Journal of Agricultural and Food Chemistry

MAY 1990
VOLUME 38, NUMBER 5

Thermal Gelation of Globular Proteins: Weight-Average Molecular Weight Dependence of Gel Strength

Cheng-Hsin Wang and Srinivasan Damodaran*

Department of Food Science, University of Wisconsin—Madison, Madison, Wisconsin 53706

Thermal gelation of various globular proteins in the presence and absence of *N*-ethylmaleimide (NEM) and cysteine was studied. For the proteins that did not contain intersubunit disulfide bonds, the relative hardness of the gels at any given protein concentration followed the order no additive > Cys > NEM. However, for the proteins that contained intersubunit disulfide bonds, the relative order was no additive > NEM > Cys. Despite the differences among the proteins, the square root of the hardness of gels of various proteins exhibited a linear relationship with the weight-average molecular weight of the polypeptides in the gel network. Evidences are presented which indicate that the hardness or gel strength of typical globular protein gels is fundamentally related to the size and shape of the polypeptides in the gel network rather than to their chemical nature such as the amino acid composition and distribution. It is also shown that the globular proteins having a weight-average molecular weight less than 23 000 cannot form a self-supporting gel network at any reasonable concentration.

Gelation refers to the transformation of the protein in the sol state into a gellike structure, in which the individual protein molecules interact with each other to form a three-dimensional network. Several factors such as pH, ionic strength, temperature, solvent composition, and reducing agents have been shown to influence the gelation properties of several globular proteins (Catsimpoolas, 1970, 1971; Oe et al., 1986; Utsumi and Kinsella, 1985; Babajimopoulas et al., 1983). Although these and other studies have facilitated a phenomenological understanding of the gelation of globular proteins (Damodaran, 1988), the fundamental relationship between the molecular properties of globular proteins and their gelation behavior has not been fully understood.

In the heat-induced gelation of proteins, first the protein in the sol state is heated above its denaturation temperature, which results in the formation of a progel state. Upon cooling, depending upon its intrinsic molecular properties, the protein in the unfolded progel state forms either an irreversible coagulum type gel or a reversible translucent type gel. Shimada and Matsuhita (1980) showed that proteins that contain above 31.5 mol % of certain hydrophobic amino acid residues form coagulum type gel, whereas those that contain less than 31.5 mol % form translucent type gels. Although such empirical rules seem to be helpful in predicting the physical appearance of protein gels on the basis of their amino acid composition, very little information is available regarding what molecular properties affect the rigidity and other rheological properties of globular protein gels. In phenomenological terms, the strength or rigidity of protein gels is related to the number of intermolecular cross-links formed in the gel network. The higher the number of cross-links, the greater would be the gel strength. However, although the number of potential cross-linkable functional groups per unit mass of the protein is almost the same in typical globular proteins, the rigidities of globular protein gels formed under similar conditions, i.e., equivalent concentration, pH, ionic strength, etc., differ very significantly. This should be related to differences in certain structural features of globular proteins.

In the present investigation, the gelation properties of several globular food proteins, under various experimen-

* Address correspondence to this author.

0021-8561/90/1438-1157$02.50/0

tal conditions, were studied. On the basis of these results, we report that the hardness or gel strength of globular protein gels is largely dependent on the weight-average molecular weight and perhaps the hydrodynamic shape of the protein rather than their amino acid composition and distribution.

MATERIALS AND METHODS

Bovine serum albumin (fraction V), *N*-ethylmaleimide (NEM), and L-cysteine (free base) were from Sigma Chemical Co. (St. Louis, MO). Electrophoretic grade sodium dodecyl sulfate (SDS) and bis(acrylamide) were from Bethesda Research Laboratory (Gaithersburg, MD). Acrylamide was from Bio-Rad (Richmond, CA). All other chemicals used in this study were of reagent grade.

Soy protein isolate (SI) and soy 7S and 11S globulins were prepared from defatted soybean flour (Central Soya, Chicago) according to the method of Thanh and Shibasaki (1976).

Phaseolin from dry bean (*Phaseolus vulgaris*) flour was extracted according to the method of Hall et al. (1977). Trypsin-digested phaseolin was prepared as follows: To a phaeolin solution in 50 mM Tris-HCl buffer, pH 8.1, was added trypsin at the ratio of 1:20 (g/g). The solution was incubated at 37 °C in a waterbath overnight. The hydrolyzed product was dialyzed and then lyophilized.

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 90 °C for 30 min in a water bath and then cooled immediately in an ice bath and stored at 4 °C for 20 h.

In the case of the studies on the effects of NEM and cysteine on gelation, these chemicals were added to the protein solutions before heat treatment. The concentrations of NEM (20 mM) and cysteine (50–400 mM) used in these experiments were selected on the basis of the results of preliminary experiments. In these preliminary experiments, the gel strength versus cysteine or NEM concentration was studied for each protein. From these experiments, the minimum cysteine/NEM concentration beyond which no further change in the gel strength occurred was determined. Approximately 2 times this minimum concentration was used in the actual experiments.

Determination of Gel Strength. Gel strength was determined by the compression 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 strength of the protein gels were measured at 5 °C (ice-bath temperature). The gel strength or hardness reported in this study corresponds to the peak force during the first compression cycle (Bourne, 1978). At least duplicate measurements were made.

Electrophoresis. To determine the molecular weights of the polypeptides in the protein gels, the gel samples were analyzed by SDS–polyacrylamide gradient gel electrophoresis according to the method of Laemmli (1970). The separating gel gradient was between 7.5% and 20%, and the stacking gel was made up of 4.5% acrylamide. A 10% protein gel (0.5 g) (5% in the case of BSA gel) was dissolved by stirring overnight in 4.5 mL of sample buffer containing 8 M urea. At the end of this period, in some protein solutions, e.g., egg white, a very small amount of fiberlike material was observed; these were presumably high molecular weight insoluble polymers. The solutions were centrifuged. Estimation of protein concentration in the supernatant indicated that while almost 100% of proteins in the BSA and soy protein gels were soluble in 8 M urea, only about 95% of the proteins in egg white gels were soluble. To determine the molecular weights of polypeptides in the gels, the supernatants were divided into two portions. One portion was electrophoresed without the addition of 2-mercaptoethanol (2-ME), and the other portion was electrophoresed after 0.3 M 2-ME was added to the sample. BSA (67 000), ovalbumin (45 000), glyceraldehyde-3-phosphate dehydrogenase (36 000), carbonic anhydrase (29 000), soybean trypsin inhibitor (20 100), and α-lactalbumin (14 200) were used as molecular weight markers. The relative amount of each protein band in a sample was determined by scanning the gel with a computerized scanning densitometer (Bio-Rad, Model 620).

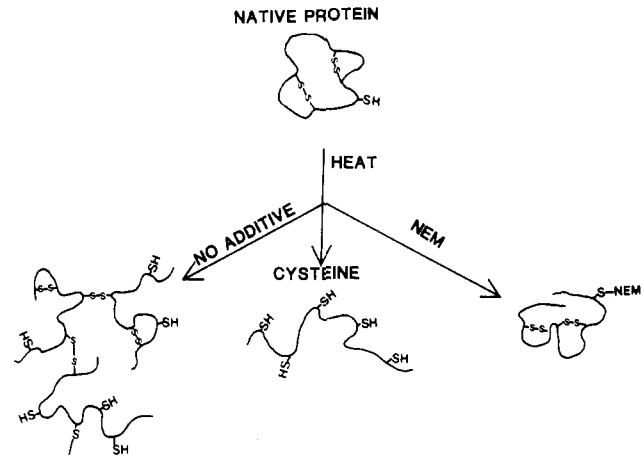

Figure 1. Schematic representation of the changes in the molecular size of a protein during heating in the presence and absence of cysteine or NEM.

RESULTS AND DISCUSSION

To elucidate the influence of the hydrodynamic size of proteins on the physical properties of thermally induced gels, the gel strength versus concentration profiles of various proteins were studied in the presence and absence of cysteine and *N*-ethylmaleimide (NEM). The rationale behind this approach is as follows: It is known that during thermal gelation proteins that contain disulfide and sulfhydryl groups undergo sulfhydryl–disulfide interchange reactions (Utsumi et al., 1984). The extents of these reactions differ among proteins and are related to the concentration and environmental conditions. Consequently, the hydrodynamic size and the weight-average molecular weight of the polypeptide species in the gelling system will be dependent upon the extent of the sulfhydryl–disulfide interchange reactions. However, when NEM is added prior to heating, it would block the free sulfhydryl groups in the protein and thus would prevent the occurrence of sulfhydryl–disulfide interchange reaction. Furthermore, if breakage of disulfide bonds occurs during heat treatment, the liberated thiol groups would be blocked immediately by the excess NEM in the solution and thus would effectively prevent any sulfhydryl–disulfide interchange reaction during gelation. Under these conditions, the protein will undergo thermal denaturation but will not form disulfide-linked polymers. Hence, the hydrodynamic size and the weight-average molecular weight distribution of the polypeptide species in this system would be smaller than that in the absence of NEM. In the case of cysteine addition, apart from blocking the sulfhydryl–disulfide interchange reaction, cysteine will cleave the intramolecular disulfide bonds in the protein and thus cause greater unfolding and denaturation during the thermal treatment. Although the weight-average molecular weight of the protein would be the same as that in the case of the NEM system, the hydrodynamic size and the conformational flexibility of the protein molecule would be expected to be greater in the presence of cysteine than in NEM. These various molecular changes are schematically shown in Figure 1.

The concentration versus gel strength profiles of various proteins in the presence and absence of excess amounts of NEM or cysteine are shown in Figures 2–5. In the

case of BSA, at any given concentration, the gel strength followed the order BSA > Cys–BSA > NEM–BSA (Figure 2A). It should be noted that although no polymerization via disulfide formation was possible in the presence of either NEM or cysteine, the gel strength of BSA in NEM was weaker than that in cysteine. This suggests that the extensive unfolding of BSA due to reduction of disulfide bonds by cysteine and the consequent change in the hydrodynamic size of the molecule was responsible for the greater gelling power. To determine the polymeric state, the weight-average molecular weight, and the hydrodynamic properties of BSA in these gels, the samples were analyzed by SDS–PAGE (Figure 2B). In Figure 2B, 1 and 2 correspond to the electrophoretic behavior of unheated BSA control in the presence and absence of 2-mercaptoethanol. It should be noted that the mobility of BSA treated with 2-ME (sample 2) was slower than that of the BSA with no 2-ME (sample 1), indicating that cleavage of disulfide bonds increased the hydrodynamic radius of the protein and thus decreased its electrophoretic mobility. Samples 5 and 6 in Figure 2B show the electrophoretic behavior of BSA from gels formed in the presence of NEM, run without and with 2-ME, respectively, in the sample buffer. It should be noted that the mobility of sample 5 was similar to that of the BSA control (sample 1), indicating that the hydrodynamic size of BSA molecules in the NEM–BSA gel was smaller because of the presence of disulfide bonds. Furthermore, the absence of any higher molecular weight band in this sample clearly indicated that there was no disulfide-induced polymerization of BSA in the NEM–BSA gel. Samples 3 and 4 in Figure 2B refer to Cys–BSA gel, run without and with 2-ME, respectively, in the electrophoretic sample buffer. It should be noted that, in contrast to the mobility of NEM–BSA (sample 5), the electrophoretic mobility of Cys–BSA (sample 3) was slower and was very similar to those samples run in the presence of 2-ME (i.e., samples 2, 4, and 6). These results indirectly indicate that in the Cys–BSA gel the BSA molecules were in a completely unfolded state with larger hydrodynamic size than in the NEM–BSA gels. The higher gel strengths of Cys–BSA gels compared to those of NEM–BSA gels might be related to these structural and hydrodynamic size differences.

In contrast to the Cys–BSA and NEM–BSA gels, the electrophoretic pattern of BSA from gels with no additives showed a higher molecular weight protein band at the top of the stacking gel (sample 7). The fact that the protein in this band could not enter the stacking and the separating gel indicated that the molecular weight of these species might be more than a million (Utsumi et al., 1984). However, when the gel sample was dissolved in the buffer containing 2-ME (0.3 M) and electrophoresed, these higher molecular weight complexes disappeared completely (sample 8, Figure 2B), indicating that these polymers were formed via sulfhydryl–disulfide interchange reaction during gelation.

The analyses of the data in parts A and B of Figure 2 clearly indicate the following: While no polymer formation occurred in the Cys–BSA and NEM–BSA gels, significant amount of disulfide-linked polymerization of BSA occurred in the gel with no additives. The higher gel strength of these gels compared to the Cys–BSA and NEM–BSA gels might be related to formation of polymers having higher hydrodynamic size and weight-average molecular weight. Conversely, the increase of gel strength in the order BSA > Cys–BSA > NEM–BSA might implicitly be due to differences in the weight-average molecular weight of the polypeptide species in the gel and their hydrodynamic size in the gel system.

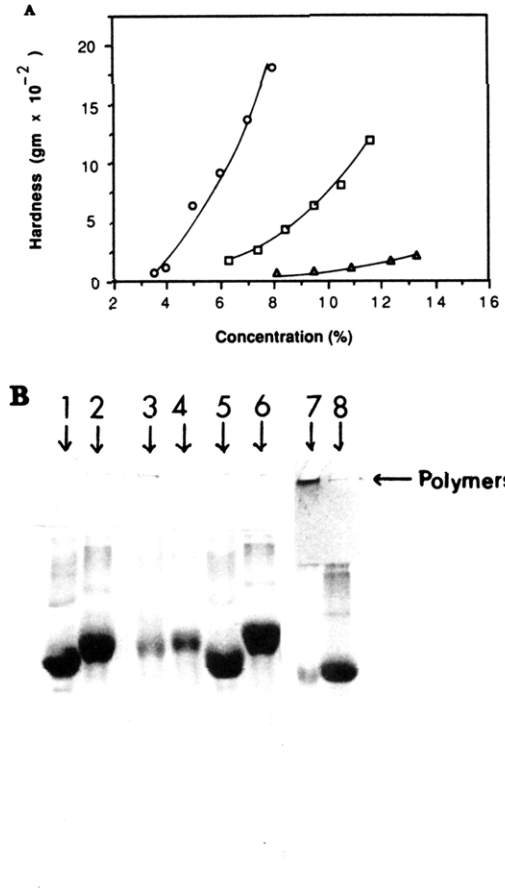

Figure 2. (A) Concentration dependence of the hardness of BSA gels. (○) BSA; (□) BSA + 400 mM cysteine; (△) BSA + 20 mM NEM. (B) SDS–PAGE of BSA gels. Samples 1 and 2 were native BSA run in the presence and absence, respectively, of 0.3 M 2-ME. Samples 3 and 4 were Cys–BSA gel run in the absence and presence, respectively, of 0.3 M 2-ME. Samples 5 and 6 were NEM–BSA gel run in the absence and presence, respectively, of 0.3 M 2-ME. Samples 7 and 8 were BSA gel run in the absence and presence, respectively, of 0.3 M 2-ME. (Note: Samples 7 and 8 were run on a separate SDS–PAGE).

The concentration versus gel strength profiles of egg white (EW) are shown in Figure 3A, and the SDS–PAGE patterns of these gels are shown in Figure 3B. The gelation behavior of egg white in the presence and absence of cysteine and NEM was very similar to that of BSA under similar conditions. That is, at any given protein concentration, the gel strength of EW gels followed the order EW > Cys–EW > NEM–EW (Figure 3A). Since the proteins present in egg white contain sulfhydryl and disulfide groups, the general explanations for the behavior of EW gels in the presence and absence of cysteine and NEM would be very similar to those provided for the BSA gels. However, the electrophoretic patterns of these EW gels might need some additional interpretations. Egg white contains two major protein components, namely, albumin and conalbumin (Figure 3B). The electrophoretic mobility of both albumin and conalbumin was slower in the 2-ME-treated sample (Figure 3B, sample 2) than that in the sample without 2-ME treatment (sample 1, Figure 3B). The electrophoretic pattern of the EW gel, run in the absence of 2-ME (sample 3, Figure 3B), showed a band at the top of the stacking gel, another band at the top of the separating gel, and a protein band corresponding to albumin; no conalbumin band was present. The intensity of the ovalbumin band

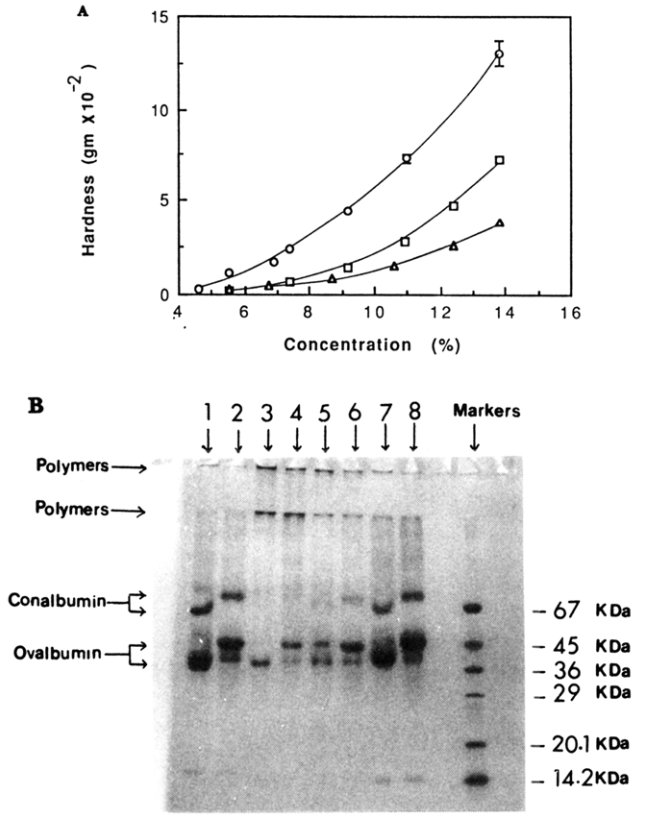

Figure 3. (A) Concentration versus hardness of egg white (EW) gels. (○) EW; (□) EW + 50 mM cysteine; (△) EW + 20 mM NEM. (B) SDS–PAGE of egg white gels. Samples 1 and 2 were native egg white run in the absence and presence, respectively, of 0.3 M 2-ME. Samples 3 and 4 were EW gel run in the absence and presence, respectively, of 2-ME. Samples 5 and 6 were Cys–EW gel run in the absence and presence, respectively, of 2-ME. Samples 7 and 8 were NEM–EW gel run in the absence and presence, respectively, of 2-ME.

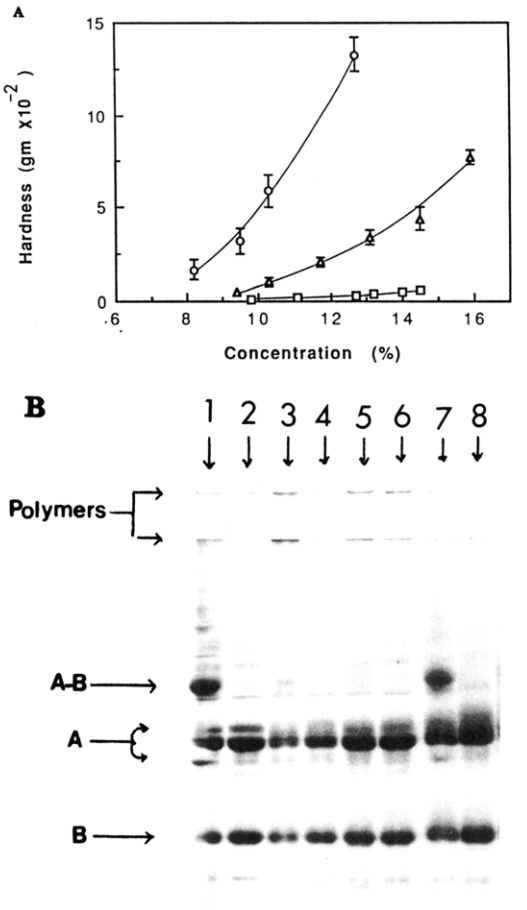

Figure 4. (A) Concentration versus hardness of soy 11S gels. (○) 11S; (□) 11S + 50 mM cysteine; (△) 11S + 20 mM NEM. (B) SDS–PAGE of soy 11S gels. Samples 1 and 2 were native 11S run in the absence and presence, respectively, of 2-ME. Samples 3 and 4 were 11S gel run in the absence and presence, respectively, of 2-ME. Samples 5 and 6 were Cys-11S gel run in the absence and presence, respectively, of 2-ME. Samples 7 and 8 were NEM–11S gel run in the absence and presence, respectively, of 2-ME. A and B are the acidic and the basic subunits of soy 11S, respectively. A–B is the acidic–basic subunit.

in this sample was much lower than that of the unheated control (sample 1, Figure 3B). These results suggested that during gelation all the conalbumin and some of the albumin were polymerized into higher molecular weight polymers which were unable to enter the stacking and separating gels. To determine whether these polymers were formed via intermolecular disulfide bond formation, 0.3 M 2-ME was added and the gels were electrophoresed. The electrophoretic pattern under these conditions still exhibited a considerable amount of polymers that were unable to enter the stacking and separating gels (sample 4, Figure 3B). Recently it has been reported that proteins, when heated at higher temperatures, undergo β-elimination of cysteine and cystine residues even at near-neutral pH, leading to formation of stable lysinoalanine linkage (Volkin and Klibanov, 1987). It is likely that the polymers present in sample 4 (Figure 3B) that were unable to enter the stacking and separating gels might be the polymers of this kind. The electrophoretic pattern, in the absence of 2-ME, of Cys–EW gel showed a considerable amount of polymers which could not enter the stacking and separating gels (sample 5, Figure 3B); these polymers did not disappear when electrophoresed in the presence of 2-ME (sample 6, Figure 3B), indicating again that these polymers were formed via covalent bonds (probably the lysinoalanine bond) other than the disulfide bond. However, in the case of NEM–EW gel, only a small amount of the polymers that were unable to enter the stacking gel was observed (sample 7, Figure 3B). Furthermore, when the NEM–EW gel sample was electrophoresed in the presence of 2-ME, these polymers disappeared (sample 8, Figure 3B), indicating that the presence of NEM permitted formation of a very small amount of polymerization via disulfide bonds but prevented formation of polymers via lysinoalanine covalent linkage.

The concentration versus gel strength profiles of 11S in the presence and absence of cysteine and NEM are shown in Figure 4A. Unlike BSA and egg white, the gel strength of 11S gels at any given protein concentration followed the order 11S > NEM–11S > Cys–11S. The molecular reasons for this deviation can be explained as follows: The soy 11S globulin contains six acidic (M_r = 35 000) and six basic (M_r = 20 000) subunits (Badley et al., 1975). The oligomeric structure of 11S globulin is made up of specific acidic–basic subunit pair (A–B subunits) and nonequimolar amounts of acidic (A) and basic (B) subunits. This is clearly evidenced from the gel electrophoretic pattern of unheated 11S globulin run without and with 2-ME treatment (samples 1 and 2, respectively, in Figure 4B). The 11S globulin contains 21 disulfide bonds and 2 free sulfhydryl groups (Badley et al., 1975). During thermal gelation, the 11S undergoes sulfhydryl–disulfide interchange, leading to formation of very high molecular weight complexes (Utsumi et al., 1984). This is evident from the gel electrophoretic pattern of the 11S gel in the absence of 2-ME treatment (sample 3, Figure 4B). It should be noted that substantial amounts

of polymers that were unable to enter the stacking and separating gels were formed at the cost of the acidic and basic subunit bands. However, when the sample was electrophoresed in the presence of 2-ME, these polymers were broken down to acidic and basic subunits (sample 4, Figure 4B). The higher strength of 11S gels in the absence of NEM and cysteine might be due to this disulfide-induced polymerization and the consequent increase in the weight-average molecular weight and the hydrodynamic size of the polypeptide species in the gel. However, when gelation occurs in the presence of NEM, the sulfhydryl–disulfide interchange reaction is inhibited. Because of this inhibition, the polypeptide species present in the NEM–11S gel system would be A–B, A and B subunits with molecular weights of 55 000, 35 000, and 20 000, respectively. The electrophoretic pattern of the NEM–11S gel, run in the absence of 2-ME (sample 7, Figure 4B) clearly indicates that this in fact is the case. Under these conditions, the weight-average molecular weight of all the polypeptide species present in the NEM–11S gel system would be much smaller than that of the 11S gel. It can be surmised that this decrease in the weight-average molecular weight and the consequent decrease in the hydrodynamic size might be responsible for the observed decrease in the gel strength (Figure 4A).

The electrophoretic analysis of the Cys–11S gel showed essentially no polymers formed via disulfide bonds (sample 5, Figure 4B); the majority (>95%) of the polypeptide species in these gels were the acidic and the basic polypeptides only. A small amount (<5%) of polymers was observed at the top of the stacking and separating gels; these polymers did not disappear when the sample was electrophoresed in the presence of 2-ME (sample 6, Figure 4B), indicating that these were non-disulfide-induced polymers. It is clear that because of the prevention of disulfide-induced polymerization and the reduction of the A–B subunits to A and B units by cysteine, the weight-average molecular weight of the polypeptide species in the Cys–11S gel system would be smaller than that of either the 11S or the NEM–11S gels. This might be the reason for the observed lower gel strength of Cys–11S gels.

To obtain more insight into the apparent relationship between the size distribution of polypeptide species in the gel and the gel strength, the concentration versus gel strength profiles of soy isolate and soy 7S also were studied. In agreement with the behavior of other proteins, the gel strengths of soy isolate and soy 7S were significantly higher than those in the presence of cysteine (Figures 5A and 6A). The electrophoretic analysis of these gels revealed that the gels formed in the absence of cysteine contained a considerable amount of disulfide-induced polymers which could not enter the stacking gel (sample 3 in Figures 5B and 6B). The gels which contained cysteine did not have any high molecular weight polymers. Since soy 7S contains no disulfide bonds (Badley et al., 1975), the small amount of polymers observed in the 7S gel sample (sample 3, Figure 6B) might have been the result of 11S contamination in this 7S preparation.

In phenomenological terms, critical analysis of the data presented here on the gelation behaviors of various proteins in the presence and absence of cysteine and NEM suggests that the variations in the concentration versus gel strength profiles of these gels could be related to differences in the weight-average molecular weight and the hydrodynamic size of the polypeptide species in these gels.

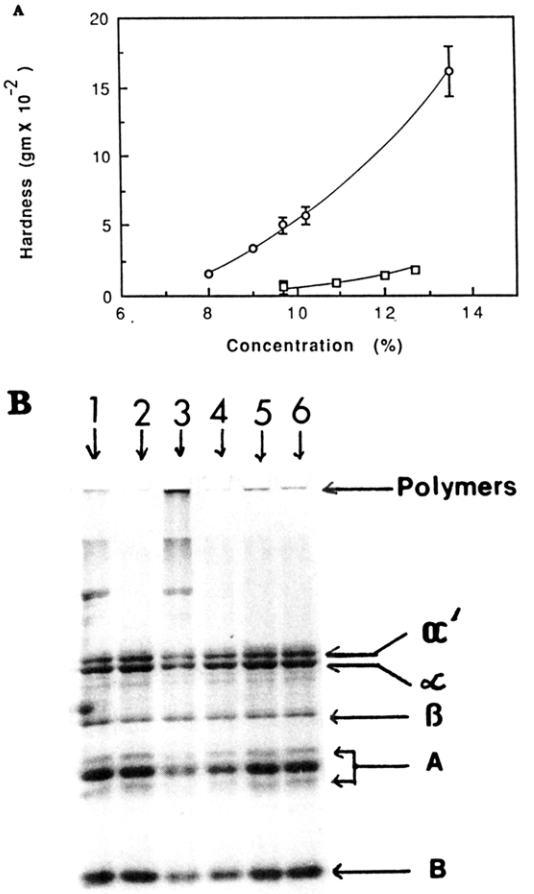

Figure 5. (A) Concentration versus hardness of soy isolate (SI) gels. (○) Soy isolate; (□) SI + 50 mM cysteine. (B) SDS–PAGE of soy isolate gels. Samples 1 and 2 were native SI run in the absence and presence, respectively, of 2-ME. Samples 3 and 4 were SI gel run in the absence and presence, respectively, of 2-ME. Samples 5 and 6 were Cys–SI gel run in the absence and presence, respectively, of 2-ME. α, α', and β are the subunits of soy 7S globulin. A and B are the acidic and the basic subunits of soy 11S globulin, respectively.

In protein gels, formations of a self-supporting gel network that is stable against thermal and mechanical motions is dependent on the number of cross-links per monomer or per unit cell of the gel. This depends on both protein concentration and the number of cross-linking loci available per molecule. Ferry (1948) showed that, under a given set of gelation conditions, such as protein concentration, pH, and ionic strength, the square root of the rigidity of gelatin gels was proportional to the molecular weight of the gelatin. This molecular weight or molecular size dependence of the gel rigidity might be related to the dependence of the extent of molecular entanglement as well as the number of cross-links formed per unit cell of the gel on the chain length of the polypeptide. Since gelatin contains neither cysteine nor cystine residues, the elucidation of the dependence of the gel rigidity to the weight-average molecular weight is straightforward. However, in the case of globular proteins (which usually contain cysteine and cystine), it is difficult to establish the relationship between the gel strength and the molecular weight because of the formation of random intermolecular disulfide bonds during heat-induced gelation, which results in formation of a heterogeneous population of high molecular weight polypeptide species of various chain lengths. However, this could be overcome by

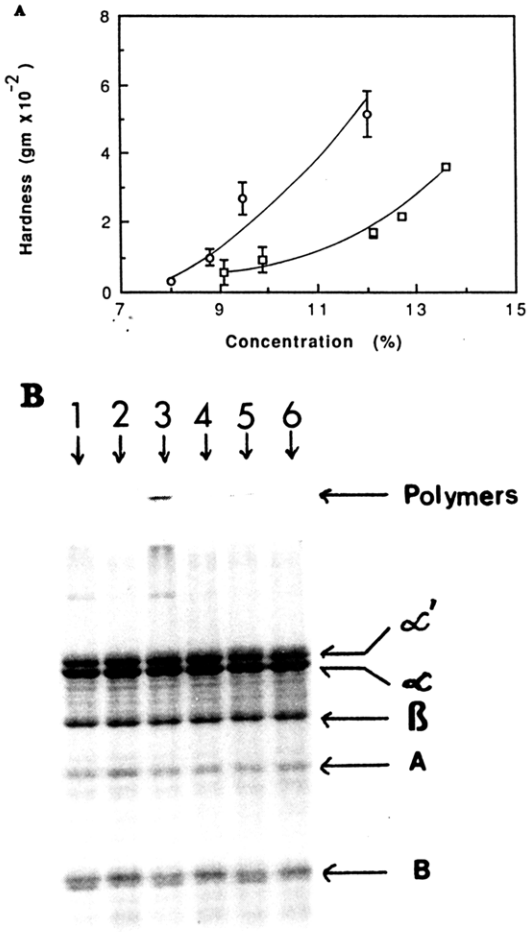

Figure 6. (A) Concentration versus hardness of soy 7S. (○) 7S; (□) 7S + 50 mM cysteine. (B) SDS–PAGE of soy 7S gels. Samples 1 and 2 were native 7S globulin run in the absence and presence, respectively, of 2-ME. Samples 3 and 4 were 7S gel run in the absence and presence, respectively, of 2-ME. Samples 5 and 6 were Cys–7S gel run in the absence and presence, respectively, of 2-ME. α', α, and β are the subunits of 7S globulin. A and B are the subunits of soy 11S globulin.

using agents, such as cysteine and NEM, which would reduce disulfide bonds and/or prevent sulfhydryl–disulfide interchange reaction. Under these conditions, it should be possible to relate the molecular weight dependence of the gel strength of various globular proteins formed under similar conditions.

To elucidate whether the gel strength of globular proteins is fundamentally related to the weight-average molecular weight of the polypeptide species present in the gel network, the data were analyzed as follows: The relative intensities of each band in SDS–PAGE of protein gels were determined by using a computerized scanning densitometer. The weight-average molecular weight of the polypeptide species in the gel was obtained from

$$M_w = \sum_i a_i M_i / \sum_i a_i$$

where a_i is the area of the ith band in the densitogram and M_i is the molecular weight of the ith band. Since the molecular weight determination of the polymers that were unable to enter either the stacking or the separating gel is very difficult, only the protein gels that contained an insignificant amount (<5%) of these polymers were selected for this purpose. The square root of the gel strength of these protein gels at two different concentrations was plotted as a function of their weight-average molecular weight (Figure 7).

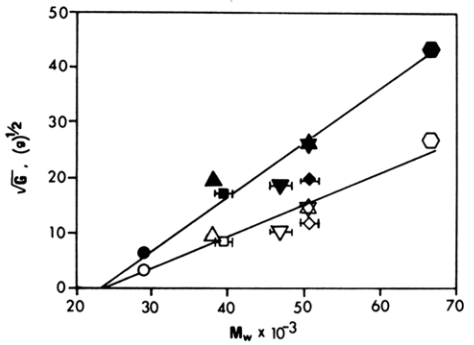

Figure 7. Relationship between the square root of the hardness of various protein gels and the weight-average molecular weight (M_w) of the polypeptides in the gel network. The open symbols correspond to 10% protein concentration, and the solid symbols correspond to 13.5% protein concentration. (○ and ●) Cys–11S; (△ and ▲) NEM–11S; (□ and ■) Cys–SI; (▽ and ▼) Cys–7S; (◇ and ◆) NEM–EW; (★ and ☆) Cys–EW; (○ and ●) Cys–BSA.

Several important conclusions can be drawn from the data in Figure 7. It should be noted that despite the inherent molecular differences among the proteins included in these plots, the M_w versus $G^{1/2}$ plots were linear with a correlation coefficient of 0.913. This clearly suggests that the rheological and textural properties of typical globular protein gels are fundamentally dependent not as much as on their chemical nature but largely on their molecular size (and shape). The concentration dependence of the slope of the curves in Figure 7 suggests that the number of stable cross-links formed per unit cell of the gel network is affected by both the molecular size and concentration, but not by the amino acid composition of the polypeptides. It should also be noted that, when extrapolated, the curves in Figure 7 converge at a common point on the x axis. The physical meaning of this point on the x axis is that to form a gel network, at any concentration, the minimum weight-average molecular weight of the protein should be greater than this critical M_w. From Figure 7, the critical M_w for globular proteins seems to be about 23 000. Conversely, the data indicate that for a globular protein to form a stable gel network at 5 °C the M_w of the protein should be greater than 23 000; below this critical M_w no globular protein would form a gel at 5 °C at any reasonable concentration.

To confirm whether or not the above prediction is correct, the gelling behavior of phaseolin and trypsin-digested phaseolin was studied. Phaseolin is the major storage protein of common bean, *P. vulgaris*. It is a trimeric protein; the molecular weight of the subunits is about 45 000, and none of the subunits contains disulfide bonds (Slightom et al., 1983). Trypsinolysis of native phaseolin results in cleavage, approximately at the center of each of these subunits, resulting in the liberation of two polypeptide fragments of about 22 000 daltons (Deshpande and Neilson, 1987). The SDS–PAGE of the native and trypsinolyzed phaseolin (Figure 8B) confirms these reports. Since the weight-average molecular weight of the polypeptides in trypsinolyzed phaseolin is about 22 000, according to the data in Figure 7 it should not be able to form a gel. In fact, as is shown in Figure 8A, while the intact phaseolin could form a gel between 7% and 14% protein concentration, the trypsinolyzed phaseolin could not form a gel under similar conditions. This positively confirms the prediction that the proteins with $M_w < 23\,000$ cannot form a thermally induced self-supporting gel at any reasonable concentration.

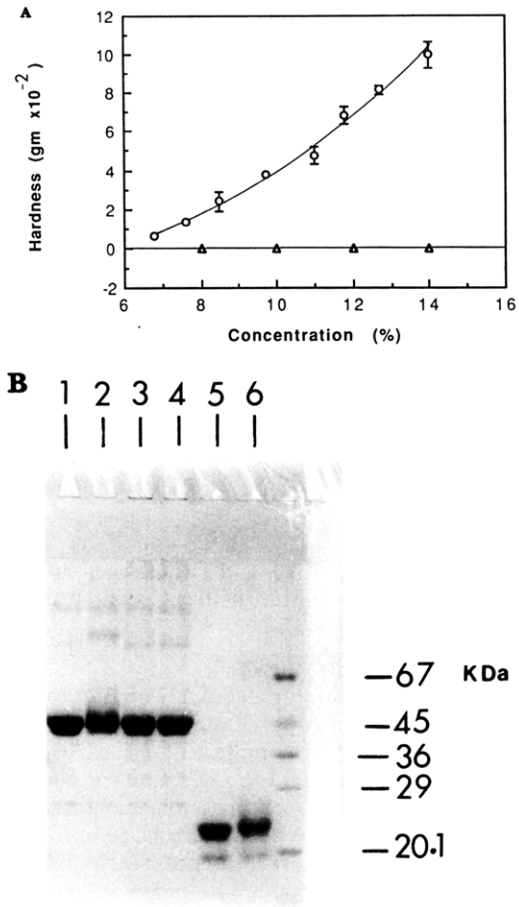

Figure 8. (A) Concentration versus hardness of phaseolin (○) and trypsinolyzed phaseolin (△) gels. In the case of trypsinolyzed phaseolin no gelation was observed. (B) SDS–PAGE of phaseolin gels. Samples 1 and 2 were native phaseolin run in the absence and presence, respectively, of 0.3 M 2-ME. Samples 3 and 4 were solubilized phaseolin gel run in the absence and presence, respectively, of 0.3 M 2-ME. Samples 5 and 6 were trypsin-digested phaseolin run in the absence and presence, respectively, of 0.3 M 2-ME.

The ability of a protein to form intermolecular disulfide bonds during heat treatment has often been considered to be a prerequisite for gelation of proteins (Utsumi and Kinsella, 1985; Huggins et al., 1951; Nakamura et al., 1984; Mori et al., 1981, 1986). However, it should be pointed out that, of all the proteins, gelatin is known to be the best gelling protein, yet this protein is devoid of cysteine and cystine residues. This clearly implies that formation of disulfide bonds is not essential for gelation of proteins. In molecular terms, the apparent role of disulfide bonds in gelation may be related to their ability to increase the weight-average molecular weight (and hence the chain length) of the protein, rather than as a specific network former in the gel. The increase in the effective chain length of the polypeptide may increase the molecular entanglement in the gel structure, which might restrict the relative thermal motions of the polypeptides. In addition, the lower diffusion coefficient of larger polypeptide species might prevent rupture of the weak noncovalent interactions and thus stabilize the gel network. Both these molecular effects might increase the total number of stable noncovalent bonds formed between the polypeptides per unit cell of the gel. In other words, it is the physical constraint imposed by the size, rather than the disulfide bond per se, that is responsible for the perceived influence of disulfide bonds on gel strength.

The results presented here clearly suggest that the hardness of a globular protein gel at a given concentration is fundamentally related to the weight-average molecular weight of the protein. The greater the M_w, the greater would be the gelling power. Globular proteins whose M_w is less than 23 000 under the gelation conditions would not form a gel. Conversely, one can improve the gelling properties of globular proteins (especially the ones that lack cysteine and cystine) by increasing their effective chain length via chemical cross-linking methods.

ACKNOWLEDGMENT

Support in part by the National Science Foundation, Grant CBT-8616970, is gratefully acknowledged.

LITERATURE CITED

Babajimopoulas, M.; Damodaran, S.; Rizvi, S. S. H.; Kinsella, J. E. Effects of various anions on the rheological and gelling behavior of soy proteins: Thermodynamic observations. *J. Agric. Food Chem.* **1983**, *31*, 1270–1275.

Badley, R. A.; Atkinson, D.; Houser, H.; Oldani, G.; Stubbs, J. The Structure, Physical and Chemical Properties of the Soy Bean Protein Glycinin. *Biochim. Biophys. Acta* **1975**, *412*, 214–228.

Bourne, M. C. Texture Profile Analysis. *Food Technol.* **1978**, 62–72.

Catsimpoolas, N.; Meyer, E. W. Gelation phenomena of soybean globulins. 1. Protein-Protein interactions. *Cereal Chem.* **1970**, *47*, 559–570.

Catsimpoolas, N.; Meyer, E. W. Gelation phenomena of soybean globulins. II. Protein-water miscible solvent interactions. *Cereal Chem.* **1971**, *48*, 150–158.

Damodaran, S. Refolding of Thermally Unfolded Soy Proteins during the Cooling Regime of the Gelation Process: Effect on Gelation. *J. Agric. Food Chem.* **1988**, *36*, 262–269.

Deshpande, S. S.; Nielsen, S. S. In Vitro Enzymatic Hydrolysis of Phaseolin, the Major Storage Protein of *Phaseolus vulgaris* L. *J. Food Sci.* **1987**, *52*, 1326–1329.

Ferry, J. D. Mechanical Properties of Substances of High Molecular Weight. IV. Rigidities of Gelatin Gels; Dependence on Concentration, Temperature and Molecular Weight. *J. Am. Chem. Soc.* **1948**, *70*, 2244–2249.

Hall, T. C.; McLeester, R. C.; Bliss, F. A. Equal Expression of the Maternal and Paternal Alleles for the Polypeptide Subunits of the Major Storage Protein of the Bean, *Phaseolus vulgaris* L. *Plant Physiol.* **1977**, *59*, 1122–1124.

Huggins, C.; Tapley, D. F.; Jensen, E. V. Sulphydryl-Disulfide Relations in the Induction of Gels in Proteins by Urea. *Nature* **1951**, *167*, 592–593.

Laemmli, U. K. Cleavage of Structural Proteins during the assembly of the head of Bacteriophage T_4. *Nature* **1970**, *227*, 680–685.

Mori, T.; Nakamura, T.; Utsumi, S. Gelation Mechanism of Soybean 11S Globulin: Formation of Soluble Aggregates as Transient Intermediates. *J. Food Sci.* **1981**, *47*, 26–30.

Mori, T.; Nakamura, T.; Utsumi, S. Behavior of Intermolecular Bond Formation in the Late Stage of Heat-Induced Gelation of Glycinin. *J. Agric. Food Chem.* **1986**, *34*, 33–36.

Nakamura, T.; Utsumi, S.; Mori, T. Network Structure Formation in Thermally Induced Gelation of Glycinin. *J. Agric. Food Chem.* **1984**, *32*, 349–352.

Oe, H.; Hirose, M.; Doi, E. Conformation changes and subsequent gelation of conalbumin by a thiol reagent. *Agric. Biol. Chem.* **1986**, *50*, 2469–2475.

Shimada, K.; Matsushita, S. Relationship between thermocoagulation of proteins and amino acid compositions. *J. Agric. Food Chem.* **1980**, *28*, 413–417.

Slightom, J. L.; Sun, S. M.; Hall, T. C. Complete Nucleotide Sequence of a French Bean Storage Protein Gene: Phaseolin. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 1897–1901.

Thanh, V. H.; Shibasaki, K. Major Proteins of Soybean Seeds: A Straightforward Fractionation and their Characterization. *J. Agric. Food Chem.* **1976**, *24*, 1117–1121.

Utsumi, S.; Kinsella, J. E. Forces involved in soy protein gelation: Effects of various reagents on the formation, hardness

and solubility of heat-induced gels made from 7S, 11S and soy isolate. *J. Food Sci.* **1985**, *50*, 1278–1282.
Utsumi, S.; Damodaran, S.; Kinsella, J. E. Heat induced interaction between soybean proteins. *J. Agric. Food Chem.* **1984**, *32*, 1406–1412.
Volkin, D. B.; Klibanov, A. M. Thermal Destruction Processes in Proteins involving Cystine Residues. *J. Biol. Chem.* **1987**, *262*, 2945–2950.

Received for review January 18, 1989. Revised manuscript received January 30, 1990. Accepted February 6, 1990.

Particular Lipid Composition in Isolated Proteins of Durum Wheat

Kàroly Kobrehel*,† and Yves Sauvaire‡

Laboratoire de Technologie des Céréales, INRA, 34060 Montpellier Cedex, France, and Laboratoire de Physiologie Végétale, Université des Sciences et Techniques du Languedoc, 34060 Montpellier Cedex, France

Gas chromatographic and mass spectrometric analyses showed that two low molecular weight proteins, extracted from glutenins of two durum wheat cultivars, contained strongly bound lipids with particular composition in comparison to semolina lipids. The percentages of palmitic acid (C16), stearic acid (C18), and oleic acid (C18:1) were high; conversely, their linoleic acid (C18:2) content was lower than 5%. Thus, semolina lipids contained about 1.5% C18, while isolated proteins contained between 23 and 28%. This fatty acid composition cannot be explained by an autoxidation of lipids during the isolation procedure. Various fatty alcohols and hydrocarbons were also present in the purified proteins; the amount of dodecanol, especially in one sample, was very high.

Previous work has shown the role of DSG proteins (durum wheat sulfur-rich glutenins) on the technological quality of durum wheats (Kobrehel and Alary, 1989a; Feillet et al., 1989). These two proteins, DSG-1 and DSG-2, had low M_s (molecular masses) of 14.1 and 17.1 kDa, respectively, and they were extractable from semolina at low concentrations of sodium tetradecanoate subsequent to the extraction of albumins, globulins, and gliadins (Kobrehel et al., 1988). Their amino acid composition was similar to that of the glutenins. However, they contained a higher amount of half-cystine than other glutenins, and they were not linked to the other glutenins through S–S bonds. There were also considerable differences between the amino acid composition and the N-terminal sequences of DSG-1 and DSG-2 (Kobrehel and Alary, 1989b). On the other hand, the amino acid sequence at the N-termini of DSG-1 and DSG-2 were found to be similar to the N-termini of CM proteins CM16 and CM3, respectively, extracted from hexaploid wheat (Shewry et al., 1984; Barber et al., 1986). The CM proteins are salt-soluble proteins that can be extracted with chloroform–methanol (2:1, v/v). Results suggest that CM proteins CM1, CM2, CM3, CM16, and CM17 are components of tetrameric α-amylase inhibitors (Garcia-Olmedo et al., 1987).

Our studies showed that some specific lipids were tightly bound to DSG proteins, which may contribute to their functional properties in the technological quality of durum wheat. These results are presented in this paper.

† INRA.
‡ Université des Sciences et Techniques du Languedoc.

MATERIALS AND METHODS

Wheat Samples. Two durum wheat (*Triticum durum*, Desf.) French cultivars, Mondur and Kidur, were studied. Semolina was obtained in 70% yield on a pilot mill. For experiments on common wheat (*Triticum aestivum*, L.), French cultivars Hardi, Talent, and Fidel were milled on a pilot mill to 74% flour yield.

Isolation of DSG Proteins (DSG-1 and DSG-2). DSG proteins were isolated under the conditions described by Kobrehel and Alary (1989b). Proteins from semolina were extracted sequentially by stirring the semolina sample for 15 h at 4 °C and then centrifuging at 38000*g* for 30 min at 4 °C. Albumins plus globulins were extracted with 0.5 M NaCl and gliadins with ethanol–water (68:32, v/v), with 1 g of semolina and 10 mL of extraction solvent. Then, 3.75 mg of sodium tetradecanoate/10 mL of distilled water was added to the residues. Sodium tetradecanoate (99% pure by gas chromatography) was prepared in our laboratory as described by Kobrehel and Alary (1989a).

About 50 mg of freeze-dried extract (glutenin 1) obtained with sodium tetradecanoate was dissolved in 0.05 M acetic acid, filtered through a lipid-free folded filter, and applied to a Bio-Gel P-30 molecular sieving chromatography column (2.5 × 100 cm). The flow rate was of 25 mL/h, and the eluate was monitored at 280 nm with a single-path monitor UV (Pharmacia). Eluted fractions (5 mL) were collected with an automatic collector; all chromatographic separations were performed at room temperature.

Electrophoresis of Proteins. SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) analyses were done under the conditions described by Payne and Corfield (1979). Proteins were not reduced before electrophoresis.

Extraction of Lipids from Semolina and from Isolated DSG Proteins. Lipids from semolina were extracted in triplicate by the method of Folch et al. (1957). To 5 g of semolina was added 25 mL of chloroform–methanol (2:1, v/v); the mixture was stirred for 5 min and then filtered through a filter previously defatted with the same solvent. Five successive extrac-

0021-8561/90/1438-1164$02.50/0