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Thermal Gelation of Globular Proteins: Influence of Protein Conformation on Gel Strength

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Circular dichroic analysis of protein fluid expressed from globular protein gels at high centrifugal force has been used to elucidate the structural state of globular proteins in gels. In the case of bovine serum albumin gels, gelation involved transconformation of α-helix and aperiodic structures into β-sheet conformation. Conditions that decreased the formation of β-sheet structure decreased the gel strength. In the case of soy proteins, which contain mainly β-sheet and aperiodic structures in the native state, only a reduction in the β-sheet content and an increase in aperiodic structure content were observed in the gel state. It is hypothesized that, in globular protein gels, intermolecular hydrogen bonding between segments of β-sheets oriented either in parallel or in antiparallel configurations may act as junction zones in the gel network.

INTRODUCTION

The important initial step in heat-induced gelation of globular proteins is heating of the protein solution above the denaturation temperature. The primary importance of the denaturation process is to expose the functional groups (such as CO and NH of peptide bonds, side-chain amide groups, and hydrophobic groups), which, under appropriate conditions, interact with each other to form a three-dimensional gel network. Since proteins undergo structural changes during thermal gelation, the extent of denaturation and exposure of functional groups may affect the extent of network formation. Conversely, the state of conformation of protein molecules in gels may have a bearing on the ultimate physical characteristics of protein gels. The structural investigations so far on protein gels have been based on the electron microscopic and low-angle X-ray scattering images (Clark and Tuffnell, 1980; Clark et al., 1981a,b; Hermansson and Buchheim, 1981). Although these techniques provide information on the macroscopic network structure of protein gels, they are incapable of providing information on the molecular structures of proteins in the gel network and their relation to gel properties. Recently, infrared and laser Raman spectroscopic methods have been used to get insight into the molecular conformation of globular proteins in heat-induced gels (Clark et al., 1981a,b). However, quantitative information regarding the type and extent of conformational changes in proteins and their relation to the physical properties of globular protein gels is very limited and much needed. Thus, the objectives of the present study are to elucidate the conformation of proteins in gels and to study the variations in the strength of these gels as a function of protein conformation.

To induce variations in the molecular conformation of a protein under a set of gelling conditions, we have chosen to use kosmotropic (structure stabilizing) and chaotropic (structure destabilizing) salts. Salts have been shown to affect the denaturation temperature and conformation of several proteins (von Hippel and Schleich, 1969; Damodaran, 1989). The effects of salts on protein structure involve two mechanisms: (1) The electrostatic shielding effect, which is usually achieved at or below 0.2 ionic strength, is only dependent on the ionic strength of the medium and not on the nature of the ion. (2) Salts exert ion-specific effect on hydrophobic interactions and affect the stability of proteins at higher concentration. This is believed to be through the modification of water structure, which subsequently causes perturbations at the protein–water interface. The effectiveness of various salts on the stability of protein follows the Hofmeister series, i.e., $F^- > SO_4^{2-} > Cl^- > I^- > ClO_4 > SCN^-$ (von Hippel and Schleich, 1969; Damodaran and Kinsella, 1982b). For this study, NaCl and $NaClO_4$ have been selected as the representatives of structure stabilizers and structure destabilizers, respectively.

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MATERIALS AND METHODS

Bovine serum albumin (fraction V) was from Sigma Chemical Co. (St. Louis, MO). Soy proteins were prepared from defatted soybean flour (Centra Soya, Chicago) according to the method of Thanh and Shibasaki (1976).

Preparation of Protein Gels. Protein solutions were prepared in 20 mM sodium phosphate buffer, pH 8.0. Protein concentration was determined by the biuret method. Aliquots (3 mL) of protein solution were placed in glass vials (21 mm × 70 mm) and closed tightly with molded screw caps. The vials were heated at 80 (for BSA) or 90 °C (for soy proteins) for 30 min in a water bath and then cooled immediately in an ice bath and stored at 4 °C for 20 h.

Gel Strength. Gel strength was determined by the puncture method (Bourne, 1978) using the Instron Universal testing machine (Model 1132). The diameter of the plunger was 9.525 mm. The moving speed of the plunger was 25.4 cm/min. The final clearance between the plunger and the plate was 3 mm. Gel strengths of the protein gels were measured at 5 °C (ice-bath temperature). The gel structure or hardness reported in this study corresponds to the peak force during the first compression cycle (Bourne, 1978). At least duplicate measurements were made.

Circular Dichroism. The following approach was employed to elucidate conformations of proteins in gels: An aliquot (0.1 mL) of soy protein solution (9.5%) or BSA solution (5.5%) was taken in a capillary tube (3 mm internal diameter), one end of which was heat sealed. The open end of the tube was closed with Teflon tape. Gelation of the sample was carried out under conditions identical with those described for the gel strength studies. The capillary tube was then placed in a centrifuge tube filled with glycerol and centrifuged at 35 000 rpm in a SW 55 rotor (Beckman Model L-2 ultracentrifuge) for 1 h at 5 °C. Care was taken not to immerse the top of the capillary tube into glycerol. 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 during centrifugation, were removed and diluted to about 0.02% protein concentration. The circular dichroic spectrum of the sample was determined by using a modified and computerized Cary Model 60 spectropolarimeter (On-Line Instrument Systems, Jefferson, GA). A 0.1-cm quartz cell was used for far-UV CD measurements. The instrument was calibrated with *d*-(+)-10-camphorsulfonic acid. The scan rate, time constant, and sensitivity of the instrument were all set so as to obtain the best signal-to-noise ratio. Ten scans of each sample were averaged, and the mean residue ellipticity [θ] values, expressed as deg·cm^2·dmol^{-1}, were calculated by using a value of 115 for the mean residue molecular weight. The secondary structure parameters were calculated by using a computer program based on a procedure described by Chang et al. (1978).

To determine whether the heat-induced conformational changes in proteins were affected by protein concentrations, experiments with 0.02% protein solutions were conducted under conditions identical with those of gelation experiments. The circular dichroic spectra and the secondary structure parameters of these samples were determined as described above.

RESULTS

The effects of 0.5 M NaCl and $NaClO_4$ on the hardness of BSA gels as a function of protein concentration are shown in Figure 1. The hardness of BSA gels in salt solutions followed the order BSA in NaCl > BSA in buffer > BSA in $NaClO_4$. Since $NaClO_4$ is generally considered to be a structure destabilizer, the gel strength of BSA in $NaClO_4$ should be expected to be greater than that in NaCl, which is a structure stabilizer. The anomaly observed in the relative gel strength may be related to unusual structural behavior of BSA in these two salt solutions. Recently, it has been shown that although $NaClO_4$ acts as a structure destabilizer for most proteins, it acted as a structure stabilizer for BSA (Damodaran, 1989). The thermal denaturation temperature (T_d) of BSA in water at pH 7.0 was 64 °C, whereas in 0.5 M $NaClO_4$ and 0.5 M NaCl the T_d values 75 and 70 °C, respectively (Damodaran, 1989), which indicated that BSA was more stable in $NaClO_4$ than in NaCl. Because of its stabilizing effect on BSA, $NaClO_4$ may lower the degree of thermal unfolding of the protein at 80 °C and thus may cause less exposure of functional groups for network formation. On the other hand, because of the lower T_d of BSA (70 °C) in 0.5 M NaCl, BSA may undergo extensive unfolding at the gelation temperature (80 °C) and thus may facilitate formation of an extensive gel network. The gels of BSA obtained in the presence and absence of $NaClO_4$ were translucent, whereas the gel formed in 0.5 M NaCl was opaque (coagulum type). Formation of a coagulum-type gel in NaCl indicates that the mechanisms of BSA gelation in NaCl and $NaClO_4$ were different. Shimada and Matsushita (1981a,b) reported that the effect of anions on coagulum formation of BSA followed the Hofmeister series SO_4^{2-} > Cl^- > Br^-, whereas I^- and SCN^- inhibited coagulation. Since the position of $NaClO_4$ in the Hofmeister series is in between I^- and SCN^- formation of a translucent gel in 0.5 M $NaClO_4$ is in agreement with the results of Shimada and Matsushita (1981a,b).

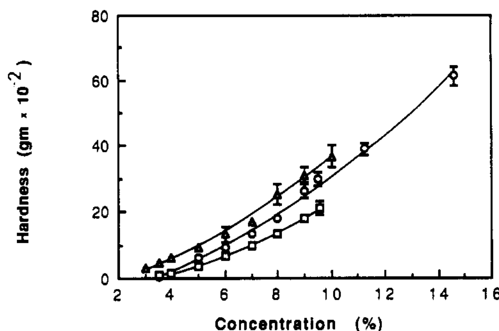

Figure 1. Effects of salts on the hardness of BSA gels. (○) Buffer only; (□) 0.5 M $NaClO_4$; (△) 0.5 M NaCl.

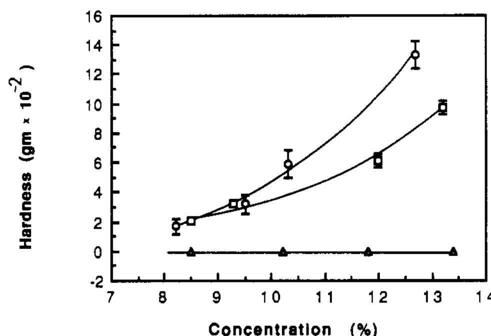

Figure 2. Effects of salts on the hardness of soy 11S gels. (○) Buffer only; (□) 0.5 M $NaClO_4$; (△) 0.5 M NaCl.

The effects of NaCl and $NaClO_4$ on the gelation of soy isolate and the two major protein fractions of soy isolate, namely soy 11S and soy 7S, are shown in Figures 2–4. It should be noted that soy 11S did not form a gel in the presence of 0.5 M NaCl at all concentrations studied. The probable reason for gelation of soy 11S in 0.5 M $NaClO_4$, but not in 0.5 M NaCl, might be the difference in the thermal denaturation temperature. The T_d of soy 11S in 0.5 M NaCl was about 100 °C, whereas that in 0.5 M $NaClO_4$ was about 90.2 °C (Damodaran, 1988). Since the T_d of soy 11S in 0.5 M NaCl was much higher than the heating temperature (90 °C), soy 11S could not form a gel in 0.5 M NaCl, owing to insufficient denaturation and exposure of functional groups. On the other hand, since the T_d of soy 11S in 0.5 M $NaClO_4$ was almost the same as the heating temperature (90 °C), the greater extent of denaturation

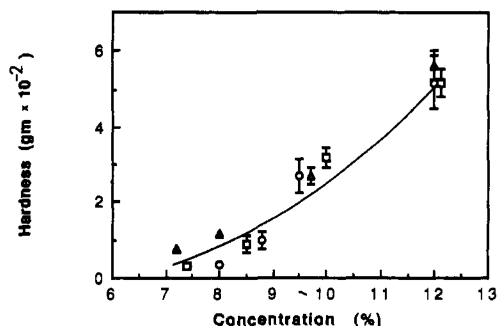

Figure 3. Effects of salts on the hardness of soy 7S gels. (O) Buffer; (□) 0.5 M $NaClO_4$; (△) 0.5 M NaCl.

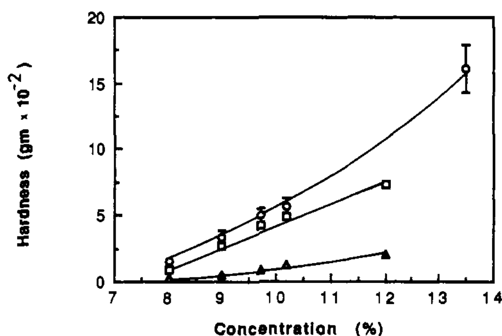

Figure 4. Effects of salts on the hardness of soy isolate gels. (O) Buffer; (□) 0.5 M $NaClO_4$; (△) 0.5 M NaCl.

and exposure of functional groups might have facilitated greater interaction between protein molecules and formation of a self-supporting gel network. However, although gelation did occur in the presence of $NaClO_4$, the strength of the gel was weaker than that of the control (no salt). This is reasonable because, since the T_d of soy 11S in the absence of added salts is about 84.5 °C (Damodaran, 1988), the extent of denaturation and exposure of functional groups when heated at 90 °C would have been far greater than in 0.5 M $NaClO_4$.

The hardness versus protein concentration profiles of soy 7S gels in the presence and absence of added salts is shown in Figure 3. Unlike soy 11S, the strength of soy 7S gels was not significantly affected by type of salt. This might probably be due to the fact that the T_d of soy 7S in the presence of 0.5 M salts used was well below the heating temperature used in this study. For example, the T_d values of soy 7S with no added salt, in 0.5 M NaCl and in 0.5 M $NaClO_4$ were 72, 82.8, and 76.6 °C, respectively (Damodaran, 1988). Since these denaturation temperatures were much lower than the 90 °C heating temperature, soy 7S would have undergone extensive denaturation in the absence as well as in the presence of salts. It should be pointed out that since the gel strength profile of soy 7S in the absence of added salt was very similar to those in the presence of 0.5 M salts, the electrostatic interactions in soy 7S apparently have no important role in the formation of soy 7S gel network. The major force that is involved in the gelation of soy 7S seems to be H-bonding interactions. Similar conclusions have been reached in other studies as well (Babajimopoulos et al., 1983; Utsumi and Kinsella, 1985).

In the case of soy isolate (SI), the relative gel strength increased in the order SI > SI–0.5 M $NaClO_4$ > SI–0.5 M NaCl (Figure 4). Since soy 7S and 11S globulins are the major protein components of soy isolate, the properties of soy isolate gels may be related to the sum total of contributions from each of these fractions. Whereas soy 11S could not form gel in the presence of 0.5 M NaCl

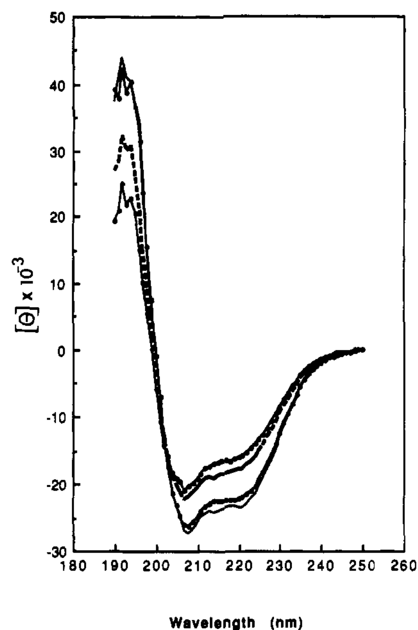

Figure 5. Far-UV CD spectra of BSA. A 0.02% BSA solution was heated at 80 °C for 30 min and stored at 4 °C for 20 h. The spectra were recorded at room temperature. (●) Native BSA in buffer; (—) BSA in 0.5 M $NaClO_4$; (O) heated BSA in buffer; (- - -) heated BSA in 0.5 M $NaClO_4$.

(Figure 2), soy isolate formed a weaker gel (Figure 4). This suggests that the gel strength of soy isolate in 0.5 M NaCl may be the result of the gelation of the soy 7S component, but not the 11S component. Since both soy 7S and 11S formed weaker gels in the presence of 0.5 M $NaClO_4$, the gel strength profile of soy isolate in 0.5 M $NaClO_4$ may represent the additivity of the contribution from gelation of the 7S and 11S components. The lower gel strength of soy isolate in 0.5 M $NaClO_4$, compared to that in the absence of salts (Figure 4), might be due to partial suppression of gelation of the 11S component, since the gelation of soy 7S was unaffected by $NaClO_4$ (Figure 3). This also suggests that the extent of denaturation and the sensitivity of gelation of the 11S component under various environmental conditions may potentially act as the modulator of the gelling properties of soy isolate in food systems.

The above results on the gelation properties of BSA and soy proteins indicate that the gel strength of these proteins is fundamentally related to the extent of denaturation and unfolding under a given set of conditions. The higher the extent of denaturation, the higher is the exposure of functional groups and the greater is the gel strength. In other words, the state of conformation of the protein at the onset of gelation apparently seems to have a major influence on the rheological properties of the final gel. To understand and to establish a quantitative relationship between the state of conformation of the proteins and the gel strength under various gelation conditions, the far-UV circular dichroism (CD) spectroscopy of proteins in gels was studied.

CD Studies of BSA Gels. The circular dichroic spectra of a dilute solution of BSA (0.02%) with and without heating and in the presence and absence of 0.5 M $NaClO_4$ are shown in Figure 5, and the secondary structure contents, estimated by using the method of Chang et al. (1978), are given in Table I. The secondary structure content of native BSA was 57.5% α-helix and 42.5% aperiodic. When 0.5 M $NaClO_4$ was added to native BSA, the helical content increased slightly to about 59%. When the 0.02% BSA solution (with no salt) was heated at 80

Table I. Effects of Heating and Cooling on the Secondary Structure of BSA[a]

BSA	% secondary structure			
	helix	β-sheet	turns	aperiodic
in buffer (no heat)	57.5	0	0	42.5
in 0.5 M $NaClO_4$ (no heat)	59.0	0	0	41.0
heated (no salt)	34.0	20.5	0.5	45.0
heated in 0.5 M $NaClO_4$	44.5	9.5	20.0	44.0

[a] The protein concentration was 0.02%. Heat treatment was at 80 °C for 30 min followed by cooling at 4 °C for 20 h.

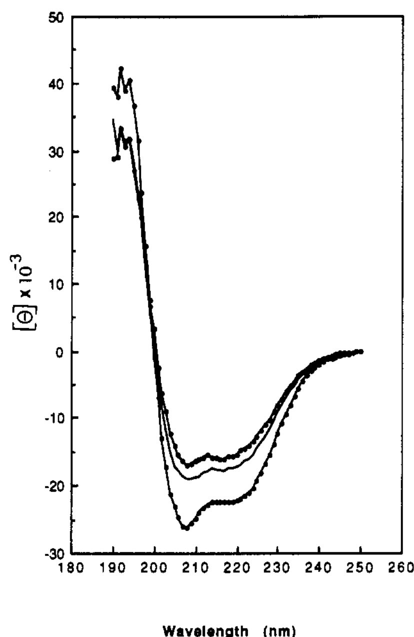

Figure 6. Far-UV CD spectra of fluids expressed from BSA gels after centrifugation at 35 000 rpm for 60 min. (●) Native BSA; (○) fluid from BSA gel with no salt; (—) fluid from BSA gel in 0.5 M $NaClO_4$.

Table II. Secondary Structure of BSA in Fluids Expressed from Gels[a]

BSA	% secondary structure			
	helix	β-sheet	turns	aperiodic
native	57.5	0	0	42.5
fluid from gel with no salt	42.0	26.5	0	31.5
fluid from gel in 0.5 M $NaClO_4$	45.0	19.0	0	36.0

[a] The concentration of BSA in gels was about 5.5%.

°C for 30 min and cooled to room temperature, the α-helix content decreased from 57.5% to 34%, but the β-sheet structure increased from 0% to 20.5%; the aperiodic structure content did not change. However, when 0.02% BSA in 0.5 M $NaClO_4$ was heated at 80 °C for 30 min and cooled to room temperature, the α-helical content decreased from 59% to 44.5% and the β-sheet structure increased slightly from 0% to 9.5%; the aperiodic structure content remained the same. These results indicate that addition of $NaClO_4$ stabilizes BSA against thermal denaturation and helps the protein retain most of its α-helical structure. However, the heat-induced structural changes observed in these dilute protein solutions may or may not reflect the actual structural changes that might occur under gelation conditions, i.e., at higher protein concentration.

To determine the conformational state of BSA in gels, the BSA gels were centrifuged and the CD of BSA in the expressed gel fluid (supernatant) was analyzed. The data are shown in Figure 6, and the estimated secondary structure contents are given in Table II. Here it is implicitly assumed that the conformation of BSA molecules in the gel network is very similar to that of the BSA molecules in the expressed gel fluid. SDS-PAGE of BSA gels dissolved in 10 M urea showed no protein band corresponding to monomeric BSA, but showed higher molecular weight polymers which could not penetrate the 4.5% stacking gel (Wang and Damodaran, 1990). On the basis of this observation, it may be assumed that the BSA molecules in the expressed gel fluid may not be in the monomeric state but in a soluble polymeric state as would be found in the clear gel.

The unheated native BSA contained 57.5% α-helix and 42.5% aperiodic structure and no β-sheet structure. However, the BSA in the fluid obtained from the BSA gel with no added salt contained about 42% α-helix, 26.5% β-sheet, and 31.5% aperiodic structure (Table II). This indicates that heating of BSA under the gelation conditions markedly decreased the α-helix content but increased the β-sheet content at the expense of α-helix and aperiodic content. The formation of β-sheet structure in BSA gels has previously been shown by infrared and Raman spectroscopy methods (Lin and Koeing, 1976; Clark et al., 1981a,b; Yasuda et al., 1986). Clark and Tuffnell (1986) showed that BSA in the gel state contained about 43% α-helix, 25% β-sheet, and 32% aperiodic structure. The results presented here are in good agreement with the reported values. The remarkable agreement of the structural analysis in situ using the infrared method (Clark et al., 1981a,b) with the results of this study clearly indicates that the circular dichroic method described here can be used to probe the conformation of globular proteins in heat-set clear gels.

In the presence of 0.5 M $NaClO_4$, BSA formed a weaker gel (Figure 1) and contained a lesser amount of β-sheet structure (Table II) compared to that of the control. This observation, coupled with previous reports (Clark et al., 1981a,b; Clark and Tuffnell, 1986) and present results, tentatively suggests that the strength of BSA gels may be directly related to the extent of transformation of α-helical and aperiodic structures into β-sheet form during the heating step. The formation of β-sheet structure may be a prerequisite for formation of aggregates and cross-linked network in a BSA gel network.

CD Studies of Soy Protein Gels. To determine the effect of heat and $NaClO_4$ on soy proteins, first the change in secondary structure content of soy proteins under nongelling conditions, i.e., at 0.015% protein concentration, was studied. The secondary structure parameters are presented in Table III. The β-sheet structure seems to be the major secondary structure in native soy proteins. Addition of 0.5 M $NaClO_4$ at room temperature caused very little change in soy protein (Table III). However, when the dilute protein solutions were heated at 90 °C for 30 min and cooled to room temperature, there was a marked decrease in β-sheet and an increase in the aperiodic structure of soy isolate and soy 7S and 11S globulins. The secondary structure contents of samples heated in the presence of 0.5 M $NaClO_4$ were almost the same as those heated in its absence (Table III), indicating that at low protein concentrations, i.e., at nongelling conditions, $NaClO_4$ does not seem to have any specific affect on the conformational states of heated soy proteins.

To determine the conformation of soy protein in the gel state, the gels were centrifuged and the CD spectra of proteins in the expressed gel fluid were analyzed. The CD spectra of soy 11S and soy isolate are shown in Figures 7 and 8, and the estimated secondary structure contents are given in Table IV. The unheated soy 11S contained about 64.5% β-sheet, 8.5% α-helix, and 27% aperiodic

Table III. Effects of Heating and Cooling on the Secondary Structure of Soy Protein[a]

protein	% secondary structure			
	helix	β-sheet	turns	aperiodic
soy isolate				
native	5.5	66.0	0	28.5
in 0.5 M $NaClO_4$	6.5	63.0	0	30.5
heated (no salt)	0.5	46.5	7.0	46.0
heated in 0.5 M $NaClO_4$	2.0	46.0	6.5	45.5
soy 7S globulin				
native	6.0	62.5	2.0	29.5
in 0.5 M $NaClO_4$	8.5	60.0	3.0	28.5
heated (no salt)	1.5	51.0	6.5	41.0
heated in 0.5 M $NaClO_4$	4.0	50.0	6.5	39.5
soy 11S globulin				
native	8.5	64.5	0	27.0
in 0.5 M $NaClO_4$	8.5	63.0	0	28.5
heated (no salt)	0	45.0	10.0	45.0
heated in 0.5 M $NaClO_4$	0.5	49.0	5.5	45.0

[a] Protein concentration was about 0.015%. Heat treatment was at 90 °C for 30 min followed by cooling at 4 °C for 20 h.

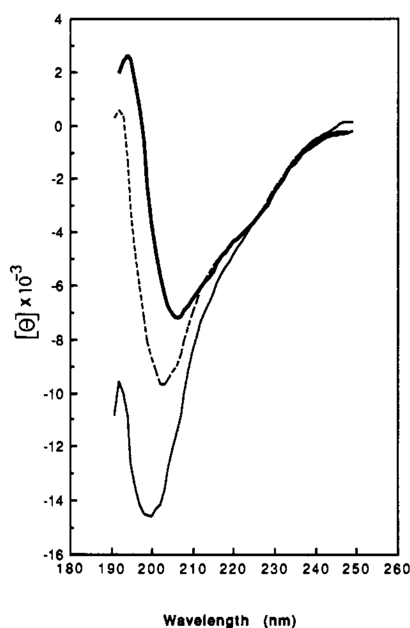

Figure 7. Far-UV CD spectra of soy 11S globulin in fluids expressed from 11S gels: (▬) native 11S; (—) fluid from 11S gels with no salt; (- - -) fluid from 11S gel in 0.5 M $NaClO_4$.

structure. However, the soy 11S in the fluid obtained from the gel containing no $NaClO_4$ contained about 26.5% β-sheet, 17.5% β-turn, and 56.5% aperiodic structure and no α-helix. This, when compared with the data at low protein concentration (Table III), indicates that at high protein concentration thermal treatment results in extensive denaturation of the secondary structures in soy 11S. It is quite probable that at low protein concentration soy 11S might indeed have undergone extensive thermal unfolding; however, upon cooling, because of lack of intermolecular interactions, the protein might have partially refolded due to intramolecular forces and thus gained most of its secondary structure. On the other hand, at high protein concentration, the propensity of intermolecular interactions apparently leads to aggregation and network formation and thus prevents refolding and regaining of secondary structures.

The β-sheet content of soy 11S in the gel containing 0.5 M $NaClO_4$ was much higher than that in the gel containing no salt (Table IV), indicating that $NaClO_4$ stabilized the secondary structure of soy 11S against thermal denatur-

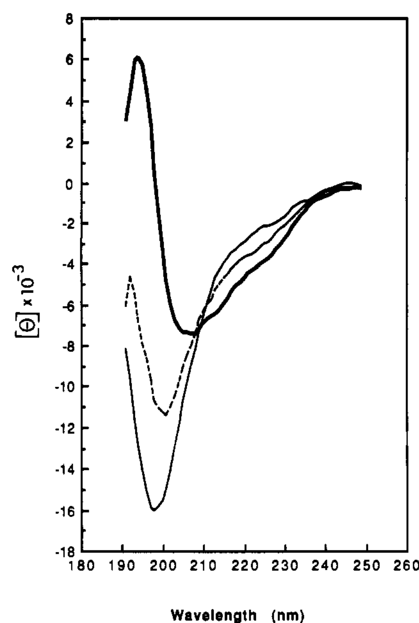

Figure 8. Far-UV CD spectra of soy isolate in fluids expressed from various soy isolate gels: (▬) native soy isolate; (—) fluid from soy isolate gel with no salt; (- - -) fluid from soy isolate gel in 0.5 M $NaClO_4$.

Table IV. Secondary Structure of Soy Isolate and Soy 11S Globulin in Fluids Expressed from Gels[a]

protein	% secondary structure			
	helix	β-sheet	turns	aperiodic
soy isolate				
native	5.5	66.0	0	28.5
fluid from gel with no salt	2.0	25.0	17.5	55.5
fluid from gel in 0.5 M $NaClO_4$	1.0	59.5	0	39.5
soy 11S globulin				
native	8.5	64.5	0	27.0
fluid from gel with no salt	0	26.5	17.0	56.0
fluid from gel in 0.5 M $NaClO_4$	0	47.0	8.0	45.0

[a] The concentration of soy protein gels was about 9.5%.

ation. Furthermore, the gel strength of soy 11S containing 0.5 M $NaClO_4$ was weaker than that of the control (containing no salt) (Figure 2), and the T_d also was higher (Damodaran, 1988). Soy isolate also exhibited weaker gels in the presence of 0.5 M $NaClO_4$, and the β-sheet content was also higher than that of the control gel (Figure 4; Table IV). These observations tentatively suggest that while β-sheet structure is involved in aggregation and gel network formation, the extent of protein denaturation and unfolding also seems to be more important in soy protein gelation. The disruption of native tertiary structure and partial destabilization of secondary structures and retention of a critical amount of β-sheet structure seem to be the critical requirements for gel network formation in soy proteins.

DISCUSSION

The results presented here clearly demonstrate that β-sheet structure is involved in aggregation and network formation in BSA and soy protein gels. Previous studies on other proteins have also showed that a decrease in α-helix content and an increase in β-sheet content is a common feature in translucent type of protein gels (Clark et al., 1981a,b; Lin and Koenig, 1976).

It is generally assumed that heating of proteins above their thermal denaturation temperature would result in total destruction of both the tertiary and the H-bonded secondary structures in proteins. It is also further believed

that such extensive denaturation and unfolding is essential for the formation of gel network. However, the data presented here do not fully support this view. At both low and high protein concentrations when either BSA or soy protein solutions are heated above their denaturation temperatures and cooled to 4 °C, the protein regains most of its secondary structure. In fact, in the case of BSA at high concentration (5.5%), the total percentage of secondary structure content (i.e., α-helix plus β-sheet) is more than that in the native BSA (Table II). In other words, some of the aperiodic structure of the native protein is actually transformed into β-sheet structure upon heating and cooling; some of the original α-helix structure is also transformed to β-sheet during the gelling process. Such transconformation of α-helix and aperiodic structures into β-sheet seems to be essential for gel network formation. In the case of soy proteins, which predominantly contain β-sheet structures, heating at either low or high protein concentration does not seem to induce transconformation of β-sheet to α-helix (Table III and IV). However, while at low concentration soy proteins regain about 70–80% of their original β-sheet structure after heating and cooling, a lesser amount of β-sheet structure was formed in higher concentration solutions. The results indicate that mere exposure of an increased number of functional groups is not the only factor that is essential, but retention of a critical amount of β-sheet structure is also a prerequisite for the formation of a translucent gel. The results presented here indicate that for both BSA and soy protein gels this critical amount of β-sheet structure seems to be about 25% (Tables II and IV; control with no salt). Evidence based on infrared and Raman spectroscopic analyses of various globular protein gels also suggests that β-sheet structure might be essential for protein–protein interactions and network formation in these gels. The intermolecular hydrogen-bonding interactions between β-sheets, oriented in either parallel or antiparallel β-sheet configurations, may act as junction zones and thus stabilize the gel network. In this respect, the globular protein gels apparently resemble the conformational states of proteins in protein fibers such as wool and hair. However, this is in marked contrast with that of gelatin gels in which gelation involves partial reformation of collagen triple helices (Ledward, 1986), which apparently act as junction zones of gelatin gel network.

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