Gelation and Gel Properties of Soybean Glycinin in a Transglutaminase-Catalyzed System

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Gelation of glycinins as catalyzed by transglutaminase was investigated. The surface lysine and glutamine residues of glycinin increased with heating. In the gelation of native and heat-treated glycinins catalyzed by transglutaminase, the formation of ε-(γ-glutamyl)lysyl cross-links was proportional to the amount of their surface lysine and glutamine residues. Both the acidic and basic subunits participated in the gelation of heat-treated glycinins, while most of the basic subunits did not in the case of native glycinin. Glycinin heated in the presence and absence of *N*-ethylmaleimide exhibited linear strands of aggregates and branched strands or small aggregates, respectively, and formed hard and elastic gels having a well cross-linked network or soft and viscous gels having a poor network structure, respectively. Modification of lysine or glutamine residues of glycinin affected viscoelastic properties of the corresponding gels. From these results, it was concluded that gel properties may be controlled not only by the amounts of cross-links formed and lysine and glutamine residues available for transglutaminase reaction but also by the nature of substrate protein.

INTRODUCTION

Transglutaminase catalyzes acyl-transfer reaction between protein-bound glutaminyl residues and primary amines (Folk and Finlayson, 1977; Clarke et al., 1959; Folk and Chung, 1973; Connellan et al., 1971). Transglutaminase was used to cross-link food proteins for texturization (Nio et al., 1985) and for the covalent attachment of essential amino acids to food proteins (Ikura et al., 1981), which produces new food materials with unique properties. Ikura et al. (1980a,b) reported that transglutaminase can also be used to polymerize bovine casein components and soybean globulins through the formation of intermolecular cross-links. However, most of these studies were done using native proteins. It is of interest to study the influence of structural changes of substrate proteins on cross-linking reactions catalyzed by transglutaminase. Heat treatment of glycinin solution at low protein concentration causes irreversible disruption of the quaternary structure of glycinin and subsequently dissociation into subunits (Wolf and Tamura, 1969; Catsimpoolas et al., 1969; Hashizume and Watanabe, 1979; Yamagishi et al., 1980). When a 5% glycinin solution was heated at 100 °C, soluble aggregates with weight-average molecular weights of 1.8×10^6, 4×10^6, and 8×10^6 were formed at 15 s, 30 s, and 1 min, respectively. In the soluble aggregates, branched and unbranched strands were present. When the glycinin solution was heated in the presence of *N*-ethylmaleimide (NEM), however, only linear strands could be found in the soluble aggregates (Nakamura et al., 1984).

On the basis of these findings, in this study, heat treatment was done to bring about structural changes of soybean glycinin, and we demonstrated that the obtained linear and branched strands of glycinin can serve as a good substrate for transglutaminase. We compared the rheological and mechanical properties of the gels from the native and heated glycinins to elucidate how those strands contribute to the physical properties of the gels formed by transglutaminase. Moreover, the rheological properties of the gels from the chemically modified glycinins were also investigated. Electon microscopy was also applied to evaluate the microstructure of the gels.

MATERIALS AND METHODS

Transglutaminase (Protein-Glutamine:Amine γ-Glutamyltransferase, EC 2.3.2.13). Transglutaminase (derived from *Streptoverticillium* sp. No. 8112) was supplied by Ajinomoto Co., Inc. (Japan).

Soybean Glycinin. Crude soybean glycinin fraction was prepared from acetone powder (Mori et al., 1981) according to the method of Thanh et al. (1976). Chromatographic purification of the crude glycinin fraction was performed on a column of DEAE-Toyopearl (Toyosoda Co., Ltd., Japan) equilibrated with 35 mM potassium phosphate buffer (pH 7.6) containing 0.15 M NaCl, 10 mM 2-mercaptoethanol, and 0.02% NaN_3. The elution was performed using a linear concentration gradient of 0.15–0.35 M NaCl. The purified glycinin fraction was dialyzed against heating buffer (35 mM potassium phosphate buffer containing 0.4 M NaCl, pH 7.6) before use.

Heat Treatment of Glycinin. The native glycinin solutions (4%) in the absence and presence of NEM (10 mM) and the modified glycinin solutions (see below) were heated in thin test tubes at 100 °C for various times. The solutions heated in the presence of NEM were subjected to transglutaminase reaction after dialyzing against heating buffer, to remove the unreacted NEM.

Modification of Lysine Residues of Glycinin. Glycinin was acetylated according to the methods using acetic anhydride (Ansari et al., 1975). Acetic anhydride was added to the glycinin solutions, and the reaction mixture was kept at pH 7.6 by the addition of 3.5 M NaOH. The temperature was held at 0–1 °C

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by immersion in an ice bath. The solution was dialyzed against half-saturated sodium acetate. The degree of acetylation was controlled by adjusting the amount of acetic anhydride added. Succinylation was performed according to the procedure of Hoagland (1968). Succinic anhydride was added to the glycinin solutions with stirring. During succinylation, the pH was maintained at 7.6 with 3.5 M NaOH and the temperature held at 0–1 °C by immersion in an ice bath. The degree of succinylation was controlled by adjusting the amount of succinic anhydride added. The extent of the modification of lysine residues was determined by the TNBS method (Fields, 1972). The acetylated and succinylated glycinin solutions were used for the experiments after being dialyzed against the heating buffer.

Modification of Glutamine Residues of Glycinin. Hydroxylamine was added to the glycinin solutions with stirring, where the molar ratio of hydroxylamine to surface glutamine residue was 0.1, assuming that the number of surface glutamines is 6 per mole of glycinin and the molecular weight of glycinin is 350 000. The pH was maintained at 7.6 with 3.5 M NaOH, and then transglutaminase (final weight ratio of transglutaminase to glycinin was 0.02 for each mixture) was added to the solutions. Incubation was performed at 37 °C for 5 h. The reaction mixture was dialyzed against the heating buffer at 5 °C before and after the heat treatment described above. These dialyzed samples refer to the unheated and heated glycinins, respectively.

Transglutaminase Reaction. Transglutaminase solution (4%) was added to glycinin solutions (4 or 6%) in the heating buffer (final weight ratio of transglutaminase to glycinin was 0.02 for each mixture), and the mixture was incubated at 37 °C in a small test tube. For the compression tests and electron microscopic observation, the mixture was poured into a 2-mm gap between two glass plates equipped with a silicon spacer (2-mm thickness) and incubated at 37 °C.

Determination of Lysine and Glutamine Residues. The number of lysine residues on the surface of glycinin molecules was determined according to the TNBS method (Fields, 1972) using 0.2% glycinin solutions. The number of glutamine residues was determined by transglutaminase-catalyzed labeling of glutamine residues of glycinin with dansylcadaverine (Cariello et al., 1990) using 0.04% glycinin solutions. Dansylcadaverine is known to compete effectively with the ϵ-amino groups of lysine in donor proteins (Lorand et al., 1968). Thus, incorporation of the dansylcadaverine inhibits cross-linking and also leads to the enzyme-directed and site-specific labeling of the participating glutaminyl residues in the acceptor protein (Lorand and Conrad, 1984; Pober et al., 1978). The reaction was carried out in the heating buffer containing excessive dansylcadaverine. The glutamine residues were labeled at 37 °C for 8 h and then dialyzed against the heating buffer containing 0.03 M hydroxylamine at 4 °C to eliminate excess dansylcadaverine not bound to glutamine residues and to inhibit further transglutaminase reaction. The dialyzed solutions were submitted to measurement of fluorescence intensities with a Hitachi F-3000 fluorescence spectrophotometer. The excitation wavelength was 340 nm, and emission was 520 nm.

The data were reproducible on duplicate runs in the determination of both lysine and glutamine residues.

Determination of ϵ-(γ-Glutamyl)lysyl Cross-Links. The glycinin solutions (4%) were reacted with transglutaminase for 2 h under the method described above and lyophilized. The contents of ϵ-(γ-glutamyl)lysine formed by transglutaminase reaction were determined according to the method of Griffin et al. (1982).

Determination of Surface Hydrophobicity. Surface hydrophobicity was determined according to the method of Kato and Nakai (1980) using 1-anilinonaphthalene-8-sulfonate (ANS) as a fluorescence probe. The concentration of ANS was 0.1 mM, and protein concentration varied from 0.005 to 0.04%. Fluorescence titrations were performed with a Hitachi F-3000 fluorescence spectrophotometer. The excitation wavelength was 375 nm, and emission was 475 nm. The initial slope (S_0) was calculated from the plot of fluorescence intensity vs protein concentration and was used as an index of the surface hydrophobicity of the protein.

Electrophoresis (SDS–PAGE). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed in a 12.5% slab gel according to the method of Laemmli (1970) at room temperature. The gels were stained with Coomassie brilliant blue R-250.

Rheological Measurement. The storage (G') and loss (G'') moduli of the reaction mixture of glycinin and transglutaminase in the heating buffer were measured by a Rheolograph Sol (Toyo Seiki Seisakusho, Tokyo, Japan) as a function of incubation time at 37 °C. Transglutaminase solution (4%) was added to glycinin solution (6%) and then mixed quickly. One part of transglutaminase was added to 50 parts of glycinin by weight. The reaction solutions (1.6 mL) were put into a cell immediately after mixing. Then, the blade was inserted into the cell and the surface of the sample was covered with silicon oil to avoid evaporation. The samples were subjected to 3-Hz sinusoidal shear oscillations with an amplitude (displacement $\pm 100\ \mu$m) which was well within the linear region and did not disturb the gelation. The detected stress was divided into the component in phase with the applied strain (storage modulus of rigidity, G') and that ahead of the strain by $\pi/2$ (loss modulus of rigidity, G''). Values of the storage and loss moduli were recorded as a function of incubation time. The storage and loss moduli represent the elastic and viscous elements, respectively, of a viscoelastic body. The reproducibility of the data was confirmed by repeated runs, two to four runs depending on the glycinin samples.

Measurement of Gel Hardness. A uniaxial compression test was carried out using a KES-FB 3 compression tester (Kato Tech Co. Ltd., Japan) equipped with a cylindrical plunger with a cross-section area of 0.25 cm^2. The tests were performed at 20 °C. The plunger descended at a rate of 1.2 mm/min. The measurement was done by compressing the sample until rupture. Hardness was evaluated from the force at rupture and expressed as gf/cm^2. The reproducibility of the data was confirmed by repeated runs, three to five runs depending on the glycinin samples.

Scanning Electron Microscopy (SEM). A three-dimensional impression of a gel structure can best be obtained by using scanning electron microscopy. In this work, the gel was sliced into small pieces by a sharp-edged knife. The gel slices were fixed in 2% glutaraldehyde at pH 7.6 and 4 °C for 2 h and then in 1% osmium tetraoxide at pH 7.6 and 4 °C for 1 h. After fixation, the gel slices were dehydrated by immersion in ethanol in a series of increasing concentrations and critical point dried with CO_2. The dried samples were mounted on an aluminum stub and coated with gold. The specimens were observed with a Hitachi S-450 scanning electron microscope.

RESULTS AND DISCUSSION

Changes in Nature of Glycinin by Heat Treatment. Since transglutaminase is an enzyme that catalyzes the formation of isopeptides between lysine and glutamine residues (Folk and Finlayson, 1977; Clarke et al., 1959; Folk and Chung, 1973), the numbers of lysine and glutamine residues on the surface of substrate proteins affect the rate of enzyme reaction. Therefore, we examined the numbers of lysine and glutamine residues on the surface of glycinin molecules. Native glycinin contained 6 glutamine and 24 lysine residues per mole on the surface of the molecules, as shown in Table I. Heating of glycinin solutions at 100 °C under 0.5 ionic strength in the absence of NEM caused the surface lysine and glutamine residues to increase with heating time. The contents of the surface lysine and glutamine residues reached about 2- and 5-fold, respectively, upon heating for 120 s. Similar increases were observed when the glycinin solution was heated in the presence of NEM. Compared with the results of glycinin heated in the absence of NEM, the contents of lysine and glutamine residues were slightly lower and higher, respectively, in the presence of NEM. Since the surface lysine and glutamine residues of glycinin increased with heating time, it can be predicted that the amount of intermolecular ϵ-(γ-glutamyl)lysyl cross-links formed by transglutaminase also would increase with the time of heat treatment. To examine this, the content of ϵ-(γ-glutamyl)-

Table I. Number of Surface Lysine and Glutamine Residues, Content of ε-(γ-Glutamyl)lysyl Cross-Links, and Surface Hydrophobicity of Native and Heated Glycinins

	lysine (no./mol)		glutamine (no./mol)		ε-(γ-glutamyl)lysine (μmol/g of dry wt)		hydrophobicity (S_0)	
glycinin	- NEM	+ NEM	- NEM	+ NEM	- NEM	+ NEM	- NEM	+ NEM
native (unheated)	24	24	6	6	2.69	2.69	949	949
heated for 30 s	29	29	8	11	4.09	3.95	1512	1619
heated for 60 s	44	37	28	30	6.86	5.19	2805	2851
heated for 120 s	47	37	29	35	7.30	6.16	3093	3233

lysyl cross-links within the gels formed by transglutaminase reaction was determined. The content of ε-(γ-glutamyl)lysyl cross-links increased with increasing time of heat treatment (Table I). This could be due to the increase of surface glutamine and lysine residues in the heated glycinin. In the glycinin heated for 120 s in the absence and presence of NEM, the content of ε-(γ-glutamly)lysyl cross-links increased by about 2.7- and 2.3-fold, respectively. These results indicate that glycinin heated with or without NEM exhibited higher reactivity in the polymerization catalyzed by transglutaminase, compared with the native glycinin. Since heat denaturation of a protein is thought to be accompanied by the unfolding of the molecule with exposure of hydrophobic groups, surface hydrophobicity was measured in the heated glycinin. With increases in heating time, the surface hydrophobicity increased (Table I). The hydrophobicity of glycinin heated in the presence of NEM is slightly higher than that of glycinin heated in the absence of NEM.

It is well-known that hydrophobic interaction plays an important role in stabilizing the network structure of various protein gels (Kinsella, 1982). These results, therefore, suggest that the heated glycinin, exhibiting higher hydrophobicity, may contribute to the formation of a rigid network structure in the glycinin gel produced by transglutaminase.

Electrophoresis of Reaction Products Formed by Transglutaminase Catalysis. The reaction products were analyzed by SDS–PAGE to confirm the formation of polymers through the intermolecular cross-links catalyzed by transglutaminase. Since the electrophoresis procedure included 2-mercaptoethanol, any polymers containing disulfide bonds were cleaved. All samples, i.e., native glycinin and glycinin heated at 100 °C in the presence and absence of NEM for various times, were diluted to 0.4% and subjected to transglutaminase reaction for 2 h, and then the reaction was stopped by adding 8 M urea–SDS solutions. Figure 1A shows the electrophoretic patterns of reaction products of all samples. Lane N shows the native glycinin with no transglutaminase reaction, exhibiting no polymer but only acidic and basic subunits. With increases in the heating time, following transglutaminase-catalyzed cross-linking, the amounts of acidic and basic subunits decreased and the polymer fraction (lanes a–d) increased. The decreases of acidic and basic subunits determined densitometrically are shown in Figure 1B. In this figure, the intensities of bands of acidic and basic subunits were plotted against the time of heat treatment. The values on the vertical axis refer to the band intensities of the native glycinin unreacted with transglutaminase as 100%. In the native glycinin, 70% of the acidic subunits disappeared, while most of the basic subunits remained. These results agreed with the previous reports demonstrating that the basic subunits are not involved in the polymerization reaction at all (Ikura et al., 1980a). Regardless of the presence or absence of NEM in the heat treatment, the acidic subunits disappeared gradually with heating time, and by 120 s of heat treatment only 5% of the acidic subunits remained. This means that the acidic subunits became more reactive by the heat treatment. It was also noted that the basic subunits of glycinin became susceptible to transglutaminase reaction by the heat treatment and were polymerized. These results together with the results shown in Table I indicate that the increase of lysine and glutamine residues at the surface of glycinin molecules by the heat treatment may be due to the exposure and unfolding of the basic subunits as well as acidic subunits. The basic subunits of glycinin heated in the presence of NEM were slightly more susceptible than those of glycinin heated in the absence of NEM. It has been demonstrated that heating causes partial dissociation of glycinin intermediary subunits into acidic and basic subunits in the absence of NEM, which results in turbid or precipitation of liberated basic subunits (Mori et al., 1982). In the presence of NEM, however, heat treatment causes no dissociation of basic subunits from acidic ones, and glycinin associates together, while retaining its substructure, i.e., the pairing of the acidic and basic subunits (Wolf and Tamura, 1969; Nakamura et al., 1984). It is likely that transglutaminase would be more reactive to soluble molecules or soluble aggregates than to precipitates. Therefore, the lower susceptibility of basic subunits to transglutaminase observed in glycinin heated without NEM (Figure 1B) may be attributed to the precipitation of liberated basic subunits.

Viscoelastic Properties of the Gels Formed by Transglutaminase. The changes in storage modulus (G') and loss modulus (G'') during transglutaminase reaction with 6% glycinin solutions (native, heated for 30 and 60 s without NEM) are shown in Figure 2. The G' of native glycinin increased rapidly with incubation time up to about 40 min and then leveled off after 60 min. This indicates that a viscoelastic gel matrix was formed rapidly by intermolecular cross-links catalyzed by transglutaminase. The heat treatment had an effect on the gelation of glycinin catalyzed by transglutaminase. With increases in the heating time, there were delays in reaction rate and decreases in maximum G', particularly in the glycinin heated for 60 s. However, the G'' of glycinin heated for 60 s increased rapidly with incubation time and showed the highest G'' values among the samples. Values of G' and G'' of native and heat-treated (30 and 60 s) glycinins after 2 h of incubation were 1.5×10^4, 1.3×10^4, and 9.8×10^3 and 1.3×10^2, 1.8×10^2, and 4.1×10^2, respectively. G' was reduced two-thirds; on the other hand, G'' was increased 3-fold by heating for 60 s. These results indicate that the soluble aggregates or strands formed by heating (without NEM) formed a less elastic but more viscous gel by transglutaminase-catalyzed cross-linking than unheated glycinin.

Figure 3 shows the results for transglutaminase-catalyzed cross-linking of native and heat-treated glycinins in the presence of NEM. G' and G'' of the native glycinin increased with incubation time gradually. Values of G' and G'' after 2 h of incubation were 9.7×10^3 and 0.9×10^2, respectively. These values were two-thirds those of native glycinin without NEM treatment. Such decreases in G' and G'' can be explained by conformational changes of glycinin caused by modification of sulfhydryl groups with NEM, which affects the susceptibility to trans-

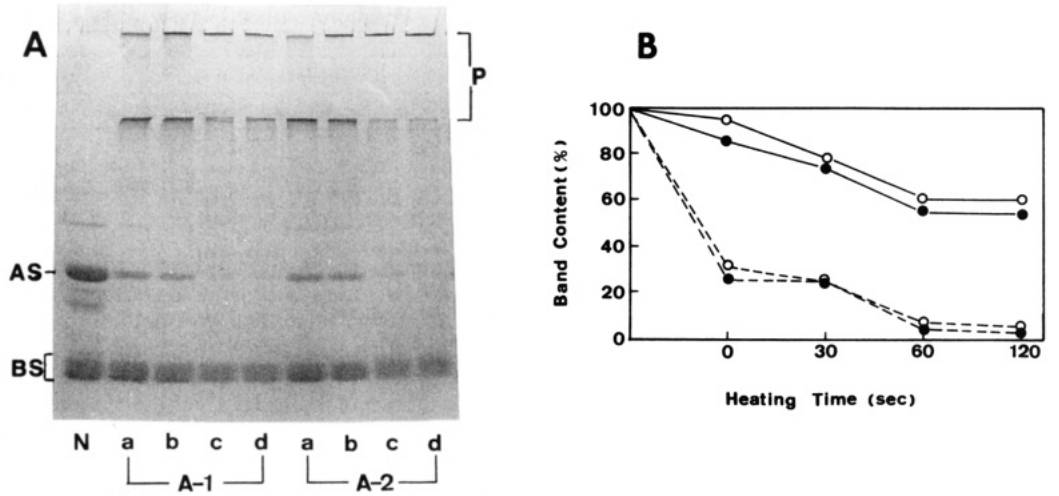

Figure 1. SDS–polyacrylamide gel electrophoresis pattern of the samples reacted with transglutaminase. Before transglutaminase reaction, samples were heated at 100 °C for 0, 30, 60, and 120 s in the absence (A-1, ○) and presence (A-2, ●) of NEM, respectively, and corresponding gels were labeled a, b, c, and d. N indicates native glycinin (no enzyme addition); P, polymer products; AS, acidic subunits; BS, basic subunits. (B) Changes in the content of acidic (- - -) and basic (—) subunits shown in A (1 and 2) were analyzed quantitatively with densitometer tracings. The content (percent) is based on the amount obtained from the lane loaded with native glycinin unreacted with transglutaminase.

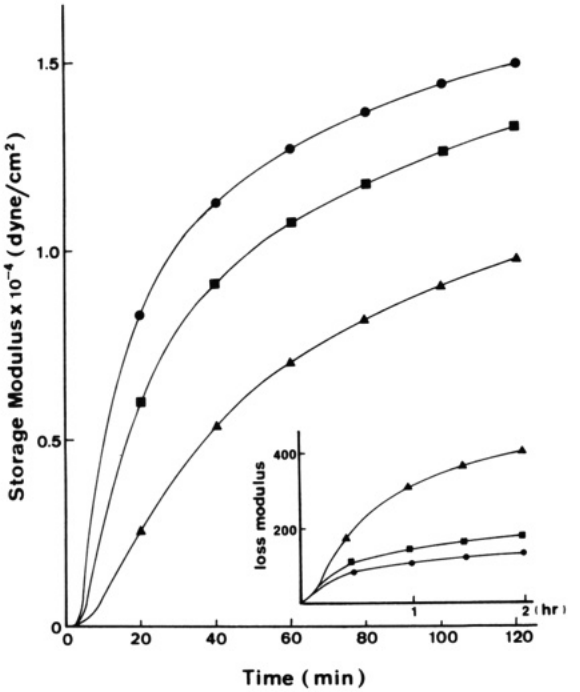

Figure 2. Changes in storage modulus (G') and loss modulus (G'') of native and heated glycinin during transglutaminase reaction. Glycinin solution (6%) was heated at 100 °C and then reacted with transglutaminase. (●) Native; (■) heated for 30 s; (▲) heated for 60 s.

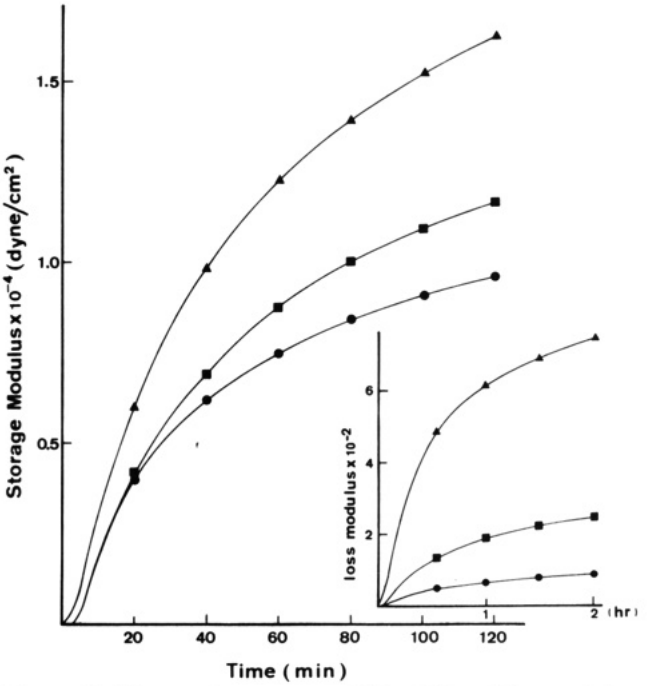

Figure 3. Changes in storage modulus (G') and loss modulus (G'') of glycinin with NEM during transglutaminase reaction. Glycinin solution (6%) was heated at 100 °C for the times shown in the presence of NEM and then reacted with transglutaminase. (●) Native; (■) heated for 30 s; (▲) heated for 60 s.

glutaminase reaction. However, heat treatment in the presence of NEM increased G' and G'' of glycinin gels formed by transglutaminase reaction. G' and G'' of glycinins heated for 30 and 60 s reached 1.2×10^4 and 1.6×10^4 and 2.5×10^2 and 7.4×10^2, respectively. It is worthwhile noting that G' as well as G'' increased by the heat treatment in the presence of NEM, although G' decreased in the absence of NEM. This indicates that the heat treatment in the presence of NEM led to the formation of more elastic and more viscous gels by the transglutaminase reaction. As pointed out in previous sections, heating with and without NEM increased ε-(γ-glutamyl)lysyl cross-links by more than 2-fold (Table I), and the acidic and basic subunits of glycinin became susceptible to transglutaminase reaction by heat treatment (Figure 1B). Therefore, increases in G'' with heating as observed in Figures 2 and 3 may be attributed to increases in ε-(γ-glutamyl)lysyl cross-links among the protein molecules. The decrease in G' by heating in the absence of NEM, however, cannot be explained by changes in the amounts of ε-(γ-glutamyl)lysyl cross-links. As discussed later (under Scanning Electron Microscopy), the G' of glycinin gels formed by transglutaminase may be closely related to the network structure of the gel.

Hardness of Gels Formed by Transglutaminase. Analysis and evaluation of food texture are often done by static and large deformation measurement of physical properties, such as compression and penetration tests (Boyd and Sherman, 1975; Kamel and DeMan, 1977). In this experiment, native and heat-treated glycinin solutions

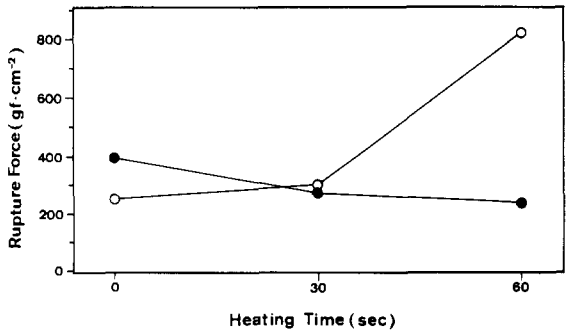

Figure 4. Effect of heat treatment of substrate on the hardness of the gels formed by transglutaminase reaction. Glycinin solution (6%) was heated at 100 °C for 30 and 60 s in the presence (○) and absence (●) of NEM and then reacted with transglutaminase for 5 h.

(6%) were reacted with transglutaminase for 5 h to make self-supporting gels, and then gel hardness was determined according to the method of Kang et al. (1991). The changes in rupture force vs the time of heat treatment in the presence and absence of NEM are shown in Figure 4. The rupture force decreased with increasing heating time of glycinin in the absence of NEM from 400 gf/cm^2 for the native glycinin in 250 gf/cm^2 with 60 s of heating. In the presence of NEM, on the other hand, rupture force increased with heating time. Particularly, by heating for 60 s, the transglutaminase-induced gel exhibited a very high rupture force, 800 gf/cm^2. These results indicate that the heat treatment in the presence or absence of NEM had contrasting effects on the rupture forces of transglutaminase-induced gels: in the absence of NEM, the heat treatment made the gel softer or more deformable, while the heating made it harder or more deformation-resistant in the presence of NEM. This contrasting effect of heat treatment with and without NEM was indicated also from the results of G' in Figures 2 and 3. The relationships between network structure and these rheological properties of gels are discussed in the following section.

Scanning Electron Microscopy. It has been accepted that there is a close relationship between the physical properties of a gel and its network structure. If a gel has a well developed three-dimensional network structure, the gel is more elastic and harder (Kitabatake et al., 1989). If a gel has a poorly developed structure, specifically an aggregation-type network, it is softer. Network structure of glycinin gels formed by transglutaminase reaction, therefore, were analyzed by SEM (Figure 5). In the case of native glycinin (Figure 5A), the microstructure of the gel was characterized as a well cross-linked network with some clumps of aggregated proteins. SEM revealed that the gel from glycinin heated for 120 s in the absence of NEM has a poor network structure, showing clumps of aggregated proteins but without the fibrillar and sheetlike structures (Figure 5B). Such poor network structure may be responsible for the decreases in rupture force (Figure 4) and G' (Figure 2) of the gel made from glycinin heated in the absence of NEM. The microstructure of the gel from NEM-modified but unheated glycinin was similar to Figure 5A (data not shown). The gel of glycinin heated for 120 s in the presence of NEM produced an extremely well cross-linked network, in which fibrillar and sheetlike protein structures are interconnected (Figure 5C). This microstructure agrees with the rheological properties of the gel, i.e., high values of rupture force (Figure 4) and G' and G'' moduli (Figure 3).

These results indicate that heat treatment with or without NEM affected differently the structure of glycinin gels formed by transglutaminase reaction. Heating without NEM led to random aggregation of proteins during the transglutaminase reaction, which caused the poor physical properties of the gel formed. On the other hand, heating with NEM caused the well-developed network structure and good physical properties of the gel formed. These differences may be attributed to the structure or shape of soluble aggregates formed by the heat treatment. As described under Introduction, in the absence of NEM, branched and unbranched strands were formed. However, in the presence of NEM, only linear strands were found in soluble aggregates. Branched strands (or small aggregates) produced by heating without NEM would associate randomly with themselves through intermolecular ϵ-(γ-glutamyl)lysyl cross-linking and hydrophobic interaction and then form aggregation-type gel networks by transglutaminase reaction. On the other hand, since cross-linking points among unbranched (linear) strands through ϵ-(γ-glutamyl)lysyl cross-linking and hydrophobic interaction would be more restricted, a well-developed network structure could be formed by transglutaminase-catalyzed cross-linking of linear strands without clumps of aggregated proteins. If this speculation is true, linear or unbranched strands should be better substrates of transglutaminase for producing hard or elastic gels. This type of strand can be obtained by heating glycinin of other cultivar soybeans or globulin of other seeds that contain fewer free sulfhydryl groups. We have already demonstrated that 11S globulin of broad bean (legumin) formed linear strands of soluble aggregates by heating because of its lower content of sulfhydryl groups (Zheng et al., 1991). In the future, we will examine the speculation described above by using such linear strands.

However, there could be another factor affecting the network structure of glycinin gel formed by transglutaminase. As described before, basic subunits are liberated from intermediary subunits by heating in the absence of NEM. It is expected that turbid or precipitated liberated basic subunits have an effect on the formation of network structure during transglutaminase reaction. Further studies are necessary to clarify this point.

Effects of Chemical Modifications of Glycinin on Transglutaminase Reaction. The effects of chemical modifications of glycinin on transglutaminase reaction were examined. Glycinin was modified with acetic anhydride, succinic anhydride, and hydroxylamine. Most of the lysine residues were modified by 10-fold molar excess of succinic anhydride (Table II, 100% succinylation). When unheated or heated (120s) 100% succinylated glycinin was incubated with transglutaminase, viscoelastic gel networks were not formed at all (Table II). This shows that 100% succinylated glycinin does not serve as a substrate for transglutaminase, since most of the reaction sites, i.e., the ϵ-amino groups of lysine residues, have been blocked by succinylation; 100% acetylated glycinin gave similar results. As is demonstrated in Table I, there were 24 lysine residues per molecule of native glycinin. When about 50% acetylated or succinylated glycinin was reacted with transglutaminase, the values of G' were 8.5×10^3 and 8.1×10^3, respectively, and those of G'' were 0.7×10^2 and 0.6×10^2, respectively. Compared with the native glycinin ($G' = 1.5 \times 10^4$, $G'' = 1.3 \times 10^2$), G' and G'' of 50% acetylated and succinylated glycinin decreased by about half. Such changes in G' and G'' were attributed to a roughly 50% decrease in lysine residues. When 50% acetylated and succinylated glycinins were heated at 100 °C, the number of exposed lysine residues increased, whereas G' decreased and G'' increased with heating time. These changes are

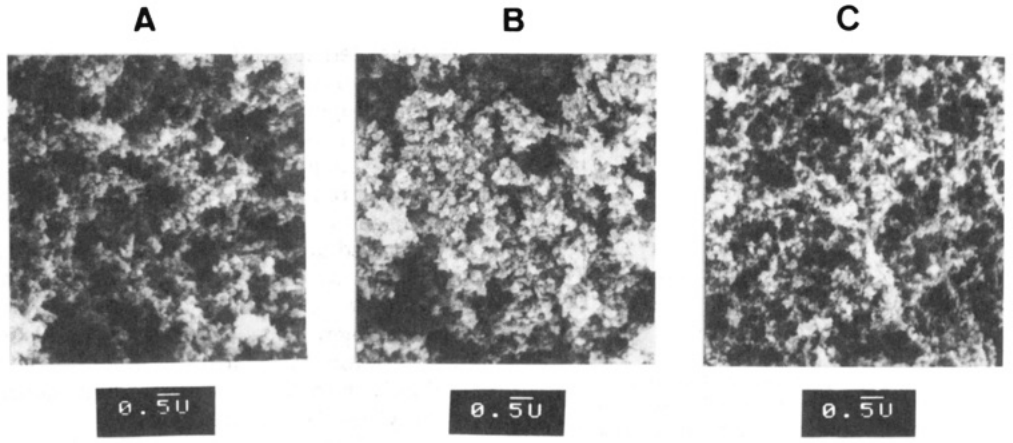

Figure 5. SEM photographs showing the effect of heat treatment of substrates on the gel network formed by transglutaminase reaction. (A) Native glycinin gel; (B) gel from glycinin heated for 120 s; (C) gel from glycinin heated for 120 s in the presence of NEM. Glycinin solution (4%) was heated at 100 °C for 120 s in the presence and absence of NEM and then reacted with transglutaminase at 37 °C for 2 h.

Table II. Storage (G') and Loss (G'') Moduli of the Glycinin Solutions Treated with Transglutaminase and the Number of Surface Lysine Residues of the Glycinins[a]

glycinin	G' (dyn/cm^2)	G'' (dyn/cm^2)	lysine (no./mol)
succinylation (100%)			
unheated	nd[b]	nd	3
heated for 120 s	nd	nd	3
succinylation (50%)			
unheated	8.1×10^3	0.6×10^2	11
heated for 30 s	6.6×10^3	1.2×10^2	16
heated for 60 s	6.5×10^3	2.5×10^2	27
heated for 120 s	6.0×10^3	2.5×10^2	27
acetylation (50%)			
unheated	8.5×10^3	0.7×10^2	13
heated for 30 s	5.9×10^3	1.1×10^2	20
heated for 60 s	5.9×10^3	2.6×10^2	32
hydroxylamine modification			
unheated	1.0×10^3	0.1×10^2	
heated for 30 s	5.9×10^3	1.0×10^2	
heated for 60 s	4.8×10^3	2.2×10^2	

[a] Transglutaminase reaction was performed for 2 h. [b] Not detectable.

similar to the case of unmodified glycinin (Figure 2). In the case of glycinin modified with hydroxylamine which blocks available glutamine residues, marked decreases in the storage modulus were observed, compared with native glycinin. However, with increased heating time, particularly for 60 s, G' increased because of exposure of interior glutamine residues. The results of chemical modification (Table II) suggest that the numbers of lysine and glutamine residues available to transglutaminase are the prime factor for determining physical properties of transglutaminase-induced gels when there is no heat treatment. However, once glycinin is heated before transglutaminase reaction, other factors, such as the shape of strands and liberated basic subunits, may also affect the rheological properties of the gels.

In this study, we investigated the effects of heat treatment and chemical modification of the substrate glycinin on the gelation and gel properties in transglutaminase-catalyzed system. The results obtained here demonstrated that not only the nature of the surface of glycinin molecules but also the shape and size of the glycinin aggregates is related to gel properties. Linear strands of aggregates obtained by heat treatment are good for making elastic and hard gels by transglutaminase reaction, while branched strands or small aggregates are also useful for soft and viscous gels. Thus, modification of original food protein providing strands as an alternate substrate may be useful for maximizing the texturization of food proteins by using transglutaminase reaction.

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