Hashizume, T.; Sugiyama, T.; Imura, M.; Cory, H. T.; Scott, M. F.; McCloskey, J. A. *Anal. Biochem.* **1979**, *92*, 111.
Heindl, J. C.; Carlson, D. R.; Brun, W. A.; Brenner, M. L. *Plant Physiol.* **1982**, *70*, 1619.
Jourdan, P. S.; Weiler, E. W.; Mansell, R. L. *J. Agri. Food Chem.* **1983**, *31*, 1249.
Kahn, S. E.; Humayun, M. Z.; Jacob, T. M. *Anal. Biochem.* **1977**, *83*, 632.
Kemp, T. R.; Andersen, R. A.; Oh, J.; Vaughn, T. H. *J. Chromatogr.* **1982**, *241*, 325.
Letham, D. S. In "Phytohormones and Related Compounds: A Comprehensive Treatise"; Letham, D. S., Goodwin, P. B., Higgins, T. J. V., Eds.; Elsevier/North Holland Biomedical Press: New York, **1978**; Vol. 1, p 205.
MacDonald, E. M. S.; Akiyoshi, D. E.; Morris, R. O. *J. Chromatogr.* **1981**, *214*, 101.
MacLeod, J. K.; Summons, R. E.; Letham, D. S. *J. Org. Chem.* **1976**, *41*, 3959.
Reeve, D. R.; Crozier, A. In "Encyclopedia of Plant Physiology: Hormonal Regulation of Development I"; MacMillan, J., Ed.; Springer-Verlag: New York, **1980**; Vol. 9, p 203.
Stafford, A. E.; Corse, J. *J. Chromatogr.* **1982**, *247*, 176.
Stewart, I.; Barthe, G. B. *Proc. Int. Soc. Citric.* **1984**, in press.
Vold, B. S.; Leonard, N. J. *Plant Physiol.* **1981**, *67*, 401.
Weiler, E. W. *Planta* **1980**, *149*, 155.
Weiler, E. W. *Biochem. Soc. Trans.* **1983**, *11*, 485.
Weiler, E. W.; Jourdan, P. S.; Conrad, W. *Planta* **1981**, *153*, 561.
Weiler, E. W.; Spanier, K. *Planta* **1981**, *153*, 326.

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Structure–Function Relationships in Food Proteins: Subunit Interactions in Heat-Induced Gelation of 7S, 11S, and Soy Isolate Proteins

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The nature of specific interactions occurring between soy protein subunits upon gelation following heating was studied by ultracentrifugation and two-dimensional gel electrophoresis. The results indicate that acidic subunit (AS) III in contrast to ASIV is an integral and essential component of glycinin and soy isolate gels, that the three subunits of β-conglycinin (7S) participate uniformly in gel matrix formation from conglycinin, and that there is a preferential interaction between the β subunit of 7S and the basic subunits of glycinin (11S) in soy isolate gels. Selective solubilization of gels indicated that hydrogen bonding and disulfide bonds were important in maintaining gel network structures.

The composition and oligomeric structure of soy proteins affect their functional behavior (Kinsella, 1979; Kinsella et al., 1985). The major globulins of soy protein are conglycinin (7S) and glycinin (11S). 7S is a trimeric glycoprotein (141 000–170 000 daltons) composed of six different combinations of three subunits, α (57 000), α' (58 000), and β (42 000) associated via hydrophobic interactions (Thanh and Shibasaki, 1978). The 11S consists of two apposed hexagonal rings each containing three hydrophobically associated pairs of disulfide-linked acidic (35 000–37 000) and basic (20 000) subunits (Badley et al., 1975). Upon being heated, these subunits may dissociate and reassociate in different ways and form gels (German et al., 1982; Damodaran and Kinsella, 1981; Mori et al., 1982a,b).

The possible mechanisms involved in the heat-induced gelation and the association–dissociation behavior soy protein isolate and its constituent proteins of soybean have been studied by many workers (Mann and Brigs, 1950; Watanabe and Nakayama, 1962; Saio et al., 1968; Wolf and Tamura, 1969; Catsimpoolas et al., 1969, 1970; Aoki, 1970; Fukushima and van Buren, 1970; Hashizume et al., 1975; Hashizume and Watanabe, 1979; Yamagishi et al., 1980; Mori et al., 1982a; German et al., 1982; Damodaran and Kinsella, 1982; Nakamura et al., 1984a,b). However, most of these studies were carried out under conditions in which gel is not formed. Considerable research has recently been done on the mechanism of gelation and the role of the constituent subunits in the formation and properties of the gels formed from 11S (Mori et al., 1982a,b; Nakamura et al., 1984a,b; Utsumi et al., 1983). These studies led to the suggestion that the 11S globulin oligomers appear to undergo minor changes upon heating, retain quaternary structure, and reveal little obvious denaturation when associating to form a gel following heating at 100 °C (Mori et al., 1982a; Nakamura et al., 1984b). The contributions of the constituent subunits to the formation and properties of the 11S gel are not equal. Thus, acidic subunit (AS) III plays an important role in increasing the hardness of the gels (Mori et al., 1982b; Nakamura et al., 1984b). Similar results were obtained with respect to 11S globulin of broad bean (Utsumi et al., 1983). These results suggest that the contribution of the various soy subunits to the interactions involved in the formation of gel may be different. Recently, we showed that the subunits of 7S globulin interact electrostatically with the basic subunits (BS) of 11S globulin forming soluble complexes at low protein concentration (German et al., 1982; Damodaran and Kinsella, 1982; Utsumi et al., 1984). Similar interactions occur at high protein concentration and the main molecular forces involved in the formation of the gels from 7S, 11S, and soybean isolates are apparently different, suggesting that the protein–protein interactions in gels made from soybean isolate may be different from those made from the constituent proteins, 7S and 11S globulins (Utsumi and Kinsella, 1985).

In the preceding paper we showed that the soluble macromolecular complexes formed upon heating of soy isolates were composed mostly of basic subunits of 11S associated with β subunits of 7S, mostly via electrostatic interactions. Association of basic subunits via disulfide bonds also occurred (Utsumi et al., 1984).

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By use of ultracentrifugal and two-dimensional gel electrophoretic analyses of proteins in the progel, gel matrix and gel supernatant information concerning specific subunit interactions in gels made from soy 7S, 11S, and soy isolate proteins was obtained. The results indicate that the ASIII and ASIV components make major and minor contributions, respectively, to the network structure of 11S globulin and soybean isolate gels. The contribution of the three subunits of 7S globulin to the network structure of 7S and soybean isolate gels is apparently uniform.

MATERIALS AND METHODS

Materials. Electrophoretic-grade sodium dodecyl sulfate ($NaDodSO_4$) was purchased from Bio-Rad (Richmond, CA). Acrylamide and bis(acrylamide) were purchased from Sigma Chemicals (St. Louis, MO). Other chemicals used in this study were reagent grade. Disposable micropipets were obtained from Scientific Manufacturing Industries, Inc. (Berkley, CA).

Preparation of Soybean Protein Isolate (SI), 7S, and 11S Globulins. Soybean protein isolate and 7S and 11S globulin rich fractions were prepared from defatted and low heat treated soybean flour according to the method of Thanh and Shibasaki (1976a). The 11S globulin fraction was further purified by Fractogel TSK HW-55 as described previously (Utsumi et al., 1984). In the case of the 7S globulin fraction, no further purification was done. The purity of these two protein fractions was >90% and >70%, respectively. Proteins were stored in lyophilized form until used.

Preparation of Gel. Gelation of 7S, 11S, and soy protein isolate samples was achieved as previously described (Utsumi et al., 1982) with a slight modification. Aliquots (30 μL) of the protein solution (12% w/v) in 30 mM Tris-HCl buffer (pH 8.0) (heating buffer) were transferred by microsyringe into sealed micropipets (2.55-mm diameter) and centrifuged at low speed to remove dissolved air. The micropipets containing the protein solution were then heated at 80 °C for 30 min in a water bath followed by cooling at 12 °C and then kept at 4 °C for 20 h to ensure complete gelation.

To isolate progel samples additional proteins heated as described above (80 °C for 30 min) were immediately diluted with 150 μL of heating buffer (80 °C) held 30 min at 22 °C and then centrifuged at 2500 rpm for 10 min. The protein in the supernatant ("nonprogel" macromolecular complexes) was analyzed by two-dimensional electrophoreses.

Ultracentrifugation of Gel. After gelation the micropipets containing the gels were centrifuged at 48 000 rpm for 1 h at 10 °C in a Sorvall ultracentrifuge (Model OTD-65:B) using a Beckman SW 65 rotor. In order to protect the micropipets from breakage during ultracentrifugation, they were placed in glycerol. The volume of the supernatant above the centrifuged gel was measured by a microsyringe, and the protein content of the supernatant was determined by the method of Lowry et al. (1951). The protein components of the supernatant and of the remaining gel matrix, solubilized by various reagents, were analyzed by two-dimensional gel electrophoresis.

Solubilization of Gel Matrix. In order to determine the nature of the forces or bonds involved and the subunits participating in the gel structure, the gel matrix was solubilized by using a number of solvents. Following centrifugation, heating buffer (150 μL) with or without 0.5 M NaCl, 0.5 M NaSCN, 20% propylene glycol, or 0.2 M 2-mercaptoethanol (2ME) was added to the micropipets containing the gel matrix, and the gel was crushed by a needle, stirred thoroughly, and then incubated for 20 h with occasional agitation at room temperature. After incubation, the samples were centrifuged at 12 000 rpm for 15 min. The protein in the supernatant was quantified by the method of Lowry et al. (1951) and analyzed by two-dimensional gel electrophoreses.

Polyacrylamide Disc Gel Electrophoresis. One-dimensional polyacrylamide gel electrophoresis (PAGE) was performed according to the method of Davis (1964) using 6.5% (w/v) polyacrylamide separating gels in glass tubes (7 × 0.5 cm). In order to separate the higher molecular weights protein complexes, two stacking gels (3 and 4% acrylamide, w/v) were used in each tube. In order to prevent leaching of the high molecular weight complexes that did not enter the stacking gel after electrophoresis, the top of the stacking gel in each tube was polymerized with 4% acrylamide. The gels were stored in 10% glycerol at −20 °C (Miyazaki et al., 1978) until used for the electrophoresis in the second dimension.

The second-dimensional $NaDodSO_4$–PAGE was performed according to the method of Laemmli (1970) using 11% (w/v) polyacrylamide slab gels (14.5 × 14.5 × 0.1 cm). Prior to the second-dimension $NaDodSO_4$–PAGE each first-dimensional disc gel was treated with 10 volumes of $NaDodSO_4$ buffer composed of 62.5 mM Tris-HCl buffer (pH 6.8), 1% $NaDodSO_4$, 0.2 M 2ME, and 20% sucrose for 30 min at 55 °C with two changes of the solution. The treated gel was placed on top of the $NaDodSO_4$ slab gel and then polymerized with 1% agarose solution containing 1% $NaDodSO_4$, 62.5 mM Tris-HCl (pH 6.8), 0.2 M 2ME, and 0.01% bromphenol blue (BPB) to fix the first-dimensional gel.

Second-dimensional urea–PAGE was performed according to the method of Davis (1964) using 7.5% (w/v) polyacrylamide gel containing 7 M urea. Prior to the second-dimensional urea–PAGE, each first-dimensional disc gel was treated with 15 volumes of urea solution composed of 10 mM potassium phosphate buffer (pH 7.6), 8 M urea, and 0.2 M 2ME for 30 min at 35 °C with two changes of the solution. The treated gel was placed on top of the second-dimensional urea slab gel and then the urea solution described above, containing 0.01% BPB, was poured. In this case the first-dimensional gel was not fixed on the second-dimensional gel. After electrophoresis (Davis, 1964), the gels were stained with Coomassie Brilliant Blue R200.

RESULTS AND DISCUSSION

In the previous paper we suggested that the contribution of different acidic subunits of 11S globulin to the network structure of 11S globulin and SI gels was variable (Utsumi et al., 1984). If this suggestion is correct, then subunits weakly involved in network formation should be relatively free or exist as small complexes, and such proteins should be released from the gels by ultracentrifugation. Furthermore, according to Catsimpoolas and Meyer (1970), heating of the protein solution converts some of the protein irreversibly to the progel state. Thus, presumably proteins not contributing to network structure should be solubilized from the progel by dilution with hot buffer. Therefore, we analyzed the supernatant proteins following ultracentrifugation of the gels and diluted progels.

The 11S and 7S globulins and SI solutions were heated at 80 °C for 30 min. The progels of 11S globulin and SI showed slight turbidity. After the heating, the progels were immediately diluted with 5 volumes of hot (80 °C) heating buffer. The diluted progels were centrifuged at 2500 rpm for 10 min, and the solubility of each progel was determined to be 60%, 50%, and 90% for 11S, 7S, and SI, respectively. The progels were cooled and kept at 5 °C

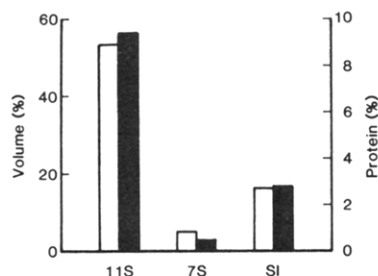

Figure 1. Volume and protein content of supernatants separated from soy protein gels by ultracentrifugation. Protein solutions of 11S and 7S globulins and SI were heated at 80 °C for 30 min in 30 mM Tris-HCl buffer (pH 8.0) at a protein concentration of 12%. After cooling at 4 °sC for 20 h, the gels were ultracentrifuged at 48000 rpm for 1 h. Open columns: volume of the gel supernatant. Solid columns: percent of total protein in the gel supernatant.

for 20 h, and the gels formed were ultracentrifuged at 48000 rpm for 1 h. The volumes and protein content of supernatants released from the 11S and 7S globulins and SI gels are shown (Figure 1). The 11S globulin gel gave a large supernatant; i.e., more than 50% of the total volume of the 11S globulin gel separated as soluble supernatant that contained 9.4% of total protein. The 7S globulin gel yielded a small supernatant, i.e., 5% of the total volume of the gel separated, and this contained only 0.5% of total protein. Only 16.7% of the total volume of the SI gel separated and this contained 2.8% of the total protein in the sample. Thus, the ease of the separation of protein from the gels is inversely proportional to the rigidity of the gels, i.e., as expected the more rigid gel should yield a small volume of supernatant. The rigidity of the gels is proportional to the hardness of the gel, and Utsumi and Kinsella (1985) observed that the order of the gel hardness is 7S ≫ SI > 11S, which corresponds to the relative ease of separation of fluid and protein from these gels.

In order to determine the size and composition of the constituent proteins of the progel and the proteins separated from the gels, these were analyzed by two-dimensional electrophoreses, on (a) PAGE to $NaDodSO_4$–PAGE and (b) PAGE to urea–PAGE. For the purpose of identification and characterization of various complexes, the first-dimensional gels of PAGE are divided into various segments as shown in Figures 2–7A and the protein bands in these parts are identified accordingly. Thus, I, II, and III represent the proteins that could not enter the 3% and 4% stacking gels and 6.5% separation gels, respectively; IV is the protein located in the top portion of the separation gel, and V corresponds to the proteins that are located in the lower portion of the separation gel. The bands thus reflect different-sized molecular complexes formed upon heating the soy proteins.

Figure 2 shows two-dimensional electrophoreses patterns from disc PAGE to $NaDodSO_4$–PAGE (Figure 2B) and to urea–PAGE (Figure 2C) of the diluted progel [Figure 2 (1)] and the gel supernatant [Figure 2 (2)] of 11S globulin. On the first-dimensional gel the diluted progel of 11S globulin gave considerable amounts of proteins corresponding to the region I and fair amounts of proteins corresponding to the regions II–V. The supernatant of the 11S globulin gel contained protein bands corresponding to regions III, IV, and V; i.e., the supernatant of 11S contained low amounts of the large complexes that could not enter the stacking gels. Thus, large complexes with molecular weights of more than few millions were not released from the 11S globulin gel presumably because they are part of the network structure of 11S gels. In order to identify the

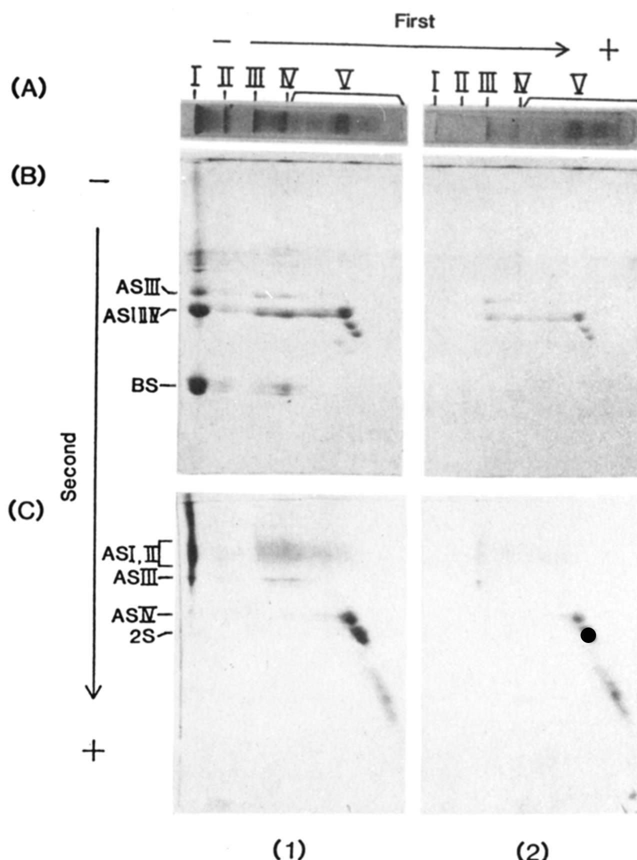

Figure 2. Two-dimensional electrophoresis of the progel (1) and the supernatant (2) of 11S globulin gels. The diluted progel and the supernatant were prepared as described under Materials and Methods. (A) First-dimensional disc PAGE. Migration is from left to right. (B) Second-dimensional $NaDodSO_4$–PAGE. Migration is from top to bottom. (C) Second-dimensional urea–PAGE. Migration is from top to bottom. AS and BS refer to acidic and basic subunits of 11S globulin, respectively.

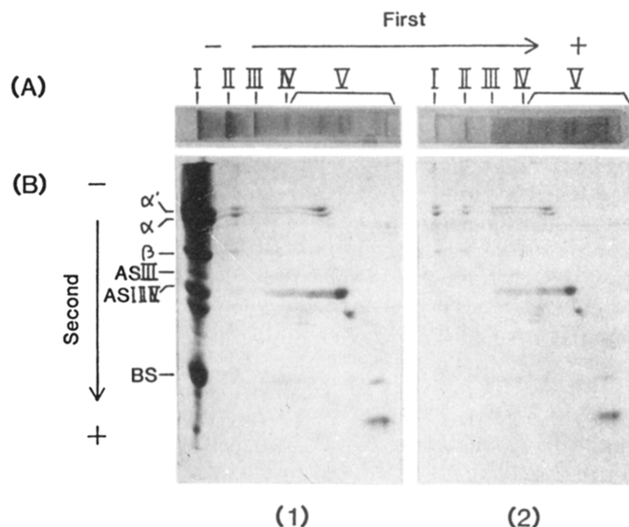

Figure 3. Two-dimensional electrophoresis of the diluted progel (1) and the supernatant (2) of 7S globulin gels. The diluted progel and the supernatant were prepared as described under Materials and Methods. (A) First-dimensional disc PAGE. Migration is from left to right. (B) Second-dimensional $NaDodSO_4$–PAGE. Migration is from top to bottom. α, α', and β refer to the three subunits of 7S globulin. Other symbols are the same as in Figure 2.

subunit composition of each of the protein bands in the first-dimensional gels, the second-dimensional $NaDodSO_4$–PAGE was carried out as shown in Figure 2B.

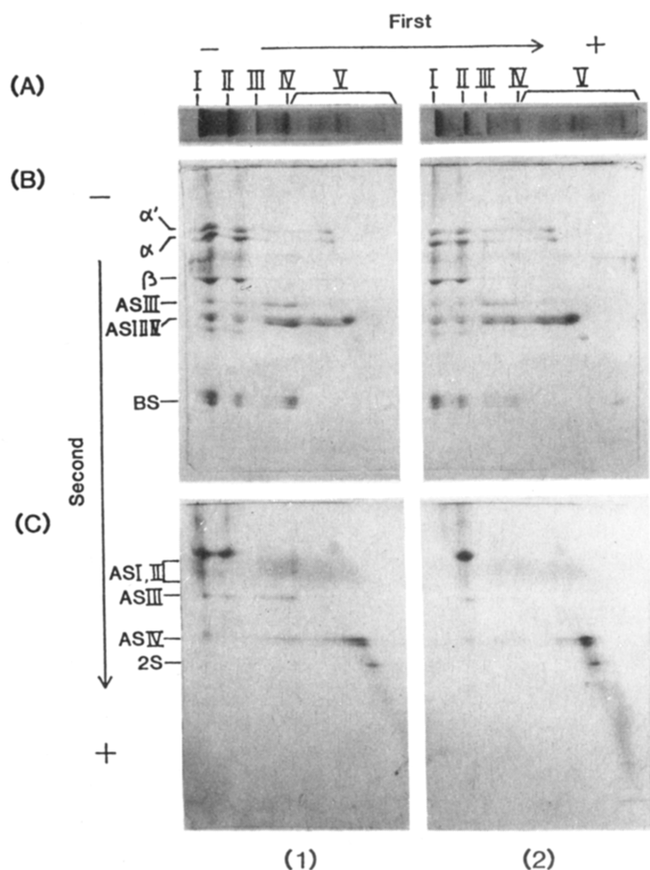

Figure 4. Two-dimensional electrophoresis of the diluted progel (1) and the gel supernatant (2) of SI gels. The diluted progel and the gel supernatant were prepared as described under Materials and Methods. (A) First-dimensional disc PAGE. Migration is from left to right. (B) Second-dimensional $NaDodSO_4$–PAGE. Migration is from top to bottom. (C) Second-dimensional urea–PAGE. Migration is from top to bottom. Symbols are the same as in Figure 3.

Identification of each subunit in the second-dimensional gel is based on previous work with known protein standards (Mori et al., 1979, 1982b; Thanh and Shibasaki, 1976b; Utsumi et al., 1984). In case of the diluted progel, the regions I, II, and IV gave bands corresponding to the acidic and basic subunits; region III predominantly contained the acidic subunits, and V contained proteins that are probably derived from the 2S fraction and the acidic subunits, except ASIII, the largest constituent acidic subunit (Mori et al., 1982b). The gel supernatant contained fewer high molecular weight complexes, and these were mostly composed of acidic subunits [Figure 2 B (2)].

In order to identify the specific acidic subunits present in each protein band in the first-dimensional gels, second-dimensional urea–PAGE was carried out as shown in Figure 2C. In case of diluted progel, the regions I, II, and III gave predominantly the bands corresponding to ASI, ASII, and ASIII and only a little of ASIV, which is noncovalently linked with a basic subunit to form an intermediary subunit (Mori et al., 1982b). Region IV contained all the acidic subunits, and V predominantly contained the bands corresponding to ASIV-contaminant proteins and a small amount of ASI and ASII but not ASIII. Similar results were obtained in the case of the gel supernatant.

These results indicate that ASIII contributes to the formation of the network structure of 11S globulin gels, whereas the ASIV contributes marginally. These results corroborate the suggestion that ASIII plays an important role in increasing the hardness of the 11S globulin gels

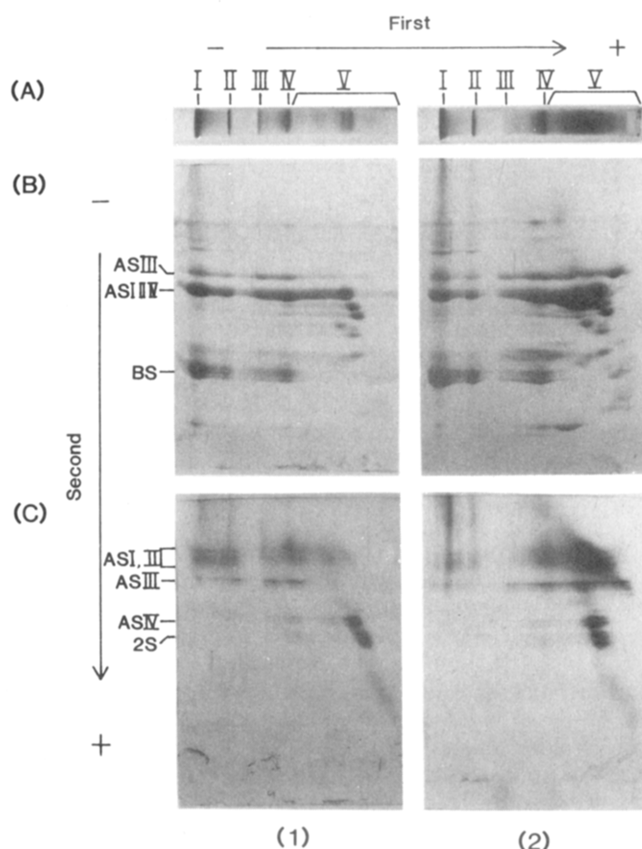

Figure 5. Two-dimensional electrophoresis of the proteins solubilized from the gel matrix of 11S globulin by the heating buffer (1) and 2ME (2). The gel was solubilized as described under Materials and Methods. (A) First-dimensional disc PAGE. Migration is from left to right. (B) Second-dimensional $NaDodSO_4$–PAGE. Migration is from top to bottom. (C) Second-dimensional urea–PAGE. Migration is from top to bottom. Symbols are the same as in Figure 2.

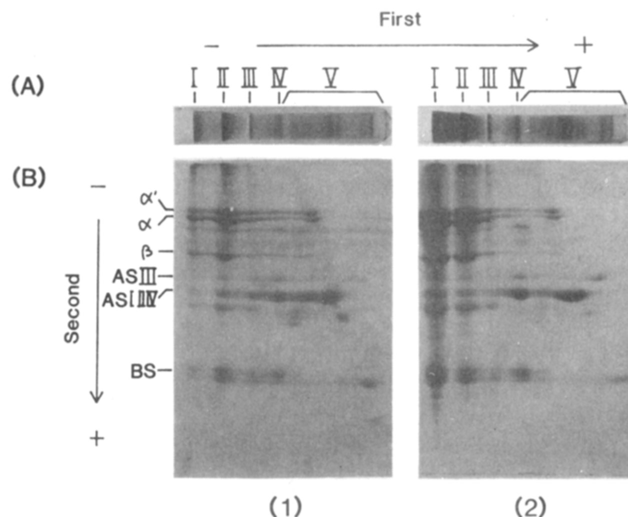

Figure 6. Two-dimensional electrophoresis of the proteins solubilized from the gel matrix of 7S globulin by the heating buffer (1) and 2ME (2). The gel core was solubilized as described under Materials and Methods. (A) First-dimensional disc PAGE. Migration is from left to right. (B) Second-dimensional $NaDodSO_4$–PAGE. Migration is from top to bottom. Symbols are the same as in Figure 3.

(Mori et al., 1982a,b; Nakamura et al., 1984a) and coincide with the observation that ASIV is liberated during the formation of the soluble aggregate (transient intermediate) during heating of 11S at 100 °C (Nakamura et al., 1984a).

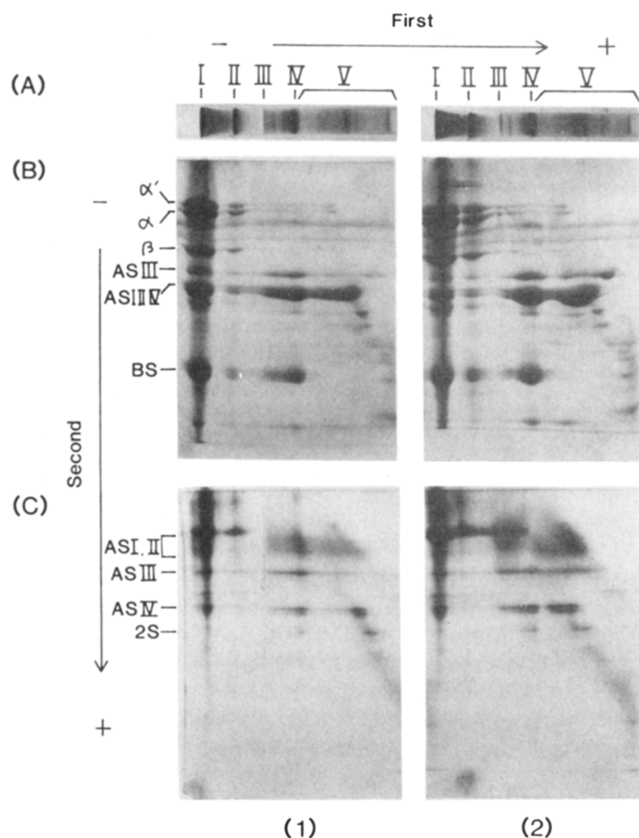

Figure 7. Two-dimensional electrophoresis of the proteins solubilized from the gel core of SI by the heating buffer (1) and 2ME (2). The gel core was solubilized as described under Materials and Methods. (A) First-dimensional disc PAGE. Migration is from left to right. (B) Second-dimensional NaDodSO$_4$–PAGE. Migration is from top to bottom. (C) Second-dimensional urea–PAGE. Migration is from top to bottom. Symbols are the same as in Figure 3.

The protein band corresponding to region IV is probably derived from undenatured 11S globulin that remained after heating, because the subunit composition and the mobility of this band are identical with those of native 11S globulin.

Two-dimensional electrophoretic patterns of the progel and supernatant of 7S globulin gel are shown in Figure 3. The diluted progel of 7S globulin gave considerable amounts of proteins corresponding to the regions I, II, and V and small amounts of the region III and IV in the first-dimensional gel (Figure 3A). In contrast, the supernatant of 7S globulin gel contained considerable amounts of the protein bands corresponding to the region V only and small amounts of bands I–IV. Thus, very little of the large complexes, with molecular weights of more than 1–2 million, separated from the 7S globulin gel, indicating that all the subunits were uniformly involved in formation of the gel matrix. The second-dimensional NaDodSO$_4$–PAGE (Figure 3B) indicates that for the diluted progel region I contained bands corresponding to α', α, and β subunits of 7S globulin and the acidic and basic subunits of the contaminant 11S globulin; region II had predominantly the three subunits of 7S globulin, and region V predominantly contained α' and α subunits of 7S globulin and the smaller acidic subunits of 11S globulin. In the case of the gel supernatant, regions I and II contained the three subunits of 7S globulin and region V was composed predominantly of α' and α subunits and the smaller acidic subunits (ASIV) of 11S globulin. The concentration of the free subunits of 7S globulin was lower than that of the acidic subunits of 11S globulin in both cases. These results and those shown in Figure 1 suggest that all of the subunits of 7S globulin contributed uniformly to the gel structure.

Table I. Relative Extent of Solubilization by Various Solvents of Proteins from Gel Matrices Formed following Heating of 11S and 7S Globulins and SI in Difference Reagents[a]

solvent	solubility, %[b]		
	11S	7S	SI
control (heating buffer)	16.7	12.4	51.4
0.5 M NaCl	11.1	8.5	38.9
0.5 M NaSCN	26.4	10.9	44.4
20% propylene glycol	6.5	4.5	7.4
0.2 M 2ME	34.7	92.1	91.3

[a] Aliquots (25 μL) of the protein solutions (12% w/v) were heated in the heating buffer at 80 °C for 30 min. After being cooled, at 4 °C for 20 h, the gels were centrifuged at 48 000 rpm for 1 h. The resultant gels were solubilized by various solvents at room temperature for 20 h and then centrifuged. The protein in the supernatant were determined by the methods of Lowry et al. [b] Percent of total protein in the original gel.

The first-dimensional gel of the diluted progel of SI showed considerable amounts of proteins corresponding to the regions I, II, IV, and V (Figure 4A). The supernatant of SI gels contained protein bands corresponding to the regions I, II, and V and small amounts of the regions III and IV. Thus, the supernatant of SI gels contained large complexes that could not penetrate the 3% or 4% stacking gels. Thus, the network structure of SI gel is more heterogeneous than those of 11S and 7S globulin gels. These results may correlate with the order of the solubility of the gels, SI > 11S > 7S (Utsumi and Kinsella, 1985). The second-dimensional NaDodSO$_4$–PAGE (Figure 4B) indicates that in the case of the diluted progel the regions I and II were composed predominantly of α', α, and β subunits of 7S globulin and the acidic and basic subunits of 11S globulin; band III contained the acidic subunits of 11S globulin; band IV had the acidic and basic subunits of 11S globulin, and band V was composed predominantly of smaller acidic subunits and small amounts of α' and α of 7S globulin and proteins derived from 2S fraction. Similar patterns were obtained in the case of the gel supernatant of SI gels. In the second-dimensional urea–PAGE, regions I, II, III, and IV of both the diluted progel and the supernatant contained the bands of ASI, ASII, ASIII, and ASIV, but region V contained mostly ASIV and proteins derived from 2S fraction but not ASIII. These results clearly indicate that in SI gels the ASIII plays an integral role in the formation of the network structure, whereas ASIV and the proteins derived from the 2S fraction does not appear to contribute to the SI gel network as in the case of the 11S globulin gel.

Solubilization of the Gel Core. In order to determine the nature of forces involved in the protein–protein interaction in the gels, the gel matrix only was solubilized following centrifugation using the heating buffer with or wiithout 0.5 M NaCl, 0.5 M NaSCN, 20% propylene glycol, or 0.2 M 2ME. The salts, NaCl and NaSCN, have a stabilizing and destabilizing effect on hydrophobic interactions in addition to charge neutralization effects, respectively (von Hippel and Schleich, 1969; Damodaran and Kinsella, 1981, 1983). Propylene glycol weakens hydrophobic interactions, but altering the dielectric constant enhances hydrogen bonding and electrostatic interactions (Tanford, 1962; Catsimpoolas and Meyer, 1971). The mercaptoethanol was used to reduce intermolecular disulfide bonds.

The extent of solubilization of protein from the gels in the different solvents is summarized in Table I. These

results are similar to those obtained by using gels extracted with these reagents without ultracentrifugation and/or crushing (Utsumi and Kinsella, 1985). Thus, the order of protein solubilization was SI > 11S > 7S in all reagents except the 2ME, which extracted the most protein from all gels. These results support the suggestion that hydrogen bonding and disulfide bonds are important in maintaining the network structure of soy protein gels (Utsumi and Kinsella, 1985); Babajimopoulas et al., 1983).

By analyzing the composition of the subunits extracted by different solvents, it should be possible to identify some of the forces involved in the association of the subunits in the gel network. The proteins extracted by buffer and 2ME from the 11S globulin gels are shown (Figure 5). Both extracts contained considerable amounts of protein in regions I, II, IV, and V, but in the case of 2ME most of the protein was in region V (Figure 5A). The second-dimensional $NaDodSO_4$–PAGE and urea–PAGE of the proteins in the heating buffer indicate that the components in regions I–IV are composed mostly of acidic and basic subunits, whereas the free proteins in region V are predominantly composed of ASIV and the contaminant 2S protein with small amounts of ASI and ASII (parts B and C of Figure 5). In the case of the 2ME extract the components in regions I and II are composed predominantly of the basic subunits and a small amount of the acidic subunits, and region V contained all the acidic subunits including ASIV (Figure 5B,C). The complexes in the region I were composed of the acidic and basic subunits in the ratio of about 1:3. Such complexes were insoluble at pH 7.6 (Yamagishi et al., 1980). Samples analyzed were centrifuged before electrophoresis to eliminate any insoluble precipitate; hence, the components in region I were not derived from the precipitate, and it is reasonable to suggest that some of the acidic subunits that were observed in region V interacted with the basic subunits before electrophoresis. During electrophoresis, the interaction was disrupted by ionic strength ($\mu = 0.12$) in the stacking gel as previously observed for the electrostatic interaction between the basic subunits (BS) and α' and α subunits of 7S globulin (Utsumi et al., 1984). The proteins solubilized by NaCl, NaSCN, and propylene glycol gave similar results to those obtained with the heating buffer (data not shown). These results indicate that the relative involvement of ASIII and ASIV in the 11S gel matrix is extensive and limited, respectively, and that (except for ASIV) disulfide linkage are involved in the association of acidic subunits as macromolecular complexes in the 11S gel matrix.

The two-dimensional electrophoretic patterns of the proteins solubilized from 7S globulin gel by the heating buffer and 2ME are shown (Figure 6). The proteins extracted by the heating buffer gave considerable amounts of the region II and small amounts of the region I and III–V proteins (Figure 6A). The proteins solubilized by 2ME gave large amounts of the region I and II and considerable amounts of region III–V proteins (Figure 6A).

The second-dimensional $NaDodSO_4$–PAGE (Figure 6B) of proteins in the heating buffer revealed that region II was predominantly composed of the α', α, and β subunits of 7S globulin and the free subunits in region V contained small amounts of α' and α and considerable amounts of the small acidic subunit of 11S globulin. The region I of the 2ME extract is predominantly composed of the three subunits of 7S globulin and the basic subunits of the contaminant 11S globulin. Region II contained the three subunits of 7S globulin and region V had small amounts of the α' and α subunits of 7S globulin and considerable amounts of the smaller acidic subunits (Figure 6B). The proteins solubilized by NaCl, NaSCN, and propylene glycol also gave similar electrophoretic patterns to those obtained for the heating buffer (data not shown).

The results indicate that the subunits of 7S globulin contribute rather uniformly to the gel structure and that large macromolecular constituent units composed of the subunits of 7S globulin, which do not penetrate into the 3% or 4% stacking gel, involve disulfide linkages. Hoshi et al. (1982) reported the involvement of disulfide linkage during the aggregation of 7S globulin. However, the content of cysteine and cystine of 7S globulin is much lower than that of 11S globulin (Derbyshire et al., 1976), and since the 7S globulin preparation used here contained about 30% 11S globulin, the formation of S–S linkages exclusively between 7S globulin subunits appears unlikely. The two-dimensional electrophoreses of the proteins solubilized by the heating buffer and 2ME gave considerable amounts of regions I, II, IV, and V. As with the 11S gel, a large amount of the region V protein was extracted with 2ME from SI gels. The second-dimensional $NaDodSO_4$–PAGE and urea–PAGE shows that in both extracts region I is composed of the three subunits of 7S globulin and all subunits of 11S globulin including ASIV; region II is predominantly composed of the three subunits of 7S globulin; region IV contains all the subunits of 11S globulin, and region V is composed of the smaller acidic subunits of 11S globulin (parts B and C Figure 7). The proteins solubilized by NaCl, NaSCN, and propylene glycol were similar to those extracted by the heating buffer (data not shown). In the case of 11S globulin gels, regions I and II did not contain much ASIV (Figure 5C) compared to the SI gels (parts B and C of Figure 7). These results strongly suggest that ASIV must interact with 7S globulin in forming the SI gel, that except for ASIV the acidic subunits are associated in the gel structure by disulfide linkages, that the three subunits of 7S globulin contribute uniformly to the formation of the gel network structure, and that the main molecular forces involved in the associations between the three subunits of 7S globulin are similar. The ratio of basic subunits to acidic subunits observed in the proteins solubilized by 2ME from SI gel was higher than that in 11S globulin gel (Figures 5B and 7B). This indicates that the basic subunits interact with 7S globulin in SI gel, though it is unclear whether the β subunit of 7S globulin exhibits affinity for the basic subunits as occurs in the case of low protein concentration (Utsumi et al., 1984).

The results obtained in this study indicate that whereas the contributions of 7S globulin subunits contribute rather uniformly to the gel structure there appears to be a preferential involvement of ASIII and a minimal involvement of ASIV in gel structures, particularly in the case of 11S gels. The contribution of ASIV to the gel structure via interactions with 7S subunits is greater in the SI gels. In order to precisely understand the nature of protein–protein interactions in the gels precisely, it is necessary to analyze the associations between isolated basic subunits of 11S globulin.

LITERATURE CITED

Aoki, H. *Nippon Shokuhin Kogyo Gakkaishi* **1970**, *17*, 129.

Babajimopoulas, M.; Damodaran, S.; Rizvi, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1983**, *31*, 1270.

Badley, R. A.; Atkinson, D.; Houser, H.; Oldani, G.; Stubbs, J. *Biochim. Biophys. Acta* **1975**, *412*, 2.

Catsimpoolas, N.; Campbell, T. G.; Meyer, E. W. *Arch. Biochem. Biophys.* **1969**, *131*, 577.

Catsimpoolas, N.; Funk, S. K.; Meyer, E. W. *Cereal Chem.* **1970**, *47*, 331.

Catsimpoolas, N.; Meyer, E. W. *Cereal Chem.* **1970**, *47*, 59.

Catsimpoolas, N.; Meyer, E. W. *Cereal Chem.* **1971**, *48*, 150.
Damodaran, S.; Kinsella, J. E. *J. Biol. Chem.* **1981**, *256*, 3394.
Damodaran, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1982**, *30*, 812.
Damodaran, S.; Kinsella, J. E. *ACS Symp. Ser.* **1983**, *No. 206*, 327.
Davis, B. J. *Ann. N.Y. Acad. Sci.* **1964**, *121*, 404.
Derbyshire, E.; Wright, D. J.; Boulter, D. *Phytochemistry* **1976**, *15*, 3.
Fukushima, D.; van Buren, J. P. *Cereal Chem.* **1970**, *47*, 571.
German, B.; Damodaran, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1982**, *30*, 807.
Hashizume, K.; Nakamura, N.; Watanabe, T. *Agric. Biol. Chem.* **1975**, *39*, 1339.
Hashizume, K.; Watanabe, T. *Agric. Biol. Chem.* **1979**, *43*, 683.
Hoshi, Y.; Yamauchi, F.; Shibasaki, K. *Agric. Biol. Chem.* **1982**, *46*, 1513.
Kinsella, J. E. *J. Am. Oil Chem. Soc.* **1979**, *56*, 242.
Kinsella, J. E.; Damodaran, S.; German, B. In "New Protein Foods. V. Oilseed Proteins"; Altschul, A.; Wilcke, H., Eds.; Academic Press: New York, 1985 (in press).
Laemmli, U. K. *Nature (London)* **1970**, *227*, 680.
Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
Mann, R. L.; Briggs, D. R. *Cereal Chem.* **1950**, *27*, 258.
Miyazaki, K.; Hagiwara, H., Yokota, M.; Kakuno, T.; Horio, T. *Protein, Nucleic Acid Enzyme* **1978**, *Suppl. 9*, 183.
Mori, T.; Nakamura, T.; Utsumi, S. *J. Food Sci.* **1982a**, *47*, 26.
Mori, T.; Nakamura, T.; Utsumi, S. *J. Agric. Food Chem.* **1982a**, *30*, 828.
Mori, T.; Utsumi, S.; Inaba, H. *Agric. Biol. Chem.* **1979**, *43*, 2317.
Nakamura, T.; Utsumi, S.; Kitamura, K.; Harada, K.; Mori, T. *J. Agric. Food Chem.* **1984b**, *32*, 647.
Nakamura, T.; Utsumi, S.; Mori, T. *J. Agric. Food Chem.* **1984b**, *32*, 349.
Saio, K.; Wakabayashi, A.; Watanabe, T. *Nippon Nogei Kagaku Kaishi* **1968**, *42*, 90.
Tanford, C. *J. Am. Chem. Soc.* **1962**, *84*, 4240.
Thanh, V. H.; Shibasaki, K. *J. Agric. Food Chem.* **1976a**, *24*, 1117..
Thanh, V. H.; Shibasaki, K. *Biochim. Biophys. Acta* **1976b**, *439*, 326.
Thanh, V. H.; Shibasaki, K. *J. Agric. Food Chem.* **1978**, *26*, 695.
Utsumi, S.; Damodaran, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1984**, *32*, 1406.
Utsumi, S.; Kinsella, J. E., unpublished results, 1985.
Utsumi, S.; Nakamura, T.; Mori, T. *Agric. Biol. Chem.* **1982**, *46*, 1923.
Utsumi, S.; Nakamura, T.; Mori, T. *J. Agric. Food Chem.* **1983**, *31*, 503.
von Hippel, P. H.; Schleich, T. In "Structure and Stability of Biological Macromolecules"; Timasheff, S. N.; Fasman, G. D., Eds.; Marcel Dekker: New York, 1969; pp 417.
Watanabe, T.; Nakayama, O. *Nippon Nogei Kagaku Kaishi* **1962**, *36*, 890.
Wolf, W. J.; Tamura, T. *Cereal Chem.* **1969**, *46*, 331.
Yamagishi, T.; Yamauchi, F.; Shibasaki, K. *Agric. Biol. Chem.* **1980**, *44*, 1575.

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Studies on Sorghum Proteins. 1. Solubilization of Proteins with Soaps

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It has been shown that up to 95% of sorghum flour proteins could be solubilized in distilled water in the presence of sodium salts of fatty acids. The most important parameters were the length of the hydrophobic chain of the soap, its concentration, and the extraction temperature. Soaps with longer hydrophobic chains (C18, C16) had lower dissolving ability than those with shorter chains (C10, C12, C14). Higher temperatures improved protein solubility, particularly at higher soap concentrations. The percentage of solubilized proteins increased with the flour protein content. Conversely, no significant difference could be observed in protein solubility between normal and high tannin sorghums. Results indicated that most of the sorghum proteins are tightly aggregated mainly through hydrophobic bonds. The main hindrance to sorghum protein solubilization would be the strong hydrophobic interactions between the proteins and the different flour components.

Most of the results reported on the solubilization of grain sorghum proteins were obtained either by the method of Osborne and Mendel (1914) or by that of Landry and Moureaux (1970). Both procedures were developed to solubilize maize proteins by using a sequence of solvents but modifications were usually introduced when the methods were applied to sorghum proteins.

According to Osborne and Mendel (1914), albumins and globulins are solubilized with a dilute salt solution, prolamins with an aqueous alcohol solution, and glutelins with a dilute alkali solution. About 50% of sorghum proteins remained insoluble by using this method (Naik, 1968; Skoch et al., 1970; Jones and Beckwith, 1970; Haikerwal and Mathieson, 1971). The procedure of Landry and Moureaux (1970) yields in addition to the albumins, globulins, and prolamins three glutelin fractions, all of them in the presence of a reducing agent: the alcohol, the alkali, and the detergent soluble reduced glutelins. About 5–10% of sorghum flour proteins were not extracted by this solvent system (Jambunathan and Mertz, 1973; Guiragossian et al., 1978; Chibber et al., 1978; Paulis and Wall, 1979; Neucere and Sumrell, 1979).

More recently it has been shown that wheat proteins, including glutenins, could be solubilized in distilled water in the presence of sodium salts of some fatty acids (Kobrehel, 1980). In the present work, the possibility of adapting this technique to solubilize sorghum flour proteins was investigated. The efficiency of different soaps was compared and optimal extraction conditions were determined. Sorghum varieties with different botanical and technological characteristics were analyzed. Results were compared to those obtained by others procedures.

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MATERIALS AND METHODS

Sorghum Samples. Most of our analyses were carried out with two french sorghum varieties, *Sorghum bicolor*

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