phytic acid in cereal grains was species specific. Dry heat (autoclaving without water in the substrate) destroyed little phytate but increased the rate of enzymatic hydrolysis in the heated sample.

Seed phytate can be hydrolyzed by an application of microbial enzyme. Improving the treatment conditions, such as elevating temperature (50 °C), adjusting to pH 4–5.5, and heating the substrate prior to enzyme treatment, facilitates the hydrolysis of phytate. Because large portions of seed phytate exist as water-soluble forms that are easily extracted by water and because they contain endogenous phytase, a practical way of phytate removal from seeds may be accomplished by utilizing a combination of these factors—water solubility of seed phytate, inherent phytase, and added microbial phytase.

Registry No. Phytate, 83-86-3; phytase, 9001-89-2.

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Refolding of Thermally Unfolded Soy Proteins during the Cooling Regime of the Gelation Process: Effect on Gelation

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The thermal denaturation and gelation of soy proteins were studied. Evidence suggests that soy 11S globulin undergoes partial refolding during the cooling regime of the thermal gelation process. At 8% protein concentration, in the absence of any additive, soy 11S refolded considerably during the cooling regime and failed to form gel. However, addition of 0.5 M $NaClO_4$ inhibited the extent of refolding and caused gelation of 11S even at 8% concentration, whereas addition of 0.5 M NaCl promoted refolding and prevented gelation. On the basis of these findings a general mechanism for thermal gelation of globular proteins is proposed. It is suggested that by controlling the extent of refolding of the protein during the cooling regime, it is possible to improve the gelation of globular proteins even at suboptimum protein concentrations. The thermal denaturation of soy proteins under various solution conditions is also discussed.

A considerable amount of research has been done on the heat-induced gelation of soy globulins and other proteins in order to understand the general mechanism of protein gelation (Catsimpoolas and Meyer, 1970, 1971; Ishino and Kudo, 1977; Babajimopoulos et al., 1983; Bello et al., 1962). Studies have shown that several factors, such as protein concentration, pH, ionic strength, reducing agents, urea, etc., affect the gelation of soy globulins and other globular proteins (Catsimpoolas and Meyer, 1970, 1971; Hermansson, 1982; Schmidt et al., 1979; Furukawa et al., 1979). The results of those studies have led to the general conclusion that the three-dimensional network in gel structures is formed via hydrogen bonds, hydrophobic bonds, and electrostatic interactions between polypeptides. However, no attempts have been made to understand the relationship between the extent of formation of gel network and the state of protein structure in the gel.

The important initial step in heat-induced reversible gelation of globular proteins is the heating of the protein solution above the denaturation temperature of the pro-

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tein. Upon cooling, the exposed functional groups in the unfolded polypeptide interact with each other, leading to formation of an intricate network that acts as the matrix for entrapping water and other components. Since the protein solution is heated above the denaturation temperature of the protein, it is often assumed that the protein would remain in the fully denatured state in the gel. However, it is quite possible that the denatured protein may undergo partial refolding and regaining of secondary structures during the cooling regime of the gelation process. It is conceivable that the extent of such refolding and formation of secondary structures during the cooling phase would affect the number of functional groups available for intermolecular interactions and thus the formation and stability of the gel network.

The present study was undertaken to elucidate the influence of specific sodium salts on the thermal denaturation of soy globulins and the gelation of soy 11S globulin. It is shown that partial regaining of secondary structures does occur during gelation of 11S globulins; the extent of refolding was dependent on the type of salt present in the system. The salts inhibiting refolding of 11S globulin facilitated formation of gel network even at suboptimum protein concentration. The effects of various sodium salts on the thermal denaturation of soy 7S and 11S globulins are also discussed.

MATERIALS AND METHODS

Protein Solutions. Soy protein isolate, soy 7S and soy 11S globulins, was prepared from defatted soybean flour (Central Soya, Chicago) according to the method of Thanh and Shibasaki (1976). The purities of 11S and 7S globulins were about 95% and 78%, respectively, as judged from SDS–polyacrylamide gel electrophoretic analysis. All the chemicals used in this study were of either reagent or ACS grade. Protein solutions were prepared in water and were adjusted to pH 8.0 with either 2 N NaOH or HCl. Protein concentration was determined by the biuret method using bovine serum albumin as standard.

Calorimetry. The thermal denaturation of soy proteins under various solution conditions was studied by differential scanning calorimetry using a Du Pont Model 990 thermal analyzer, equipped with a DSC cell, as described previously (DeWit and Swinkles, 1980). In a typical experiment, 10–15 μL of about 7% protein solution was sealed in a preweighed hermetic aluminum pan and weighed again. Another aluminum pan containing water/salt solution with no protein was used as the reference pan. In studies on the effects of neutral salts on protein stability, microliter amounts of stock solutions of protein, salt solution, and water (all preadjusted to pH 8.0) were measured directly into the sample pans to give the desired final salt and protein concentrations. The pans were sealed, and the contents were mixed well and allowed to stand for 30 min. The pans were heated for 30 to 160 °C in the DSC cell at a programmed rate of 10 °C/min. The DSC instrument was calibrated with indium. The calibration coefficient, E, of the DSC cell was determined from the thermogram of weighed amount of indium with known heat of fusion (6.79 mcal/mg of indium). The areas under the endothermic peaks were measured with a planimeter. The enthalpy of denaturation, ΔH, was calculated by eq 1, where A is the area under the endothermic

$$\Delta H = \frac{A}{W} \times \mathrm{TB} \times E \times S \qquad (1)$$

peak (in.^{2}), W is the weight of protein (mg), TB is the time base (s/in.), E is the calibration coefficient, and S is the sensitivity (mcal/in.·s). The denaturation temperature T_D was obtained from the temperature at the peak of the endotherm.

Gelation. Thermal gelation of soy 11S globulin was studied by the method described previously (Babajimopoulos et al., 1983; Bello et al., 1962). Protein dispersions were heated at 90 °C for 30 min, then cooled immediately to 4 °C in an ice bath, and stored at 4 °C for 24 h. The melting point of the gel, i.e., the transition temperature at which the gel is transformed to liquid progel state, was determined by the method described previously (Babajimopoulos et al., 1983; Bello et al., 1962). The reproducibility of the melting temperature was about ±1.0 °C.

Circular Dichroism. Circular dichroic measurements were made with a Jasco Model J-41C spectropolarimeter. The path length of the cell was 1 mm, and protein concentration was about 0.015%. The mean residue ellipticity, $[\theta]$ (deg·cm^2/dmol), was calculated from eq 2, where d is

$$[\theta] = \frac{100 d \bar{M}}{C} \qquad (2)$$

the rotation (deg/cm path length), $\bar{M}$ is the average molecular weight of amino acid residues in the protein, and C is the protein concentration (mg/mL). In our calculations we assumed a value of 115 for the average molecular weight of amino acid residues in proteins.

To elucidate the conformation of soy 11S in the gel network, we employed the following approach: 30-μL aliquots of soy 11S solution (8%) containing 0.5 M salts or no salts were taken in a capillary tube (3-mm internal diameter), one end of which was heat sealed previously. The open end of the tube was closed with a small rubber cap. Gelation of these samples was carried out under conditions exactly similar to those described for other experiments. The capillary tubes were then placed in centrifuge tubes filled with glycerol and centrifuged at 35 000 rpm in a T-40 rotor (Beckman Model L-250 centrifuge) for 30 min at 5 °C. The purpose of glycerol was to protect the capillary tube from breaking during centrifugation. Microliter aliquots of the supernatant, i.e., the solution that has been squeezed out of the gel matrix during centrifugation, was removed for protein determination and circular dichroic analysis.

RESULTS AND DISCUSSION

The DSC thermograms of soy isolate, soy 7S and soy 11S globulins, are shown in Figure 1. Two endothermic peaks were observed for soy isolate corresponding to the denaturation of 7S (low-temperature endotherm) and 11S (high-temperature endotherm) components. At 10 °C/min heating rate, the denaturation temperatures of 7S and 11S globulins were 72 and 84.5 °C, respectively. While the 11S sample exhibited apparently only one peak, the 7S sample exhibited two endothermic peaks, presumably because of presence of 11S globulin as a contaminant in the 7S sample. SDS–polyacrylamide gel electrophoretic analysis of soy isolate sample (Figure 2) indicated that the 7S and 11S contents of soy isolate were about 33% and 56%, respectively, whereas the 7S and 11S globulin contents of the crude 7S preparation were 73% and 21%, respectively. The purity of 11S sample was about 95%.

Because of the difficulties in obtaining pure 7S and 11S globulins from soy isolate, and also because of poor resolution of 7S and 11S endothermic peaks in soy isolate, it has been difficult to determine accurately the enthalpies of denaturation of soy 7S and 11S globulins (Hermansson, 1979b). However, to determine the enthalpies of denaturation of 7S and 11S globulins from the thermograms in Figure 1, we employed the following approach: The total enthalpy of denaturation of soy isolate, calculated from

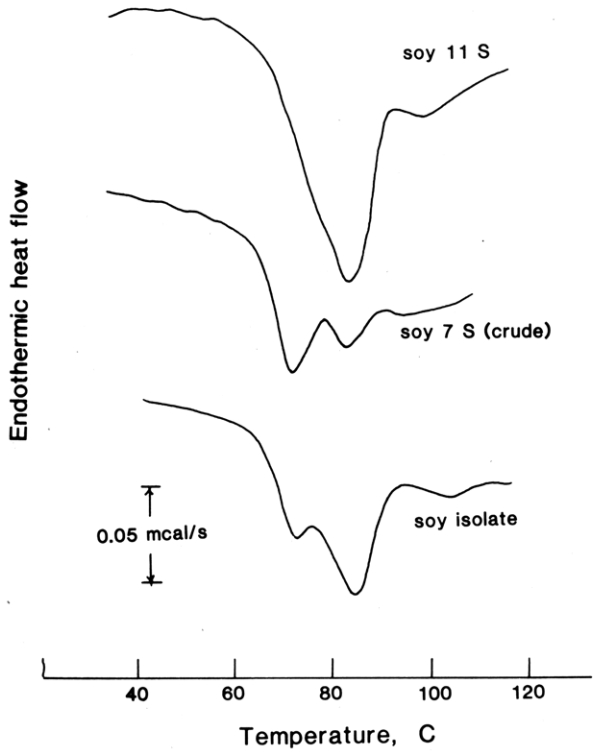


Figure 1. DSC thermograms of soy isolate, soy 7S and soy 11S globulins, in water at pH 8.0. Heating rate was 10 °C/min.

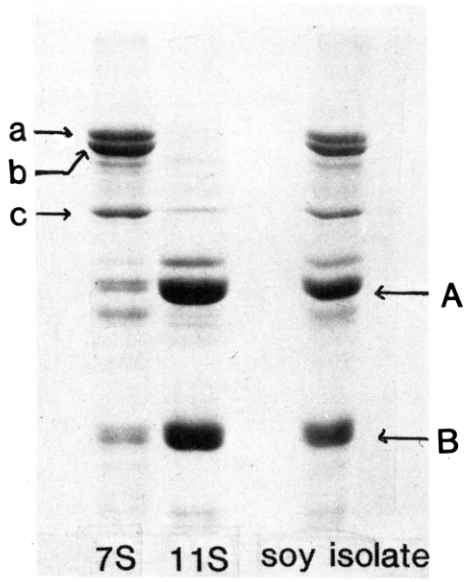


Figure 2. Urea–SDS–polyacrylamide gel electrophoretic patterns of soy isolate, soy 11S and 7S preparations. Bands a–c correspond to α', α, and β subunits of soy 7S globulin; bands A and B represent the acidic and basic subunits of soy 11S globulin. The gels were scanned with a densitometer to determine the contents of 7S and 11S globulins in each sample.

the area of the entire thermogram, can be expressed as in eq 3, where A_1 and B_1 are the weight fractions of 7S and

$$A_1x + B_1y = \Delta H_{SI} \quad (3)$$

11S proteins and x and y are the enthalpies of denaturation of 7S and 11S, respectively, in the soy isolate sample. Similarly, the total enthalpy of denaturation of the crude 7S sample can be expressed as eq 4, where A_2 and B_2 are

$$A_2x + B_2y = \Delta H_{7S} \quad (4)$$

weight fractions of 7S and 11S proteins, respectively, in the crude 7S preparation. It is assumed that the contribution of proteins other than 7S and 11S (mainly the minor 2S components) to the endotherm of soy isolate is very negligible. By solving the simultaneous equations (1) and (2) for x and y, it is possible to calculate accurately the enthalpies of denaturation of 7S and 11S globulins.

Estimation of ΔH by the above method gave values of 3.08 and 4.56 cal/g for 7S and 11S globulins, respectively. The significant difference in the ΔH values of 7S and 11S might be attributed to the differences in the molecular structures of these globulins. The 11S globulin contains 21 disulfide bonds, of which 15 are intrasubunit and 6 are intersubunit bonds. On the other hand, there are no intra- and intersubunit linkages in soy 7S globulin (Badley et al., 1975). The extensive intra- and intersubunit disulfide bonds in the 11S globulin may provide greater stability against thermal denaturation, which is reflected in its higher temperature and enthalpy of denaturation.

Effects of Neutral Salts. In order to elucidate the magnitude of contribution of the major noncovalent forces to the stability of soy 7S and 11S globulins, we studied the thermal denaturation of soy proteins in the presence of various neutral salts. The basic principle in this approach is that at low concentrations neutral salts affect electrostatic interactions between charged groups in a protein, whereas at higher concentrations, in addition to nonspecific charge neutralization effects, neutral salts have ion-specific effects on hydrophobic interactions (von Hippel and Schleich, 1969; Damodaran and Kinsella, 1981; Danliker and deSaussure, 1971). These ion-specific affects on hydrophobic interactions arise from perturbations in bulk water structure, which in turn affect segment–solvent and segment–segment interactions (von Hippel and Schleich, 1969). In this respect NaCl and NaSCN act as promoter and destabilizer, respectively, of hydrophobic interactions.

Sample thermograms of soy isolate in the presence of NaCl and NaSCN at various salt concentrations are shown in Figure 3. It can be seen that the thermal denaturation of both 7S and 11S components of soy isolate was affected by salt concentration as well as the type of salt used. The magnitude of changes in the denaturation temperatures of 7S and 11S globulins in the presence of NaCl, NaBr, $NaClO_4$, and NaSCN at various concentrations is shown in Figure 4. At any given salt concentration the relative order in which the T_D of 7S and 11S globulins were affected followed the series NaCl > NaBr > $NaClO_4$ > NaSCN. This order is the same as the Hofmeister series for anions describing the relative stabilizing or destabilizing effects of anions on hydrophobic interactions (von Hippel and Schleich, 1969).

Although $NaClO_4$ and NaSCN are considered to be structure destabilizers, these salts caused an increase in the T_D values of both 7S and 11S globulins up to about 0.25 M concentration, indicating stabilization of soy globulins against thermal denaturation. This initial increase in the T_D values at low concentrations can be attributed mainly to nonspecific ionic effects on electrostatic surface charge interactions in proteins that are effectively neutralized at 0.2 M ionic strength (Eagland, 1975). Such neutralization of both inter- and intrasubunit electrostatic repulsive forces by salts may account for the enhanced stability of the tertiary and quaternary structures of soy globulins. However, it should be pointed out that even at low concentrations there is considerable difference in the magnitude of increase in T_D by various salts. This suggests that even at low concentrations the anions seem to exert their ion-specific stabilizing or destabilizing effects on hydrophobic interactions. These ion-specific effects become more pronounced above 0.5 M salt concentration. At

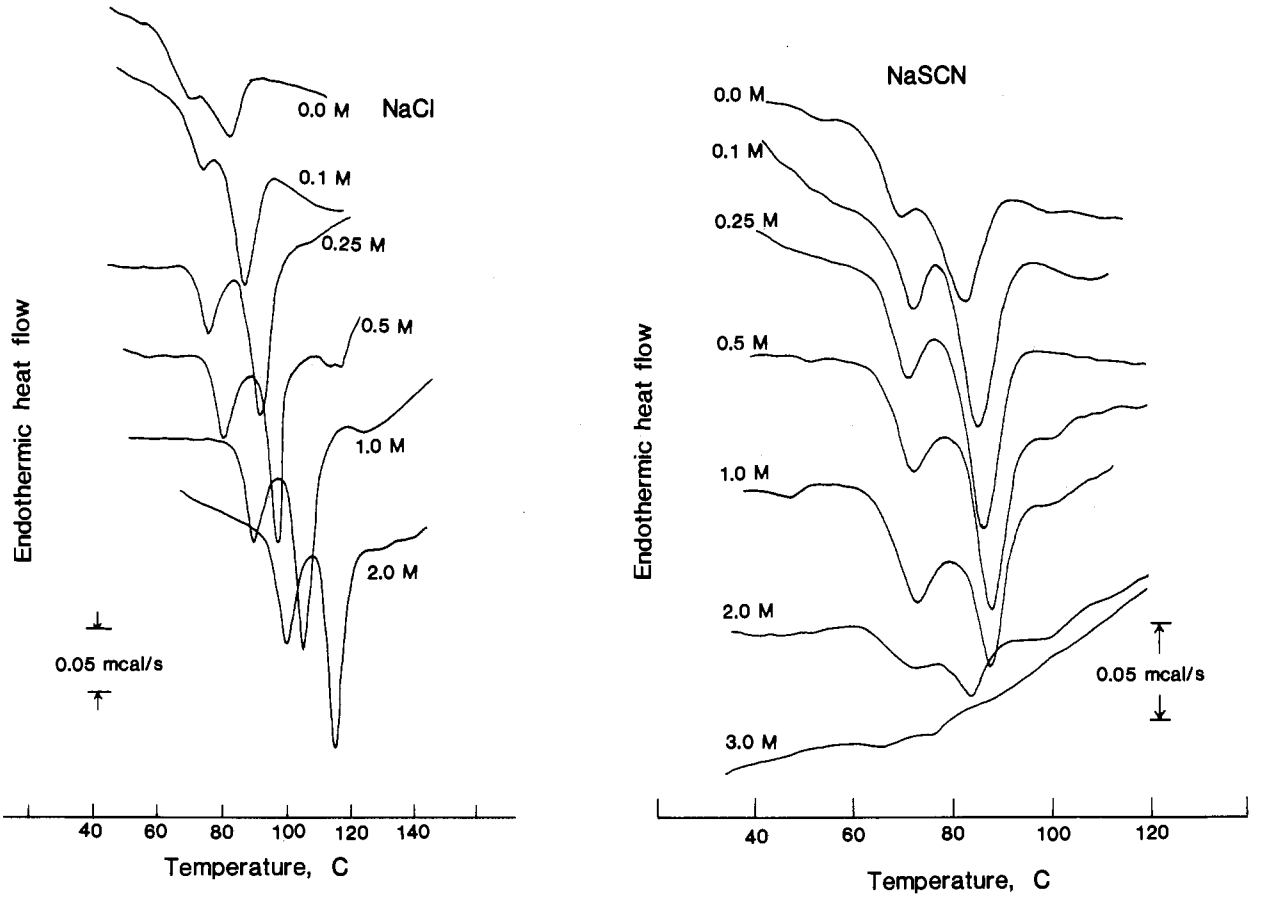


Figure 3. Effects of NaCl (left) and NaSCN (right) on the DSC thermograms of soy isolate at pH 8.0. The endothermic peak at higher temperature corresponds to soy 11S, and the one at lower temperature corresponds to soy 7S. Heating rate was 10 °C/min.

higher salt concentrations while NaCl and NaBr progressively increased the T_D values, $NaClO_4$ and NaSCN decreased the thermostability of both 7S and 11S globulins. However, it should be noted that the decrease in T_D of 7S and 11S in the presence of either $NaClO_4$ or NaSCN was not dramatic even at 2 M concentration.

Analysis of the data in Figure 4 indicates that even though quaternary structures of 7S and 11S globulins are very different, addition of salts affects the denaturation temperatures of these globulins almost to the same extent. For example, the ΔT_D in the presence of 2 M NaCl (i.e., the difference in the denaturation temperature between control and 2 M NaCl) is about 33 °C for 11S globulin compared to about 30 °C for 7S globulin. Similarly, the ΔT_D in the presence of 2 M NaSCN is about 1.5 °C for 11S and about 2.5 °C for 7S. Similar studies with β-lactoglobulin gave a ΔT_D value of about 12 °C in the presence of 2 M NaCl and about −10 °C in the presence of 2 M NaSCN (unpublished data). These observations apparently indicate that even though the quaternary structures of 7S and 11S globulins are different, the magnitude of salt-induced net effect on the stability of these globulins is almost the same.

The effects of various neutral salts on the enthalpy of denaturation of soy isolate are shown in Figure 5. The ΔH values were calculated from the total area of the endothermic peaks of 7S and 11S components. Up to about 0.5 M salt concentration the enthalpy of denaturation of soy isolate increased in the presence of all the salts studied, indicating that the proteins were stabilized against thermal denaturation under these conditions. However, the magnitude of increase in ΔH depended on the type of anion and followed the order NaCl = NaBr > $NaClO_4$ > NaSCN. This was similar to the behavior of T_D of 7S and 11S in the presence of these salts under similar conditions. At higher salt concentrations the enthalpy of denaturation of soy isolate decreased with salt concentration irrespective of the type of salt used, apparently suggesting that the proteins were destabilized at higher salt concentrations. It should be pointed out that while the decrease in ΔH at high concentrations of $NaClO_4$ and NaSCN is reasonable because of decrease in T_D values, the decrease in ΔH at high concentrations of NaCl and NaBr is contradictory to the behavior of T_D under similar conditions. It is geneally believed that the higher the temperature of denaturation, the higher would be the enthalpy of denaturation (Privalov and Khechiashvili, 1974). Since the denaturation temperature of both 7S and 11S globulins increased progressively with NaCl and NaBr concentrations (Figure 4), it is reasonable to expect that the enthalpy of denaturation would also increase with concentration of these salts. The deviation from the expected behavior apparently suggests that although higher T_D values of 7S and 11S globulins in NaCl and NaBr reflect increased resistance to thermal denaturation, the tertiary and quaternary structures of 7S and 11S components at high NaCl and NaBr concentrations might not be the same as those of low salt concentrations. These nonnative structures might possess smaller enthalpy of denaturation than the native but might possess greater thermal transition temperatures. Alternatively, the apparent decrease in the enthalpy of denaturation at high NaCl and NaBr concentrations might be due to some type of aggregation of the denatured protein. The exothermic heat effects of such aggregation process may partly off-set the endothermic heat flow of denaturation.

Gelation. To elucidate whether there is any relationship between the thermal denaturation of soy proteins in the presence of neutral salts and subsequent gel-forming

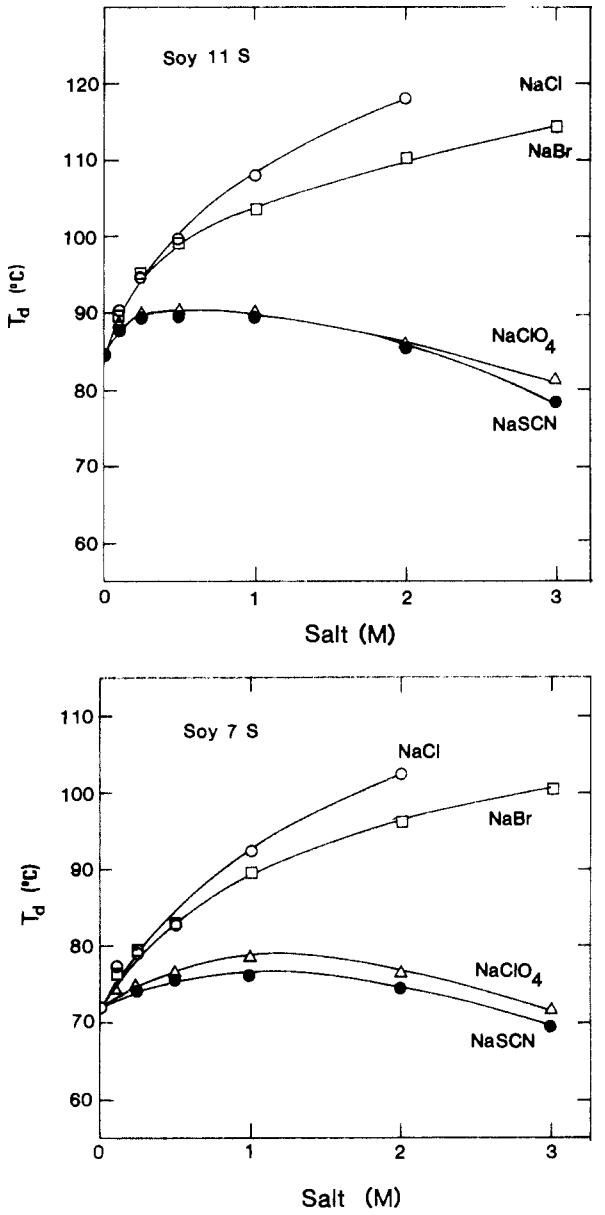


Figure 4. Effects of concentration of various sodium salts on the denaturation temperature (T_d) of soy 11S (top) and soy 7S (bottom) globulins in soy isolate. Heating rate was 10 °C/min.

characteristics, the melting properties of soy 11S gels formed in the presence and absence of 0.5 M NaSCN or $NaClO_4$ were studied. The data were analyzed by the relationship (Eldridge and Ferry, 1954) (eq 5), where C is

$$\log C = \frac{\Delta H^\circ}{2.303RT_m} + \text{constant} \qquad (5)$$

the protein concentration (g/L), T_m is the melting point of the gel, i.e., the temperature at which the gel state is converted to liquid progel state, R is the gas constant, and ΔH° is the enthalpy of gelation. According to the above relationship a plot of log C versus $1/T_m$ would exhibit a straight line, the slope of which would be $\Delta H^\circ/2.303R$.

The plots of log C versus $1/T_m$ for soy 11S in the presence and absence of 0.5 M NaSCN and $NaClO_4$ are shown in Figure 6. The enthalpies of gelation are given in Table I. It may be noted that while there was no difference between enthalpies in the presence of either $NaClO_4$ or NaSCN, the ethalpy of gelation was smaller in the absence of salts. Furthermore, the concentration of soy 11S needed to form a gel having a particular melting temperature decreased upon addition of either NaSCN or

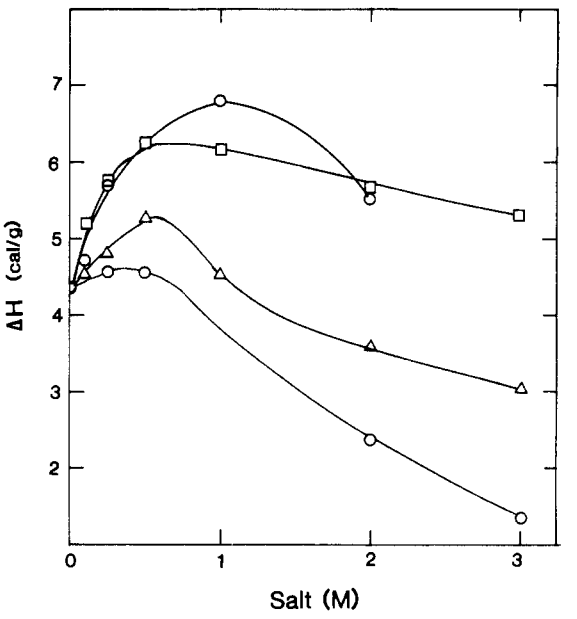


Figure 5. Effects of various sodium salts on the enthalpy of denaturation of soy isolate at pH 8.0. The ΔH values were calculated from the entire thermogram (both 7S and 11S components) of soy isolate at various salt concentrations.

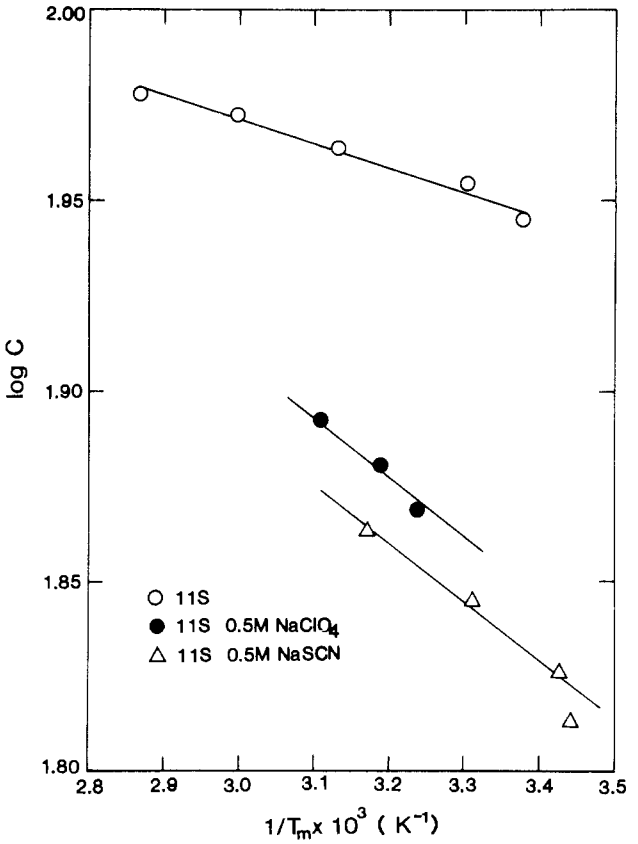


Figure 6. log C versus $1/T_m$ plots for soy 11S protein gels in the presence of 0.5 M $NaClO_4$ and 0.5 M NaSCN. C is in grams/liter.

Table I. Enthalpy of Gelation of Soy 11S Globulin under Various Conditions

treatment[a]	ΔH, kcal/mol
soy 11S in water	−0.247
soy 11S in 0.5 M NaSCN	−0.696
soy 11S in 0.5 M $NaClO_4$	−0.696

[a] The pH of protein dispersions was 8.0.

$NaClO_4$. For example, to obtain an 11S protein gel with a melting temperature of 37 °C (310 K), a minimum of

about 9.1% protein concentration was required in the absence of salts; however, in the presence of 0.5 M $NaClO_4$ the minimum protein concentration required to form a gel with 37 °C melting temperature was about 7.2% (Figure 6). Since both the enthalpy of gelation and the melting temperature of protein gels are dependent on the number of noncovalent cross-links in the gel network (Eldridge and Ferry, 1954), the results apparently indicate that a comparable number of cross-links formed in the control (with no salts) could be obtained with less protein concentration in the presence of either 0.5 M NaSCN or $NaClO_4$.

One of the probable reasons for higher enthalpy of gelation and reduction in the protein concentration needed to form gel could be the influence of electrostatic forces on the formation of gel network. In the absence of added salts the electrostatic repulsive interactions between protein subunits might hinder the formation of noncovalent cross-links and thus destabilize the gel network; however, in the presence of 0.5 M added salt such electrostatic repulsive forces might be neutralized and thus facilitate formation of an increased number of noncovalent cross-links in the gel network. In addition, it is also probable that, in the presence of 0.5 M NaSCN and $NaClO_4$, soy 11S may undergo extensive thermal unfolding at 90 °C, which would also expose many functional groups to form cross-links in the gel network. However, it should be pointed out that the denaturation temperature (at 10 °C/min heating rate) of 11S in the presence of 0.5 M $NaClO_4$ or NaSCN was about 90 °C compared to about 85 °C in the absence of these salts (Figure 4). Since the heating condition in the gelation studies was 90 °C for 30 min, one would expect complete unfolding of soy 11S subunits in either case; hence, the thermal unfolding of the protein might not be the limiting factor for the observed differences in the gelation behavior of soy 11S in the presence and absence of $NaClO_4$ or NaSCN.

Experiments on gelation of soy 11S in the presence of 0.5 M NaCl were unsuccessful, because no gelation was observed up to 9.2% protein concentration. The probable reason for this might be the elevation of T_D by NaCl. At 0.5 M NaCl the apparent T_D of soy 11S was about 100 °C (Figure 4). Under these conditions, heating at 90 °C might result only in partial unfolding of the protein, which may not be sufficient enough to form critical number of cross-links that may be essential to maintain a gel network. However, this argument alone may not be completely valid because of two reasons: First, the T_D of 11S in 0.5 M NaCl in DSC studies represents the apparent denaturation temperature at 10 °C/min heating rate. At 0 °C/min heating rate the T_D of soy 11S in 0.5 M NaCl would be less than 100 °C and probably only few degrees above 90 °C (German et al., 1982; Hermansson, 1979a,b). Second, even if the T_D was a few degrees higher than 90 °C, by holding the protein solution at 90 °C for 30-min duration, one would expect substantial denaturation and unfolding of the protein (Arntfield and Murray, 1981). In other words, the inability of soy 11S to gel in the presence of 0.5 M NaCl can not be attributed solely to elevation in T_D. It may be also due to other molecular events that might occur during gelation.

In heat-induced reversible gelation of proteins, the primary role of initial heating at higher temperatures is to cause unfolding and exposure of functional groups (mainly hydrogen-bonding groups). The structural state of the protein under these conditions is usually termed as progel, which upon cooling and incubating at low temperatures (usually 4 °C) facilitates formation of intermolecular interactions between the exposed functional groups.

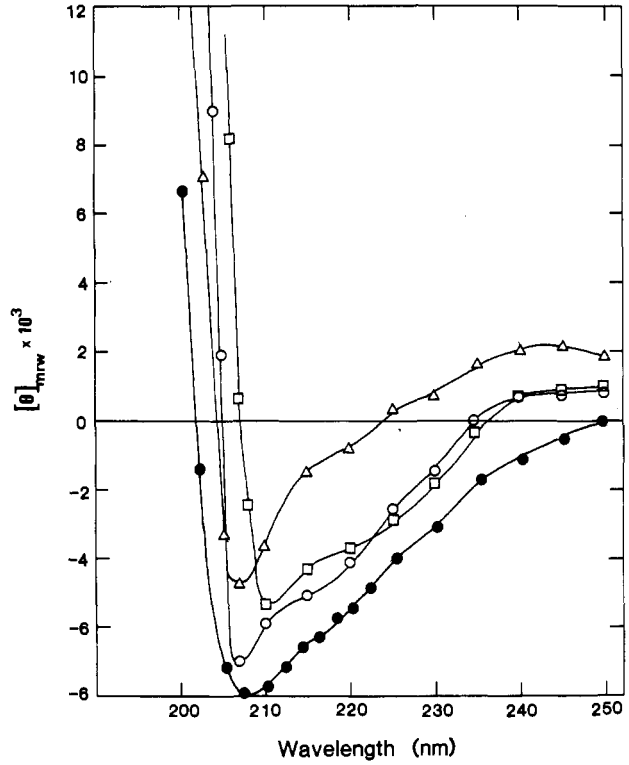


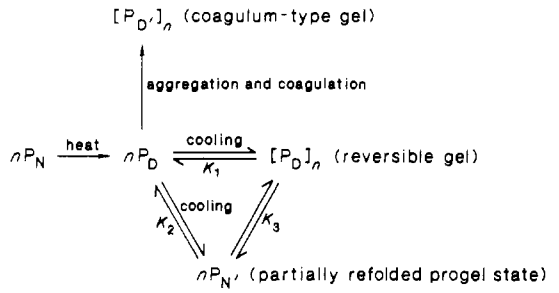
Figure 7. Far-UV–CD spectra of soy 11S globulin in gels made under different treatments: ●, unheated, native 11S; ○, sample from gel containing no added salt; □, sample from gel containing 0.5 M NaCl; △, sample from gel containing 0.5 M $NaClO_4$. (See Materials and Methods for details.) All gels were prepared by heating the protein solution in a capillary tube at 90 °C for 30 min and then aging at 4 °C for 24 h.

However, it should be pointed out that the structural state of the protein in the progel state (i.e., at high temperature) may be different from that of the protein at low temperature before the onset of gelation. In other words, it is possible that partial refolding of the protein might occur during the cooling regime. The extent of refolding or formation of secondary structures in the subunits of the protein would depend on the solution conditions such as pH, ionic strength, type of salts, and also the rate of cooling of the solution. The extent of refolding in turn would affect the number of functional groups available for intermolecular cross-linking and thus would affect the enthalpy of gelation, T_m, and other properties of the gel.

To elucidate the conformational state of soy 11S in the gel structure and relate the differences in the conformation to differences in the properties of gels formed under various conditions, we employed the procedure described in Materials and Methods.

The far-UV–CD spectra of the fluids (supernatants) obtained from soy 11S gels prepared in the presence and absence of 0.5 M $NaClO_4$ and NaCl are shown in Figure 7. The CD spectrum of the unheated soy 11S is also shown. In the case of sample containing no added salt and the sample containing 0.5 M NaCl, no typical gelation was observed at 8% protein concentration. These samples, upon shaking and tilting at 5 °C, flowed like a clear viscous fluid. Nonetheless, these samples were centrifuged, and the liquid layers at the top of the tubes were taken for CD analysis. The CD spectra shown in Figure 7 indicate that even though the gelation conditions were the same, the conformational state of the protein in the gel matrix varied depending on the salt species in the system. The exhibition of negative ellipticities at wavelengths 220–222 and 216–218 nm indicate that the protein molecules in the gel

Scheme I



matrix possess a significant amount of secondary structures. In other words, the data suggest that although soy 11S is denatured and/or unfolded at high temperature, partial refolding and regaining of secondary structures seem to occur during the cooling regime of the gelation process.

The shape of the CD spectra for unheated (native) soy 11S and the gel supernatant samples containing no salt (control) and 0.5 M $NaClO_4$ were very similar; they exhibited negative shoulders at 217–218 nm and a trough at 207 nm. The shoulder at 217 nm is indicative of presence of β-sheet structure (Townend et al., 1966). However, the negative ellipticity at 217 nm decreased in the order native 11S > no salt > 0.5 M $NaClO_4$. This indicates that in the presence of 0.5 M $NaClO_4$ the conformation of the protein in the gel was in a highly unfolded structure compared to that in the absence of $NaClO_4$. In other words, although soy 11S is denatured when heated at 90 °C for 30 min in the presence or absence of $NaClO_4$, upon cooling the extent of partial refolding seems to be inhibited by 0.5 M $NaClO_4$ but not in its absence. Under these conditions, the greater number of exposed functional groups might facilitate formation of greater number of cross-links and thus stabilize the gel network. On the other hand, the greater extent of refolding of the protein in the case of no salt might reduce the number of functional groups available for cross-link formation and thus destabilize the gel network. This might be, in addition to the electrostatic effect discussed earlier, the reason for gelation of soy 11S in the presence of 0.5 M $NaClO_4$ but not in the absence of $NaClO_4$ at 8% protein concentration.

The CD spectrum of the supernatant from the gel prepared in the presence of 0.5 M NaCl was remarkably different from the CD spectra of other samples (Figure 7). This sample exhibited a shoulder at 222 nm and a trough at 210 nm, characteristic of α-helical structure (Townend et al., 1966). This is significantly different from the native 11S, which has been reported to contain mainly β-sheet and random-coil structures (Sakakibara and Noguchi, 1977; Koshiyama and Fukushima, 1973). The data suggest that, upon heating and cooling of soy 11S in the presence of 0.5 M NaCl, the unfolded protein partially refolds during the cooling phase to an ordered structure containing more α-helix than β-sheet conformation. From the comparison of the CD spectra of 0.5 M $NaClO_4$ and 0.5 M NaCl samples, it is apparent that the extent of refolding of the protein in the presence of NaCl was greater than that in the presence of $NaClO_4$. It should be pointed out that while soy 11S gelled in the presence of $NaClO_4$, no gelation occurred in the presence of 0.5 M NaCl. This might be attributed to greater extent of refolding of the protein, which might reduce the number of exposed functional groups (e.g., hydrogen bond donors and acceptors) to form cross-links and thus prevent formation of the gel network.

The present study clearly indicate that heating the protein solution above the T_D is not the only critical step in the thermal gelation process. The tendency of the unfolded protein to refold during the cooling regime of the gelation process seems to play a critical role in the formation of the gel network. On the basis of the present study, the general mechanism of thermal gelation of globular proteins can be described as shown in Scheme I, where n is the number of protein molecules, P_N is protein in native state, P_D is protein in denatured state, $P_{N'}$ is protein in partially refolded state (formed during the cooling phase), $[P_D]_n$ is protein in the gel state, and K_1, K_2, and K_3 are the equilibrium constants. In the above scheme, P_D actually represents the progel state of the protein at high temperature and $P_{N'}$ can be characterized as the progel state at low temperature. In the case of reversible gels, the extent of gel network formation upon cooling, at a given protein concentration, depends on the relative magnitude of K_1 and K_2. In extreme situations, if $K_1 \gg K_2$, the system would set readily to a translucent gel with greater elasticity and strength. On the other hand, if $K_1 \ll K_2$, partial refolding of the protein would decrease the availability of the number of functional groups for intermolecular cross-linking and thus prevent formation of a self-supporting gel network. In the intermediate situations, the strength and elasticity of the gel would depend on the relative magnitudes of K_1 and K_2.

Proteins that contain high levels of apolar amino acid residues undergo hydrophobic aggregation upon thermal unfolding; at higher protein concentrations these insoluble aggregates might form an irreversible, coagulum-type gel. It is possible that a translucent gel forming protein might form a coagulum-type gel under certain conditions of pH and ionic strength. In such types of gels, it is the protein concentration, rather than partial refolding of the protein, that would be the limiting factor in gel network formation.

It should be pointed out that in the case of reversible gels it is possible to promote gelation by controlling the magnitude of K_2. This can easily be done by adding substances that would specifically inhibit refolding of the protein and maintain it in the unfolded state. It can also be predicted that in the presence of such an additive the minimum protein concentration required for gelation would decrease (Figure 6). The $P_{N'}$, i.e., the progel at low temperature, can be converted to gel state by heating and cooling in the presence of the additive. Further research is needed to elucidate the relationship between the physical properties of the gel network and the structure of the protein in the gel network.

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Registry No. $NaClO_4$, 7601-89-0; NaCl, 7647-14-5; NaSCN, 540-72-7; NaBr, 7647-15-6.

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Isolation and Characterization of β-Globulin Low Molecular Weight Protein Fraction from Sesame Seed (*Sesamum indicum* L.)

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The low molecular weight protein fraction from the proteins of sesame seed has been isolated in a homogeneous form and is termed β-globulin or consesamin. The protein has an $S°_{20,w}$ of 2.0 ± 0.1, $D_{20,w}$ of 8×10^{-7} cm²/s, and a partial specific volume of 0.725 mL/g. The intrinsic viscosity of the protein was determined to be 4.1 mL/g. The molecular weight determined by various approaches gives a value of 15000 ± 500. The evaluation of frictional ratios using Stokes radius and other hydrodynamic parameters indicates that the protein is elongated. The secondary structure of the protein indicates it to be rich in α helix. The protein is rich in acidic amino acids, especially glutamic acid, and also hydrophobic amino acids.

Sesame seed (*Sesamum indicum* L.) contains nearly 25% protein, and the defatted meal contains about 50% protein (Prakash and Narasinga Rao, 1986). Except for the presence of oxalates and phytic acid in the seed, there is no known toxic, antinutritional, or coloring principles in sesame seed (Prakash and Nandi, 1978). The work on sesame seed total proteins has mostly centered around nitrogen solubility and fractionation of proteins (Prakash, 1986). The total protein consists of mainly two fractions: One is the high molecular weight protein fraction, α-globulin (nearly 60%), which has been isolated and characterized under various solution conditions (Prakash and Nandi, 1976, 1977a–c, 1978; Prakash, 1985). Its quaternary structure is well established and is one of the seed proteins whose structure is well understood (Plietz et al., 1986). The other fraction, the low molecular weight protein fraction, β-globulin, constitutes nearly 25% of the total protein. No information is available on this protein fraction. In this study we report the isolation and char-

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