Role of Constituent Subunits in the Formation and Properties of Heat-Induced Gels of 11S Globulins from Legume Seeds

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Attempts were made to prepare various hybrid 11S globulins by combining broad bean legumin basic subunits (L-BS) with each soybean glycinin acidic subunit (G-ASI, G-ASII, and G-ASIII) and glycinin basic subunits (G-BS) with each legumin acidic subunit (L-ASI, L-ASII, and L-ASIII). In these combinations, half-molecules of the 11S component were formed in the case of G-ASII and L-BS, but neither half-molecule nor 11S component was formed in the case of G-ASIII and L-BS. Properties of heat-induced gels of the native legumin and glycinin, pseudolegumins (prepared by recombining acidic and basic subunits of legumin), and hybrid globulins were investigated. The results indicated that L-ASII and L-ASIII play an important role in increasing the hardness and the transparency, respectively, and that the superior gelling ability of glycinin as compared to that of legumin depends on the properties of both the acidic and basic subunits of glycinin.

Gelling ability of soybean proteins is one of the most significant functional properties with respect to their usage in food systems. It is an important problem to elucidate what kind of chemical and structural properties of soybean proteins are responsible for their gelling ability. This understanding would further the adoption in foods of soybean proteins and other grain legume proteins which are similar in structure to soybean proteins. However, at present sufficient information is lacking.

11S globulin, a legumin-like protein, is one of the most predominant storage proteins in various seeds (Danielsson, 1949). Of various 11S globulins, those from soybean (Badley et al., 1975; Kitamura et al., 1976; Mori et al., 1979; Staswick et al., 1981), broad bean (Wright and Boulter, 1974; Utsumi and Mori, 1980), and pea (Casey, 1979; Gatehouse et al., 1980) have common intermediary subunits (AB), disulfide-bonded acidic (A) and basic (B) subunits, and the 6(AB) structure.

In a previous paper (Utsumi et al., 1980), we obtained preliminary observations that artificially reconstituted 11S globulins which have different subunit compositions than the native 11S globulins, i.e., pseudo and hybrid 11S globulins, could be formed from combinations of the native acidic and basic subunits of soybean 11S globulins (glycinin) and broad bean 11S globulin (legumin). Utilizing various pseudoglycinins, we suggested that in the glycin molecule the acidic subunit with the highest molecular weight (G-ASIII) mainly contributes to the hardness of glycinin heat-induced gel (Mori et al., 1982b). It is important to elucidate whether such a phenomena would be observed with respect to other legume 11S globulins, which have fundamental subunit structures that are similar to that of glycinin. This elucidation may make it possible for us to use legume proteins logically and intentionally.

In the present work, in order to elucidate how the subunits of legumin contribute to the physical properties of the gel, we compared the physical properties of gels of legumin and pseudolegumins. Moreover, we investigated such points using hybrid globulins from the subunits of glycinin and legumin.

MATERIALS AND METHODS

Materials. Urea and 2-mercaptoethanol (2-ME), specially prepared for biochemical research, were obtained from Nakarai Chemicals. Sodium dodecyl sulfate ($NaDodSO_4$), specially prepared for electrophoresis, was purchased from Wako Pure Chemical Industries. Disposable micropipets were obtained from Drummond Scientific Company. Dry seeds of broad bean var. Sanuki-Nagasaya and soybean var. Tsuru-no-ko were purchased from Mizuno Seed Co.

Preparation of Native Subunits of Glycinin and Legumin. The acidic and basic subunits of glycinin and legumin were isolated by DEAE-Sephadex A-50 column chromatography in the presence of 6 M urea and 0.2 M 2-ME as described previously (Mori et al., 1979; Mori and Utsumi, 1979). The acidic subunits used in this investigation were AS_{1-3} (ASI, M_r 34800), AS_4 (ASII, M_r 34800), and AS_5 (ASIII, M_r 38000) of glycinin (Mori et al., 1979) and peak II (ASI, M_r 36000), peak III (ASII, M_r 36000), and peak IV (ASIII, M_r 49000 and 51000) of legumin (Mori and Utsumi, 1979). The basic subunits of glycinin and legumin used in this investigation were the flow-through fractions, which contained all the basic subunits (Mori et al., 1979; Mori and Utsumi, 1979).

Preparation of Pseudolegumins and Hybrid Globulins from Isolated Native Subunits. The isolated acidic subunits were mixed with the basic subunits at a weight ratio of approximately 1:2.5 and allowed to stand for 1 h at room temperature. The mixtures were dialyzed against 35 mM potassium phosphate buffer (pH 7.6) containing 0.4 M NaCl, 40% (v/v) glycerol, and 0.02% NaN_3 for 24 h and for another 24 h with a change of the buffer. After dialysis, the sample was centrifuged on a linear sucrose density gradient (10–30% w/v). After centrifugation, the gradient was divided into 0.4-mL fractions and the absorption was measured at 280 nm with an ISCO density gradient fractionator. The details of the procedure have been described in the previous paper (Utsumi et al., 1980).

Electrophoresis. $NaDodSO_4$–polyacrylamide gel electrophoresis was performed according to the method of Laemmli (1970) at room temperature with 10% polyacrylamide gels in the presence and absence of 2-ME as described previously (Mori and Utsumi, 1979). A total of 0.03 A_{280} unit of protein was subjected to the electrophoresis.

Method of Protein Gelation. Twenty-microliter aliquots of the protein solution in 35 mM potassium phosphate buffer (pH 7.6) containing 0.4 M NaCl (heating buffer) were placed in disposable micropipets (200 μL with 1.68-mm inside diameter), and sealed at one end with poly(vinylidene chloride) film. The micropipets containing

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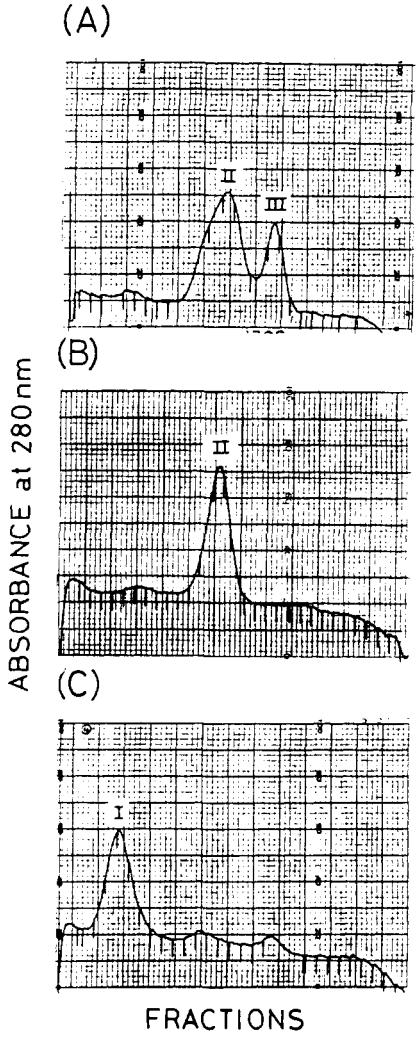

Figure 1. Sucrose density gradient centrifugation of the reconstituted products from the combinations of G-AS and L-BS. The isolated acidic and basic subunits were combined and reconstituted in combinations of L-BS and G-ASI (A), G-ASII (B), or G-ASIII (C). Sedimentation is from left to right. Absorbance range is 0–0.2.

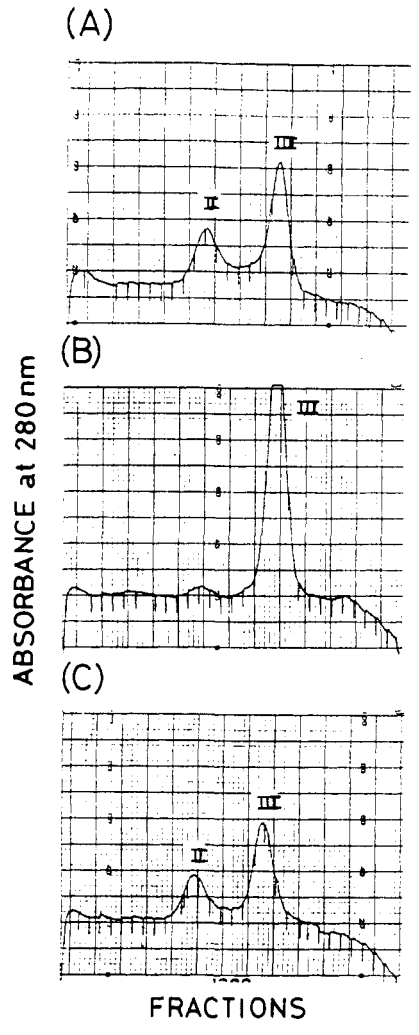

Figure 2. Sucrose density gradient centrifugation of the reconstituted products from the combinations of L-AS and G-BS. The isolated acidic and basic subunits were combined and reconstituted in combinations of G-BS and L-ASI (A), L-ASII (B), or L-ASIII (C). Other conditions are the same as in Figure 1.

the protein solution were then heated at 100 °C in a water bath for 20 min followed by rapid cooling to room temperature by immersing in tap water.

Determination of Turbidity and Hardness of Gel. The gel formed in the micropipets as described above was scanned as such on a Shimadzu dual-wavelength chromatoscanner, Model CS-910. Scanning was carried out at 600 and 750 nm for "sample" and "reference", respectively.

Gel hardness was measured with a texturometer (General Foods Corp., GXT-2) by using a Lucite plunger of 18-mm diameter and a cup of 24-mm diameter. The protein gels formed in the micropipets as described above were taken out, and they were cut into 5-mm lengths and put on the cup. The clearance between plunger and plate was adjusted to 0.1 mm. Hardness was measured from the height of the first chew profile, and all values were normalized to a 1-V input.

RESULTS AND DISCUSSION

Preparation of Hybrid Globulins. The reconstituted product from the combination of legumin basic subunit (L-BS) and each glycinin acidic subunit (G-ASI, G-ASII, and G-ASIII) was fractionated by sucrose density gradient centrifugation (Figure 1). In the absorbance patterns peaks I, II, and III correspond to unreacted acidic subunit and/or free intermediary subunit, 7S component [half-molecule of the 11S component, i.e., 3(AB) structure], and 11S component (M_r 300 000–350 000), respectively. As shown in Figure 1, the formation of the 11S components by the combinations of G-AS and L-BS was low. This was especially so in the cases of the latter two combinations (Figure 1B,C). From the combination of G-ASII and L-BS, only the 7S component was formed. On the other hand, in the case of G-ASIII and L-BS, the formation of both 7S and 11S components was less than 10%.

The reconstituted products from the combinations between G-BS and L-ASI, L-ASII, or L-ASIII were fractionated by sucrose density gradient centrifugation (Figure 2). In Figure 2, peaks II and III correspond to the 7S and 11S components, respectively. In contrast with the combinations of G-AS and L-BS, the formation of the 11S components by the combinations of L-AS and G-BS was high. As shown in parts A and C of Figure 2, the formation of 11S components from the combinations of L-ASI and G-BS and L-ASIII and G-BS were 60–70%. On the other hand, in the case of L-ASII and G-BS (Figure 2B), more than 90% of L-ASII was converted. Unreacted acidic subunits or free intermediary subunits formed from the reconstitution reaction were scarcely detected in any case.

These results indicate that the hybrid 11S globulins can be formed from the isolated subunits of glycinin and legumin except in the cases of the combinations of L-BS and G-ASII or G-ASIII.

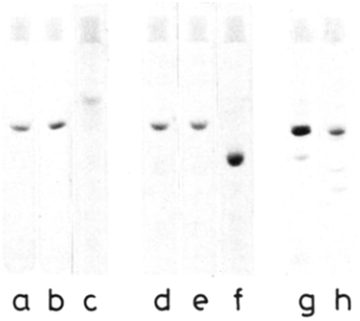

Figure 3. $NaDodSO_4$–polyacrylamide gel electrophoresis in the absence of 2-ME of the reconstituted products obtained by sucrose density gradient centrifugation. Migration is from top to bottom. Gels a–d, 11S components from L-ASI and G-BS, L-ASII and G-BS, L-ASIII and G-BS, and G-ASI and L-BS, respectively; gel e, 7S component from G-ASII and L-BS; gel f, peak I from G-ASIII and L-BS; gel g, native legumin; gel h, native glycinin.

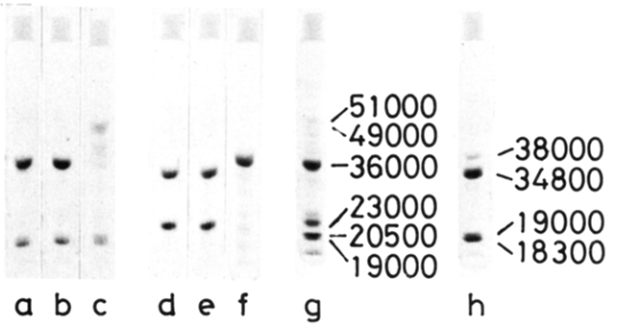

Figure 4. $NaDodSO_4$–polyacrylamide gel electrophoresis of the reconstituted products in the presence of 2-ME. Electrophoretic conditions and gel symbols are the same as in Figure 3 except that electrophoresis was performed in the presence of 2-ME.

The hybrid globulins obtained from the sucrose density gradient centrifugation were analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis. The results of $NaDodSO_4$–polyacrylamide gel electrophoresis in the absence and presence of 2-ME are shown in Figures 3 and 4, respectively. In the absence of 2-ME (Figure 3), all the hybrid globulins, except that formed from the combination of L-ASIII and G-BS, gave only one band each with a molecular weight of approximately 50 000 (gels a, b, d, and e), while the hybrid 11S globulin formed from the combination of L-ASIII and G-BS gave two bands with molecular weights of about 60 000 (gel c).

On the other hand, in the presence of 2-ME (Figure 4), all the hybrid globulins gave bands corresponding to the acidic and basic subunits used and no band corresponding to those observed in the absence of 2-ME. Peak I of Figure 1C contained only unreacted G-ASIII (Figures 3 and 4, gel f), which implies that this combination did not form a hybrid globulin or therefore a corresponding intermediate. The molar ratio of the acidic and basic subunits given by each hybrid globulin was approximately 1:1. These results suggest that the bands with molecular weights ranging from 50 000 to 60 000, observed in the absence of 2-ME, correspond to hybrid intermediary subunits composed of acidic and basic subunits which are linked by disulfide bridges in the ratio of 1:1. Moreover, the molecular weight of each hybrid 11S globulin was evaluated to be about 6 times that of the intermediary subunit from the sucrose density gradient centrifugation pattern. Therefore, the hybrid 11S globulins are similar to the native 11S globulins, glycinin and legumin, with respect to the 6(AB) structure (Badley et al., 1975; Kitamura et al., 1976; Mori et al., 1979; Staswick et al., 1981; Wright and Boulter, 1974; Utsumi and Mori, 1980).

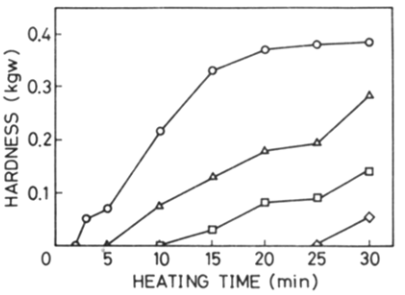

Figure 5. Effect of heating time and protein concentration on hardness of legumin gels. The symbols on the abscissa correspond to samples referred to as gel-like materials, the hardness of which could not be measured because they were not self-supporting. Concentration of protein: (○) 17.5%; (△) 15%; (□) 12.5%; (◇) 10%.

A comparison of the $NaDodSO_4$–polyacrylamide gel electrophoresis patterns of the hybrid globulins with those of the native glycinin and legumin in the presence of 2-ME indicates that all the glycinin acidic subunits except G-ASIII (which showed no reaction with the L-BS, Figure 1) preferentially selected the legumin basic subunit with a molecular weight of 23 000 in the formation of the hybrid intermediary subunits. L-ASI and L-ASII do not exhibit such specificity for one of the two glycinin basic subunits with molecular weights of 19 000 and 18 300. However, L-ASIII seems to exhibit the affinity for the glycinin basic subunit with a molecular weight of 19 000 to some extent, as is evident from the basic subunits higher presence in the hybrid 11S globulin formed from the combination of L-ASIII and G-BS, in comparison with the situation that exists in the native glycinin. Further studies on these points may shed light on evolutionary relationships between soybean and broad bean with respect to construction of the 6(AB) structure and genetic relationships among the subunits of each.

Gel Formation of Legumin, Pseudolegumins, and Hybrid Globulins. The native legumins were heated to give a gel, and the hardness of the gels formed was determined. As shown in Figure 5, the lowest concentration of gelation of native legumin within 30-min heating was 10%. The gel hardness increased with an increase in protein concentration and/or heating time. The higher the concentration of protein, the shorter the time required for gel formation. These tendencies coincide with those of glycinin, although at the same protein concentration it tends to gel and produce a harder gel than legumin (Utsumi et al., 1982).

It is noteworthy that legumin forms a transparent gel at high protein concentrations and does not form precipitates even at the low protein concentration where gel formation does not occur, although glycinin forms a turbid gel or precipitates at the low protein concentration (Mori et al., 1982a; Utsumi et al., 1982). This difference between glycinin and legumin may be due to the difference in ease of disaggregation of their basic subunits, since free basic subunits of both glycinin and legumin are insoluble in the heating buffer. The detailed explanation for the difference is not available at present.

The reconstituted 11S components (pseudolegumins) prepared by recombining acidic and basic subunits of legumin were heated for 20 min at various protein concentrations, and the hardness of their gels formed was compared with that of the native legumin as shown in Figure 6. The pseudolegumin from the combination of L-ASIII and L-BS did not form a self-supporting gel at a 17.0% concentration but formed a sol with high viscosity and formed a gel when heated for 60 min. The pseudolegumin

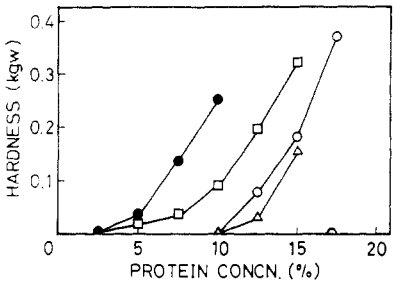

Figure 6. Hardness of pseudolegumin gel. The symbols on the abscissa are similar to those in Figure 5. (●) Glycinin; (○) legumin; (△) L-ASI and L-BS; (□) L-ASII and L-BS; (◇) L-ASIII and L-BS.

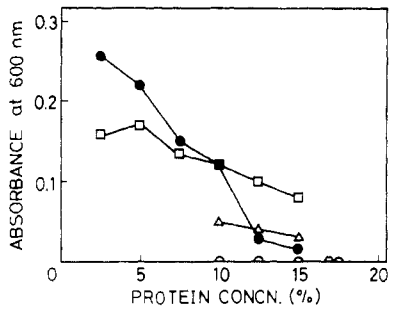

Figure 7. Turbidity of pseudolegumin gel. The symbols are the same as in Figure 6.

from the combination of L-ASI and L-BS exhibited gelling ability and hardness similar to those of the native legumin. The pseudolegumin from the combination of L-ASII and L-BS tended to form a gel at lower protein concentration and formed a significantly harder gel than did the native legumin. These results suggest that L-ASII of the native legumin plays an important role for determining the hardness of legumin gels. With respect to glycinin heat-induced gel, G-ASIII with the highest molecular weight mainly contributes to hardness of the gel (Mori et al., 1982b). Thus, there is no such commonness between glycinin and legumin that the largest one among the acidic subunits contributes to hardness of the gel. Therefore, in order to elucidate structural factors contributing to the hardness of gel, it may be important to investigate the primary and higher structures of L-ASII and G-ASIII.

As shown in Figure 7, the pseudolegumins from the combinations of L-ASI and L-BS and L-ASII and L-BS formed turbid gels, and the turbidity decreased with the increase of the protein concentration. The turbidity of the gel formed from the former pseudolegumin was significantly higher than that of the latter one. On the other hand, the pseudolegumin from L-ASIII and L-BS did not become turbid. These results suggest taht L-ASIII plays an important role in rendering the legumin gel transparent.

The hybrid globulins obtained from the sucrose density gradient centrifugation were heated for 20 min at various protein concentrations, and the hardness of the gels obtained was compared with that of the native glycinin and legumin as shown in Figure 8. The hybrid globulins formed form all combinations, except that from L-ASIII and G-BS, exhibited similar gelling ability and gel hardness, the values of which were located between glycinin and legumin. However, the hardness of the gel formed from the combination of G-ASI and L-BS was slightly harder than the others. The hybrid 11S globulin from the combination of L-ASIII and G-BS did not form either a self-supporting gel or a sol below 9.6% concentration. These results suggest that the superior gelling ability of glycinin as compared to legumin depends on the properties of both the acidic and basic subunits of glycinin.

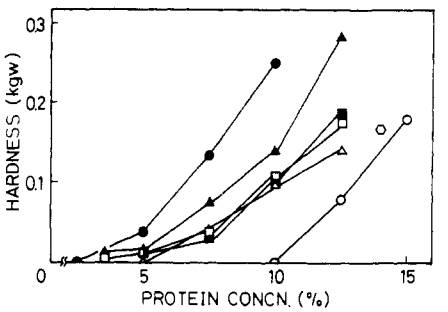

Figure 8. Hardness of hybrid globulin gel. (●) Glycinin; (○) legumin; (▲) G-ASI and L-BS; (■) G-ASII and L-BS; (△) L-ASI and G-BS; (□) L-ASII and G-BS; (◇) L-ASIII and G-BS.

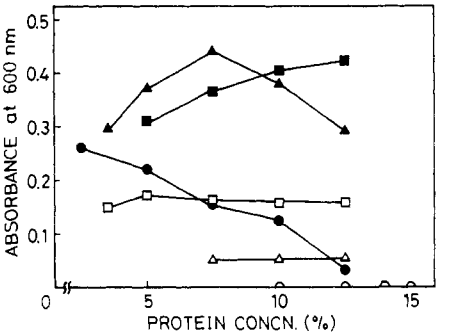

Figure 9. Turbidity of hybrid globulin gel. The symbols are the same as in Figure 8.

As shown in Figure 9, the turbidity of the gel formed by the G-AS and L-BS combination is higher than those of the native glycinin, and the turbidity of hybrid globulins did not exhibit strong dependency on the protein concentration contrary to the case of the native glycinin. Although this might suggest that the hybrid 11S globulin from the former combination is more labile than that from the latter one, the reason is unclear at present.

Since we used unfractionated basic subunits in this investigation, we could not deduce the role of each basic subunit. In order to elucidate this point, it is necessary to prepare pseudo and hybrid 11S globulins which each contain only one kind of basic subunit of glycinin and legumin. Studies are currently under way in these respects.

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