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Thermal Coagulation of Egg Albumin

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Turbidity was used to study thermocoagulation of egg albumin as a function of pH and protein concentration. Coagulation was dependent on pH and protein concentration. The net charge of egg albumin at the critical pH ($h_{T=0.5}$) upon coagulum formation increased linearly with protein concentration. The first step of coagulation reaction involved the formation of disulfide bonds and the exposure of hydrophobic groups. During further heating, egg albumin was polymerized by intermolecular sulfhydryl–disulfide exchange and the protein network structure was formed. The high net charge of proteins prevented the matrix from forming mainly by hydrophobic interaction. Succinylated egg albumin acted in a similar way with unmodified protein regarding relationship between protein concentration and the critical pH ($h_{T=0.5}$) upon coagulation.

The utilization of proteins as food is largely determined by their functional properties such as emulsifying activity, emulsion stability, foaming capacity, water-holding property, and gel formation.

Egg albumin is a key ingredient in many food products because of its ability to coagulate upon heating. The major proteins of egg white, ovalbumin and conalbumin, are heat coagulable and they are constituted of almost 70% of whole protein (Parkinson, 1966). Painter and Koening (1976) reported the Raman spectra of heat-coagulated ovalbumin. The spectral changes demonstrated the formation of extensive regions of antiparallel β-sheet between ovalbumin molecules. The heat denaturation of egg white and its component proteins was studied by differential scanning calorimetry (Donovan et al., 1975). At a heating rate of 10 °C/min, egg white at pH 7.0 showed two major endotherms, 65 and 84 °C, which were produced by the denaturation of conalbumin and ovalbumin, respectively. Seideman et al. (1963) reported that pH affected the coagulation temperature of egg white. Nakamura et al. (1978) confirmed that the heat-induced aggregation of ovalbumin depended on the degree of electrostatic repulsion on the denatured protein molecules. To prevent thermoaggregation and coagulation, an attempt was made to add anionic detergents (Hegg and Löfqvist, 1974, 1977) and sucrose (Seideman et al., 1963) to ovalbumin solution. In spite of these studies, the mechanism of coagulation is still not well understood.

In this paper, coagulum of egg albumin, an opaque and heat-irreversible gel, was investigated with respect to pH and protein concentration. The mechanism of three-dimensional network formation was also speculated.

MATERIALS AND METHODS

Materials. Egg albumin was purchased from Nakarai Chem. Ltd., Kyoto. This protein consisted of 80% ovalbumin and 20% conalbumin which were detected by NaDodSO$_4$–polyacrylamide gel electrophoresis method as described below. Other chemicals were reagent grade.

Succinylation of Egg Albumin. Succinylation was performed by the procedure of Groinger (1973). The succinylated protein was dialyzed against distilled water

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and lyophilized. The extent of succinylation of the protein was determined by the method of Kakade and Liener (1969).

Heat Treatment and Turbidimetry. Heat treatment for 7 mL of egg albumin solution was done in Coleman glass tubes (150 × 18 mm). The glass tubes were submerged in a water bath at 80 °C, and after termination of heating, the tubes were immediately cooled in ice–water. Turbidity was measured with Coleman Model 6-20 Junior 2 spectrophotometer at 600 nm. The protein concentration was determined by drying the samples to constant weight at 110 °C.

Determination of Sulfhydryl Groups. The determination of sulfhydryl groups was performed by the procedure of Habeeb (1972). Coagulums were dissolved in 0.1 M sodium phosphate buffer containing 2% sodium dodecyl sulfate and 0.05% EDTA (pH 8.0) using a glass homogenizer and diluted with the same buffer. To 3 mL of the sample solution was added 0.1 mL of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) solution (40 mg of DTNB in 10 mL of 0.1 M sodium phosphate buffer, pH 8.0) and the color absorbance was read at 412 nm. A molecular extinction coefficient of 1.36×10^4 M^{-1} cm^{-1} (Habeeb, 1972) was used to calculate moles of SH/10^5 g of protein. The protein concentration was derived from the absorbance at 280 nm using $E_{1cm}^{0.1\%} = 0.764$.

NaDodSO$_4$–Polyacrylamide Gel Electrophoresis. Gels of 7.5% (w/w) polyacrylamide were prepared as described by Shapiro et al. (1967). The electrophoresis buffer was 0.1 M phosphate with 0.5% NaDodSO$_4$ (pH 7.1). The samples were run at 8 mA/gel for 3 h. The gels were stained with a solution of 0.2% Coomassie Brilliant Blue G-250 in water/methanol/acetic acid (5:5:1, v/v/v) and destained by 7% acetic acid. Ovalbumin fraction on polyacrylamide gel was determined by scanning the gel with a Shimadzu dual-wavelength chromatoscanner CS-910.

Texturometer Measurement. The textural properties of coagulum were determined by a texturometer (General Food Co. GXT-2). The clearance between plunger and plate was adjusted to 2 mm. Hardness was measured from the profile of the first chew.

Electrometric Titration. Continuous titration under nitrogen was carried out in a water-jacketed vessel at 25 °C on a Radiometer PHA 943 pH meter using G2222C glass electrode and K4112 calomel electrode. Three milliliters of 1% egg albumin in 6 M guanidine hydrochloride (GuHCl) was placed in a jacketed vessel with a capacity of about 20 mL and adjusted below pH 2 with HCl. The system was stirred for 30 min in an atmosphere of nitrogen for temperature equilibration and removal of carbon dioxide. Then 0.2 N KOH was added from an autoburet ABU 12 of 0.500-mL capacity. Similar titration was performed on 3 mL of 6 M GuHCl. The titration curve of true protein was obtained by subtraction of the solvent curve from that of the protein solution.

Isoionic point measurement was performed by the procedure of Nozaki and Tanford (1967). The h value is defined as the net charge in 100 amino acid residues.

RESULTS

Denaturation of Egg Albumin by Heating. A 4.5% solution of egg albumin in water, pH 8.0, was heated at 80 °C. Figure 1A shows the changes in the amount of sulfhydryl groups by heating. The amount of free sulfhydryl groups decreased rapidly 1 min after the start of heating and reached a plateau after 5 min. For measuring amounts of the monomer of ovalbumin, the same solution used for sulfhydryl group determination was submitted to NaDodSO$_4$–polyacrylamide gel electrophoresis. Figure 2

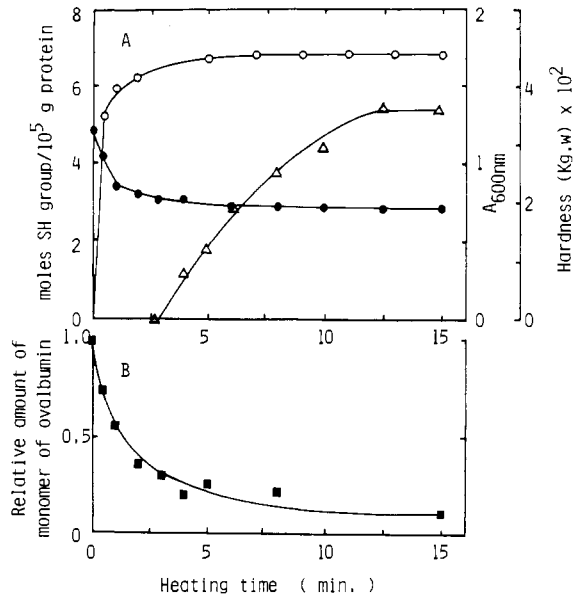


Figure 1. Denaturation of egg albumin by heating. (A) (○) Absorbance at wavelength of 600 nm, (●) changes in amount of SH groups, and (△) hardness of coagulum. (B) Changes in relative amount of egg albumin fraction.

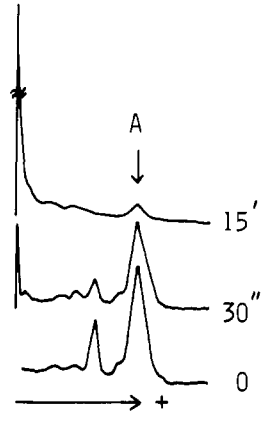


Figure 2. Densitometric scanning of NaDodSO$_4$–polyacrylamide gel electrophoresis pattern for egg albumin heated at 80 °C for period of 0 s, 30 s and 15 min. A indicates the monomeric fraction of ovalbumin.

shows the typical NaDodSO$_4$–gel electrophoretic profiles. The relative amount of monomer was obtained from results of densitometry. The relative amount of ovalbumin fraction (monomeric form) decreased rapidly 5 min after the start of heating and continued to decrease slowly up to 15 min (Figure 1B). The amounts of polymerized proteins increased with heating time (Figure 2). In the initial step of heating, a definite amount of free sulfhydryl groups formed disulfide bonds, and in the next step the proteins seemed to be polymerized gradually by sulfhydryl–disulfide exchange with additional heating time.

Turbidity increased rapidly 1 min after heating began and this increase occurred simultaneously with the decrease of free sulfhydryl groups. This turbidity may indicate the extent to which hydrophobic amino acid residues are exposed on the surface of protein molecules. The coagulum began to form 3 min after heating and the hardness reached its highest degree about 12 min after heating (Figure 1). This coagulum was a white, opaque, and heat-irreversible gel.

Effect of pH on Coagulation. When the pH of the system was varied, the changes in turbidity and the hardness of coagulums were measured (Figure 3). Above

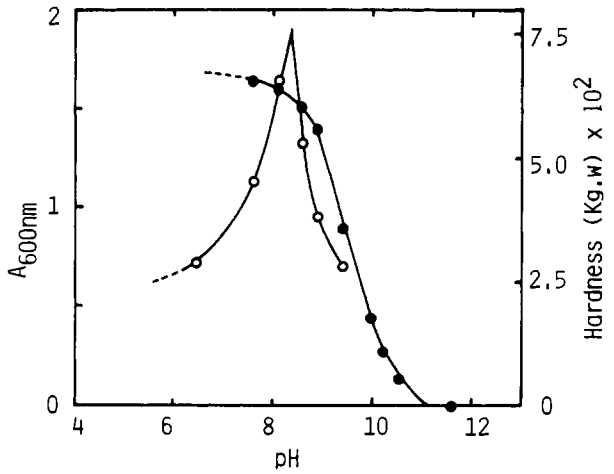


Figure 3. Effect of pH on coagulation. A 4.5% egg albumin solution was heated at 80 °C for 15 min: (●) absorbance at wavelength of 600 nm and (○) hardness of coagulum.

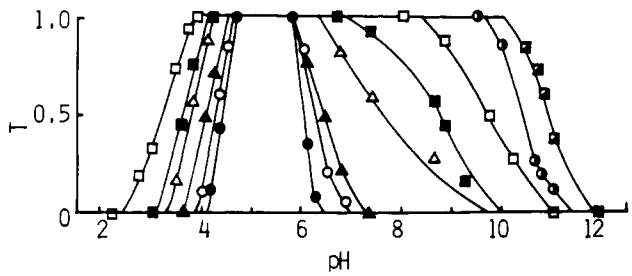


Figure 4. Effect of protein concentration on coagulation. Protein solutions were heated at 80 °C for 15 min. T indicates relative turbidity: alkaline side; (●) 0.09%, 0.23% protein concentration, (○) 0.45%, (▲) 0.92%, (△) 1.84%, (■) 2.66%, (□) 4.53%, (◑) 8.54% and (◪) 12.5%, acidic side; (●) 0.09%, 0.46%, (○) 0.93%, (▲) 1.84%, (△) 2.91%, (■) 4.47% and (□) 7.11%.

pH 11, the turbidity of heated egg albumin solution was not observed, but below pH 11, it increased, and at about pH 8, it reached a plateau. Coagulum began to form at about pH 9.5, and hardness reached its highest at pH 8.5 and decreased gradually below pH 8.5. These observations suggest that the coagulums exclude water and become fragile as the pH approaches the isoelectric point. When the hardness of the coagulum reached its highest, the absorbance at 600 nm was 1.55. This absorbance may indicate the point that the formation of coagulum was completed. Similar results were obtained with various protein concentrations, and the relative turbidity was expressed by the absorbance 1.55 at 1 in the following experiments.

Effects of Protein Concentration on Coagulation. The effects of protein concentration on coagulation are shown in Figure 4. The higher the protein concentration was, the more acidic or alkaline was the tendency of the turbidity. At low protein concentration, turbidity was observed only within the narrow pH range around the isoionic point, but at high concentration, it was observed in a wider pH range. The pH when the relative turbidity is 0.5 is expressed as the critical pH for coagulaum formation at some protein concentration.

The net charges of the critical pH ($h_{T=0.5}$) were derived from the data of Figure 4 and the net charges of egg albumin at each pH which was obtained from electrometric titration. The $h_{T=0.5}$ values were plotted as the protein concentrations (Figure 5A). Figure 5B shows the relationship between the hardness of coagulums and protein concentrations. In the coagulum forming range, $h_{T=0.5}$ increased linearly with protein concentration in both alkaline and acidic pH sides.

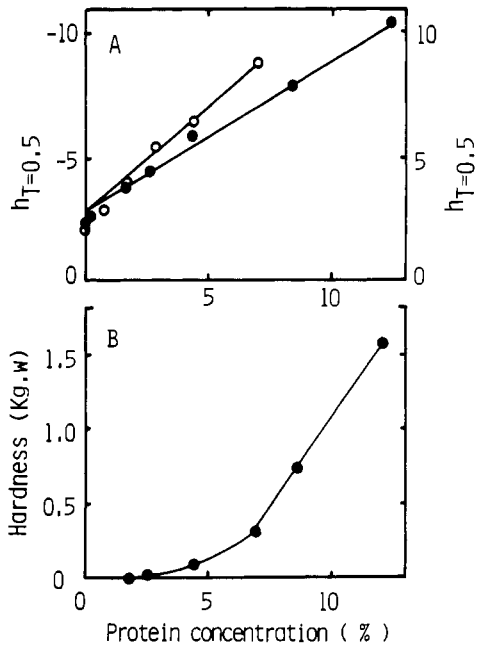


Figure 5. Relationship between (A) $h_{T=0.5}$ and protein concentration [(●) alkaline side and (○) acidic side] and (B) hardness of coagulum and protein concentration. Hardness refers to only the coagulum formed on the alkaline side and the maximal value that was measured at each protein concentration.

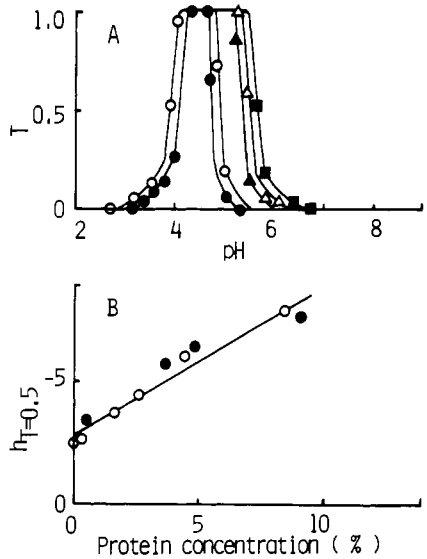


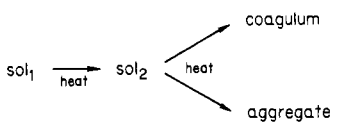
Figure 6. Coagulation of succinylated egg albumin. (A) Effect of protein concentration. T indicates relative turbidity: (●) 0.09% protein concentration, (○) 0.47%, (▲) 3.70%, (△) 4.91%, and (■) 9.21%. (B) Relationship of $h_{T=0.5}$ to protein concentration. The $h_{T=0.5}$ refers to only alkaline side: (●) 22.6% succinylated egg albumin, (○) native egg albumin.

Effect of Protein Concentration on Coagulation of Succinylated Egg Albumin. Succinylated egg albumin was coagulated by heating, and the effect of protein concentration was investigated. The isoionic point of 22.6% succinylated egg albumin shifted to pH 4.0 from pH 4.85 for unmodified egg albumin. The results of the turbidimetry experiment showed that succinylated egg albumin seems to coagulate only in the acidic region compared with unmodified protein (Figure 6A). The electrometric titration of succinylated egg albumin was carried out, and the $h_{T=0.5}$ of succinylated egg albumin was plotted with the protein concentration (Figure 6B). The $h_{T=0.5}$ of modified protein increased linearly with protein concentration as

in the case of the unmodified one, and the slopes for both were similar.

DISCUSSION

The thermocoagulation of proteins is formed by intermolecular interactions which produce a continuous, three-dimensional network exhibiting structural rigidity. First of all, protein denaturation occurs by heating and then protein–protein interactions take place with a three-dimensional gel network resulting. The overall scheme of the coagulation is illustrated below:



The native protein solution (sol_1) is converted to a denatured protein solution (sol_2) and then set to coagulum or aggregate by heating. The conditions for forming either coagulum or aggregate are determined by various factors such as protein concentration, molecular weight, heating time, and others. In the present study, the first step of coagulation, the $sol_1 \rightarrow sol_2$ reaction most likely involves the formation of disulfide bonds and the exposure of hydrophobic amino acid residues (Figure 1). Many investigators have reported that the changes of sulfhydryl groups by heating are closely related to the formation of protein gel (Jensen et al., 1950; Circle et al., 1964; Saio et al., 1971). During further heating, the egg albumin was cross-linked by sulfhydryl–disulfide exchange and then a continuous, three-dimensional network was produced. Since hydrophobic interaction is favored by a rise in temperature (Nèmethy et al., 1963), this type of bonding may mainly form an associated structural network, a coagulum.

In the case of egg albumin, the formation of coagulum was related to the increase of turbidity (Figure 3). Therefore, coagulum formation can be estimated by turbidity. The formation of coagulum was dependent on pH and protein concentration (Figures 3, 4, and 5). The higher the protein concentration, the wider the pH region of the formed coagulum. When protein concentration is high, the network structure formed can maintain a higher net charge. That is, the protein network becomes dense and the charged amino acid residues are separated individually, and consequently electrostatic forces may be suppressed.

Succinylation converts cationic amino groups to anionic residues and shifts the isoionic point to a more acidic side. The pH region for coagulum formation on modified egg albumin shifted to the acidic side compared to unmodified egg albumin (Figure 6A). But, the $h_{T=0.5}$ of modified egg albumin showed a dependency on the concentration of protein as did the unmodified one (Figure 6B). The $h_{T=0.5}$ was the same value when the protein concentration of modified and unmodified egg albumin is equal. Thermoaggregation of the dilute ovalbumin solution was affected by the net charge of protein molecules (Nakamura et al., 1978; Holme, 1963). Consequently, it seems likely that the network structure of egg albumin is formed by hydrophobic interactions and other bonds rather than ionic attractions, and the net charge of protein inhibits thermocoagulation.

As mentioned above, coagulation of egg albumin requires a low net charge but with a lower net charge, namely in the pH region near the isoionic point, a water-excluded and fragile coagulum is formed. In order to form a homogeneous coagulum, it is necessary to provide a proper balance between attractive and repulsive forces on protein structures.

The reaction of sol_2 to coagulum (or aggregate) is more complicated than the reaction of sol_1 to sol_2. The network structure is formed by protein–protein interactions and the interaction between protein and water may also play an important role (Chou and Morr, 1979). Since the coagulum is formed during heating, it seems likely that hydrophobic groups largely contribute to network formation. Egg albumin contains many hydrophobic amino acids that may be related to concentration-dependent coagulation.

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