Thermal Denaturation of Soy Glycinin in the Presence of 2-Mercaptoethanol Studied by Differential Scanning Calorimetry

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ABSTRACT

The effect of 2-mercaptoethanol on the thermal properties of soy glycinin was determined at protein concentrations of 1 and 10% in buffered salt solution. Heating glycinin at 1% concentration in a buffered salt solution resulted in an endothermic denaturation; heating it in the presence of 2-mercaptoethanol, ethanol, or 2-propanol resulted in an exothermic transition. Increasing 2-mercaptoethanol concentration from 0.01 to 10% lowered the exothermic denaturation temperature (T_d) from 94.8 to 46.2°C. The denaturation enthalpy (ΔH) remained relatively unchanged (30 J/g). High concentrations of glycinin (10% suspensions) always exhibited an endotherm during denaturation. Increasing concentrations of 2-mercaptoethanol lowered the T_d and the ΔH of denaturation. Our data indicate that both disulfide bonds and hydrophobic interactions affect the thermal behavior of soy glycinin.

Soy proteins are incorporated in many food systems. Most of these food systems require some heat treatment. Heating has a strong effect on most of the functional properties of soy proteins. Several authors have studied soy protein heat denaturation and gelation (Wolf and Tamura 1969; Hashizumi and Watanabe 1979; Hermanson 1985, 1986; Yamagishi et al 1980; Sheard et al 1986, 1987; Bibkov et al 1986; German et al 1982).

Soy glycinin is reported to have a molecular weight of 360,000, consisting of six subunits devoid of sugar. Each glycinin subunit consists of two polypeptide components, one with an acidic and the other with a basic isoelectric point. The two polypeptide components are linked by a single disulfide bond (Nielsen 1985). Pure glycinin has poor solubility in water, although its solubility increases in solutions of relatively high salt concentrations. Heating of proteins can result in several reactions such as association, dissociation, aggregation, and gelation. At low protein concentrations (1%), as would be found in dry coffee whiteners and protein-fortified beverages, gelation of glycinin does not occur. A recent study by Marshall and Zarins (1989) showed that 1% glycinin heated in the presence of 0.05*M* 2-mercaptoethanol exhibited an exothermic transition, but 10% glycinin heated under the same conditions showed an endothermic transition. The presence of the exotherm was dependent upon glycinin concentration and disappeared at about 5% glycinin. Also, little change was observed in the denaturation temperature of glycinin at the one specific 2-mercaptoethanol concentration (0.05*M*) examined, which was similar to the results of Harwalkar and Ma (1987) for oat globulin in the presence of 0.01*M* dithiothreitol. However, when these authors used a high level (5%) of 2-mercaptoethanol, the denaturation temperature of oat globulin decreased markedly. Prompted by our previous observations and those of Harwalkar and Ma, the objective of this study was to examine the effect of 2-mercaptoethanol concentration on heat denaturation of glycinin at protein concentrations of 10 and 1% where gelation does and does not occur, respectively.

MATERIALS AND METHODS

Materials

All chemicals were reagent grade. Sepharose 6B, diethylaminoethyl Sepharose CL 6B and Con A Sepharose 4B were obtained from Pharmacia Fine Chemicals.

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Isolation of Soy Glycinin

Glycinin (11S globulin) was isolated from defatted Nutrisoy 7B flakes (Archer Daniels Midland Company, Decatur, IL). The flakes were ground in a Wiley mill. The 11S globulin was extracted from the flour and concentrated by the method of Thanh and Shibasaki (1976). The crude 11S globulin was further purified by a modified method described by Kitamura et al (1976). This purification consisted of passing the 11S globulin (1 g) through a Con A Sepharose 4B column (2.6 × 90 cm), and eluting with 0.035*M* phosphate/0.4*M* NaCl buffer, pH 7.6. The fraction that was not adsorbed was concentrated by ultrafiltration and chromatographed on a Sepharose 6B column (10 × 80 cm). The eluate in the main peak from the Sepharose 6B column was concentrated, dialyzed against 0.035*M* phosphate buffer (pH 7.6) containing 0.2*M* NaCl and 0.01*M* 2-mercaptoethanol, and then fractionated on a diethylaminoethyl Sepharose CL 6B (5×50cm) column, eluted with a linear ionic strength gradient (0.2–0.5*M* NaCl). A fraction of the purified glycinin was concentrated by ultrafiltration to be used for analysis by ultracentrifugation. During ultrafiltration a precipitate formed; this precipitate was removed and analyzed by gel electrophoresis. The rest of the glycinin was dialyzed against deionized water and lyophilized.

Ultracentrifugation

Ultracentrifugation patterns were obtained by W. J. Wolf, NRRC, Peoria, IL, with a Spinco model E ultracentrifuge, equipped with Schlieren optics, using the analytical rotor at a speed of 48,000 rpm, at an angle of 70° in a standardized cell. The buffer was 0.035*M* sodium phosphate/0.4*M* sodium chloride, pH 7.6, containing 0.01*M* 2-mercaptoethanol.

Gel Electrophoresis

Gel electrophoresis was carried out using a Tris-glycine buffer system containing 1% sodium dodecyl sulfate on polyacrylamide gels (SDS-PAGE) as described by Fling and Gregerson (1986). Heated glycinin samples from differential scanning calorimetry (DSC) ampoules were centrifuged, and the supernatant and precipitate were dialyzed, lyophilized, and redissolved in electrophoresis buffer, as described by Fling and Gregerson (1986).

DSC

The thermal behavior of the 11S globulin was examined with a Hart Scientific model 707 differential scanning calorimeter (Hart Scientific, Provo, UT). The calorimeter was equipped with four cylindrical 1-ml Hastelloy screw-top ampoules. One of the ampoules was used for the reference cell, which was filled with buffer corresponding to that in which the samples were dissolved. The remaining three ampoules were used for samples. The protein (8 mg) was weighed directly into the ampoule, and buffer, or buffer containing additives, was added to achieve the desired concentration. The ampoules were agitated and then weighed again.

Preliminary tests indicated that incubation of glycinin at ambient temperature with additives for several hours did not cause significant changes in DSC response.

The samples were heated from 20 to 106°C, held at 106°C for 10 min, cooled to 20°C, and then reheated and cooled in the same manner. The heating and cooling rate was 60°C/hr. Because glycinin exhibited irreversible denaturation under all conditions investigated, the second heating was subtracted from the first heating to obtain the DSC profiles.

The enthalpy of denaturation was calculated by integration of the thermal curve using a baseline formed by extending a straight line between the point where the excess heat capacity deviates from the initial baseline and the point where the excess heat capacity merges with the final baseline. The integration was done by a computer routine employing trapezoidal summation, with an interval of 0.167°C (points taken at 10-sec intervals with a scan rate of 60°C/hr). Programs for the collection and analysis of thermal data are part of the software supplied by Hart Scientific.

The temperature of denaturation (T_d) was determined as the peak maximum temperature. The values of T_d and ΔH were an average of at least two separate determinations. To determine the reproducibility of the DSC results, six separate determinations were carried out using 1% glycinin dissolved in 0.035*M* phosphate/0.4*M* NaCl, pH 7.0, containing 0.01% 2-mercaptoethanol.

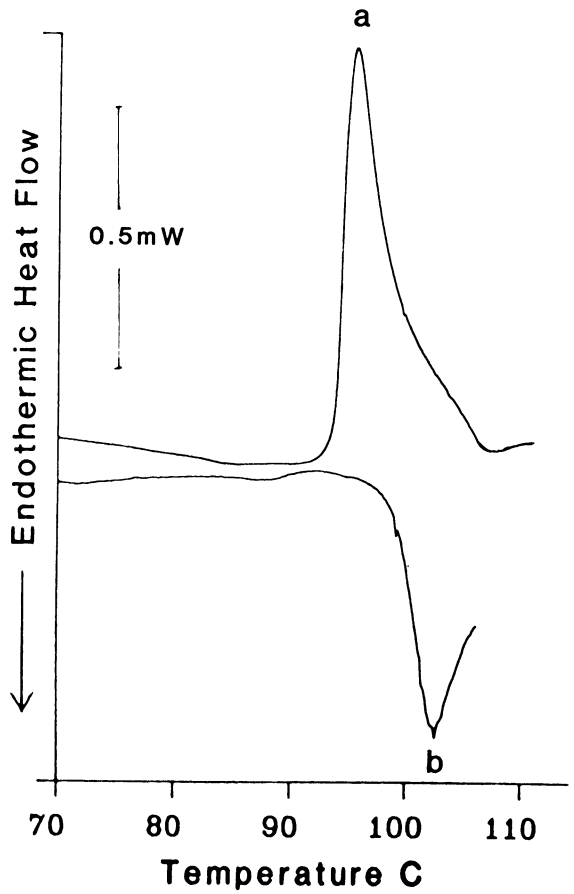

Fig. 1. Differential scanning calorimetry thermal curves of 1% glycinin heated in 0.035*M* phosphate, pH 7.0, containing 0.4*M* NaCl with 0.01% 2-mercaptoethanol added (**a**) or no additive (**b**).

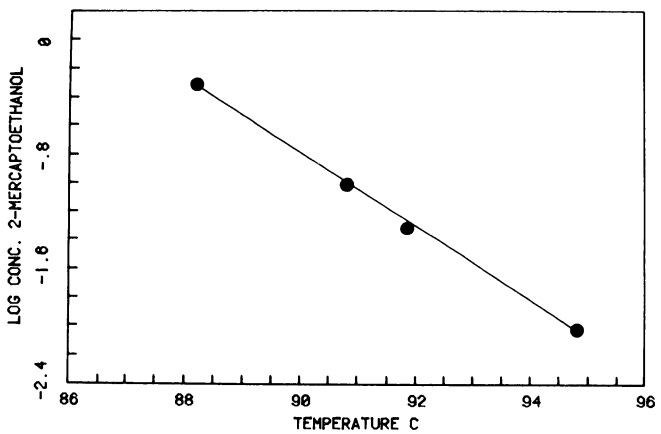

Fig. 2. Effect of 0.01–1% 2-mercaptoethanol on exothermic transition temperatures of 1% glycinin solutions.

RESULTS AND DISCUSSION

The purified glycinin (11S globulin) analyzed by ultracentrifugation showed the following results: 2.2% 7S, 92.5% 11S, and 5.3% 15S. However, when the aggregate obtained by concentrating the glycinin by ultrafiltration through an Amicon XM-300 membrane was analyzed by SDS-PAGE, the only bands obtained were the acidic and basic polypeptides of glycinin. This lead to the conclusion that the 15S was actually aggregated glycinin. Thus our sample was about 98% glycinin.

DSC Analysis

A 1% solution of glycinin heated in pH 7.0, ionic strength (μ) 0.5 buffer exhibited an endotherm at T_d 102.4°C in the absence of 2-mercaptoethanol (b in Fig. 1), and an exotherm in the presence of 0.01% 2-mercaptoethanol at T_d 94.8°C (a in Fig. 1). A value of 30 J/g was obtained for the exothermic denaturation from six separate determinations with a standard deviation of 0.61 and coefficient of variation of 2.0%. Increasing the 2-mercaptoethanol concentration decreased the temperature of denaturation, but had little effect on the enthalpy of denaturation (ΔH = 30 J/g, SD = 0.87, CV = 2.87). When the denaturation temperature was plotted against the logarithm of 2-mercaptoethanol concentration, a straight line was obtained for 2-mercaptoethanol concentrations 0.01–0.5% (Fig. 2). The relationship between 2-mercaptoethanol concentration above 0.5% and T_d of glycinin is shown in Figure 3; the curve becomes almost linear as the 2-mercaptoethanol concentration increases. A similar curve was obtained for the relationship of 2-mercaptoethanol concentration to T_d of the denaturation endotherm for the 10% glycinin suspensions (Fig. 3).

To determine if other alcohols besides 2-mercaptoethanol would have a similar effect on glycinin denaturation, 1% glycinin was heated in pH 7.0 buffer μ 0.5 in the presence of 10% 2-propanol and also in the presence of 10% ethanol. In both cases an exotherm was obtained upon heating. Ethanol slightly decreased glycinin solubility, and an exotherm at T_d 89.3°C and a ΔH of 15 J/g were obtained. The sample heated in the presence of 10% 2-propanol exhibited an exotherm at T_d 77.4 with a ΔH of 30

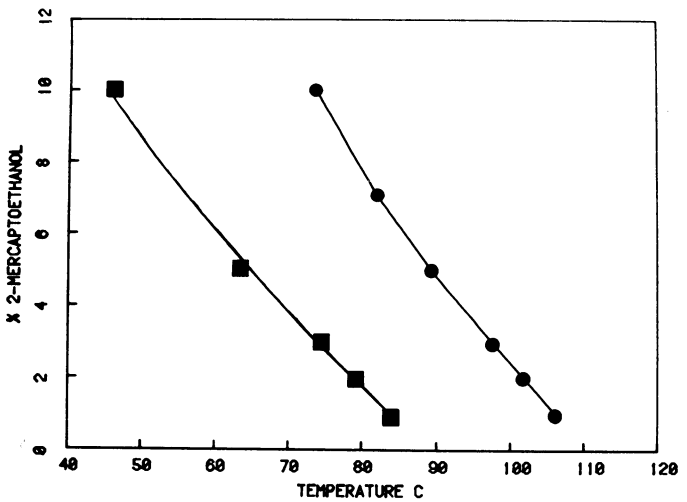

Fig. 3. Effect of 1–10% 2-mercaptoethanol on transition temperatures (T_d) of glycinin: 1% glycinin solutions (■); 10% glycinin slurries (●).

J/g; this effect is about the same as would be produced by 2.5% 2-mercaptoethanol (Fig. 3). Vellicelebi and Sturtevant (1979) reported that in thermal denaturation of lysozyme, the most obvious effect of alcohols is the lowering of T_d with higher alcohol concentration and longer alkyl chain.

Crude 1% glycinin (prior to the three-column purification) solution at pH 7.0 μ 0.5 heated in the absence of 2-mercaptoethanol also exhibited an irregular exotherm at 91.4°C, 5.6 J/g. The crude glycinin preparation contained a small amount of hemagglutinin, which, at low concentrations, exhibits an exotherm during heating whether 2-mercaptoethanol is absent or present (*unpublished results*). However, the T_d and the joules of this exotherm were too high to be attributed to a hemagglutinin impurity. Sheard et al (1987) observed an exothermic transition when soy isolate of greater than 70% moisture content was heated and monitored by DSC. They referred to this as a postdenaturational transition, because it was preceded by two endotherms. However, our results showed that when purified glycinin was heated in dilute solution in the presence of a perturbant, the denaturation was an exothermic transition (Fig. 1), and not preceded or followed by an endotherm.

However, unlike the results obtained at 1% glycinin concentrations, all 10% suspensions of glycinin exhibited endothermic transitions during heating (Fig. 4). No exotherms were observed within the temperature range covered. A small endotherm was observed that preceded the main endotherm at 2-mercaptoethanol concentrations of 5% and higher (Fig. 4). The ΔH for the main endotherm decreased from 17.8 J/g at 3% 2-mercaptoethanol concentration to 9.0 J/g at 10% 2-mercaptoethanol. The ΔH at 2-mercaptoethanol concentrations of 2% and lower could not be calculated due to the absence of a concluding baseline for the transitions. Harwalkar and Ma (1987) observed similar effects of 2-mercaptoethanol on 10% oat globulin suspensions. Addition of 5% 2-mercaptoethanol lowered T_d from 108°C to 96.7°C and caused a reduction in ΔH. They attributed these effects to 2-mercaptoethanol acting as a monohydric alcohol that destabilized proteins by weakening hydrophobic interactions.

Privalov and Pfeil (1979) also observed similar effects on lysozyme heated in solutions of different pH monitored by microcalorimetry. Lowering the pH of the solution lowered the denaturation temperature as well as the enthalpy of denaturation. Their most important discovery from calorimetry was that the enthalpy of a conformational transition, i.e., the denaturation enthalpy, corrected for ionization and solvation effects, does not depend on the mode of denaturation, because denaturation by temperature, pH, or denaturants has the same enthalpy value at a given temperature (Pfeil and Privalov 1976).

Gel Electrophoresis

Examination by SDS-PAGE of supernatant and precipitate fractions obtained by heating 1% glycinin solutions (Fig. 5) showed that in all cases where an alcohol was added, the supernatant consisted mostly of the acidic subunits. This is in agreement with results obtained by other researchers (Hashizumi and Watanabe 1979; Yamagishi et al 1980, 1987; Mori et al 1982). The supernatant of glycinin heated in pH 7.0 buffer, μ 0.5 without additives still contained some basic subunits (Fig. 5) as seen from the band in the basic subunit region.

When glycinin was heated in the standard buffer containing 1% 2-mercaptoethanol, 10% 2-propanol, or 10% ethanol, the supernatant (Fig. 5) did not contain any basic subunits. Yamagishi et al (1982) reported that sulfhydryl compounds increased the level of basic subunits in the thermal aggregate. We have found that the hydroxyl groups of alcohols have effects similar to those of sulfhydryl groups on thermal denaturation of glycinin.

The supernatant and the precipitate contained high molecular weight aggregated material, which caused streaking of the bands and protein remaining in the sample cell on top of the PAGE gel. Yamagishi et al (1980, 1987) have shown that the soluble oligomers and polymers result from covalent polymerization of the acidic subunits. In contrast, the disulfide bonds of oat globulin were not disrupted by heat treatment (Ma and Harwalkar 1987), only the noncovalent forces holding the subunits together were disrupted. Oat globulin formed soluble and insoluble aggregates during heating, but there was little difference in the subunit composition between the supernatant and the precipitate.

Marshall and Zarins (1989) provided evidence to show that glycinin exotherms, observed at 1% concentration in the presence of 0.05*M* 2-mercaptoethanol, resulted from the aggregation of basic polypeptides. The same mechanism could apply in the present case, not only with 2-mercaptoethanol but also with ethanol and 2-propanol as well. At 1% glycinin, exotherms were seen in every sample where no basic polypeptides existed in the supernatant of the denatured samples (Fig. 5). In the absence of additives, no exotherm was observed, and basic polypeptides were noted in the supernatant (Fig. 5).

No exotherms were seen at 10% protein concentration (Fig. 4). Marshall and Zarins (1989) noted that the presence of the exotherm in 0.05*M* 2-mercaptoethanol was concentration dependent. The exotherm disappeared at about 5% protein. They

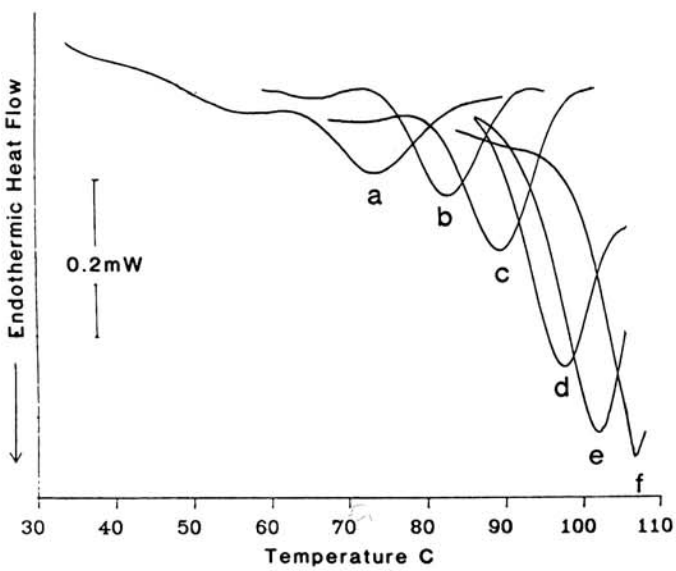

Fig. 4. Differential scanning calorimetry thermograms of 10% glycinin slurry denaturations in 0.035*M* phosphate, pH 7.0, containing 0.4*M* NaCl in the presence of 2-mercaptoethanol at concentrations of 10% (**a**), 7% (**b**), 5% (**c**), 3% (**d**), 2% (**e**), and 1% (**f**).

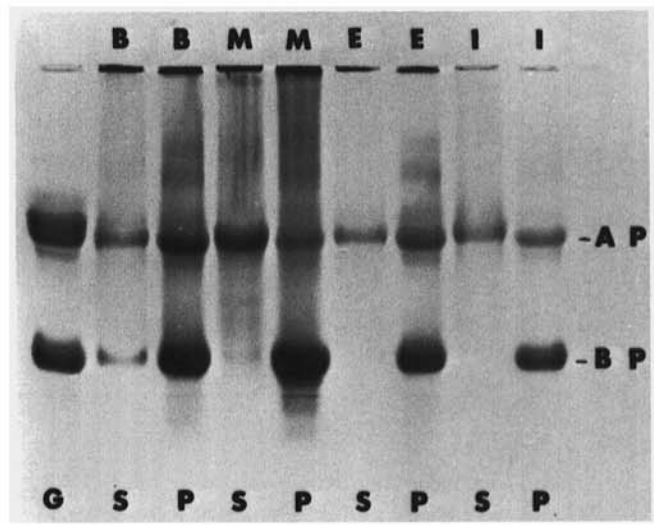

Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of 1% glycinin heated in 0.035*M* phosphate, pH 7.0, containing 0.4*M* NaCl (**S**) supernatant, and precipitate (**P**). Native glycinin (**G**), heated in buffer (**B**), heated in buffer containing 0.1% 2-mercaptoethanol (**M**), heated in buffer containing 10% ethanol (**E**), heated in buffer containing 10% 2-propanol (**I**), acidic polypeptides (**AP**), basic polypeptides (**BP**).

attributed the concentration dependence of the exotherm to a spatial effect. At high (10%) protein concentrations, glycinin dissociation into acidic and basic polypeptides may occur, but the basic polypeptides become sufficiently constrained and hindered from thermally aggregating with each other.

CONCLUSION

Glycinin thermal stability and the nature of the glycinin denaturation process was altered by the presence of alcohols at both 1 and 10% protein concentrations. Heat denaturation of 1% glycinin, in the presence of alcohols, yielded an exothermic transition and lowered the denaturation temperature of the protein. The denaturation temperature was also lowered at 10% protein concentration but an endotherm was observed. The appearance of the exotherm is probably associated with the aggregation and precipitation of the basic polypeptides. The reduction in denaturation temperature as a consequence of increased 2-mercaptoethanol is likely due to the destabilization of hydrophobic bonds in the protein interior.

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LITERATURE CITED

BIBKOV, T. M., DANILENKO, A. N., PLETENKO, M. G., GERMAN, M. L., VERFOLOMEEVA, E. P., GRINBERG, V. Y., LEONTYEV, A. L., and TOLSTOGUZOV, B. T. 1986. Selective thermal denaturation as a method of preparative isolation of 11S globulins from plant seeds. J. Agric. Food Chem. 34:297.

FLING, S. P., and GREGERSON, D. S. 1986. Peptide and protein molecular weight determination by electrophoresis using a high-molarity Tris buffer system without urea. Anal. Biochem. 155:83.

GERMAN, B., DAMODARAN, S., AND KINSELLA, J. E. 1982. Thermal dissociation and association behavior of soy proteins. J. Agric. Food Chem. 30:807.

HARWALKAR, H. R., and MA, C.-Y. 1987. Study of thermal properties of oat globulin by differential scanning calorimetry. J. Food Sci. 52:394.

HASHIZUMI, K., and WATANABE, T. 1979. Influence of heating temperature on conformational changes of soybean proteins. Biol. Chem. 4:683.

HERMANSSON, A. M. 1985. Structure of soya glycinin and conglycinin gels. J. Sci. Food Agric. 36:832.

HERMANSSON, A. M. 1986. Soy protein gelation. JAOCS, J. Am. Oil Chem. Soc. 63:658.

KITAMURA, K., TAKAGI, T., and SHIBASAKI, K. 1976. Subunit structure of soybean 11S globulin. Agric. Biol. Chem. 40:1837.

MA, C.-Y., and HARWALKAR, V. R. 1987. Thermal coagulation of oat globulin. Cereal Chem. 64:212.

MARSHALL, W. E., and ZARINS, I. M. 1989. Exothermic transitions of glycinin determined by differential scanning colorimetry. J. Agric. Food Chem. In press.

MORI, T., NAKAMURA, T., and UTSUMI, S. 1982. Gelation mechanisms of soybean 11S globulin: Formation of soluble aggregates as transient intermediates. J. Food Sci. 47:26.

NIELSEN, N. C. 1985. The structure and complexity of the 11S polypeptides in soybeans. JAOCS, J. Am. Oil Chem. Soc. 62:1680.

PFEIL, W., and PRIVALOV, P. L. 1976. Thermodynamic investigations of proteins. II. Biophys. Chem. 4:33.

PRIVALOV, P. L., and PFEIL, W. 1979. Thermodynamics of protein folding. Proc. FEBS Meet. 59:159.

SHEARD, P. R., FELLOWS, A., LEDWARD, D. A., and MITCHELL, J. R. 1986. Macromolecular changes associated with the heat treatment of soya isolate. J. Food Technol. 21:55.

SHEARD, P. R., LEDWARD, D. A., and MITCHELL, J. R. 1987. Post-denaturational exothermic transition in soya isolate. Int. J. Food Sci. Technol. 22:139.

THAN, V. H., and SHIBASAKI, K. 1976. Major proteins of soybean seeds. A straight-forward fractionation and their characterization. J. Agric. Food Chem. 24:117.

VELICELEBI, G., and STURTEVANT, J. M. 1979. Thermodynamics of the denaturation of lysozyme in alcohol water mixtures. Biochemistry 18:1180.

WOLF, W. J., and TAMURA, T. 1969. Heat denaturation of soybean 11S protein. Cereal Chem. 46:331.

YAMAGISHI, T., YAMAUCHI, F., and SHIBASAKI, K. 1980. Isolation and partial characterization of heat-denatured products of soybean 11S globulin and their analysis by electrophoresis. Agric. Biol. Chem. 44:1575.

YAMAGISHI, T., YAMAUCHI, F., and SHIBASAKI, K. 1982. Effect of sulfhydryl and disulfide compounds on the formation and quality of thermal aggregates of soya bean 11S globulin. J. Sci. Food Agric. 33:1092.

YAMAGISHI, T., TAKHASHI, N., and YAMAUCHI, P. 1987. Covalent polymerization of acidic subunits on heat-induced gelation of soybean glycinin. Cereal Chem. 64:207.

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