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Received for review November 26, 1984. Revised manuscript received June 17, 1985. Accepted August 30,1985. Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Effects of Temperature on the Different Stages in Thermal Gelling of Glycinin

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The thermal gelation of soybean 11S globulin (glycinin) proceeds at 100 °C, pH 7.6, and ionic strength 0.5 through the two stages, i.e., the association of glycinin molecules to form soluble aggregates in the form of strands (stage 1) followed by interaction of the strands to form the gel network (stage 2). When the effects of heating temperature on the progress of each stage in the gelling process were examined, it was revealed that 100 °C was required for the stage 1 but not for stage 2. When stage 1 had proceeded sufficiently at 100 °C, stage 2 proceeded by subsequent heating even at 80 °C. The two stages in thermal gelling of glycinin were revealed to be different and characterized with regard to the requirement of heating temperature. Thus, it is not necessary to maintain the temperature at 100 °C from the beginning to the end of the process to make gels of glycinin.

INTRODUCTION

The gelation phenomena of soybean protein and its main components have been studied by many workers (Hermansson, 1978; Saio and Watanabe, 1978; Kinsella, 1979; Shimada and Matsushita, 1980; Babajimopoulos et al., 1983). The 11S globulin (also referred to as glycinin), one of the major components of the soybean storage proteins, has the ability to form thermally induced gels (Mori et al., 1982a; Utsumi et al., 1982; Mori et al., 1982b; Nakamura et al., 1984). Since the gelling process proceeds continuously under continued heating, it might be considered to be a single-stage process. In previous work (Mori et al., 1982a; Nakamura et al., 1984), it has been shown that glycinin forms gels as a result of aggregation to form soluble aggregates (in the form of strands) with a molecular weight of 8×10^6 (stage 1) followed by interaction of the strands to form soluble macroaggregates and finally the gel network (stage 2). Further, when the heating was stopped at an appropriate time, e.g., at 1 min of heating of 5–10% glycinin solution, the gelling process was terminated by the formation of the soluble aggregates and the stage 2 did not proceed. Thus, the thermal gelling process of glycinin can be regarded as a two-stage process.

In the present study, we attempted to characterize these stages by investigating the effects of heating temperatures on each stage and found that the requisite of the temperature of heating differs depending on the stages. Also, the significance of the results obtained here is discussed relating to the use of soybean proteins in food systems.

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MATERIALS AND METHODS

Materials. Soybean seeds (*Glycine max*, var. Tsuruno-ko) were purchased from Mizuno Seed Co., Ltd. DEAE-Sephadex A-50 was purchased from Pharmacia Co., Ltd. 2-Mercaptoethanol, extra pure reagent, was obtained from Nakarai Chemicals (Japan). Other chemicals were guaranteed reagent grade.

Preparation of Glycinin. A crude glycinin fraction was prepared from soybeans according to the method of Thanh et al. (1975). Chromatographic fractionation of the crude glycinin fraction was performed on a column of DEAE-Sephadex A-50 as described previously (Mori et al., 1979), where the column was eluted with 35 mM potassium phosphate buffer (pH 7.6) containing 10 mM 2-mercaptoethanol, 0.02% NaN_3, and NaCl in a linear gradient of 0.25–0.5 M.

Heat Treatment of Glycinin. Thirty-three microliter aliquots of 7.5% glycinin solution in 35 mM potassium phosphate buffer (pH 7.6) containing 0.4 M NaCl (heating buffer) were heated in thin test tubes as described previously (Mori et al., 1982a).

Sucrose Density Gradient Centrifugation. The heat-treated glycinin solutions were centrifuged at 20 °C in 12 mL of 15–40% (w/v) linear sucrose gradient in the

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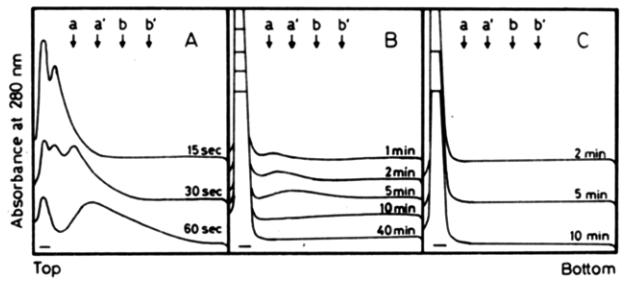

Figure 1. Sucrose density gradient centrifugation of glycinin heated under different temperatures: A, 100 °C; B, 90 °C; C, 80 °C. The arrows (a, a', b, and b') denote the sedimentation position of the markers (Mori et al., 1982a) with a molecular weight of 4.3×10^6, 8.6×10^6, 39.4×10^6, and 78.8×10^6, respectively.

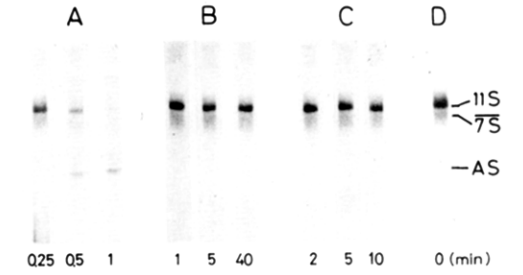

Figure 2. Polyacrylamide gel electrophoresis of the top fraction of the sucrose density gradient centrifugation. A, B, and C represent the top fractions of 100, 90, and 80 °C of Figure 1, respectively. 11S, $\overline{7S}$, and AS represent glycinin, a half-molecule of glycinin, and the acidic subunits of glycinin, respectively.

heating buffer at 152000*g* for 60 min in a Hitachi RPS 40T rotor. After centrifugation, the gradient was examined for absorbance at 280 nm with an ISCO density gradient fractionator. For sucrose gradient centrifugation, 2.5 mg of protein was used per experiment.

Electrophoresis. Polyacrylamide gel electrophoresis was performed according to the method of Davis (1964) with a slight modification as described previously (Utsumi and Mori, 1980). Fifty micrograms of each sample was used for electrophoresis.

Method of Gelation and Determination of Hardness of the Gel. Twenty-microliter aliquots of the 7.5% protein solution in the heating buffer were taken in micropipets (Drummond Scientific Co., 200 μL) and heated under the conditions as indicated, and the hardness of gels formed was then measured with a texturometer (General Foods Corp., GXT-2). The hardness of gels was expressed as a texturometer unit (kgw). The details of the procedure have been described in the previous papers (Mori et al., 1982b; Utsumi et al., 1982).

Differential Scanning Calorimetry (DSC). The measurements were made with a Daini Seikosha SSC-560 differential scanning calorimeter by using a heating rate of 0.5 °C/min. Samples of 33 μL of 7.5% glycinin in the heating buffer (native glycinin) was pressure sealed in a hermetic pan. The reference material was a sealed in a pan containing the same buffer as the protein solution. In the case of the heated glycinin, the glycinin solution was heated at 100 °C for 1 min prior to DSC measurement.

Protein Determination. Protein was determined by the method of Lowry et al. (1951).

RESULTS AND DISCUSSION

The 7.5% glycinin solution when heated at 100 °C for 2 min formed gels, but not at and below 90 °C for up to 60 min. The glycinin solutions heated for varying times were analyzed by sucrose density gradient centrifugation. As shown in Figure 1, part A, soluble aggregates with a molecular weight of 1.8×10^6, 4.0×10^6, and 8.0×10^6 were formed by heating at 100 °C for 15 s, 30 s, and 1 min, respectively. This result is consistent with that obtained at a protein concentration of 5% in the previous paper (Nakamura et al., 1984). By heating of the protein solution at 90 °C (Figure 1, part B), soluble aggregates were formed only slightly at 2 min and 5 min. However, as shown in Figure 1, parts B and C, no soluble aggregate formation was observed by heating at 90 °C for 10 min and 40 min and at 80 °C for up to 10 min, respectively. Also, the formation of soluble aggregates was not observed at 80 °C for 40 min (data not shown). When each peak at the top of sucrose gradients (the underlined part) shown in Figure 1 was analyzed by polyacrylamide gel electrophoresis, the behavior of glycinin during heating at each temperature became apparent (Figure 2). In the case of heating at 100 °C, glycinin decreased with time and then disappeared with the concomitant appearance of the acidic subunits (Figure 2, part A). This indicates that all glycinin molecules were converted to the soluble aggregates accompanied by the liberation of some acidic subunits, which has been suggested also in the previous papers (Mori et al., 1982a; Nakamura et al., 1984); the most acidic subunit was liberated much more than the other acidic subunits. The acidic subunits are linked to the basic subunit counterpart by disulfide bridges except for the most acidic subunit which is linked only through noncovalent interactions (Mori et al., 1981). On the other hand, in the case of heating at 90 and 80 °C, neither such marked changes in the disappearance of glycinin nor in the appearance of acidic subunits was observed (Figure 2, parts B and C). The bands denoted by $\overline{7S}$ in the gels may correspond to the half-molecules of glycinin which are derived from the dissociation of the glycinin molecules during the electrophoresis as shown by Kitamura et al. (1974); the possibility of occurrence of $\overline{7S}$ at the heating procedure was eliminated by sucrose density gradient centrifugation analysis (data not shown). The results of the centrifugation and electrophoresis analyses indicate that glycinin molecules remain unchanged at 90 °C and below and therefore no gelation occurs.

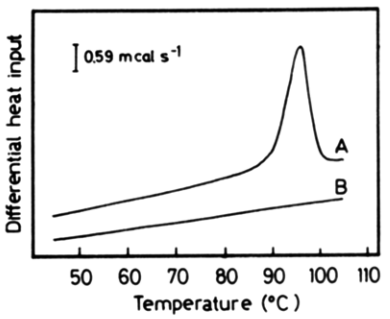

Figure 3. DSC thermograms of glycinin. A, native glycinin; B, preheated glycinin.

These temperature-dependent behaviors of glycinin may be associated with its thermal denaturation temperature. As shown in Figure 3, the thermogram of the native glycinin gave only one peak, which appeared between temperatures of 89 and 99 °C and exhibited a maximum at 94 °C (Figure 3, part A). When glycinin previously heated at 100 °C for 1 min was examined, the peak was no longer observed in the calorimetric scan (Figure 3, part B). The thermal denaturation temperature of native glycinin obtained here is slightly higher than that reported by Hermansson (1978), Koshiyama et al. (1980), and Bikbov et al. (1981). This may be due to that the protein concentration of the glycinin solution used here is 7.5% and higher than that of the others. The conformational change

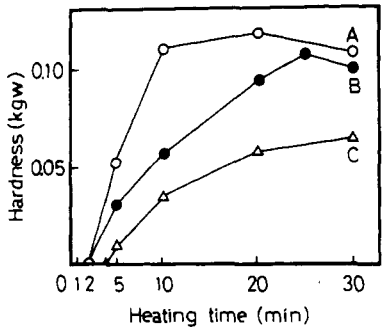

Figure 4. Effect of heating time and temperature on gel hardness. A, heated at 100 °C; B and C, heated at 100 °C for 1 min followed by subsequent heating at 90 and 80 °C, respectively.

of glycinin molecule may occur by heating at and above the denaturation temperature. Below the denaturation temperatures, neither the formation of soluble aggregates (Figure 1, parts B and C) nor the liberation of acidic subunits (Figure 2, parts B and C) occurred. These results demonstrate that the conformational change and/or denaturation of glycinin molecule caused at and above 94 °C is required for the formation of soluble aggregates and the liberation of acidic subunits. With certain conformational changes of glycinin, a steric strain and alterations in the environment of sites for noncovalent interactions and disulfide exchange on the glycinin molecules may occur, thereby facilitating the liberation of some subunits and the association reaction of glycinin molecules to form the soluble aggregates. Thus, it was revealed that a heating temperature of 100 °C is required for stage 1 of the gelling process of glycinin, however, this may not be necessarily true for stage 2 of the gelling process. In order to make this clear, effects of changing the heating temperature in stage 2 of gel formation following completion of stage 1 were examined. As shown in Figure 4, gels were formed by heating at below 100 °C down to 80 °C as expected. The gel hardness in the case of 90 °C heating increased with time of heating and finally approached that of 100 °C. In the case of 80 °C heating, similarly the gel hardness increased, but it did not reach the level of higher heating temperature. The results clearly demonstrate that stage 2, i.e., the network formation due to aggregation of the soluble aggregates, can proceed below 100 °C which is the requisite temperature for stage 1.

The process of thermal gelation of glycinin has been shown to consist of two characteristic stages (stages 1 and 2) with regard to the extent of polymerization as described above. Additionally, it was shown that each stage is characterized by a specific temperature of heating for proceeding of the stage. The lower temperature of heating in stage 2 suggests that the interaction of strands to form networks proceeds without the remarkable conformational changes of glycinin molecules observed in stage 1, and that the detailed mechanisms involved in the polymerization reaction are different between stages 1 and 2. No further interpretation of the difference of the required heating temperature between the two stages for the mechanisms involved in each stage can be offered, since details of the polymerization reaction during the course of gel formation are not well understood at present.

Apart from the considerations of gelation mechanisms, the results obtained here have some significance in regard to practical aspects of soybean protein usage provided glycinin is representative of a significant portion of soybean protein isolates concerning their functional properties. Firstly, a heating temperature of 100 °C was necessary only for an initial short period (stage 1) of the gelling process and thereafter (stage 2) one could lower the temperature to 80 °C. It may therefore be worthwhile from the standpoint of saving energy to follow up whether such two-step heating is applicable to whole soybean protein materials such as commercial soy protein isolates. Secondly, when the temperature of heating was lower, the rate of change in the gel hardness was more gradual than that at 100 °C. This may facilitate the timing of termination of the gelling process and suggests the ability to control gel hardness and probably texture, too, by choosing adequate heating conditions, i.e., combinations of the temperature and times of heating. The gelling process may possibly be terminated at any period in the course of the process besides the period shown here (1 min at 100 °C) by stopping the heating, since the gelation process of glycinin is a series of polymerization reactions in which mainly hydrophobic interactions and disulfide exchange reactions are likely to be involved (Mori et al., 1982a; Nakamura et al., 1984). Thirdly, when the termination is performed, particularly in the course of the initial period of the process where gel has not yet been formed, various kinds of soluble aggregates with different degrees of polymerization may become available as a material for food production. Such soluble aggregates probably exhibit characteristic physical and functional properties depending on the degree of polymerization. When these soluble aggregates with different properties are used as ingredients, one should take into consideration their thermal histories for setting up the conditions of food processing. Alternatively, one may give the material an appropriate thermal history in advance and thereby make it suitable for a fixed condition of processing.

Thus, there may be flexibility for controlling the thermal treatment, i.e., a fine control, in production of both the ingredients and finished products in the use of soybean proteins for food systems.

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Received for review December 7, 1984. Accepted June 4, 1985.