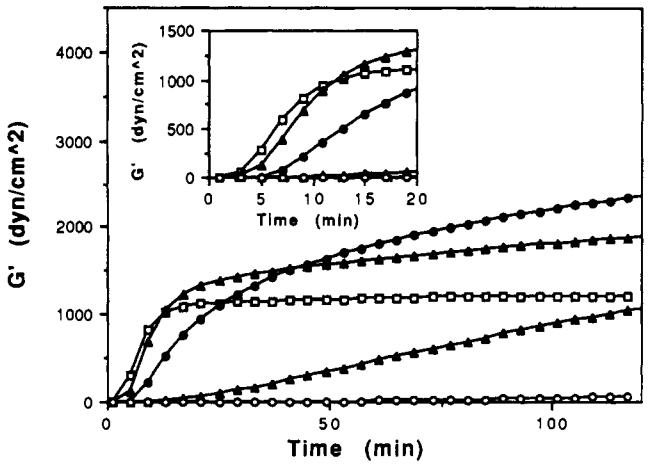

Figure 5. Gelation curves of 10% 7S globulin solution in 35 mM potassium phosphate buffer, pH 7.5, at different temperatures. (□) 80 °C; (△) 75 °C; (●) 70 °C; (▲) 65 °C; (○) 62 °C.

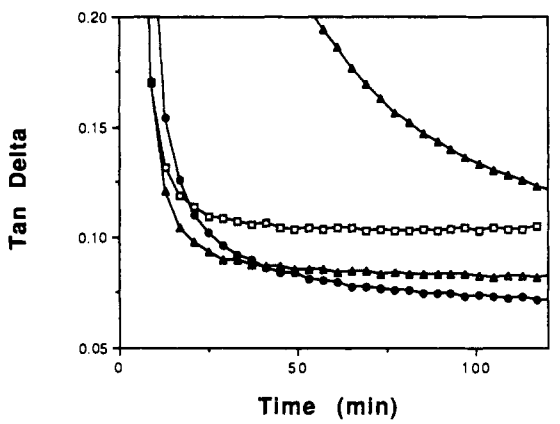

Figure 6. Mechanical loss tangent tan δ 10% 7S globulin solution in 35 mM potassium phosphate buffer, pH 7.6, at differential temperatures. (□) 80 °C; (△) 75 °C; (●) 70 °C; (▲) 65 °C.

different temperatures. The storage modulus G' of 7S globulin solution did not rise with heating below T_{i0} (62 °C) for 120 min and began to rise only above T_{i0}. The gel was formed faster with increasing heating temperatures. The storage modulus G' of 7S globulin gel heated for 120 min increased with increasing heating temperature and showed maximum around T_{p0} (70 °C) and then decreased.

Figure 6 shows the mechanical loss tangent tan δ of 7S globulin as a function of time at different heating temperatures. The decreasing of tan δ indicates that the system tends to a more solidlike state with the formation of the gel networks.

Gelation curves of 7S globulin were approximated well by the empirical equation (Kaibara, 1973; Beveridge et al., 1984)

$$G'(t) = G'_{sat}\{1 - \exp[-k(t - t_0)]\} \quad (1)$$

where G'_{sat} is the saturated value of G', k is the rate constant of gelation, t_0 is the gelation time, and t is the time. The gelation time was taken as the point where G' began to deviate from the baseline. This equation can easily be cast in linear form:

$$\ln (1 - G'(t)/G'_{sat}) = -k(t - t_0) \quad (2)$$

To determine two unknown constants (G'_{sat} and k), an initial trial value of G'_{sat} was selected. Then, k and the correlation coefficient (r) of the regression line were calculated. Values of G'_{sat} and k yielding maximum r were adopted.

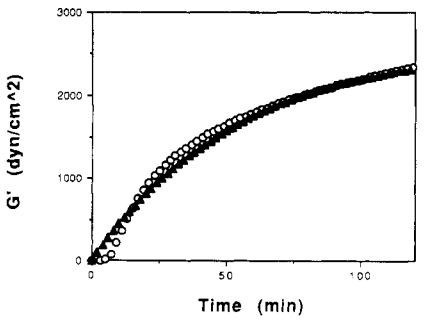

Figure 7. Experimental storage modulus G' data (○) for 7S globulin gelation curve and kinetic model representation (▲) at 70 °C heating.

Table I. Parameters of the Kinetic Model for the Gelation of 7S Globulin[a]

heating temp, °C	t_0, min	$G'(120)$, dyn/cm²	G'_{sat}, dyn/cm²	k, min⁻¹	r
70	5	2340	2600	0.0188	0.994
75	3	1880	1940	0.0293	0.958
80	2	1210	1210	0.0523	0.942

[a] t_0, gelation time; $G'(120)$, storage modulus heated for 120 min; G'_{sat}, saturated storage modulus; k, rate constant; r, correlation coefficient.

Figure 7 shows that the empirical equation fitted the experimental data for the gel heated at 70 °C with considerable accuracy. Table I shows G'_{sat}, k, t_0, and r at different heating temperatures. The gelation time (t_0) became shorter and the rate constant (k) increased with increasing heating temperatures. The correlation coefficient (r) showed that the gelation curve at 70 °C fitted better than those at 75 or 80 °C.

DISCUSSION

We described a simple method to isolate 7S and 11S globulins of high purity from soybeans (Figure 1). It is considered that the sample which shows only one endothermic peak by DSC measurement is very useful to investigate the gelation properties of soy proteins. Two endothermic peaks which originate from 11S and 7S globulins make it difficult to clarify the gelation process. Although the method of Thanh and Shibasaki (1976) is widely used for the isolation of 7S and 11S globulins from soybeans, Damodaran (1988) reported that the 7S fraction which was isolated according to their method exhibited two endothermic peaks because of the presence of the 11S globulin as a contaminant in the 7S fraction. In this study, the 7S fraction obtained by the present method exhibited only one endothermic peak (Figure 3).

The contamination by β-subunit of 7S globulin in 11S globulin fraction was detected by SDS-PAGE (Figure 2). This is unavoidable, if 11S globulin is obtained by precipitation at pH 6.4 as mentioned by Yamauchi et al. (1981). However, the purity of the 11S globulin fraction obtained according to our method was more than 90% measured by densitometry and also showed only one endothermic peak by DSC measurement (data were not shown). It is considered that the purity of 11S globulin fraction is enough high.

In this investigation, the incipient temperature and the endothermic peak temperature of 7S globulin were determined: $T_{i0} = 64$ °C, $T_{p0} = 70$ °C. Hermansson (1978) and Damodaran (1988) studied the endothermic peak of 7S globulin at a heating rate of 10 °C/min. Hermansson (1978) reported that T_i was 72 °C and T_p was 78.5 °C. It is well-known that endothermic peak temperatures in heating DSC curves shift to higher temperatures with

increasing scanning rates (Ozawa, 1970; Relkin and Launay, 1990).

This investigation clearly showed that the thermal profile of DSC measurements and rheological properties of 7S globulin are closely related. The storage modulus G' of 7S globulin solution was very small below T_{i0}, even after 120 min of heating, and showed an apparent increase by only heating above T_{i0} (Figure 5). This result supports the statement that the denaturation process is a prerequisite for the heat-induced gelation of globular proteins (Clark and Tuffnell, 1986; Damodaran, 1988).

On the other hand, the storage modulus showed a maximum with heating at T_{p0} (70 °C) after 120 min. A less rigid gel was formed with heating above 75 °C (Figure 5 and Table I). It is believed that the high-temperature heating generated more reaction sites for gel networks on the surface of protein molecule (mainly hydrophobic sites) and formed the disordered gel. Similar results were also observed by Furukawa et al. (1979) when they studied the effect of heating temperature on the gel strength of commercial soy protein isolate. They found that the gel strength as a function of the heating temperature increased with increasing heating temperature up to 80 °C and then decreased. Moreover, Wu et al. (1991) reported similar results in the case of myosin. They observed less rigid gels at heating temperatures higher than 58 °C. They mentioned that myosin was either polymerized to form large molecular weight clusters or unfolded to increase volume at high temperatures.

The rheological properties of protein gels must be explained on the molecular level. Detailed investigation related to protein gel structure is required.

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