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α-Casein Improves the Gel Properties of Dried Egg White

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The effects of addition of α-casein (α-CN) to dried egg white (DEW) were investigated by measuring transparency, hardness, and water-holding capacity (WHC) of the heat-induced gels. A DEW concentration of 8% (w/w) was required for formation of a self-supporting gel following heating at 80 °C for 20 min at pH 7. Solutions of α-CN, even up to a protein concentration of 12% (w/w), did not gel under the same conditions. The addition of α-CN (0.5−4%) to 8% DEW caused the increase in gel hardness gels, as compared with DEW gels alone at a total amount of protein concentrations, and the mixed gels became transparent with the increase of added α-CN concentrations. The 10% mixed protein solutions of α-CN (3−6%) and DEW (4−7%) formed transparent gels, although each protein did not gel individually at their protein concentrations. Mixture with 2:8 mixing ratio of α-CN to DEW at a total protein concentration of 10% showed synergistic effects in improving DEW gel properties above pH 7 and below 25 mM NaCl. The improvements (hardness, transparency, and WHC) of DEW gel by α-CN seem to be caused mainly by the inhibition of α-CN against heat coagulation of DEW protein.

KEYWORDS: Dried egg white; α-casein; mixed gel; gel properties; transparent gel

INTRODUCTION

Egg white (EW) is extensively utilized as functional food materials in food processing. Heat-induced gelation is one of its important functional properties with respect to EW usage in food systems (*1*). The gel formation of EW is sensitively affected by various factors, including pH, ionic strength, and salts (*2*−*5*). Most commercially available EW gives turbid products on heating. Thus, the nature of EW may not always be suitable for food materials. Preparation of transparent products from EW on heating would be favorable to further applications of EW as functional food materials.

Milk proteins are also used in food products for nutritive value and emulsifying, foaming, and gelling properties (*6*, *7*). Many studies have been published on the blends of food proteins for the improvement of nutritive values and food functionality. Such blends seem to cause protein−protein interactions on foaming, emulsifying, and heat-set gelling (*8*−*13*). Accordingly, physicochemical properties of a food protein gel may be altered by blending a different protein during heating for gelling. Milk proteins and egg proteins are usually mixed in food formulas; however, the rheological studies of the blend gels of EW proteins and casein proteins are quite few (*13*). We have found that a transparent and firm gel could be prepared from EW by the incorporation of α-casein (α-CN) on heating, suggesting that EW proteins and α-CN interact each other during gelling. In this paper, we report the characteristics of heat-induced gels made from a mixed system of dried egg white (DEW) and α-CN. The possible mechanism by which the mixture forms a transparent, firm gel is discussed.

MATERIALS AND METHODS

Materials. DEW, spray-dried at an exhaust temperature of 60−70 °C after decarbohydrate treatment by a glucose oxidase−catalase enzyme system, was provided by Q. P. Corp. (Tokyo, Japan), dialyzed extensively against distilled water, and then centrifuged at 10 000*g* for 20 min at 4 °C to remove small amounts of insoluble materials. The supernatant was then freeze-dried. After they were freeze-dried, samples were stored in a desiccator at 4 °C. α-CN (from bovine milk containing minimum 70% as α-CN) was purchased from Sigma Chemicals (St. Louis, MO) and was used without further purification. All other chemicals used in this study were of reagent grade.

Method of Gelation and Measurement of Gel Properties. A stock solution (20% protein, w/w) of DEW was prepared in distilled water, the stock solution was diluted with water to give the desired protein concentration, and then, the pH was adjusted to the indicated pH with 1 M NaOH or HCl. On mixed system, various amounts of α-CN were added directly to the DEW solution of the desired protein concentration as a solid and stirred gently for 10 min at room temperature. Finally, the DEW solution containing α-CN was adjusted to the indicated pH before the following gelation step. A solution (1 mL) of DEW with or without α-CN was put into glass tubes (6.0 mm in diameter) previously treated with Sigmacote (Sigma Chemical Co.). The content of each tube was deaerated by placing the tube in a Sharp sonicator (model UT-205, Tokyo) under vacuum for 1 min. The tubes were heat-sealed and heated for 20 min in a water bath at 80 °C for gelling. After they were heated, the tubes were removed from the water bath and held overnight at 4 °C before testing. After the tubes were tempered at room temperature, the protein gel was taken out of each tube without disrupting the gel surface. Each gel was cut into uniformly flat 5.0 mm thick sections and compressed to 40% of its original height by a

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tensile tester (Tensilon UTM-II, Toyo Baldwin Co., Tokyo) as previously described (*14*). The force required to compress the gel to 40% was expressed as gel hardness. None of gels were fractured under the compression conditions. Water-holding capacity (WHC) of protein gel was measured by centrifugation as reported previously (*15*). After the gel was centrifuged at 2000*g* for 15 min at 20 °C using a 2 mL centrifugal tube with a 0.45 μm filter (Advantec MF045, Tokyo), the amount of water dropped from the gel was then weighed. The WHC of the gel was given by [1 − (weight of water separated)/(weight of initial gel)] × 100.

Gel Solubilization. The solubilization of proteins in the gel matrix was conducted using various agents, which differ from each other according to their ability to cleave intermolecular bonds: electrostatic and hydrogen bonds (0.6 M NaCl), hydrogen bonds (1.5 M urea), hydrogen and hydrophobic bonds (8 M urea), and disulfide bonds (10 mM dithiothreitol, DTT) (*16*, *17*). The gels were treated independently with 0.6 M NaCl (solution A), 0.6 M NaCl plus 1 M urea (solution B), 0.6 M NaCl plus 8 M urea (solution C), and 0.6 M NaCl and 8 M urea plus 10 mM DTT (solution D). The gel containing each solution was homogenized with a Polytron homogenizer (Kinematica PT10-35, Luzern, Switzerland) at 2000 rpm for 1 min below 25 °C and then centrifuged. The supernatant fractions were analyzed for protein solubility. Protein solubility was calculated as a percentage of protein content of the supernatant as compared with the total protein content. The composition of protein thus solubilized was next analyzed by electrophoresis.

Protein Determination. Protein concentration was determined by the method of Lowry et al. (*18*) as modified by Bensadoun and Weinstein (*19*).

Gel Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was performed by using a thin slab gel electrophoresis apparatus (Advantec EP-080, Tokyo) with a slab gel made from 5% stacking gel and 15% separating gel, according to the method of Laemmli (*20*). The electrophoresis was done in both reducing and nonreducing conditions in the presence and absence of 2-mercaptoethanol (2-ME), respectively. To assess structural changes of protein and protein interaction, native PAGE without SDS and reducing agent was also performed in 7.5% polyacrylamide gel sheet, as described by Davis (*21*). All gels were fixed and stained with Coomassie Brilliant Blue Stain. Molecular weight protein standards (phosphorylase b, 97 kDa; bovine serum albumin, 66 kDa; aldorase 42 kDa; carbonic anhydrase, 30 kDa; trypsin inhibitor, 20 kDa; lysozyme, 14 kDa; Daiichi Pure Chemicals, Tokyo) were applied on each SDS gel for comparison.

Protein−Protein Interaction. Two milliliters of a dilute DEW solution (1−3%) in 10 mM sodium phosphate buffer (pH 7.0, heating buffer) and α-CN at a weight ratio of 0.5 to the DEW protein were placed in glass vials and then heated at 80 °C for 20 min. The turbidity of the solution in each vial was measured at 500 nm in a Hitachi spectrophotometer (model U-2000, Tokyo). In some cases, insoluble aggregates formed in 1% DEW solution after heating were collected by centrifugation. The precipitate was washed twice with the heating buffer and then dispersed in electrophoretic solvent for electrophoresis.

Experimental Design. DEW powders used in this study were commercial products from two different lots provided by Q. P. Corp. in January 2001. Those powders had been stored at 4 °C in a sealed container prior to use. No differences were observed on the electrophoretic patterns and protein band intensities of those DEW powders, and gels from those samples presented very similar gel characteristics at pH 7 and protein concentration of 10% (w/w) (data not shown). All experiments were carried out with samples from two different lots. Each data value represents the mean of at least four determinations.

RESULTS AND DISCUSSION

Effects of Protein Concentration and Added α-CN on Gel Hardness. The minimum concentration of protein required for gel formation is an important criterion of the gel-forming ability of specific protein (*22*). The hardness and visual appearance of protein gels formed by heating DEW, α-CN, and their mixtures at different concentrations at pH 7 are shown in **Figure 1**. α-CN/DEW mixtures were prepared by mixing 8% (w/w) DEW and α-CN with various concentrations. A DEW concentration of 8% (w/w) was required for the formation of a self-supporting gel, and the firmness and turbidity of DEW gels alone increased with increasing protein concentrations. α-CN did not gel under the heating conditions for gelling even up to a protein concentration of 12% (w/w). α-CN/DEW mixtures formed transparent gels when the proportion of α-CN was increased. The addition of α-CN (0.5−3%) to 8% DEW caused a remarkable increase of gel hardness as compared with DEW gel alone at the total amount of proteins, although α-CN itself would not form a gel at the protein concentrations in the mixture. However, the addition of 5% α-CN to 8% DEW resulted in a reduction of gel hardness when compared with 13% DEW gel alone, suggesting the formation of a phase-separated gel in the large volume fraction of α-CN. We found from the result of **Figure 1** that replacing DEW by α-CN within a restricted range of 9−12% DEW, in which the DEW protein concentration was kept constant at 8%, was able to change a turbid DEW gel to a firmer and transparent gel. Such gel properties of the mixed gel were considered to have reflected a positive interaction between α-CN and proteins in DEW on heating.

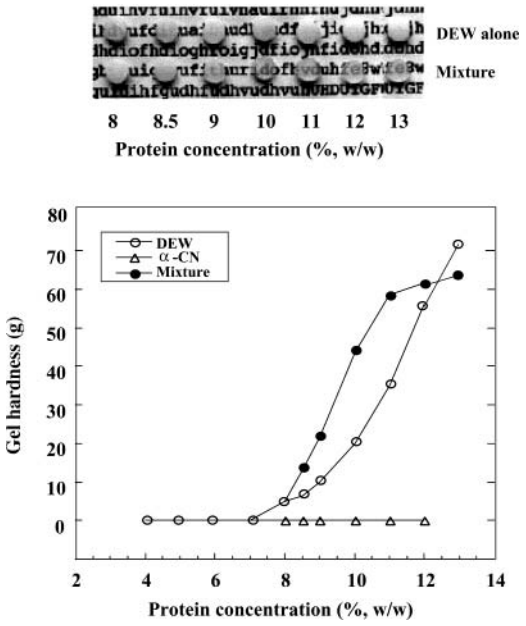

Figure 1. Effect of protein concentration on hardness and appearance of various protein gels. The mixed gels were made from mixtures of 8% (w/w) DEW and α-CN with various concentrations.

Figure 2 shows the effect of the mixing ratio of α-CN to DEW at constant total protein concentration (10%, w/w) on the gel hardness and visual appearance of mixed gels. The gel hardness behavior of mixed gel was apparently different from that of DEW gel alone, and the firmness of mixed gel was much higher when compared with the same concentrations of DEW. In addition, the mixtures of DEW below 7% (w/w) and α-CN formed transparent gels, although each protein did not individually gel at their protein concentrations. The result suggested that DEW and α-CN might cause synergistic interactions on gel formation. However, a DEW concentration above 4% in the mixed system at a total protein concentration of 10% (w/w) was required for the formation of a self-supporting gel. At the 2:8 mixing ratio of α-CN to DEW, the gel hardness gave the maximum value, and the visual appearance of the gel became transparent, while gels from 8 and 10% (w/w) DEW solutions were still turbid (**Figure 1**). It was found that improvements of

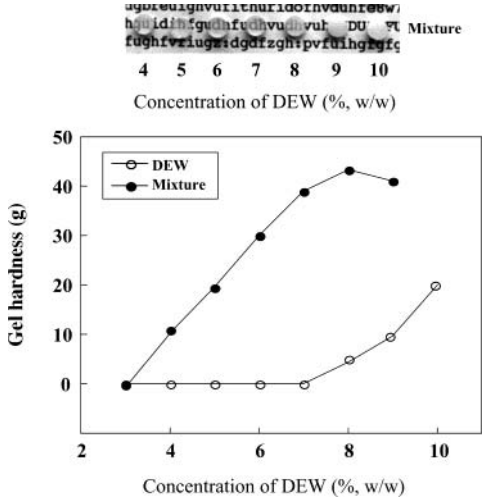

Figure 2. Effect of the mixing ratio of α-CN to DEW on hardness and appearance of the mixed gel. The mixed gels were made from mixtures of DEW and α-CN at various concentrations; the total protein concentration was kept constant at 10% (w/w).

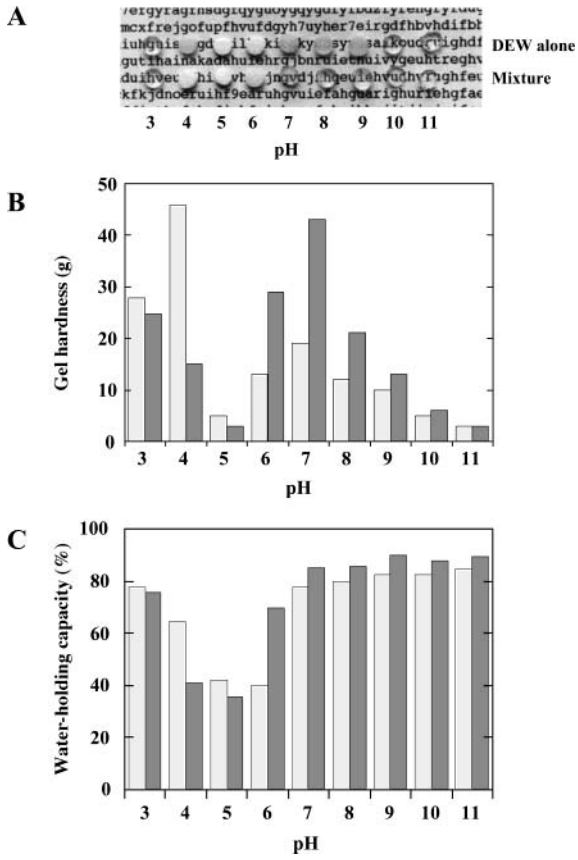

Figure 3. Effect of pH on properties of heat-induced gels from 10% DEW (light gray) and the mixture of 2% α-CN and 8% DEW (dark gray).

hardness and turbidity for DEW gel at pH 7 could be achieved by the replacement or addition of α-CN. Thus, the 2:8 mixing ratio of α-CN to DEW at total protein concentration of 10% (w/w) was used for the following experiments in which the effects of pH and ionic strength on gel properties were investigated. To elucidate the functional role of α-CN, 10% DEW solution was used as the control experiment.

Effects of pH and NaCl Concentration on Gelation. The properties of heat-induced gels from EW protein are sensitively affected by various factors, including pH and ionic strength (*2*−*5*). The effect of pH on the characteristics (gel hardness, transparency, and WHC) of heat-induced gels from DEW alone and α-CN/DEW mixture is shown in **Figure 3**. Protein solutions (10%, w/w) were adjusted to pH values of 3−11 and then heated at 80 °C for 20 min. As shown in **Figure 3A**, control DEW samples formed milky white gels at pH values of 4−6 and turbid gels at pH values of 7−9, except that samples of pH 3 and above pH 10 gave transparent gels. Most commercially available EW gives milky white gels on heating when pH was close to the isoelectric point (pI 4.5) of ovalbumin (*23*), a major protein in EW (*4*). On the other hand, the mixed samples formed much clearer gels at pH values of 7−9 when compared with the DEW gel alone, whereas no effect of α-CN on gel transparency was observed at the pH values of 4−6 that were close to the pI of α-CN (pI 5.1) (*24*). The effect of pH on gel hardness and WHC is also shown in **Figure 3B**,**C**, respectively. The firmness of DEW gel alone gave maximum values at pH 4 and 7, showing minimum value at pH 5. A similar behavior was observed in the hardness of α-CN/DEW mixed gel, and the mixed gel resulted in the increased gel hardness at the alkaline pH region above 6 and the decreased hardness at the acidic pH region below 5, as compared with DEW gel alone. The result indicates that α-CN had synergistic effects on firmness of the mixed gel only when the pH was above the pI of α-CN. This would be related to the less soluble nature and the shift to positive charge of α-CN when the pH was close and below the pI of α-CN, respectively. Similarly, WHC of the mixed gel was high at pH above 6 and low at pH below 5, as compared with that of DEW gel alone. WHC of transparent or translucent gels was higher than that of turbid gels. The higher WHC of transparent gels

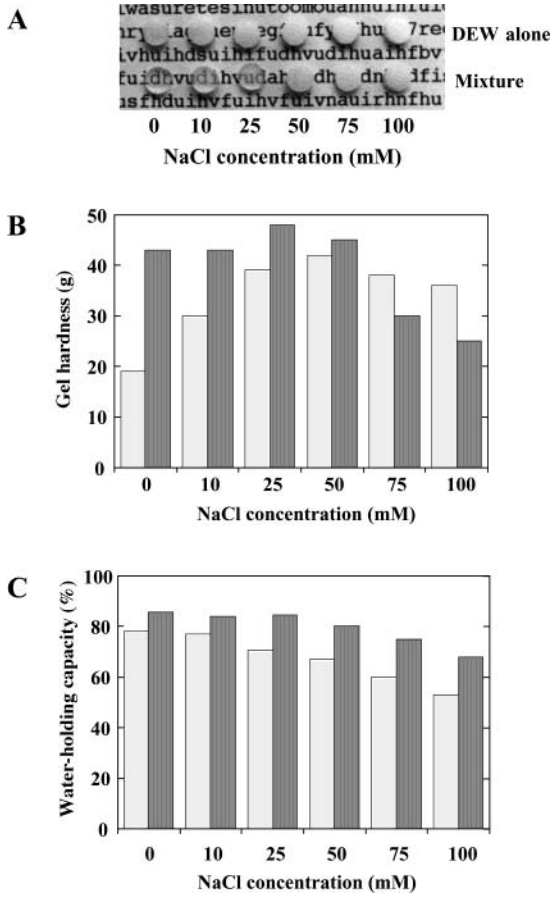

Figure 4. Effect of NaCl concentration on properties of heat-induced gels from 10% DEW (light gray) and the mixture of 2% α-CN and 8% DEW (dark gray).

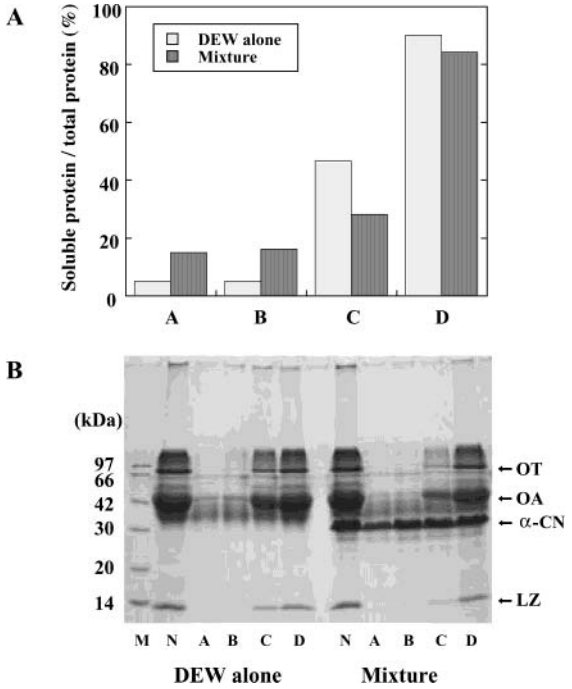

Figure 5. Solubilization of heat-induced gels from 10% DEW and the mixture of 2% α-CN and 8% DEW by various protein-denaturing reagents [0.6 M NaCl (A), 0.6 M NaCl plus 1.5 M urea (B), 0.6 M NaCl plus 8 M urea (C), or 0.6 M NaCl and 8 M urea plus 10 mM DTT (D)] and SDS–PAGE profiles of protein solubilized from gels by the indicated reagents. Lanes M and N indicate marker proteins (phosphorylase, 97 kDa; bovine serum albumin, 66 kDa; aldolase, 42 kDa; carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 20 kDa; and lysozyme, 14 kDa) and nonheated samples of DEW alone and α-CN/DEW mixture, respectively. The main proteins in DEW are represented as OT (ovotransferrin), OA (ovalbumin), and LZ (lysozyme).

from the mixed system might likewise be due to the construction of a fine gel network (*25*) by interactions of DEW proteins with α-CN.

The effect of NaCl concentration on the gel properties of heat-induced gels from DEW alone and α-CN/DEW mixture is shown in **Figure 4**. Protein solutions (10%, w/w) were adjusted to NaCl concentrations from 0 to 100 mM at pH 7 and then heated at 80 °C for 20 min. After the solutions were heated, the turbidity of DEW gel alone increased and became milky white with an increase in NaCl concentration. On the other hand, the mixed gel remained transparent at NaCl concentrations of 25 mM, although above 50 mM NaCl the mixed gels became turbid. As shown in **Figure 4B**, firmness of the mixed gels was higher at NaCl concentrations up to 50 mM and lowered above 75 mM NaCl, when compared with that of DEW gel alone. Thus, the enhancing effect by α-CN on gel hardness of the mixed gel was observed only in NaCl concentrations below 50 mM. WHC of heat-induced gels is shown in **Figure 4C**. WHC of DEW gel alone decreased gradually as NaCl concentration was increased. On the mixed gel, a distinguishable difference in WHC was not observed at NaCl concentrations below 25 mM, while above 50 mM NaCl the WHC decreased gradually. However, WHC of the mixed gel was higher than that of DEW gel alone in the range of NaCl concentrations tested. The strengthening and transparency of DEW gel due to the partial replacement of DEW by α-CN were lost at NaCl concentrations above 75 and 50 mM, respectively. The results of effects of pH and ionic strength on gel properties of the mixed gel

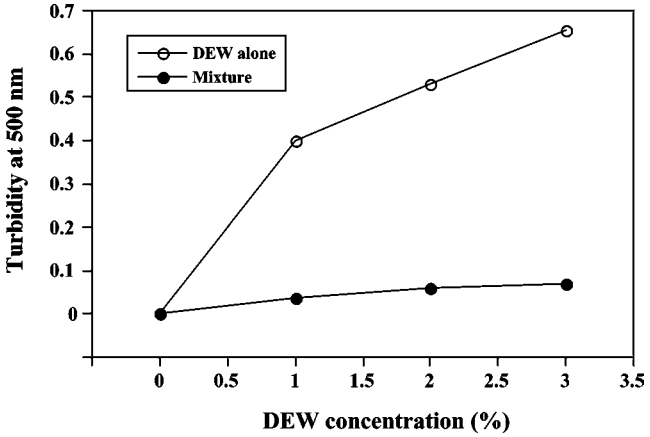

Figure 6. Changes in turbidity of DEW solutions (1–3%, w/w) with and without a weight ratio of α-CN to DEW of 0.5 following heating at 80 °C for 20 min and pH 7.

suggested weak electrostatic interactions between α-CN and DEW protein.

Gel Solubility. Our result showed that the α-CN/DEW mixture formed a transparent, firm gel with a high WHC at pH 7. Therefore, α-CN might affect denaturation and aggregation of DEW proteins during heating for gelling. To elucidate the role of added α-CN and the major forces involved in the formation and stabilization of the mixed gel matrix, protein solubility in solutions A−D in gels is shown in **Figure 5A**. The fractions soluble in solution A (0.6 M NaCl) and solution B (0.6 M NaCl plus 1.5 M urea) would be related to proteins bound weakly to the gel network through noncovalent bondings such as electrostatic interactions or hydrogen bonds or to proteins that were not involved in the formation of gel network. Gels treated with solutions A and B gave protein solubility values of around 15% of protein in the mixed gel and around 5% in the DEW gel alone. When gel was treated with solution C (0.6 M NaCl plus 8 M urea), the mixed gel exhibited much less solubility (about 27%) than did DEW gel alone (about 46%), suggesting that the structure of the mixed gel was more stable to solution C than that of DEW gel alone. On the other hand, solution D (0.6 M NaCl and 8 M urea plus 10 mM DTT) almost solubilized both gel samples. These results imply that the contribution of disulfide cross-linkages to the formation and stability of gels might be a large extent in the mixed gel as compared with DEW gel alone.

To identify the composition of protein fractions solubilized with the different solutions, the solubilized proteins were analyzed by SDS−PAGE with 2-ME (**Figure 5B**). In the profiles of protein fractions soluble in solutions A and B, apparent differences were not observed between DEW gel alone and the mixed gel, except that most of the added α-CN had been solubilized from the mixed gel. The result indicated that α-CN had bound weakly to the gel network in the mixed gel. The electrophoretic profile of protein fraction solubilized from the mixed gel with solution C (0.6 M NaCl plus 8 M urea) was apparently different from that of DEW gel alone, showing the decreased band intensity in order of ovotransferrin, ovalbumin, and lysozyme, which accounted for the lesser solubility of the mixed gel in solution C (**Figure 5A**). The result suggested that α-CN might cause the reinforcement and stabilization of gel matrix by enhancing disulfide cross-links formation among EW proteins through weak electrostatic interactions with DEW protein on heating.

Heat-Induced Interaction of DEW with α-CN. Protein gelation is thought to consist of multiphase reactions involving

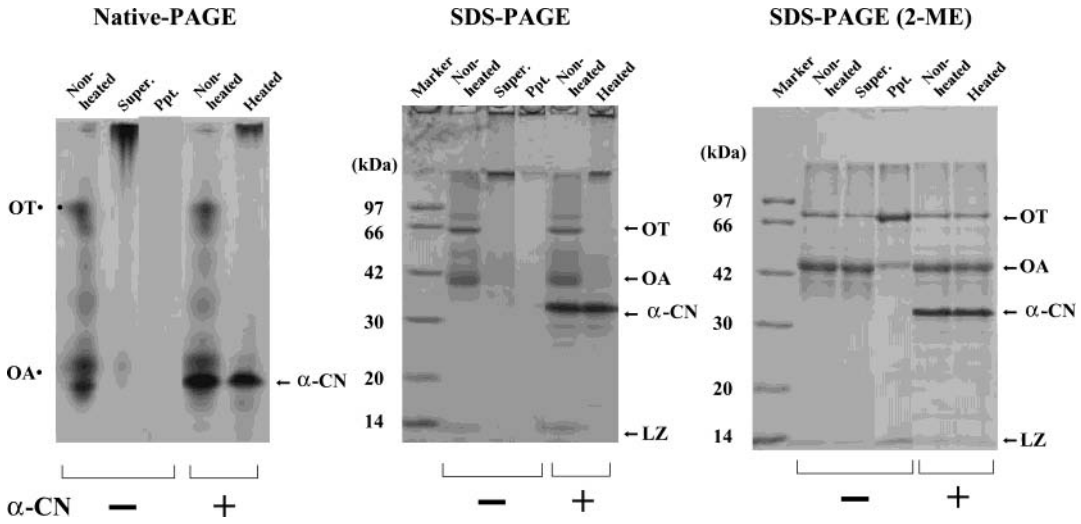

Figure 7. Native PAGE and SDS–PAGE analysis of 1% DEW solution with or without α-CN (0.5%) following heating at 80 °C for 20 min and pH 7. The turbid solution of DEW without α-CN after heating was separated into supernatant and precipitate fractions by centrifugation, and then, those fractions were examined by electrophoretic analysis. The marker proteins (phosphorylase, 97 kDa; bovine serum albumin, 66 kDa; aldolase, 42 kDa; carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 20 kDa; and lysozyme, 14 kDa) were applied on the SDS gel for comparison. The main proteins in DEW are represented as OT (ovotransferrin), OA (ovalbumin), and LZ (lysozyme).

the initial protein structure unfolding (denaturation) followed by the aggregation of polypeptide, which gradually proceeds to form an infinite gel network (*26*−*28*). To determine possible causative factors responsible for altered gel characteristics of the α-CN/DEW system, the aggregation step during DEW gelation was monitored in the presence and absence of α-CN (**Figure 6**). The weight ratio of α-CN to DEW was kept constant at 0.5. When the dilute solutions (1−3%) of DEW were heated at 80 °C for 20 min, the solutions resulted in increased turbidity with increasing protein concentration and produced insoluble aggregates. However, when DEW solution containing α-CN was heated at the same conditions, the turbidity development was almost completely prevented. In other words, clear solution can be prepared by the addition of α-CN even when DEW solution was heated at 80 °C and pH 7.

To elucidate the possible mechanism for the inhibition by α-CN against DEW protein coagulation, the mixed solution of DEW (1%) with α-CN (0.5%) was heated at 80 °C for 20 min, and the resulting clear solution was analyzed by native PAGE and SDS−PAGE in the presence and absence of 2-ME (**Figure 7**). In the native PAGE, polymer bands were observed at the top of the separating gel, with the disappearance of DEW proteins, while α-CN was dissociated almost completely. On SDS−PAGE under nonreducing conditions, the heated protein mixture was found to be composed of α-CN and soluble aggregates with higher molecular weights on each top of the stacking gel and separating gel. In the presence of SDS−PAGE and 2-ME, most soluble aggregates were dissolved into the constituent proteins of DEW. On the other hand, the precipitate fraction that was formed in heated DEW solution alone gave a band of aggregates remaining on the top of the stacking gel in SDS−PAGE under nonreducing conditions, while this protein band was dissociated almost completely into ovotransferrin, ovalbumin, and lysozyme on treatment with 2-ME, indicating that the precipitate had been formed through disulfide cross-links among their proteins. These results indicated that α-CN would inhibit the formation of insoluble aggregates induced through disulfide cross-links among DEW protein during heating. It is possible that the inhibiting effects of α-CN on the insoluble aggregate formation are responsible for the formation of a firmer and transparent gel from DEW at pH 7 and low ionic strength. The mechanism of formation of such gels from DEW in the presence of α-CN is being investigated in detail from heat-induced interactions between α-CN and EW proteins.

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