Effect of Conglycinin on the Thermal Aggregation of Glycinin

Srinivasan Damodaran* and John E. Kinsella

The effect of conglycinin on the thermal aggregation of glycinin was studied. The thermal aggregation of glycinin in the presence of a reducing agent was due to aggregation of the basic subunits. Addition of isolated conglycinin prevented thermal aggregation of both glycinin and the isolated basic subunits of glycinin. Succinylation of conglycinin enhanced its inhibitory effect on glycinin aggregation. Further, addition of other highly negatively charged proteins also prevented thermal aggregation of glycinin, indicating that the effect of conglycinin on glycinin aggregation is nonspecific. Evidence is presented to suggest that the absence of glycinin aggregation in the presence of conglycinin is due to formation of a soluble complex between the subunits of conglycinin and the basic subunits of glycinin via electrostatic interactions.

One of the problems limiting the utilization of soy protein in certain food products is its resistance to heat coagulation. This thermal stability behavior is usually attributed to the structural properties of this protein. However, it has been previously reported that isolated glycinin, one of the major storage proteins in soybean seed, undergoes thermal aggregation when heated at 80 °C (Catsimpoolas et al., 1970; Wolf and Tamura, 1969). Hashizume and Watanabe (1979) have shown that heating of acid-precipitated soy protein at 80 °C results in the disappearance of glycinin and the concurrent appearance of soluble aggregates and protein components of 2–4 S in the ultracentrifugal pattern. However, the protein solution did not show turbidity even at 100 °C. This implied that although glycinin in acid-precipitated soy protein can undergo thermal denaturation, such denaturation does not result in precipitation as it does when glycinin is heated alone at 80 °C (Wolf and Tamura, 1969). This raises the question as to why glycinin does not precipitate when heated at 80 °C in the presence of conglycinin, which is the other major storage protein in soybean. This could be due to certain interactions between conglycinin and glycinin that may lead to formation of a soluble complex. In a preceding paper evidence for the interaction of basic components of the 11S with the 7S fraction was presented (German et al., 1982). The purpose of this communication is to elucidate the mechanism of such interactions and to analyze the nature of the soluble complexes formed.

EXPERIMENTAL SECTION

Materials. Soy protein fractions were isolated from defatted and low heat treated soy flour (Central Soya, Chicago, lot 878, Code 3040). Bovine serum albumin and egg albumin were obtained from Sigma Chemical Co. All other chemicals used in this study were reagent grade.

Isolation of Soy Proteins. Soy conglycinin (7S) and glycinin (11S) fractions were isolated from defatted soy flour as described by Thanh and Shibasaki (1976). The purity of 11 S as checked by sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gel electrophoresis was more than 95%. The 7S fraction was about 90% pure. The acidic and basic subunits of 11S protein were separated by DEAE-Sephadex ion-exchange chromatography, essentially as described by Kitamura and Shibasaki (1975). DEAE-Sephadex was suspended in 0.02 M phosphate buffer, pH 7.6, containing 6 M urea and 10 mM 2-mercaptoethanol and filtered through Watman No. 1 filter paper in a funnel. Soy 11S protein was dissolved in the above buffer and poured through the DEAE-Sephadex bed in the funnel. The protein in the DEAE-Sephadex bed was eluted with the same buffer containing 6 M urea until the absorbance of the eluent at 280 nm was zero. The eluent, which contained basic subunits, was collected and dialyzed exhaustively, first against very dilute acetic acid (pH 3.5) and then against water at pH 3.0. The DEAE-Sephadex bed in the funnel was then eluted with 0.02 M phosphate buffer, pH 7.6, containing 0.5 M NaCl until the absorbance of the eluent at 280 nm was zero. The eluate that contained acidic subunits was dialyzed against water at pH 8.0.

Protein Concentration. Protein concentrations of various solutions were estimated by biuret method.

Heating Experiments. Heating experiments were carried out in a water bath with 2-mL portions of protein solutions in glass vials. In experiments involving two proteins, the protein solutions were mixed at various volume ratios and the final volume was adjusted to 2 mL with buffer. The vials containing protein solutions were closed tightly with screw caps to prevent evaporation of water during heating. The vials were shaken slightly in the water bath during incubation. At the end of the heating period, the vials were removed and immediately cooled in an ice bath. The turbidity of the solutions in the vials were measured at 540 nm in a Spectronic 700 spectrophotometer. Each vial was shaken well before measuring the turbidity to ensure uniform suspension of particles.

Gel Electrophoresis. $NaDodSO_4$–polyacrylamide slab gel electrophoresis of the proteins was done essentially as described by Brinegar and Kinsella (1981). Protein bands were stained with Coomassie brilliant blue. The gels were scanned in a gel scanner, and the absorbances of the stained bands were recorded.

Chemical Modification. Succinylation of soy 7S protein, bovine serum albumin, and egg albumin was done as described by Shetty and Kinsella (1979). Methylation of bovine serum albumin was done as described by Mandell and Hershey (1960). Under the conditions employed, the extent of both succinylation and methylation was assumed to be 100%.

RESULTS AND DISCUSSION

The thermal aggregation of acid-precipitated soy protein, conglycinin (7 S), and glycinin (11 S) at 80 °C is summarized in Figure 1. The protein solutions were made in 30 mM Tris–HCl buffer, pH 8.0, containing 10 mM 2-mercaptoethanol. Glycinin readily aggregated at 80 °C within 3–5 min, whereas conglycinin and the whole soy protein solutions remained clear even after heating for 1

Institute of Food Science, Stocking Hall, Cornell University, Ithaca, New York 14853.

0021-8561/82/1430-0812$01.25/0

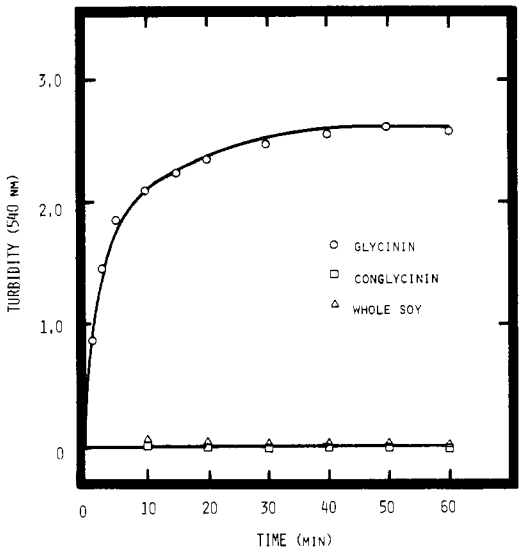

Figure 1. Thermal aggregation of acid-precipitated soy protein and the two major soy protein fractions, glycinin (11 S) and conglycinin (7 S) at 80 °C. Protein solutions (0.5%) were made in 30 mM Tris-HCl buffer, pH 8.0, containing 10 mM 2-mercaptoethanol. Two-milliliter portions of the protein solutions were heated for required amount of time and cooled in an ice bath, and the turbidity at 540 nm was measured.

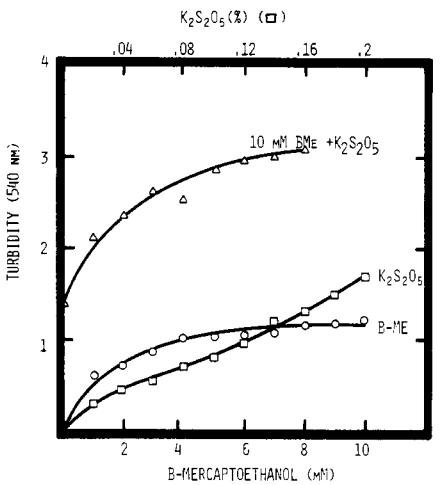

Figure 2. Effect of 2-mercaptoethanol and potassium metabisulfite ($K_2S_2O_5$) on the thermal aggregation of glycinin at 80 °C for 30 min. Protein solution (0.25%) was made in 30 mM Tris-HCl buffer, pH 8.0.

h. The differences in the thermal behavior of glycinin and conglycinin may be due to differences in the subunit composition and oligomeric nature of these two proteins. Glycinin is made up of twelve subunits, of which six subunits are acidic and six subunits are basic in nature (Badley et al., 1975), whereas conglycinin contains three subunits and these subunits are acidic in nature (Thanh and Shibasaki, 1979). For determination of whether the acidic or basic subunits are involved in the thermal aggregation of glycinin, the supernatant and precipitate of the heated glycinin solution was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. As shown in Figure 2 the precipitate contained only the basic subunits and the soluble supernatant contained acidic subunits. This indicated that the thermal aggregation of the glycinin at 80 °C was mainly due to aggregation of the basic subunits after they are thermally dissociated from the oligomeric structure of glycinin.

The effect of reducing agents on the thermal aggregation of glycinin is shown in Figure 3. The glycinin solutions

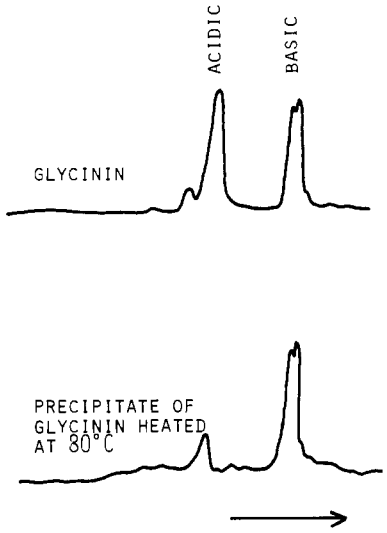

Figure 3. NaDodSO$_4$–polyacrylamide gel electrophoretic patterns of glycinin and the precipitate obtained after heating glycinin at 80 °C for 30 min.

were heated at 80 °C for 30 min in the presence of various concentrations of 2-mercaptoethanol. The turbidity of the glycinin solution increased with 2-mercaptoethanol concentration up to about 5 mM and remained the same above this concentration, whereas in the presence of potassium metabisulfite the turbidity increased monotonically in the concentration range shown in Figure 3. These results indicate that for the aggregation of the basic subunits to take place, a reducing atmosphere in the medium is necessary. This may be because in the oligomeric structure of glycinin, the subunits exist as units of acidic and basic subunits linked together by a disulfide bond (Badley et al., 1975). For the thermal aggregation of the basic subunits to take place, apparently it is essential to cleave this disulfide bond by using a reducing agent, either 2-mercaptoethanol or potassium metabisulfite. At room temperature, this disulfide linkage may be protected from the reducing agent. But when heated at 80 °C, the thermal perturbation of the glycinin molecule may facilitate the reduction of this disulfide bond. In the presence of 10 mM 2-mercaptoethanol, increasing concentrations of potassium metabisulfite further increased the turbidity (Figure 3). This enhanced turbidity by potassium metabisulfite may be due to further reduction of the intramolecular disulfide bonds in the basic subunits. In other words, while 2-mercaptoethanol has the ability to cleave only the disulfide bond linking the acidic and basic subunits, potassium metabisulfite has the ability to cleave the intramolecular disulfide bonds in the basic subunits. This may result in complete unfolding of the basic subunits, which may further facilitate thermal aggregation.

Although soy protein contains about 30% glycinin (Thanh and Shibasaki, 1976), when the whole soy protein was heated at 80 °C for 30 min, no thermal aggregation of glycinin was observed (Figure 1). But it is known that glycinin in whole soy protein does undergo thermal dissociation when heated at 80 °C (Hashizume and Watanabe, 1979). Differential scanning calorimetry studies with soy protein reveal two enthalpy transitions in the thermograms (Hermansson, 1979; German et al., 1982). The transition at about 75 °C is due to conglycinin, and the transition at about 90 °C is due to glycinin. These results evidently indicate that glycinin in whole soy protein does undergo thermal dissociation but fails to aggregate as it does when it is heated in the isolated form. In other words, the basic subunits dissociating from the oligomeric structure of

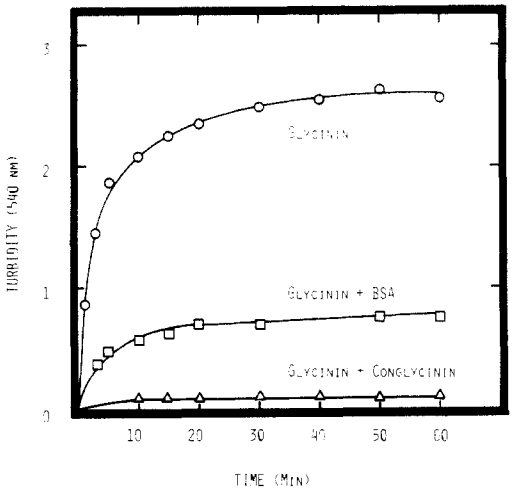

Figure 4. Effect of bovine serum albumin(□) and conglycinin (△) on the heat aggregation of glycinin. One milliliter of either bovine serum albumin (0.5%) or conglycinin (0.5%) was mixed with 1 mL of glycinin (0.5%) in glass vials and heated at 80 °C, and the turbidity at 540 nm was measured as a function of time.

glycinin are prevented from forming aggregates in the presence of the other soy protein fractions. Conceivably, this is because of certain interactions between conglycinin and the basic subunits of glycinin, which may lead to formation of soluble complexes.

If the solubilization of the basic subunits of glycinin is due to certain structure specific interactions between conglycinin and the basic subunits, then one might expect that other proteins that are structurally different from conglycinin, e.g., bovine serum albumin or egg albumin, would not have any effect on the aggregation of the basic subunits of glycinin when heated at 80 °C.

The effect of conglycinin and bovine serum albumin on the heat aggregation of glycinin is shown in Figure 4. Both conglycinin and bovine serum albumin suppressed the heat aggregation of glycinin. Although the degree to which bovine serum albumin suppresses the glycinin aggregation differed compared to that of conglycinin, the data nonetheless indicate that the interaction between conglycinin and the basic subunits of glycinin is not a structure-specific one but may involve nonspecific electrostatic or hydrophobic interactions that result in the formation of a soluble complex.

To determine if either electrostatic or hydrophobic forces are responsible for the interaction, we studied the effect of succinylated proteins on the heat aggregation of glycinin. The rationale for this approach is that if the interaction is hydrophobic in nature, then the succinylation of conglycinin that disrupts the hydrophobic regions (Damodaran and Kinsella, 1981) should prevent the complex formation and result in aggregation of the basic subunits. On the other hand, if the interaction is electrostatic in nature, then the increase in the net electronegativity of conglycinin following succinylation should increase its ability to interact with the basic subunits of glycinin.

The effect of native and succinylated conglycinin and egg albumin on the thermal aggregation of glycinin is shown in Figure 5. The abscissa represents the weight ratio of either conglycinin or egg albumin to glycinin (gram/gram), and the ordinate represents the turbidity of the solution at 540 nm when heated at 80 °C for 30 min. As the ratio of conglycinin to glycinin is increased, the turbidity decreases progressively and reaches a minimum at a ratio of 0.4. The same trend is reflected in the case of egg albumin. Furthermore, succinylation of both con-

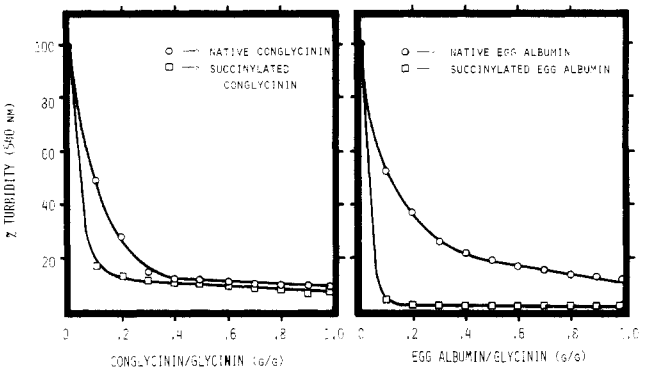

Figure 5. Effects of native and succinylated conglycinin and egg albumin on thermal aggregation of glycinin. To 1 mL of glycinin solution in glass vials increasing amounts of either egg albumin or conglycinin were added, and the total volume was made up to 2 mL by adding buffer (30 mM Tris-HCl, pH 8.0, containing 10 mM 2-mercaptoethanol). The vials were heated in a water bath at 80 °C for 30 min and cooled, and the turbidity at 540 nm was measured.

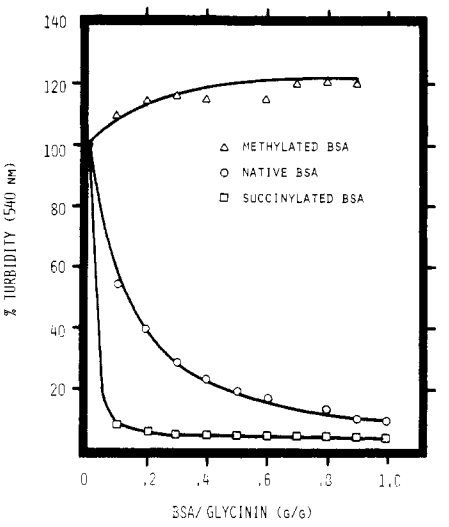

Figure 6. Effects of native, succinylated, and methylated bovine serum albumin on the thermal aggregation of glycinin. The experimental details are as described in Figure 5.

glycinin and egg albumin increased their ability to suppress the aggregation of the basic subunits of glycinin. In other words, increasing the net charge on these proteins increased their ability to solubilize the basic subunits of denatured glycinin. This evidently indicates that the interaction between the positively charged basic subunits of glycinin and the negatively charged conglycinin, which results in the formation of a soluble complex, is electrostatic in nature. This is further reflected from the effects of native, succinylated, and methylated bovine serum albumin on thermal aggregation of glycinin (Figure 6). In the case of succinylated bovine serum albumin, the increase in the net negative charge on the protein increased its ability to interact electrostatically with the basic subunits of glycinin and thus prevent its precipitation, whereas in the case of methylated bovine serum albumin, in which the carboxyl groups are esterified, the protein has net positive charge and hence did not interact with the positively charged basic subunits of glycinin. Thus heating results in the precipitation of the basic subunits in the presence of methylated bovine serum albumin.

To further confirm that there is indeed an interaction between conglycinin and the basic subunits of glycinin upon heating, we studied the effect of conglycinin on the pH insolubility profile of the isolated basic subunits.

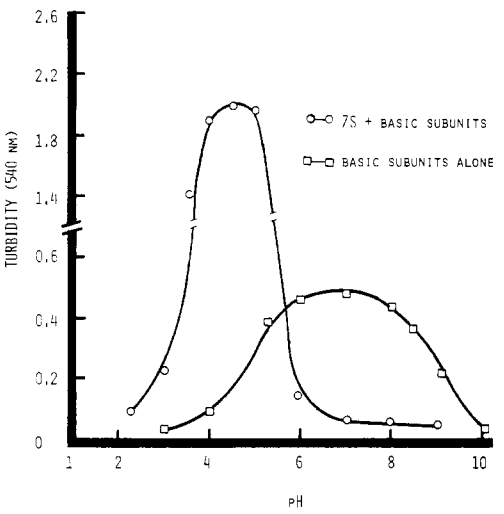

Figure 7. Effect of conglycinin on the pH–insolubility profile of the basic subuinits of glycinin. The molal ratio of conglycinin to basic subunits in the solution was about 3. The insolubility was measured as turbidity at 540 nm. The pH was adjusted by adding microliter quantities of either HCl or NaOH (2 N) to minimize the dilution factor.

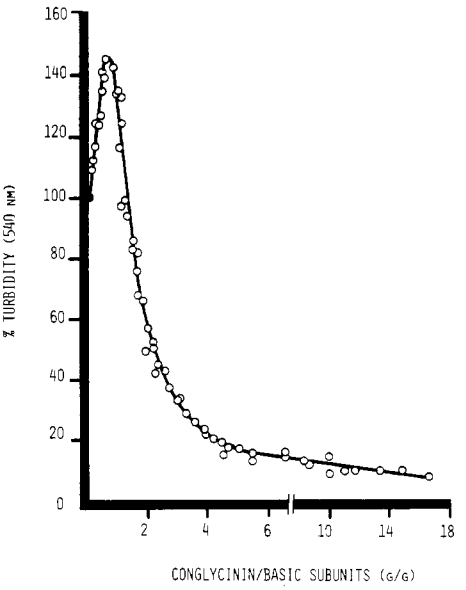

Figure 8. Effect of conglycinin on the thermal aggregation of isolated basic subunits of glycinin at 80 °C. The experimental conditions were as described in Figure 5. The concentration of the basic subunit stock solution was 0.45 mg/mL. To 1 mL of basic subunit increasing amounts of conglycinin solution were added and the total volume was adjusted to 2 mL.

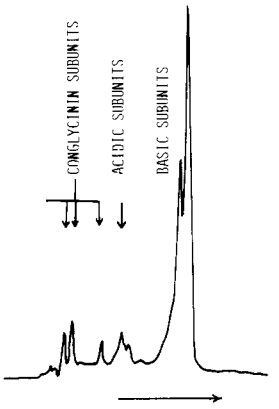

Figure 9. NaDodSO$_4$–polyacrylamide gel electrophoresis pattern (densitogram) of the precipitate obtained from heating a mixture of conglycinin and the basic subunits (0.6 weight ratio) at 80 °C.

The pH–insolubility profile of the basic subunits in the presence and absence of conglycinin is shown in Figure 7. The concentration of basic subunits in both the cases was the same. In the absence of conglycinin the basic subunits exhibited least solubility at the pH range 6–8. But in the presence of conglycinin, the basic subunits were solubilized in this pH range. Ideally, in a noninteracting two-component system, one would expect two peaks in the pH–insolubility profile, each corresponding to the isoelectric point of the individual components. For example, in soy protein the two major protein fractions, conglycinin and glycinin, which apparently do not interact with each other, exhibit individual isoelectric pH values (Thanh and Shibasaki, 1976). Hence the observed solubilization of the basic subunits at pH 6–8 in the presence of conglycinin is probably due to formation of a complex between conglycinin and the basic subunits. The isoelectric pH of this complex is about 4.6, which is same as that of isolated conglycinin (Thanh and Shibasaki, 1976).

Using isoelectric focusing techniques, Catsimpoolas (1969) reported that the isoelectric pH of the glycinin basic subunits was between 8 and 8.5. But the pH–insolubility profile of the basic subunits, shown in Figure 7, exhibits least solubility at pH 6–8. At this pH range one would expect the basic subunits to possess net positive charge, and hence repulsive interaction between them would be expected. However, the experimental data suggest that the hydrophobic interactions between the basic subunits more than overcome the residual net positive charge at this pH and hence lead to formation of aggregates. However, in the presence of conglycinin, the electrostatic interaction between conglycinin and basic subunits may be energetically more favorable than the hydrophobic interaction between the basic subunits and hence lead to formation of soluble complexes.

For determination of the exact molecular nature of the complex formation between conglycinin and the basic subunits of glycinin, further thermal aggregation studies were done with isolated basic subunits. The effect of conglycinin on the thermal aggregation of the basic subunits at 80 °C is shown in Figure 8. The abscissa in Figure 8 represents the weight ratio of conglycinin to basic subunit, keeping the concentration of the basic subunits constant. As the ratio of conglycinin to basic subunit is increased, the turbidity increased initially up to a ratio of 0.9. Above this ratio the turbidity decreased monotonically up to a ratio of 4. Above the ratio of 4 there was no dramatic decrease in the turbidity.

The increase in the turbidity of the solution upon heating (Figure 8) at low concentrations of conglycinin may be due to some glycinin contamination in conglycinin. Electrophoretic analysis of the conglycinin preparation indicated that the glycinin basic subunit contamination in conglycinin was less than 10% at most. But the increase in turbidity at a basic subunit to conglycinin ratio of 0.9 (g/g) is about 45% over that of the control (Figure 8). Hence, such high increase in turbidity cannot be attributed solely to aggregation of the basic subunit contaminant in conglycinin. Alternatively, the complex formed between conglycinin and the basic subunits of glycinin up to a 0.9 (g/g) ratio may possess zero net charge at pH 8.0 and hence aggregate via hydrophobic interactions. In other words, at a low conglycinin to basic subunit ratio the residual positive charges may be titrated by the addition of negatively charged conglycinin, and hence conglycinin may have precipitated along with the basic subunits under these conditions. In fact, electrophoretic analysis of the pre-

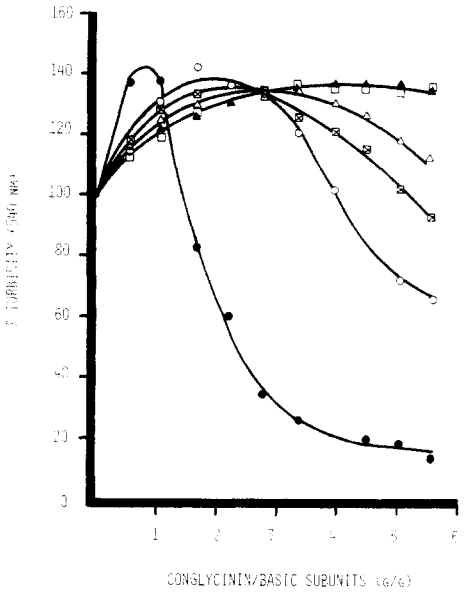

Figure 10. Effect of ionic strength on the interaction between conglycinin and the basic subunits. The experimental conditions were as described in Figure 5. NaCl concentrations were as follows: (●) no salt; (○) 0.1 M; (⊠) 0.2 M; (△) 0.3 M; (□) 0.4 M; (▲) 0.5 M.

cipitate (washed twice with buffer to remove any contamination from the supernatant) obtained at a conglycinin to basic subunit ratio of about 0.6 (g/g) exhibited the presence of conglycinin along with the basic subunits (Figure 9). This supports the speculation that a part of the increase in turbidity at low conglycinin to basic subunits may be caused by the aggregation of conglycinin. However, above the ratio of 0.9 (g/g), the complex formed may have a net negative charge and hence due to electrostatic repulsion the aggregation tendency between the complexes may decrease, resulting in a progressive decrease in turbidity, i.e., solubilization.

When the initial negative slope in Figure 8 is extrapolated to the abscissa, a conglycinin to basic subunit ratio (gram/gram) of about 3 is obtained. If a molecular weight of 170 000 for conglycinin (Koshiyama, 1969) and 22 000 for the basic subunits (Catsimpoolas et al., 1971) is assumed, the weight ratio of 3 corresponds to a molal ratio of about 1/3. Since conglycinin is made up of three subunits (Thanh and Shibasaki, 1979) that readily undergo thermal dissociation at 75 °C (Hermansson, 1979), the stoichiometry of interaction between the subunits of conglycinin and the basic subunits is about 1/1. Under these conditions, the complex formation between conglycinin subunits and the basic subunits presumably has a net negative charge. Due to charge repulsion the aggregation tendency of these complexes at pH 8.0 would decrease and hence would result in decreased turbidity. In other words, for complete solubilization of the basic subunits (zero turbidity) the minimum molal ratio of conglycinin to basic subunit is about 1/3. But the observed deviation from linearity in Figure 8 suggests that even at higher conglycinin concentrations the basic subunits are not completely solubilized. This might be due to a state of thermal equilibrium between various forms of complexes formed between conglycinin and basic subunits.

If the interaction between conglycinin and the basic subunits of glycinin is electrostatic in nature, then such electrostatic interaction may be suppressed by increasing the ionic strength of the medium. In other words, at higher ionic strength the suppression of the electrostatic interaction would result in the aggregation of the basic subunits at pH 8.0.

The effect of ionic strength on the thermal aggregation of the basic subunits is summarized in Figure 10. The abscissa in Figure 10 represents the weight ratio of conglycinin to basic subunits, and the curves were obtained with various concentrations of NaCl in the medium. In the absence of NaCl, the turbidity curve has a maximum at the conglycinin to basic subunit ratio of 0.9. But in the presence of NaCl, at all ionic strengths studied, the curves exhibit an isosbestic point at a conglycinin to basic subunit ratio of 2.75 (g/g). Apparently, the isosbestic point appears to be the maximum in the turbidity curves. As mentioned earlier, the initial increase in turbidity may either be due to basic subunit contamination in conglycinin or be due to insoluble complex formation between conglycinin and basic subunits. However, at a conglycinin to basic subunit ratio of 2.75 (g/g), while the basic subunits are solubilized by conglycinin at zero ionic strength, at higher ionic strengths the basic subunits are precipitated. This indicates that at high ionic strength the interaction between the basic subunits and conglycinin is inhibited, which results in the aggregation of the basic subunits due to hydrophobic interaction between them. This clearly dem-

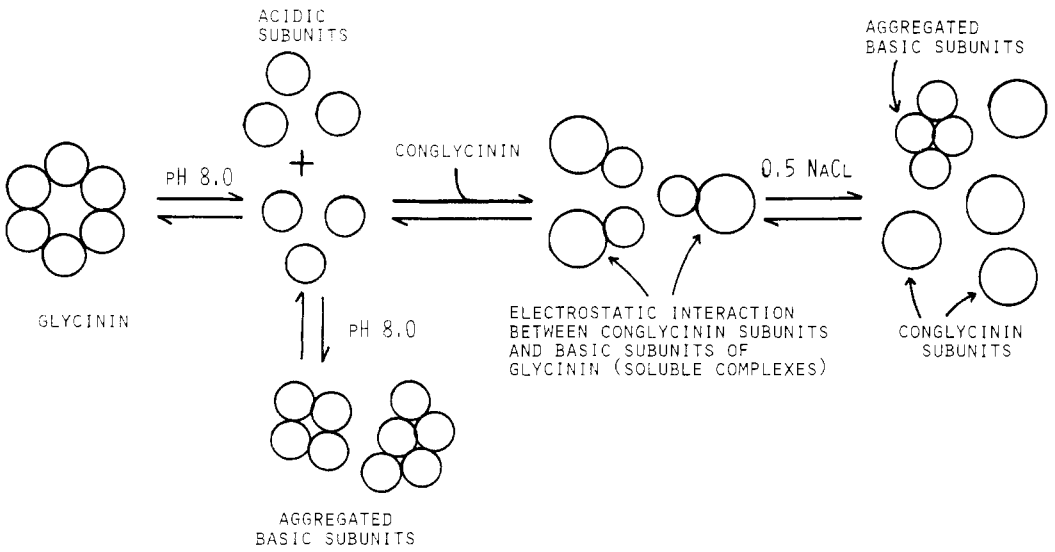

Figure 11. Schematic representation of various molecular changes in soy proteins following heating at 80 °C in the presence of 2-mercaptoethanol.

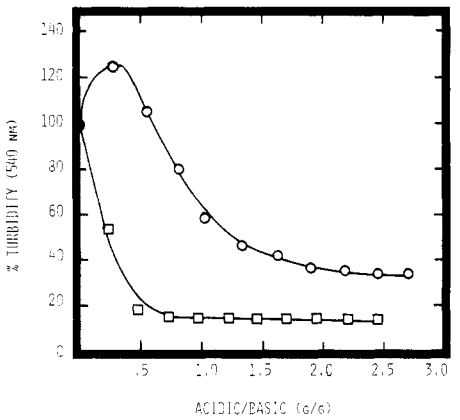

Figure 12. Effect of native and succinylated acidic subunits of glycinin on the thermal aggregation of the basic subunits of glycinin. The experimental conditions were as described in Figure 5. (○) Native acidic subunits; (□) succinylated acidic subunits.

onstrates that the interaction between conglycinin and basic subunits is basically electrostatic in nature. This is further reflected in the turbidity curves above the isosbestic point that show increased basic subunit aggregation with increased ionic strength. Above 0.4 M ionic strength there is no further increase in the turbidity, indicating that the minimum ionic strength required to completely inhibit the electrostatic interaction between conglycinin and basic subunit is about 0.4 M.

These observations are consistent with some of the indirect observations reported in the literature. Hashizume and Watanabe (1979) reported that heating of acid-precipitated soy protein did not result in precipitation at low ionic strength, whereas high ionic strength or addition of NaCl to the heated soy protein solution resulted in formation of a precipitate. Further, ultracentrifugal analysis showed complete disappearance of glycinin at 80 °C. On the basis of these reports and from our data, we interpret the various molecular changes in soy protein following heating at 80 °C as follows: When soy protein is heated at 80 °C in the presence of 2-mercaptoethanol and at low ionic strength, both conglycinin and glycinin undergo thermal dissociation. The dissociated basic subunits of glycinin interact electrostatically with the subunits of conglycinin, forming a soluble complex. But when the ionic strength of the solution is increased by adding NaCl, the electrostatic interaction between the basic subunits and conglycinin is suppressed. This results in the aggregation of the basic subunits via hydrophobic interaction between them. This is schematically illustrated in Figure 11.

All the above data suggest that the basic subunits of glycinin can be prevented from thermal aggregation by the presence of any acidic protein at low ionic strength, and the absence of thermal aggregation of glycinin in whole soy protein is principally due to the interaction between the subunits of conglycinin and the basic subunits of glycinin. But a logical question arises at this point as to why the acidic subunits of glycinin do not have the ability to prevent the aggregation of the basic subunits when glycinin alone is heated at 80 °C (Figure 1). This may be because either the acidic subunits of glycinin may not be acidic enough to electrostatically interact with the basic subunits or the concentration of the acidic subunits may not be sufficient to solubilize the basic subunits once they are dissociated from the oligomeric structure of glycinin. This is supported by the observation that at higher molal ratios of acidic to basic subunits, the basic subunits are solubilized by the acidic subunits of glycinin (Figure 12). Further, succinylation of the acidic subunits also profoundly increases their ability to solubilize the basic subunits (Figure 12).

In the light of the data presented here we propose that the observed stability of soy protein to heat coagulation may be attributable to the ionic character of the individual constituent subunits of various protein fractions. The resistance of conglycinin to heat coagulation may be due to its electrophilic/hydrophobic ratio. Even under denatured states, the electrostatic repulsion between conglycinin molecules may be greater than the hydrophobic interaction between the exposed hydrophobic residues. Thus, neutralization of the negative charges on conglycinin by interacting them with various positively charged proteins may provide a method for inducing thermal aggregation of soy protein.

ACKNOWLEDGMENT

The technical assistance of Y. T. Huang is acknowledged.

LITERATURE CITED

Badley, R. A.; Atkinson, D.; Houser, H.; Oldani, G.; Green, J. P.; Stubbs, J. M. *Biochim. Biophys. Acta* **1975**, *412*, 214.
Brinegar, A. C.; Kinsella, J. E. *Int. J. Pept. Protein Res.* **1981**, *18*, 18.
Catsimpoolas, N. *FEBS Lett.* **1969**, *4*, 259.
Catsimpoolas, N.; Funk, S. K.; Meyer, E. W. *Cereal Chem.* **1970**, *47*, 331.
Catsimpoolas, N.; Keeney, J. A.; Meyer, E. W.; Szuhaj, B. F. *J. Sci. Food Agric.* **1971**, *22*, 448.
Damodaran, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1981**, *29*, 1249.
German, B.; Damodaran, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1982**, preceding paper in this issue.
Hashizume, K.; Watanabe, T. *Agric. Biol. Chem.* **1979**, *43*, 683.
Hermansson, A. M. In "Functionality and Protein Structure"; Pour-El, A., Ed.; Americal Chemical Society: Washington, DC, 1979; ACS Symp. Ser. No. 92, pp 81–103.
Kitamura, K.; Shibasaki, K. *Agric. Biol. Chem.* **1975**, *39*, 945.
Koshiyama, I. *Agric. Biol. Chem.* **1969**, *33*, 281.
Mandell, J. D.; Hershey, A. D. *Anal. Biochem.* **1960**, *1*, 66.
Shetty, K. J.; Kinsella, J. E. *J. Food Sci.* **1979**, *44*, 133.
Thanh, V. H.; Shibasaki, K. *J. Agric. Food Chem.* **1976**, *24*, 1117.
Thanh, V. H.; Shibasaki, K. *J. Agric. Food Chem.* **1979**, *27*, 805.
Wolf, W. J.; Tamura, T. *Cereal Chem.* **1969**, *46*, 331.

Received for review December 16, 1981. Revised manuscript received May 3, 1982. Accepted May 11, 1982. This work was supported in part by National Science Foundation Grant CPE 80-18394.