Intermolecular Forces Involved in the Gelation and Gel Stability of Sesame 13S Globulin

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Received January 20, 1988

The effect of various reagents on the formation and stability of heat-induced gels of sesame 13S globulins were investigated. Electrostatic interaction, the hydrophobic bond and the disulfide bond were important for forming the network structure of gels, and the hydrogen bond also had an influence on the formation of the gel. Hydrophobic bonds mainly contributed to the stability of the gel. Subunit analyses of the proteins solubilized from the gels showed the presence of a free acidic subunit (AS) and basic subunit (BS), a polymer of AS, a dimer of BS and the dimer of a fragment from AS or BS. From the results, sulfhydryl-disulfide exchange reactions during gelation are suggested.

Sesame protein has not been much utilized as food resources, although being rich in sulfur-containing amino acids such as methionine and cystine when compared to soybean protein. We have already studied the subunit structure and functional properties of sesame 13S globulin to develop the utilization of this protein, comparing it with soybean protein, which is a major food protein in plants. We have shown that the gel characteristics and emulsifying properties of sesame 13S globulin were different from those of soybean 11S globulin, although both the subunit structure and amino acid composition were similar to each other.[1～5] The water retention of the gel and the emulsifying stability of sesame globulin have been reported to be lower than those of soybean globulin,[3,4] which might be due to the higher hydrophobicity of this protein than that of soybean 11S globulin. Therefore, the mechanism of gelation and emulsification of sesame protein may be different from that of soybean 11S globulin. In this paper, we have physicochemically studied the mechanism of gelation of sesame globulin; namely, the intermolecular forces which contribute to the formation and stability of the gel, and the changes in subunit structure during the gelation process, comparing to those of soybean 11S globulin.

MATERIALS AND METHODS

Materials. Bovine serum albumin, ovalbumin, α-chymotrypsinogen *A* and β-lactoglobulin were purchased from Sigma (St. Louis). The other reagents were purchased from Wako Pure Chemicals, Osaka, and Nakarai Chemicals, Kyoto. Sesame seeds were purchased from Takii Ltd., Kyoto.

Preparation of sesame 13S globulin. Sesame 13S globulin was purified essentially according to the procedure of Hasegawa *et al.*[5] Sesame seeds were milled and defatted by several extractions with *n*-hexane. From the 40- to 60-mesh defatted meal, the 2S and 5S components were extracted twice with 2% NaCl, and then the 13S globulin was extracted from the resulting precipitate with 10% NaCl. After dialyzing against distilled water, the 13S globulin was lyophilized. The homogeneity of the protein was confirmed by ultracentrifugal analysis with a Hitachi UCA-1 analytical ultracentrifuge.

Preparation of gels. The gelation of 13S globulin was

achieved by modifying the method of Utsumi *et al.*[6] Aliquots (300 μl) of the protein solution (10% w/v) in a 0.5 M NaCl solution (pH 10.0) were transferred to glass tubes (55 mm × 5 mm i.d.) which were sealed at one end with polyvinylidene chloride film. The glass tubes containing the protein solution were then heated at 90°C for 10 min in a water bath, cooled at 0°C for 30 min, and then kept at room temperature for 30 min. After the formed gels had been removed, the hardness of the gels was measured by the method described later. In the experiments concerning the effects of reagents on the formation of gels, various reagents (NaCl, NaSCN, urea, propylene glycol (PG) and 2-mercaptoethanol (2-ME)) were added to the protein solution prior to its heat treatment.

Treatment of the gels. This treatment was carried out according to the method of Mori *et al.*[7] with some modifications. To examine the stability of the gels, the gels formed from native globulin in the glass tubes (55 mm × 5 mm i.d.) were removed and transferred into a test tube (90 mm × 12 mm i.d.), in which 2.4 ml of one of the following four kinds of solutions containing the denaturants was placed: 0.5 M NaCl at pH 10 (solution 1), 0.5 M NaCl at pH 10 containing 0.2 M 2-ME (solution 2), 0.5 M NaCl at pH 10 containing 8 M urea (solution 3), and 0.5 M NaCl at pH 10 containing 0.2 M 2-ME and 8 M urea (solution 4). The test tubes were shaken gently for the periods as indicated in each figure. The supernatant liquid (solubilized material) was removed with a pipette, and the extracts and gels were subjected to further experiments.

Determination of the hardness of the gels. The hardness of the gles (15 mm × 5 mm i.d.) was measured with a rheometer (Fudoh NRM-2010J-CW) and is expressed in rheometer units (g).

Protein determination. The protein concentration was determined by the method of Lowry *et al.*[8] When 2-ME was contained in the sample, the protein concentration was determined according to the method of Ross and Schatz.[9]

SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The extracts from gels formed were dialysed against distilled water and then lyophilized, and the resulting lyophilized materials were analyzed by SDS-PAGE according to the method of Laemmli.[10] SDS-PAGE was carried out using 12.5% polyacrylamide slab gel. When the protein was analyzed by two-dimensional gel electrophoresis,[11] the protein (20 ~ 25 μg) which had not been incubated with 2-ME was first electrophoresed on 12.5% polyacrylamide tube gel (0.2 cm i.d. and 17 cm length) containing 0.1% SDS. The gel was removed from the tube according to the method of Hu and Esen,[12] and then incubated with 0.2 M 2-ME at 38°C for 30 min. The gel was fixed with 1% agarose on the top of a slab gel (16 cm width, 17.5 cm height and 0.2 cm thickness), before the protein was electrophoresed under a constant voltage of 100 V for 17 hr.

RESULTS

Effects of various reagents on the sesame 13S globulin gel formation and its hardness

It is known that the best condition for the gelation of sesame 13S globulin is on the alkaline side (pH 10.0) in the presence of 0.5 M NaCl, and that the properties of the gel are more viscoelastic and harder than those of soybean 11S globulin gels.[2,3] To investigate the intermolecular forces in the protein that contributes to the gel formation, various reagents (NaCl, NaSCN, urea, PG and 2-ME) were added to the protein solution prior to heat treatment, before the hardness of the gel that had been formed was measured (Fig. 1). The effects of various reagents are summarized in Table I. The hardness of 13S globulin gel decreased with the increasing concentration of NaCl, and in the presence of more than 2.0 M of NaCl, the gel did not form (Fig. 1(a)). These results indicate that electrostatic interaction may contribute to the formation of 13S globulin gel, since NaCl has a charge neutralization effect. In the presence of NaSCN, the hardness of gel gradually decreased with the increasing concentration of NaSCN, and more than 0.4 M

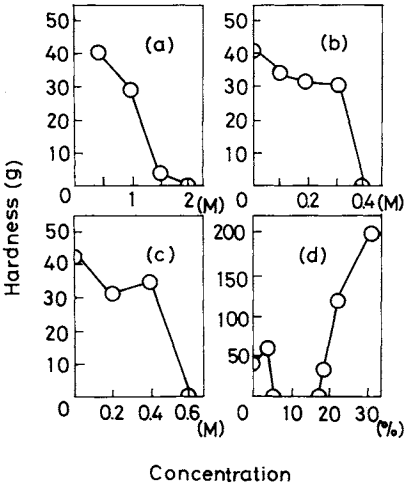


FIG. 1. Effects of Various Reagents on the Hardness of Sesame 13S Globulin Gels.
(a) NaCl; (b) NaSCN; (c) urea; (d) PG.

TABLE I. EFFECTS OF REAGENTS ON THE INTERMOLECULAR FORCES

	Noncovalent bonds			Covalent bond
	Electrostatic interaction	Hydrophobic bond	Hydrogen bond	Disulfide bond
NaCl[13]	Decrease	Increase		
NaSCN[13~15]	Decrease	Decrease		
Urea[16]		Decrease	Decrease	
PG[17]	Increase	Decrease	Increase	
2-ME				Decrease

of NaSCN completely inhibited the gelation (Fig. 1(b)). In the presence of urea, the hardness decreased at 0.2 M, and above 0.6 M, a gel did not form (Fig. 1(c)). In the presence of a concentration between 5 and 17.5% of PG, the globulin did not form a gel, whereas above 17.5%, the globulin formed the gel, and the hardness rapidly increased (Fig. 1(d)). NaSCN has destabilizing effects on both the electrostatic and hydrophobic interactions, while urea has destabilizing effects on the hydrophobic interaction and hydrogen bonds.[16] PG diminishes the hydrophobic interaction at low concentrations, but enhances hydrogen bonds and electrostatic interaction at high concentrations.[17] Therefore, these results (Fig. 1(b~d)) indicate that hydrophobic interaction and hydrogen bonds contributed to the gel formation. However, it is unclear whether hydrogen bonds alone were sufficient for gel formation. A 13S globulin gel was not formed in the presence of 2 mM 2-ME or 20 mM *N*-ethylmaleimide (data not shown), showing that the contribution of disulfide bonds to the gel formation was necessary.

Effect of various reagents on the stability of heat-induced gels

In order to examine the factors which contribute to the stability of heat-induced gels, the gels which had been formed under the best conditions for the gelation were shaken gently in 0.5 M NaCl solutions (pH 10) containing various reagents at 37°C.

(a) *Effects of various reagents on the hardness of gels.* Changes in the hardness of gels after incubating with the solution containing various reagents are shown in Fig. 2. In the case of solution 1 (0.5 M NaCl, pH 10), the gel hardness increased with increasing incubation time, and the hardness reached 1.5 times the initial value after 120 min. When the gel was treated with solution 2 (+2-ME), the hardness and the shape of the gel hardly changed. On the other hand, the hardness of gel was markedly decreased by treating with solution 3 (+urea), and the gel was broken into small particles within 15 min. Treatment with solution 4 (+urea and 2-ME) for 5 min solubilized the gel, and its hardness could not be

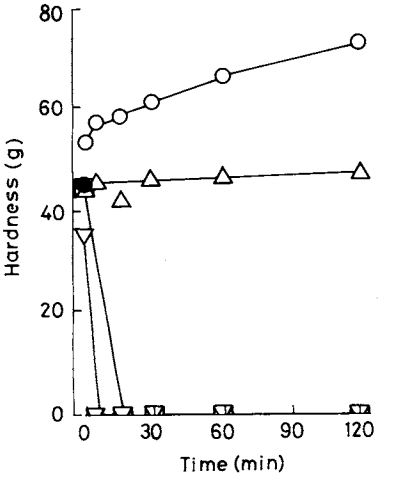


FIG. 2. Effects of Denaturants on the Hardness of Native Sesame 13S Globulin Gels.

Treatment of the gel: ●, no treatment; ○—○, solution 1 (0.5 M NaCl, pH 10); △—△, solution 2 (0.5 M NaCl, pH 10, 0.2 M 2-ME); □—□, solution 3 (0.5 M NaCl, pH 10, 8 M urea); ▽—▽, solution 4 (0.5 M NaCl, pH 10, 0.2 M 2-ME, 8 M urea).

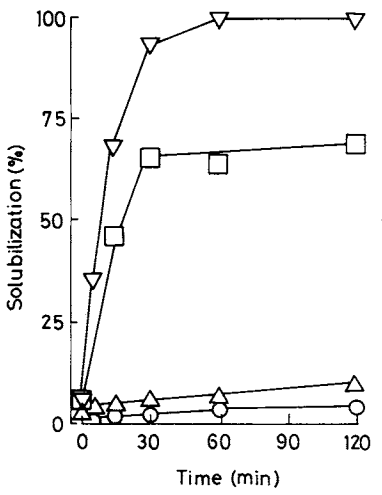


FIG. 3. Effects of Denaturants on the Solubilization of Native Sesame 13S Globulin Gels.

The protein amount in the materials solubilized by each treatment was determined as described in MATERIALS AND METHODS, and is expressed as the percentage of solubilization against the initial protein concentration of the gel. Symbols are the same as those in Fig. 2.

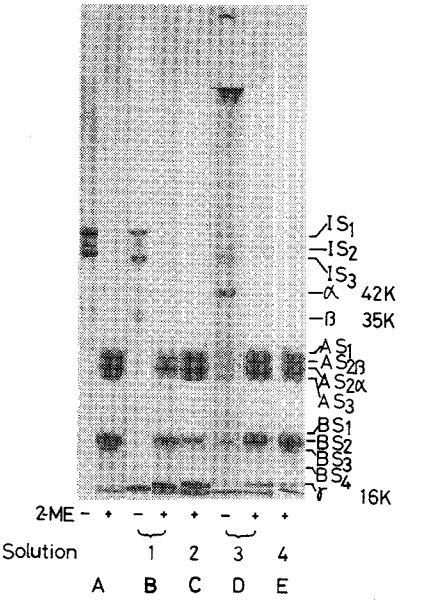


FIG. 4. SDS-PAGE of the Solubilized Materials.

A, control (native 13S); B, solution 1 (0.5 M NaCl, pH 10); C, solution 2 (0.5 M NaCl, pH 10, 0.2 M 2-ME); D, solution 3 (0.5 M NaCl, pH 10, 8 M urea); E, solution 4 (0.5 M NaCl, pH 10, 0.2 M 2-ME, 8 M urea).

measured.

(b) *Effects of various reagents on the solubilization of gels.* Figure 3 shows the degree of solubilization of the gels after incubating with the solution containing various reagents. In the case of treatment with solution 1 (0.5 M NaCl, pH 10), the solubility was low (around 4%), but the solubility gradually increased with the increase of incubation time. Treatment with solution 2 (+2-ME) also gave a low solubility (about 10% after 120 min). When the gel was treated with solution 3 (+urea), 73% of the gel was solubilized after 120 min. However, the solubility was saturated to 75% even after 240 min. By treating with solution 4 (+urea and 2-ME) for 60 min, 98% of the gel was solubilized.

Relationship between the stability and subunit structure of heat-induced gels

(a) *SDS-PAGE of extracts from the gels.* Figure 4 shows SDS-PAGE patterns, with and without 2-ME, of the extract from the gel by each solution. In the cases of solutions 2 and 4, only the patterns in the presence of 2-ME are shown, because these solutions contained 2-ME.

Native 13S globulin gave three bands (intermediary subunits IS_1, IS_2 and IS_3) in the absence of 2-ME, and gave eight bands (acidic subunits AS_1, $AS_{2\beta}$, $AS_{2\alpha}$ and AS_3 and basic subunits BS_1, BS_2, BS_3 and BS_4) in the presence of 2-ME (Fig. 4A). In the previous report,[1] we found three kinds of ASs (AS_1, $AS_{2(\alpha,\beta)}$ and AS_3) on one-dimensional SDS-PAGE. But in this study, $AS_{2\alpha}$ could be completely separated from $AS_{2\beta}$ even in the one-dimensional electrophoresis, because the concentration of polyacrylamide was changed to 12.5% from 10% (the previous condition) to get higher resolution. In the case of solution 1 (0.5 M NaCl, pH 10, Fig. 4B), a new band (β, M.W. 35,000) and a trace of an AS band were observed with three kinds of IS bands in the absence of 2-ME, and the SDS-PAGE pattern in the presence of 2-ME was very similar to that of native 13S except for the detection of a new band (γ, M.W. 16,000), which had a lower

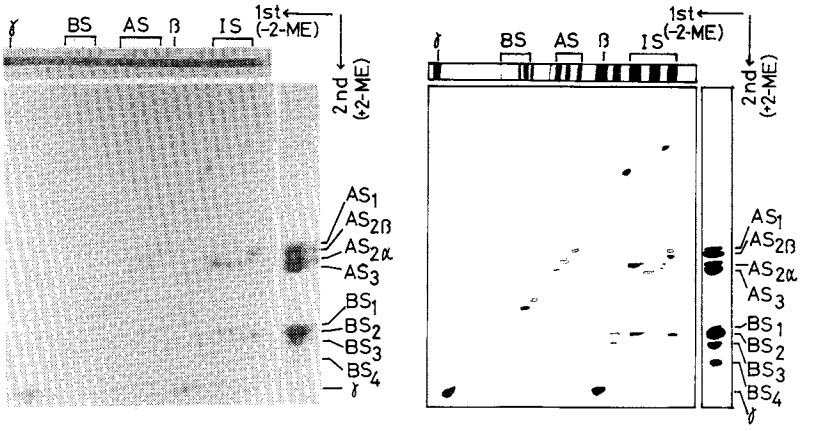


FIG. 5. Two-Dimensional SDS-PAGE of Solubilized Materials by Solution 1.

The solubilized materials from the gel by solution 1 (0.5 M NaCl, pH 10) were prepared as described in MATERIALS AND METHODS. Migration of the first-dimensional SDS-PAGE is from right to left, and the second-dimensional SDS-PAGE is from top to bottom.

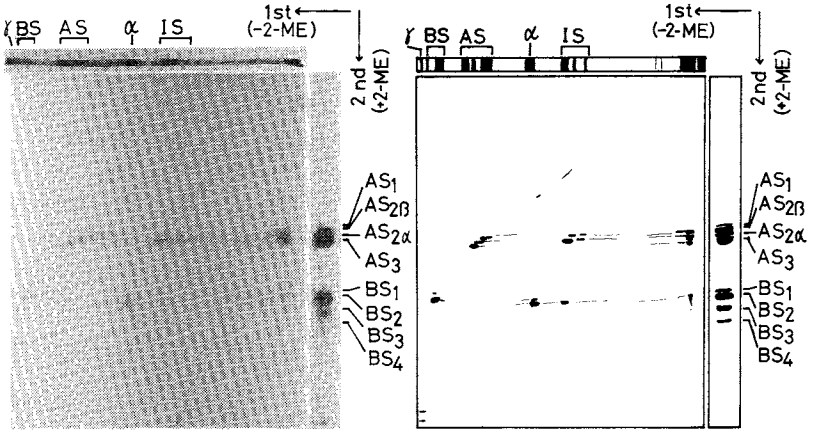


FIG. 6. Two-Dimensional SDS-PAGE of Solubilized Materials by Solution 3.

The solubilized materials from the gel by solution 3 (0.5 M NaCl, pH 10, 8 M urea) were prepared as described in MATERIALS AND METHODS. Migration of the first-dimensional SDS-PAGE is from right to left, and the second-dimensional SDS-PAGE is from top to bottom.

molecular weight than BS_4. The new bands (β and γ) were not detected in the pattern for native 13S globulin. In the extract by solution 2 (+2-ME, Fig. 4C), AS and BS were observed, whereas the ratio of AS to BS was higher than that of native 13S globulin. In the case of solution 3 (+urea, Fig. 4D), the solubilized materials consisted of aggregates which did not migrate into the stacking or separating gel, bands α, IS, AS and BS. The molecular weight of band α was 42,000, while that of the aggregates was presumed to be more than 660,000 by a gradient gel electrophoresis[18] (data not shown). All of these bands observed in the absence of 2-ME were dissociated into smaller components (AS and BS) in the presence of 2-ME. The solubilized materials of solution 4 (+urea and 2-ME, Fig. 4E) gave an electrophoretic pattern similar to that in solution 3 in the presence of 2-ME. Furthermore, in the presence of 2-ME, band γ was detected in all the extracts by each solution.

(b) *Two-dimensional SDS-PAGE.* In order to investigate the subunit compositions of the new bands (β and α) and of the aggregates, the extracts by solutions 1 (0.5 M NaCl, pH 10) and 3 (+urea) were subjected to two dimensional SDS-PAGE. The extracts by solutions 1 and 3 were first electrophoresed in the absence of 2-ME on tube gels, before being incubated with 0.2 M 2-ME, and the treated gels were further subjected to a second electrophoresis on the slab gels (Figs. 5 and 6). The electrophoretic pattern of the extract by solution 1 (0.5 M NaCl, pH 10) showed that band β, which can be seen in Fig. 4-B, consisted of band γ (Fig. 5). Furthermore, the subunit composition of each IS in Fig. 5 was similar to that of native 13S. Simultaneously, a few bands could not dissociate even in the presence of 2-ME. On the other hand, band α, which can be seen in Fig. 4D, was a dimer of BS, and each subunit composition of the intermediary subunits was markedly different from that of native 13S. Futhermore, the aggregates, which did not migrate into the separating gel in the absence of 2-ME, were mainly composed of the ASs (AS : BS = 90 : 10, estimated by a densitometer) (Fig. 6).

DISCUSSION

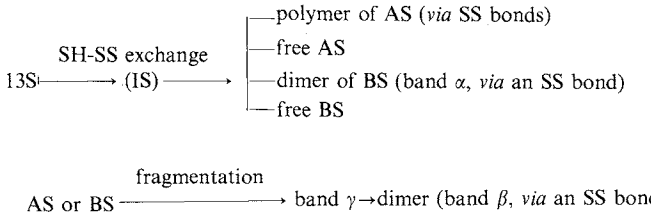
We found that the electrostatic interaction, the hydrophobic bond and the disulfide bond contributed to the formation of sesame 13S globulin gel, and that the hydrogen bond had some effect on the gel formation (Fig. 1). Utsumi and Kinsella have reported[19] that the contribution of hydrophobic bonds to the formation of soybean 11S globulin gel was not clear, although electrostatic interaction, hydrogen bonds and disulfide bonds contributed to the gel formation. This suggests that the difference in the properties of gels between sesame 13S and soybean 11S may be due to the difference in the contribution of hydrophobic bonds. Both gels were solubilized in the solution containing urea (Fig. 3 and ref. 7), showing the contribution of hydrophobic bonds to the stabilization of the gels. We have also previously reported that the chemical properties (subunit structure and amino acid composition) of 13S and 11S globulins were very similar,[1] but that surface hydrophobicity of 13S globulin was higher than that of 11S globulin.[4] The difference in the gel properties and the intermolecular forces contributing to the formation and stability between 13S and 11S globulin gels may be explained by the difference in surface hydrophobicity of both globulins. The 13S globulin in the presence of 2 mM 2-ME did not form a gel, indicating that disulfide bonds were necessary for gelation. On the other hand, when the gel were treated with the solution including 2-ME (solution 2), the hardness and solubility of the gel were hardly influenced by this treatment (Figs. 2 and 3). This appears to show an insignificant contribution from the disulfide bond to the stability of the gel. However, the treatment by solution 4 (+2-ME and urea) resulted in complete solubilization (Fig. 3), although the solubility of the gel with solution 3 (+urea) was 75% at the most, even after 240 min. These facts could be explained by disulfide bonds in the 13S globulin gel being located inside the globulin molecule, where a reducing agent has difficulty in making contact with the bonds, but treatment by urea causing the agent to make contact with the bonds. Therefore, disulfide bonds also contributed to the stability of the gels. Mori *et al.* have reported[7] that soybean 11S gel was solubilized by 30% of the protein in the presence of urea. The difference between the effect of the hydrophobic bond on the stability of each gel is consistent with the electron microscopic observation that sesame 13S gel is constructed with a hydrophobic aggregate of small spherical particles.[2]

When the 13S gel was incubated in an NaCl solution, the hardness of the gel was increased gradually with increasing incubation time (Fig. 3). It is not clear why this increase of hardness occurred.

Other than the components already described, trace amounts of some components, which were not identified, were detected at the position of the intermediary subunits on the

pattern (in the presence of 2-ME). These components may have been due to the subunit crosslinked by lysinoalanine, because the condition for gelation was alkaline (pH 10).[20]

The electrophoretic patterns (Figs. 4, 5 and 6) indicated that new components were formed from 13S globulin through sulfhydryl–disulfide exchange reactions during the gelling process, including peptide fragmentation. The newly formed components, namely a polymer of AS, dimer of BS, free AS, free BS and the dimer of a fragment from AS or BS, seem to have made up the matrix of the gel through non-covalent bonds, mainly hydrophobic bonds and partly electrostatic and hydrogen bonds. It is not clear yet, however, whether such an exchange reaction occurred in the aggregates formed or whether the dissociation of 13S into ISs occurred prior to the exchange reactions.



Nakamura *et al.* indicated[21] that during the gelling process of soybean 11S globulin, the globulin molecules associated by forming a string of beads, as they retained the globular structure of 11S globulins. The mechanisms for the coagulation of sesame[22] and oat globulins[23] and for the gelation of soybean protein[24,25] have been studied. Yamagishi *et al.*[24] reported that ASs of soybean 11S globulin were polymerized by disulfide bonds during the gelation process, and that the resulting polymer of AS mainly contributed to the gelation. In the present study, we found that the AS of sesame 13S globulin was also polymerized by disulfide bonds, although it is not clear to what extent the polymer contributed to the gelation.

REFERENCES

1) N. Yuno, T. Matoba, M. Hirose and K. Hasegawa, *Agric. Biol. Chem.*, **50**, 983 (1986).
2) S. Fujino, Y. Kamiguchi, Y. Yokoi and K. Hasegawa, *Nippon Nōgeikagaku Kaishi*, **55**, 975 (1981).
3) S. Fujino, S. Matsumoto, T. Matoba and K. Hasegawa, *Nippon Nōgeikagaku Kaishi*, **60**, 829 (1986).
4) N. Yuno, T. Matoba and K. Hasegawa, *Agric. Biol. Chem.*, **52**, 685 (1988).
5) K. Hasegawa, K. Maeda, Y. Fujino, T. Wakinaga and S. Fujino, *Nippon Nōgeikagaku Kaishi*, **52**, 341 (1978).
6) S. Utsumi, T. Nakamura and T. Mori, *Agric. Biol. Chem.*, **46**, 1923 (1982).
7) T. Mori, T. Nakamura and S. Utsumi, *J. Agric. Food Chem.*, **34**, 33 (1986).
8) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
9) E. Ross and G. Schatz, *Anal. Biochem.*, **54**, 304 (1973).
10) U. K. Laemmli, *Nature*, **227**, 680 (1970).
11) P. H. O'Farrell, *J. Biol. Chem.*, **250**, 4007 (1975).
12) B. Hu and A. Esen, *J. Agric. Food Chem.*, **30**, 21 (1982).
13) S. Damodaran and J. E. Kinsella, *J. Biol. Chem.*, **256**, 3394 (1981).
14) P. H. von Hippel and T. Schleich, "Structure and Stability of Biological Macromolecules," Marcel Dekker, New York, 1969, p. 417.
15) S. Damodaran and J. E. Kinsella, "Food Protein Deterioration Mechanisms and Functionality," American Chemical Symposium Series 206, ACS, New York, 1982, p. 327.
16) J. A. Gordon and W. P. Jencks, *Biochemistry*, **2**, 47 (1963).
17) C. Tanford, *J. Am. Chem. Soc.*, **84**, 4240 (1962).
18) J. Margolis, K. G. Kenrick, *Anal. Biochem.*, **25**, 347 (1968).
19) S. Utsumi and J. E. Kinsella, *J. Food Sci.*, **50**, 1278 (1985).
20) K. Hasegawa, N. Okamoto, H. Ozawa, S. Kitajima and Y. Takado, *Agric. Biol. Chem.*, **45**, 1645 (1981).
21) T. Nakamura, S. Utsumi and T. Mori, *J. Agric. Food*

Chem., **32**, 349 (1984).
22) T. S. Lakshmi and P. K. Nandi, *J. Agric. Food Chem.*, **27**, 818 (1979).
23) C.-Y. Ma and V. R. Harwalkar, *Cereal Chem.*, **64**, 212 (1987).
24) T. Yamagishi, N. Takahashi and F. Yamauchi, *Cereal Chem.*, **64**, 207 (1987).
25) A.-M. Hermansson, *J. Am. Oil Chem. Soc.*, **63**, 658 (1986).