Role of Electrostatic Forces in the Interaction of Soy Proteins with Lysozyme

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ABSTRACT

The nature of the interaction between lysozyme with soy protein isolate, soy 11S, and soy 7S fractions was studied using a turbidity method. Complete dissociation of soy protein lysozyme complexes occurred at an ionic strength of 0.12*M*; however, below this ionic strength the extent of dissociation was affected by pH. These observations suggested that electrostatic forces were involved in the interaction between lysozyme and soy proteins. The interaction between lysozyme and soy 11S was stronger than that of lysozyme and soy 7S, suggesting that the surface electronegativity of soy 11S was greater than that of soy 7S. It is proposed that similar protein-protein interactions may occur in protein blends during processing. Such interactions may affect the functional behavior of individual protein components in protein blends.

Oilseeds are an abundant source of food proteins; however, the lack of certain functional properties limits their maximum utilization in conventional foods (Kinsella et al 1985). Appropriate chemical modification of oilseed proteins is a promising approach to improve the functional properties (Kinsella and Shetty 1979). However, the economic feasibility and nutritional safety of chemically modified proteins is yet to be determined. To overcome some of these obstacles and improve the functional properties of oilseed proteins, research on the physicochemical and nutritional properties of mixed proteins and coprecipitated protein blends is being conducted (Berardi and Cherry 1979, 1981; Hayes et al 1978; Schmidt et al 1978). Berardi and Cherry (1979) reported that coprecipitation of proteins from blends of cottonseed, soybean, and peanut flours produced new forms of protein preparations. These new proteins conceivably resulted from dissociation and subsequent reassociation of the subunit components of various protein sources used. The hybrid proteins formed under the conditions of coprecipitation influenced the functional properties of the resulting mixture when compared to the individual native proteins (Cherry et al 1978). Incorporation of soy, whey, and yeast proteins in meat as meat extenders affected the solubility, heat denaturability, and emulsifying capacity (Porteous and Quinn 1979). The changes in the functional behavior were attributed to complex formation between myosin and the soy protein components of the meat extenders (King 1977).

To better understand and predict the functional properties of protein blends in food systems, an understanding of the mechanisms as well as the factors influencing the interaction between proteins is desirable. Previous studies reported that during thermal treatment of soy protein isolate, the subunits of soy 7S fraction interact with the basic subunits of soy 11S via electrostatic forces and formed soluble complexes that reduce the thermal coagulation of soy 11S (German et al 1982, Damodaran and Kinsella 1982). To better understand the electrostatic properties of soy proteins that affect their behavior in protein blends, we studied the interaction of soy proteins with positively charged lysozyme under nondenaturing conditions.

MATERIALS AND METHODS

Preparation of Soy Proteins

Whole soy protein was isolated from defatted and low-heat treated soy flour (Central Soya, Chicago) as described elsewhere (Damodaran and Kinsella 1981). The soy 11S and 7S globulins were isolated as described by Thanh and Shibasaki (1976). These two protein fractions were about 90 and 70% pure, respectively, as judged from sodium dodecyl sulfate polyacrylamide gel electrophoresis (Utsumi et al 1984). Lysozyme was purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used in this study were of reagent grade.

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Lysozyme-Soy Protein Interaction

A turbidometric method was used to study the interaction between lysozyme and soy proteins. The method is based on the observation that the interaction of the positively charged lysozyme with the negatively charged soy proteins leads to formation of an insoluble complex at pH 8.0 in 10 m*M* Tris-HCl buffer. The turbidity development under these conditions is used as a measure of the extent of interaction between lysozyme and soy proteins. In a typical experiment, 1-ml aliquots of soy protein solutions (0.1% in 10 m*M* Tris-HCl buffer) were treated with increasing amounts of lysozyme (0.1% in Tris-HCl buffer) and the total volume was made up to 2.0 ml by adding appropriate amounts of buffer. The solutions were held for 5 min at room temperature, vortexed vigorously, and the turbidity was measured at 540 nm with a Spectronic 700 spectrophotometer.

In studies involving the effect of ionic strength, a constant ratio of lysozyme to soy protein was maintained in the final mixture, and the level of ionic strength was varied by adding calculated amounts of 1.0*M* NaCl stock solution and buffer. In these experiments, various solutions were added during mixing in the sequence buffer, lysozyme, soy proteins, and NaCl stock solution. The tubes were held at room temperature for 5 min, vortexed vigorously, and the turbidity at 540 nm was measured. Protein concentrations were quantified by the biuret method.

RESULTS AND DISCUSSION

The interaction of lysozyme with soy isolate, soy 11S, and soy 7S fractions is shown in Figure 1. Addition of increasing amounts of lysozyme to soy proteins resulted in increased turbidity. For both soy isolate and soy 7S, the turbidity reached saturation value at a lysozyme-to-soy 7S weight ratio of about 1.2. In the case of soy 11S, the turbidity attained saturation at a lysozyme-to-11S weight ratio of about 0.5. The extent of turbidity development was used as a measure of the extent of interaction between lysozyme and soy proteins.

The development of turbidity upon addition of lysozyme to soy proteins may possibily be caused by electrostatic interaction between the positively charged lysozyme and the negatively charged soy proteins. This interaction, which is analogous to pH titration of proteins, decreases the net charge of the soy protein-lysozyme complex at pH 8.0. The apparent elimination of electrostatic repulsion promoted aggregation of these complexes via hydrophobic interaction between the nonpolar patches on the surface of the protein molecules.

To confirm the involvement of electrostatic interactions in complex formation between soy proteins and lysozyme, the extent of turbidity development at various NaCl concentrations was studied. The extent of interaction of lysozyme with soy isolate and soy 7S decreased at higher ionic strength (Fig. 2A and B). Above 0.1*M* NaCl the development of turbidity was completely suppressed even at higher lysozyme-to-soy isolate or soy 7S ratios. Because the major noncovalent force that is affected in the range of 0–0.2*M* ionic strength is the electrostatic interaction (Eagland

1975), the results suggest that the mode of interaction between lysozyme and soy isolate or soy 7S is electrostatic in nature. This nonspecific interaction apparently reduces the net surface charge density of the soy protein-lysozyme complex, which promotes hydrophobic aggregation and development of turbidity in the system.

Unlike soy isolate or soy 7S, the ionic strength dependence of lysozyme-soy 11S interaction was more complex (Fig. 2C). At zero NaCl concentration, the development of turbidity as a function of lysozyme to soy 11S exhibited a hyperbolic curve and reached maximum turbidity at a ratio of lysozyme to soy 11S of about 0.5. At 0.075*M* NaCl the turbidity development was sigmoidal, and the turbidity did not reach saturation value even at the lysozyme-to-soy 11S ratio of 1.3. However, turbidity development was markedly suppressed at 0.1*M* NaCl concentration. The sigmoidal nature of lysozyme-soy 11S interaction at 0.05*M* and 0.075*M* NaCl concentrations may be attributed to changes in the oligomeric structure of soy 11S at various ionic strengths (Eldridge and Wolf 1967, Koshiyama 1972). It is known that at 0.5*M* ionic strength, the soy 11S protein exists as an oligomeric species that contains six acidic and six basic subunits and has a sedimentation velocity of 11S. When the ionic strength is decreased to 0.1*M*, two molecules of the 11S species associate to form a 15S species. When the ionic strength is further decreased below 0.1*M*, the 11S species dissociates into two identical units having a sedimentation coefficient of 7S. Such changes in the oligomeric structure of soy 11S protein between 0.01 and 0.1*M* ionic strength may alter its electrostatic properties, which may affect the extent of its interaction with lysozyme. Nonetheless, the data show that above 0.1*M* ionic strength the lysozyme-soy 11S interaction is suppressed, indicating involvement of electrostatic forces.

The effect of pH on the dissociation of lysozyme-soy 7S and lysozyme-soy 11S complexes as a function of ionic strength is shown in Figure 3. In these experiments, both the lysozyme-to-soy 7S and lysozyme-to-soy 11S ratios were maintained at a constant value of 0.8:1, and the development of turbidity at various ionic strengths in the medium was taken as the extent of complex formation. The 0.8:1 protein ratio was chosen on the basis of the data presented in Figure 1, in which both lysozyme-soy 7S and lysozyme-soy 11S systems exhibited near saturation turbidity values at the 0.8:1 protein ratio. Because precipitation of soy 11S occurs below pH 7.0 in Tris-HCl buffer (Thanh and Shibasaki 1976), the dissociation experiments in the case of lysozyme-soy 11S

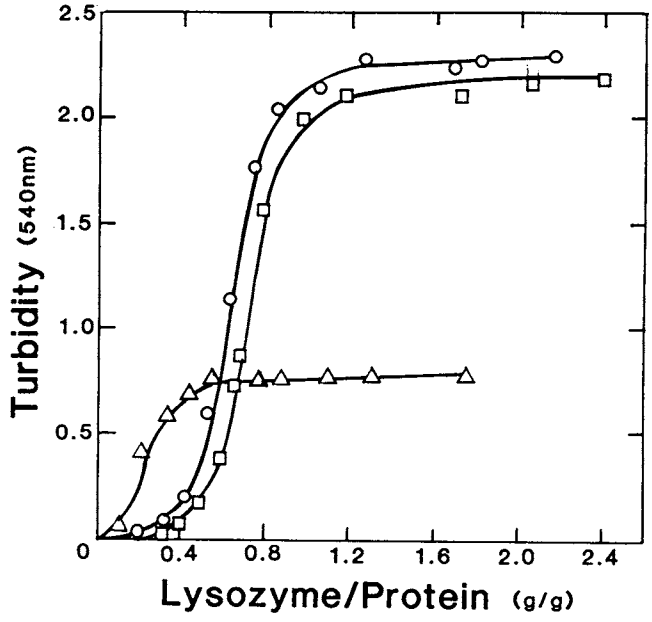

Fig. 1. Interaction of lysozyme with soy isolate (o—o), soy 7S (□—□), and soy 11S Δ—Δ) in 10 m*M* Tris-HCl buffer, pH 8.0. Increasing amounts of lysozyme stock solution were added to soy protein solutions. The total volume of the reaction mixture was 2 ml.

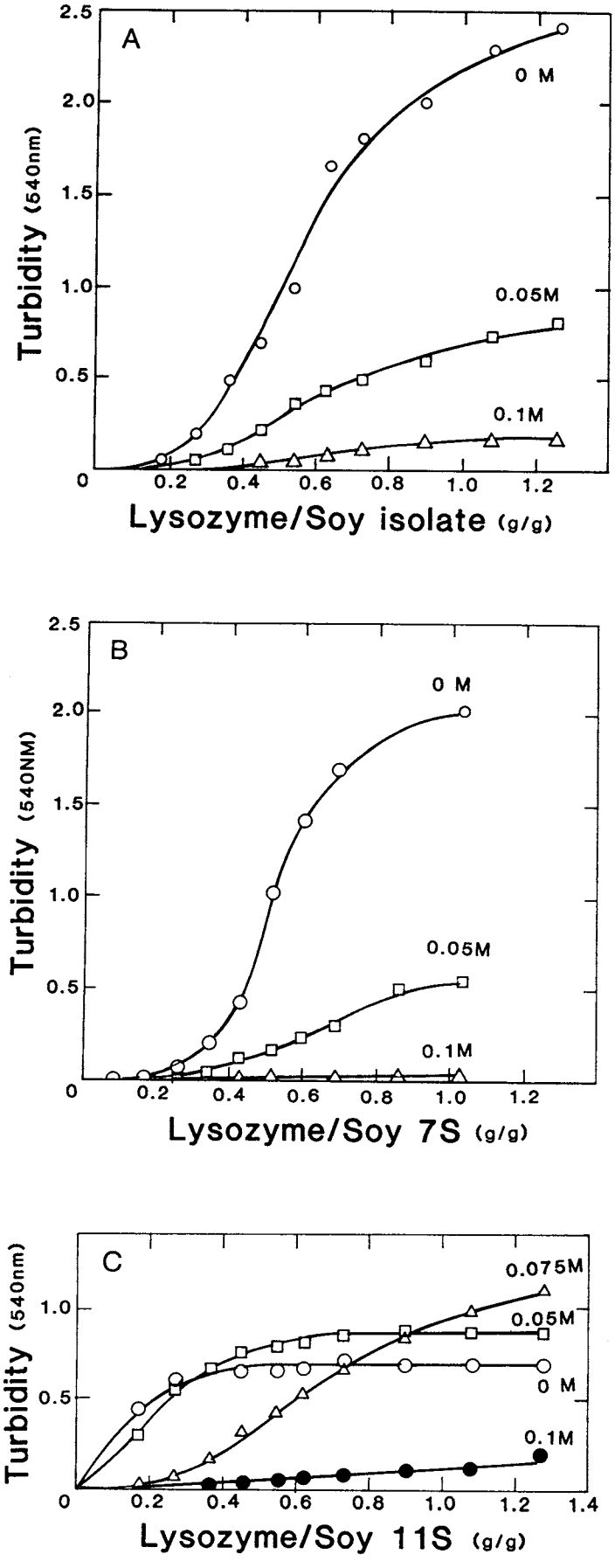

Fig. 2. Effect of ionic strength on the interaction of lysozyme with soy isolate (**A**), soy 7S (**B**), and soy 11S (**C**) at pH 8.0 in 10 m*M* Tris-HCl buffer. The ratio of lysozyme to soy proteins was increased by adding increased amounts of lysozyme stock solution. The total volume of the reaction mixture was adjusted to 2 ml with the buffer.

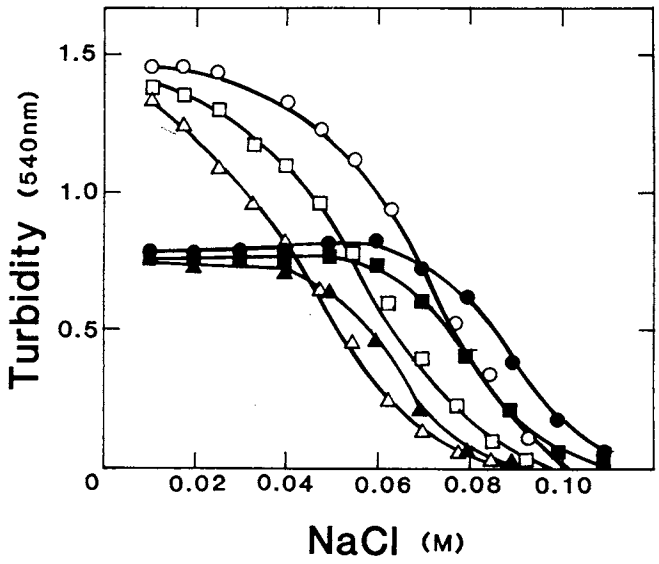

Fig. 3. Effect of pH on the ionic-strength-induced dissociation of lysozyme-soy 7S complex (open symbols) and lysozyme soy 11S (filled symbols). The experiments were done at a constant lysozyme-to-protein ratio of 0.8:1 (w/v). The symbols represent: o—o, pH 7.0; □—□, pH 8.0; Δ—Δ, pH 8.5; ●—●, pH 8.0; ■—■, pH 8.5; and ▲—▲, pH 9.0.

were carried out only between pH 8 to 9.

The ionic-strength-induced dissociation of the lysozyme-soy 7S complex was apparently complete at 0.1*M* NaCl at all pH levels studied (Fig. 3). At any given ionic strength the extent of dissociation was greater at pH 8.5 than at 7.0, suggesting that the interaction between lysozyme and soy 7S is stronger at pH 7.0. Because the isoelectric pH of lysozyme is 10.7, at pH 7.0 lysozyme is highly positively charged, and hence the complexation with soy 7S may involve more electrostatic bonds. As the pH is increased towards the isoelectric point of lysozyme, the decrease in electropositivity of lysozyme may decrease the number of electrostatic bonds formed in the complex; this will alter the equilibrium in favor of dissociation. Similar results were also obtained with the lysozyme-soy 11S system (Figure 3). However, up to about 0.04*M* NaCl, apparently no dissociation was observed with the lysozyme-soy 11S complexes, indicating that the complexation of lysozyme with soy 11S is stronger than that with soy 7S. This apparent higher affinity of lysozyme for soy 11S suggests that the negative charge density on the surface of soy 11S is greater than that of soy 7S, which requires higher ionic strength to neutralize the electrostatic interactions.

The results presented here clearly show that the interaction of lysozyme with soy proteins occurs predominantly via electrostatic forces, and these interactions are effectively neutralized at about 0.1–0.12*M* ionic strength in the medium. It may be postulated that similar protein-protein interactions occur in protein blends in which some acidic subunits of one protein component interact with basic subunits of other protein components during processing. Such protein-protein interactions may affect the functional behavior of each protein component in the system. Further studies are needed to characterize specific interactions that may occur in coprecipitated protein blends (Berardi and Cherry 1979, 1981) and the impact of such interactions on a specific functional property.

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