Characteristics of Heat-Induced Transparent Gels from Egg White by the Addition of Dextran Sulfate

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Heat-induced egg white gels containing polysaccharides were formed at pH 6.5, and the physicochemical properties were investigated. The hardness, water-holding capacity, and transparency of egg white gel markedly increased in the presence of dextran sulfate (DS) at a weight ratio of 0.05 to the protein. The effects were not observed by the addition of dextran (DX). Scanning electron microscopy showed that a very fine network structure had been formed in the gel containing DS. The action of DS on heat gelation of egg white appeared mainly due to chemical interactions rather than physical entanglement. Dilute solutions of egg white alone and egg white containing DX formed insoluble aggregates by protein interactions through disulfide cross-links during heating. From SDS−PAGE analysis with 2-mercaptoethanol, the insoluble aggregates consisted of ovalbumin, ovotransferrin, and lysozyme. The addition of DS inhibited formation of insoluble aggregates in egg white solution during heating. The inhibitory effect of DS would be ascribed to the formation of firmer and transparent egg white gels.

Keywords: *Egg white; dextran sulfate; transparent gel; microstructure; aggregation*

INTRODUCTION

Egg white is extensively utilized as a functional food material in food processing. Heat-induced gelation of egg white protein is one of its important functional properties with respect to usage in food systems (Kinsella, 1982). The gel properties are strongly affected by various factors of the medium, including pH, ionic strength, and salts (Woodward and Cotterill, 1986; Kitabatake et al., 1988). Egg white normally forms an opaque gel on heating. Therefore, the nature of egg white gel may limit greater utilization of the protein as gelling ingredients.

Interactions between proteins and polysaccharides have been shown to be an essential element in the study of food texture or processing (Stainsby, 1980). Many studies have been made on how polysaccharides alter the physicochemical properties of food protein gels (Circle et al., 1964; Imeson et al., 1977; Ustunol et al., 1992; etc). In some cases, salt linkages between both macromolecules can result in the formation of complex gels. It has been reported by Tolstoguzov (1986) that alginate−caseinate mixtures form complex gels under the conditions where both macromolecules did not gel. Watase and Nishinari (1983) reported that strong electrostatic forces between acylated gelatin and κ-carrageenan inhibited gel formation. Overall, the gelation properties of proteins can be modified or controlled through interactions with polysaccharides. We have found that transparent and firm gels could be prepared from egg white by the addition of dextran sulfate, an anionic polysaccharide, on heating. The objective of this study was to examine the physicochemical properties of gels formed from a composite system of egg white and dextran sulfate at selected pH (pH 6.5) and to elucidate the functional role of dextran sulfate.

MATERIALS AND METHODS

Materials. Egg white (EW), spray-dried at 60−70 °C after decarbohydrate treatment, was provided by QP Corp. (Tokyo, Japan), dialyzed extensively against distilled water, and then centrifuged at 10000*g* for 20 min at 4 °C to remove small amounts of insoluble materials. The supernatant was then freeze-dried. After freeze-drying, samples were stored in a desiccator at 4 °C. Dextran (DX) and dextran sulfate (sodium salt, DS) with molecular mass of 500 kDa were purchased from Sigma Chemical Co. (St. Louis, MO). These polysaccharides were soluble in cold water, and 2% (w/v) polysaccharide solution did not gel at pH 6.5 upon heating.

Gel Preparation. An aqueous dispersion (20% protein, w/v) of EW was prepared in deionized water and adjusted to 6.5 with 1 M NaOH. Various amounts of DS or DX were added to the protein solution as a solid. Finally, 10% (w/v) EW solution containing polysaccharides was stirred gently for 10 min at room temperature and readjusted to pH 6.5 before the following gelation step. Mixtures (1.0 mL) of EW and polysaccharide were put into glass tubes (6.0 mm in diameter) previously treated with Sigmacote (Sigma Chemical Co.). The contents of each tube were deaerated by placing in a Sharp sonicator (model UT-205, Tokyo) under vacuum for 1 min. The tubes were heat-sealed and heated for 30 min in a water bath at 80 °C. After heating, tubes were removed from the water bath and held overnight at 4 °C.

Determination of Gel Hardness. The tubes were tempered at room temperature for 20 min, and the gel was removed from each tube without disrupting the gel surface. Each gel was cut into uniformly flat 5.0 mm thick sections and compressed to 50% of its original height by a tensile tester (Tensilon UTM-II, Toyo Baldwin Co., Tokyo) as previously described (Matsudomi et al., 1994). The force required to compress the gel to 50% was expressed as gel hardness.

Measurement of Water-Holding Capacity. Water-holding capacity of intact gels was measured by centrifugation (Xiong and Brekke, 1989). After the gel was centrifuged (2000*g*) at 20 °C for 15 min using a 2 mL centrifugal tube with a 0.45 μm filter (Advantec, Tokyo), the amount of water dropped from the gel was then measured. Water-holding capacity was calculated by dividing the liquid weight by the initial gel weight and multiplying by 100.

Gel Microstructure. Samples of the gelled material were frozen in dry ice at −80 °C and then freeze-dried. Small pieces of each dried sample were mounted on aluminum stubs, using epoxy glue, and coated with a gold−platinum alloy using a sputter-coating device. The microstructural properties of gel samples thus prepared were examined by scanning electron microscopy (JEOL JSM-6100, Tokyo) at an accelerating voltage of 20 kV.

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Gel Solubilization. The solubilization of the 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) (Kauzmann, 1959; Tsuchiya et al., 1980; Wall and Huebner, 1981; Careche et al., 1995). The gels were treated independently with 0.6 M NaCl (solution A), 0.6 M NaCl plus 1.5 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 protocol followed for each kind of gel was as follows: A 0.1 mL aliquot of 10% (w/v) EW solution with or without 0.5% polysaccharides at pH 6.5 was placed into a glass vial (Pyrex culture tube 9826, Corning Glass Co., Corning, NY). Each vial was tightly closed with a screw cap, heated at 80 °C for 30 min, and cooled in water at 4 °C before 1.9 mL of one of the solutions (pH 6.5) indicated above was added. The gel containing each solution was homogenized with a Polytron homogenizer (Kinematica, 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 compared with the total protein content. The composition of protein thus solubilized was next analyzed by electrophoresis. The same treatment without heating was the control experiment.

Gel Electrophoresis. The SDS−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 (1970). Samples were prepared in Tris-glycine buffer (pH 8.3) containing 1% SDS in the presence and absence of 2-mercaptoethanol (ME). Electrophoresis was at a constant current of 15 mA for 3 h in Tris-glycine buffer containing 0.1% SDS. After electrophoresis, the gels were stained with Coomassie brilliant blue R250 (Wako Pure Chemical Co., Osaka, Japan). Molecular weight standards (phosphorylase B, serum albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, and lysozyme; Daiichi Pure Chemicals, Tokyo) were applied on each slab gel for comparison.

Protein Determination. Protein concentration was determined by the method of Lowry et al. (1951) as modified by Bensadoun and Weinstein (1976).

Protein−Protein Interaction. Two milliliters of a dilute EW (0.1−0.5%) solution in 10 mM phosphate buffer (pH 6.5, heating buffer) and polysaccharides at a weight ratio of 0.05 to the protein were placed in glass vials (Pyrex culture tubes, Corning 9826). The vials were tightly closed with screw caps to prevent evaporation of water during heating at 80 °C for 30 min. After heating, the vials were removed and immediately cooled in an ice bath. The turbidity of the solution in each vial was measured at 500 nm in a Hitachi spectrophotometer (model U-2000, Tokyo), using water as a standard. Each vial was shaken well before measuring the turbidity to ensure a uniform suspension. In some cases, the insoluble aggregate formed in 0.2% EW solution was collected by centrifugation. The precipitate was washed twice with the heating buffer and then dissolved in electrophoretic solvent for SDS−PAGE.

Experimental Design and Statistical Analysis. The EW powders used in this study were commercial products from two different baths provided by QP Corp. (Tokyo, Japan) in October 1995. These 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 these freeze-dried EW samples obtained after dialysis, and these EW samples presented very similar gel characteristics at pH 6.5 and protein concentration of 10% (w/v) during heating (data not shown). All experiments were carried out with samples from two different lots. Each data value represents the means of at least four determinations, and the error bars indicate standard deviation.

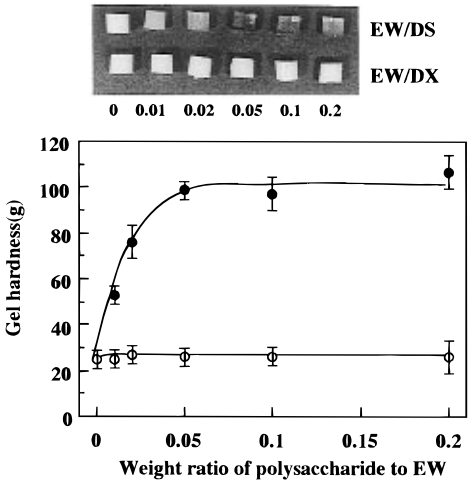

Figure 1. Hardness and appearance of gels made from 10% (w/v) egg white (EW) solution with different levels of polysaccharides [DS (●), DX (○)] following heating at 80 °C for 30 min and pH 6.5. Error bars denote standard deviation based on means of four determinations.

RESULTS AND DISCUSSION

Characteristics of DS-Induced EW Gel. Effects of added polysaccharide (DX or DS) on properties of heat-induced EW gel were investigated. The preparation of gel was performed at 10% (w/v) protein, pH 6.5, and heating at 80 °C for 30 min. Figure 1 shows changes in hardness and visual appearance of gels with an increase in the weight ratio of polysaccharide to EW. The EW gel formed in the absence of polysaccharides under these conditions was opaque, had a soft texture, and exhibited considerable syneresis. In the presence of DX, no significant changes in hardness and transparency of the gels were observed as compared to the EW alone gel. Addition of DS had a profound effect on the gel-forming ability of EW. DS led to a dramatic increase in gel hardness, leveling off at a weight ratio of the polysaccharide to EW of 0.05, and the gel hardness reached a value about 4 times high as compared to the hardness value of the EW alone gel. Depending on the concentration of added DS, the appearance of the gels changed surprisingly and became transparent at the weight ratio of 0.05. At the weight ratio of 0.2, hardness of the EW/DS gel remained the maximum value; however, the visual appearance of the gel became slightly turbid. The addition of a suitable amount of DS (at a weight ratio of 0.05%) to EW (10%, w/v) could increase markedly the transparency and hardness of the protein gel.

The water-holding capacity of the EW (10%, w/v) gel was investigated at a weight ratio of polysaccharides to the protein of 0.05 (Figure 2). The gel formed in the presence of DS had high water-holding capacity. When gel samples were centrifuged at 2000*g* for 15 min, the percentage syneresis was decreased from over 40% for the control (no additive) to less than 10% in the presence of DS. Addition of DX had no effect on the water-holding capacity of the EW gel. Such significant improvements (transparency, hardness, and water-holding capacity) of the EW gel at pH 6.5 were observed in the presence of DS, suggesting possible EW-DS interactions on heating. The action of DS on heat gelation of EW appeared mainly due to chemical interactions rather than physical entanglement.

Our result showed that the gel containing DS was transparent and firm and had high water-holding

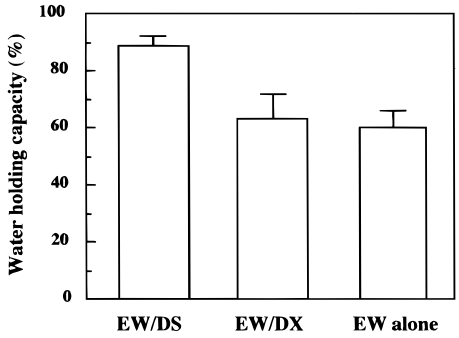

Figure 2. Water-holding capacity of gels made from 10% (w/v) EW solution with a weight ratio of polysaccharides to the protein of 0.05. Heating conditions were as in Figure 1. Error bars denote standard deviation based on means of four determinations.

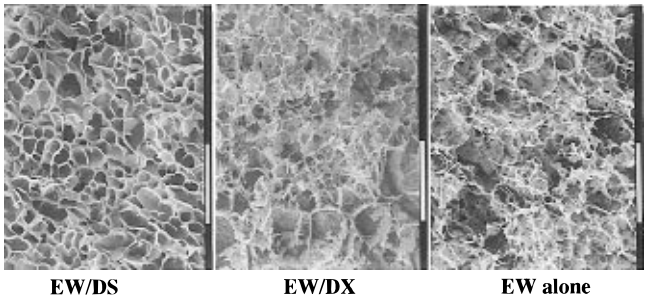

Figure 3. Scanning electron micrographs of gels made from 10% (w/v) EW solution with a weight ratio of polysaccharides to the protein of 0.05. Heating conditions were as in Figure 1. The bar represents 100 μm (figure is reproduced here at 45% of original).

capacity. The EW/DS gel characteristics markedly differed from the EW alone and EW/DX gels. Research on the microstructure of turbid and transparent bovine serum albumin gels was also reported by Clark et al. (1981). As this difference seemed to be affected by the fine structure of the gel, microstructural changes in the gel from the EW/DS system were compared with the EW alone gel or EW/DX gel, using a scanning electron microscope (Figure 3). A 300-fold magnification revealed a very fine network structure only in the gel containing DS, while the EW alone gel and EW/DX gel showed a rough surface. The microstructure of the transparent EW/DS gel was homogeneous and characterized by a uniform distribution of densely thick matrix, leading to a firm gel having high water-holding capacity. Opaque EW alone and EW/DX gels, however, displayed a nonhomogeneous gel microstructure consisting of a roughly thin matrix. Results from the present study and observations by Clark et al. (1981) indicate that gel clarity is related to the homogeneity of the gel matrix. DS might affect denaturation and aggregation of EW proteins during heating.

Effects of Heating Temperature on Gelation. The ability of a protein solution to gel is influenced by the extent of heat-induced protein denaturation (Nakamura et al., 1986; Kitabatake et al., 1989). The hardness and appearance of gels made from 10% (w/v) EW solution with or without polysaccharides by heating for 30 min at different temperatures are shown in Figure 4. The weight ratio of polysaccharide to the protein was kept constant at 0.05. The hardness of all types of gels increased progressively with an increase in heating temperature and reached a maximum value at 80 °C, leveling down slightly at around 85 °C. In the case of the EW/DX system or EW alone (no additive),

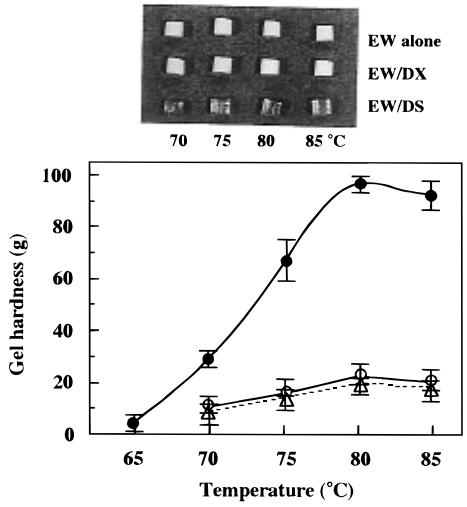

Figure 4. Hardness and appearance of gels made from 10% (w/v) EW solution with a weight ratio of polysaccharides to the protein of 0.05 following heating at pH 6.5 and for 30 min at different temperatures. Error bars denote standard deviation based on means of four determinations: (●) EW/DS, (○) EW/DX, (△) EW alone.

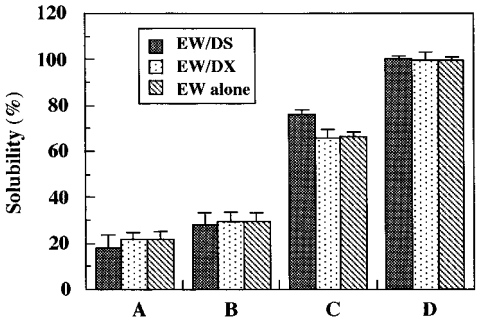

Figure 5. Solubilization of gels by various protein-denaturing reagents [0.6 M NaCl (A), 0.6 M NaCl plus 1.5 M urea (B), 0.6 M NaCl plus 8 M urea (C), or 0.6 M NaCl and 8 M urea plus 10 mM DTT (D)]. Gelling conditions were as in Figure 2. Error bars denote standard deviation based on means of five determinations.

no gelation occurred below 65 °C, although these solutions became viscous. There were no significant differences in turbidity and hardness between the EW alone gel and the EW/DX gel prepared at indicated temperatures above 70 °C. However, DS addition caused formation of a very weak gel at 65 °C and induced formation of a harder gel at heating temperatures from 65 to 85 °C, as compared to the case of EW/DX or EW alone. This result suggests that DS may promote protein unfolding at the lower temperature and strengthen the gel structure, facilitating favorable protein−protein and/or protein−DS interactions to lead to gelation.

Gel Solubility. In order to elucidate the major forces involved in the formation and stabilization of the gel matrix, protein solubility in solutions A−D in gels is shown in Figure 5. The fractions soluble in solution A (0.6 M NaCl) would be related to proteins bound weakly to the network through noncovalent bondings such as electrostatic interactions or hydrogen bonds or to proteins that were not involved in the formation of the network. Gels treated with solution A gave protein solubility values of around 20% of protein of gel. There were no significant differences among all types of gel, although the EW/DS gel did display a lower solubility in general. When gel was treated with solution B (0.6 M NaCl plus 1.5 M urea), approximately 30% of protein

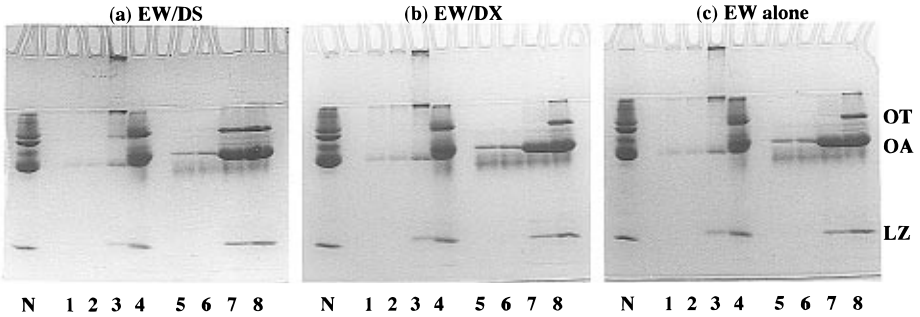

Figure 6. SDS−PAGE profiles of protein solubilized from gels by various protein-denaturing reagents indicated in Figure 5. Gelling conditions were as in Figure 2. The main proteins in EW are represented as OA (ovalbumin), OT (ovotransferrin), and LZ (lysozyme): lane N, native EW; lanes 1 and 5, protein solubilized with solution A; lanes 2 and 6, protein solubilized with solution B; lanes 3 and 7, protein solubilized with solution C; lanes 4 and 8, protein solubilized with solution D. The right lanes (lanes 5−8) of each slab gel were treated with ME.

was solubilized from all types of gel, and no significant differences were found among the gels. When gel was treated with solution C (0.6 M NaCl plus 8 M urea), about 65−77% of protein was solubilized from the gel, and the EW/DS gel exhibited more solubilization than did the EW alone or EW/DX gel. The result suggests that the protein gel network forming in the presence of DS had involved mainly hydrophobic interactions that were more exposed in these solubilization conditions. With solution D (0.6 M NaCl and 8 M urea plus 10 mM DTT), most proteins were solubilized completely in all types of gels, indicating that these gels have been stabilized partly by disulfide bonds.

In order to identify the composition of the protein fractions solubilized with the different solutions, the solubilized proteins were analyzed by SDS−PAGE (Figure 6). In the profiles of protein fractions solubilized with the four different solutions, no significant differences were found between the EW alone gel and the EW/DX gel. The electrophoretic profile of protein fraction solubilized from the EW/DS gel with solution C (0.6 M NaCl plus 8 M urea) was different from that of the other gel types, showing a band corresponding to ovotransferrin besides bands of ovalbumin and lysozyme (lane 3 in Figure 6a). The band corresponding to ovotransferrin was more pronounced after reduction with ME (lane 7 in Figure 6a), which accounted for the greater solubility of the EW/DS gel in solution C (Figure 5). This would suggest that part of ovotransferrin had been incorporated into the EW/DS gel network through noncovalent bondings including hydrophobic interactions and that most of the ovotransferrin was polymerized with ovalbumin and/or lysozyme by disulfide cross-links and then incorporated into the gel matrix through hydrophobic interactions. On the other hand, in the case of the protein fraction solubilized from the EW alone or EW/DX gel with solution C, no ovotransferrin was detected even on the slab gel in the presence of ME (lane 7 in Figures 6b,c), suggesting that ovotransferrin had been incorporated covalently into gel matrix through disulfide cross-links with the other protein of EW.

Heat-Induced Interaction of EW. Protein gelation is thought to consist of multiphase reactions involving the initial protein structure unfolding (denaturation) followed by the aggregation of polypeptides which gradually proceeds to form an infinite gel network (Nakamura et al., 1986; Ziegler and Foegeding, 1990; Matsudomi et al., 1993). In order to determine possible causative factors responsible for altered gel characteristics of the EW/DS system, the aggregation step during EW gelation was monitored in the presence and absence

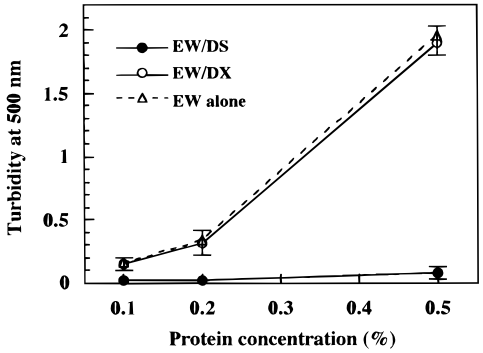

Figure 7. Changes in turbidity of EW solutions (0.1−0.5%, w/v) with a weight ratio of polysaccharides to the protein of 0.05 following heating at 80 °C for 30 min and pH 6.5. Error bars denote standard deviation based on means of four determinations.

of both polysaccharides (Figure 7). The weight ratio of polysaccharide to protein was kept constant at 0.05. When the dilute solutions (0.2%) of EW alone or EW/DX were heated at 80 °C for 30 min, these solutions became turbid, and insoluble aggregates were formed. However, when EW solution (0.2%) containing DS was heated at the same condition, the solution did not show any change in turbidity. In EW alone and EW/DX solutions, the turbidity dramatically increased with an increase of protein concentration. However, the development of turbidity was depressed in the presence of DS when compared at the same protein concentration. Addition of DS might inhibit formation of insoluble aggregates in EW during heating.

In order to elucidate the role of DS, the insoluble aggregates obtained from solutions (0.2%) of EW alone and EW/DX systems were examined by electrophoretic analysis. The turbid solutions were separated into soluble and insoluble fractions by centrifugation, and these fractions obtained were dissolved separately in electrophoretic solvent for SDS−PAGE. Figure 8 shows the SDS−PAGE profiles of the supernatant and precipitate fractions, comparing with the case of the EW/DS solution (0.2%). The SDS−PAGE profile of the heat-treated EW/DS solution that did not form insoluble aggregates revealed the presence of ovotransferrin and very small amounts of lysozyme (lane 1 in Figure 8A), although these bands were less apparent in the supernatant fraction of EW alone and EW/DX systems (lanes 2 and 3 in Figure 8A). Both these bands were intense in the SDS−PAGE profile after reduction with ME (lane 1 in Figure 8B). The presence of ovotransferrin and lysozyme in the soluble fraction of the EW/DS system may reflect the fact that these proteins could be more

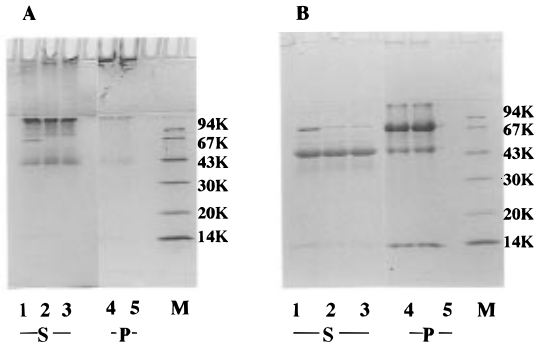

Figure 8. SDS−PAGE profiles of the supernatant (S) and precipitate fractions (P) obtained from 0.2% EW solution with a weight ratio of polysaccharides to the protein of 0.05 following heating at 80 °C for 30 min and pH 6.5: lane M, molecular marker proteins; lane 1, EW/DS; lanes 2 and 4, EW/DX; lanes 3 and 5, EW alone. Panels A and B show SDS−PAGE profiles in the absence and presence of ME, respectively.

easily released from the gel matrix of the EW/DS gel against solution C (0.6 M NaCl plus 8 M urea) (lanes 3 and 7 in Figure 6a). In EW alone and EW/DX systems, the protein in the precipitate did not enter the slab gel and, in the absence of ME, remained on top of the stacking gel (lanes 4 and 5 in Figure 8A), while this protein band was dissociated almost completely into ovotransferrin, ovalbumin, and lysozyme in the presence of ME (lanes 4 and 5 in Figure 8B), suggesting that the insoluble aggregate had been formed by their protein interaction through disulfide bonds. These results indicate that DS would inhibit the formation of insoluble aggregates induced by disulfide cross-links during heating. It is possible that the inhibitory effect of DS on insoluble aggregate formation is responsible for the formation of a firmer and transparent gel from EW. Mechanism of formation of such gels from EW in the presence of DS is being investigated in detail.

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