Inhibition against Heat Coagulation of Ovotransferrin by Ovalbumin Dry-Heated at 120 °C

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Ovalbumin (OVA) was aggregated stepwise by dry heating at 120 °C with a gradual increase in its heating times (10 min−6 h). The inhibiting effects of DHOVAs (OVAs dry-heated for various times up to 6 h) on the heat coagulation of ovotransferrin (OT) were studied. DHOVAs and OT were solubilized at 5% (w/w) concentration with 10 mM sodium phosphate buffer, pH 7.4. Their solutions were mixed at the volume ratio of 1:1 and reheated at 60 °C for 3.5 min. Some remarkable differences according to dry-heating time were observed: coagulum formations were greatly inhibited in the solutions mixed with DHOVAs treated for more than around 2.0 h, with decreasing turbidity as dry-heating time increased. In addition, the effects of reheating time and temperature, as well as those of pH and ionic strength, were also examined on coagulum formation and turbidity development in connection with dry-heating time. Thus, the inhibiting effects of dry-heated egg white on the heat coagulation of fresh egg white previously described were confirmed on the molecular level of OVA and OT.

Keywords: *Ovalbumin; ovotransferrin; dry heating; heat coagulation*

INTRODUCTION

Pasteurization of egg white prior to its utilization as a food material is generally necessary for reducing or eliminating the load of *Salmonella* or other organisms without significant impairment of its functional properties. The pasteurization of egg white is usually carried out for a few minutes at a temperature near 60 °C because of the high heat sensitivity of egg white proteins. However, functional properties such as whipping were also reported to begin deteriorating at temperatures as low as 54 °C (Cunningham and Lineweaver, 1965). It was shown that ovotransferrin (OT) was the most thermolabile protein in the egg white, and heating of egg white (pH 7) at 60 °C for 5 min caused the aggregation only of OT (Matsuda et al., 1981). Moreover, egg white was described as forming a soft opaque gel which depended on the aggregation of OT at a lower temperature of around 65 °C (Yamashita et al., 1998). Therefore, inhibition of OT aggregation/coagulation−gelation at heating around 60 °C is necessary for the pasteurization of egg white. This called for the development of a method of heat pasteurization of egg white without any loss of functional properties or the formation of coagulum or gel.

In previous studies, we showed that when solutions of spray-dried (60−70 °C) and then heat-treated egg white (55−65 °C, 3 days in a dry state to reduce microbial numbers) were reheated at 60 °C at various times, the insoluble materials in their solutions almost failed to form. Such a phenomenon was also found to be mainly attributable to changes in proteins occurring in the process of heating in a dry state (Xu et al., 1997). It was also indicated that a transparent solution without any coagula in dry-heated egg white (DHEW) when reheated (60 °C, 3.5 min) could be prepared by using a procedure of shorter dry-heating time and higher dry-heating temperature. When 10% (w/w) DHEW (120 °C, 2−6 h) solutions or their mixtures with fresh egg white at the volume ratio of 1:1 were reheated at 60 °C for 3.5 min, coagulum formation was inhibited. Soluble aggregates of ovalbumin (OVA) formed in the dry-heating process were suggested to inhibit the formation of OT coagulum (Xu et al., 1998). In our study with DHEW and fresh egg white (Watanabe et al., 1999), the possible mechanism by which DHEW inhibits the heat coagulation of OT during dry heating and reheating was examined.

The aim of the present study was to obtain a better understanding on a molecular basis of the phenomena and mechanism described above. Therefore, we determined the inhibiting effects of OVAs dry-heated at 120 °C for various times up to 6 h on the formation of OT coagulum under various reheating conditions of time and temperature, as well as pH and ionic strength of the solvent.

MATERIALS AND METHODS

Materials. OVA (Grade V) and OT (Type I, from chicken egg white that was substantially iron-free) were purchased from Sigma Chemicals, St. Louis, MO, and used without further purification. All other reagents were of analytical grade.

Preparation of Samples. Reagents of OVA and OT were dissolved at a concentration of 7% (w/w) with distilled water, and freeze-dried after adjusting pH to 7.4, centrifuging (2000*g*, 5 min) and membrane-filtrating (0.45 μm) to remove the insoluble proteins. The freeze-dried OVA (FDOVA, moisture content: 4.5%) in a sealed vial was dry-heated in the oven at 120 °C for various durations up to 6 h, and the samples prepared were designated DHOVAs. The freeze-dried OT was termed FDOT.

Gel Filtration. A gel filtration experiment using a Sephacryl S-400 column (2.0 × 75 cm) was carried out to examine the degree of aggregate formation in DHOVAs (dry-heating condition, 120 °C, 30 min and 6 h) for comparisons with OVA and

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FDOVA. Samples were solubilized at the concentration of 150 mg in 3 mL of 20 mM phosphate buffer, pH 7.4, applied to the column, and eluted with the same phosphate buffer. The 3.7-mL fractions were collected and monitored at 280 nm.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). SDS−PAGE was carried out by the procedures of Laemmli (1970) using 12.5% acrylamide separating gel as described in a previous paper (Xu et al., 1997), and by Weber and Osborn (1969) and Davies and Stark (1970) using 3.5% acrylamide separating gels as outlined in Technical Bulletin No. MWS-877X (Sigma).

Heat Aggregation or Coagulation of DHOVA. The DHOVAs (up to 6 h) were solubilized to obtain a concentration of 5% (w/w) with 10 mM sodium phosphate buffer at pH 7.4. Each sample (1.5 mL) of the prepared DHOVA solutions in stoppered test tubes [11.7 mm (i.d) × 2 mm] was reheated (60 °C, 3.5 min) in a water bath under set conditions, cooled immediately in tap water, and centrifuged at 2000*g* for 5 min. Soluble protein content in the resultant supernatant was measured following appropriate dilution with 10 mM phosphate buffer, pH 7.4, according to the method of Lowry et al. (1951), and the coagulated protein ratio (%, CPR) was obtained as follows: CPR = (SPCBH − SPCAH)/SPCBH × 100, SPCBH (soluble protein content in supernatant before reheating), SPCAH (soluble protein content in supernatant after reheating). Turbidities of their supernatants diluted 40 times with 10 mM phosphate buffer (pH 7.4) were measured at 540 nm. All measurements were done in triplicate.

Inhibiting Effects of DHOVA on Heat Coagulation of FDOT. The inhibiting effects of DHOVAs on the heat coagulation of FDOT in their mixtures of 1:1 in volume were determined under reheating of 60 °C for 3.5 min at pH 7.4 (10 mM sodium phosphate buffer), for comparison with heat coagulation in a single system of FDOVA or FDOT. Such inhibiting effects were also determined in the samples solubilized with distilled water, and various buffers [10 mM sodium phosphate buffers (pH 5.5, 6.5, 7.4, and 9.0), and 20 and 100 mM phosphate buffers (pH 7.4)] at reheating of 60 °C for 3.5 min, and in the samples solubilized with 20 mM phosphate buffer, pH 7.4 at 60 °C for various times (3.5−60 min) and temperatures (65−85 °C) for 30 s. All measurements were done in triplicate.

RESULTS

First of all, gel filtration profiles of OVA, FDOVA, and DHOVAs (30 min and 6 h) solutions were also compared (Figure 1). The elution pattern of OVA indicated a single peak. On the other hand, the pattern of FDOVA showed the formation of aggregates of more than dimer with monomer of OVA as major component, and the samples of DHOVAs (30 min and 6 h) depicted a broader peak, indicating the formation of various aggregates. Aggregates having a higher molecular weight in sample dry-heated for 6 h than for 30 min were observed. That is, DHOVA (6 h) was found to contain aggregates having apparent weights ranging more than 669 kDa (molecular marker: thyroglobulin), although the monomer of OVA in the DHOVA also remained as the main component.

The SDS−PAGE patterns, stained with Coomassie brilliant blue R-250, of the DHOVAs (up to 6 h) in the absence (Figure 2A) and presence (Figure 2B) of 2-mercaptoethanol (2-ME) are shown in Figure 2. The SDS−PAGE patterns of DHOVA (120 °C, 1.5 h), stained with a silver staining kit, corresponding to the sample of Figure 2A, lane 4, were also shown in Figure 2C. The molecular weights of these samples were determined by comparing the electorophoretic mobilities with cross-linked phosphorylase b SDS molecular weight markers (Sigma). In the absence of 2-ME, FDOVA was found to already contain the dimer and trimer with the monomer

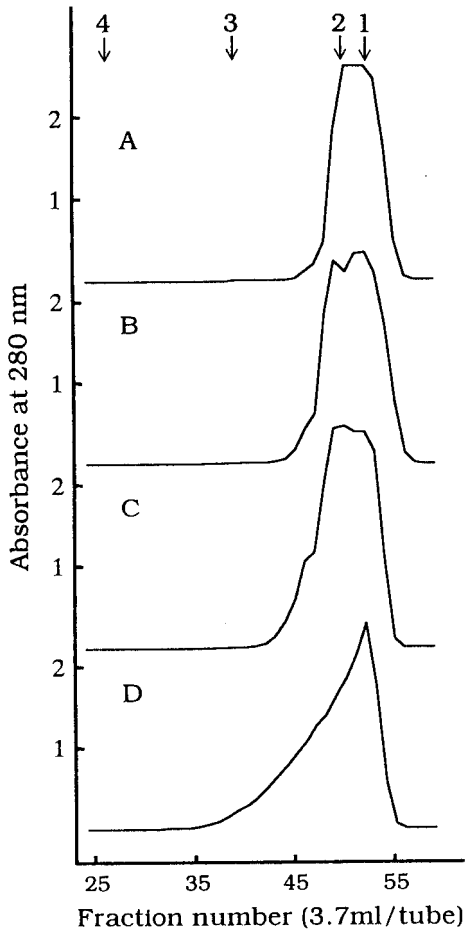

Figure 1. Gel filtration profiles of OVA (A), FDOVA (B), and DHOVA (C) (dry-heating conditions, 120 °C, 30 min), and DHOVA (D) (dry-heating conditions, 120 °C, 6 h) solutions. The gel filtration experiment was carried out with Sephacryl S-400 (2.0 × 75 cm). The samples (150 mg/3 mL of 20 mM phosphate buffer, pH 7.4) were applied and eluted with the same buffer at a flow rate of 0.6 mL/min. Arrows indicate elution volumes of standard proteins: (1) OVA, 45 kDa; (2) OT, 78 kDa; and (3) thyroglobulin, 669 kDa, as well as void volume (4) (Vo, blue dextran).

of OVA. In the samples dry-heated for less than 1.5 h, the aggregates reaching the pentamer were detected stepwise in the patterns (Figure 2A). Such aggregated patterns were clearly confirmed in Figure 2C. Aggregates reaching from the pentamer to an apparent decamer in the samples dry-heated for 3 and 6 h were also detected, although the intensities of their bands were faint and indistinct (Figure 2A, lanes 5 and 6). In the presence of SDS and 2-ME, most aggregates having higher molecular weights were reduced to aggregates having lower weights, although some aggregates of the dimer and trimer of OVA were also observed in the samples dry-heated for 1.5, 3, and 6 h. These results indicated that a disulfide bond was partly responsible for the formation of aggregates, and that binding among aggregates in DHOVAs treated for longer than 1.5 h was so strong that some aggregates did not dissociate into monomers in the cooperative treatment of SDS and 2-ME. The exact nature of the bonds which prevented the aggregates from dissociation into monomers is unclear.

The turbidities and coagulated protein ratios were examined on the solutions of FDOVA and DHOVAs (30 min−6 h) solubilized at 5% (w/w) levels with 20 mM

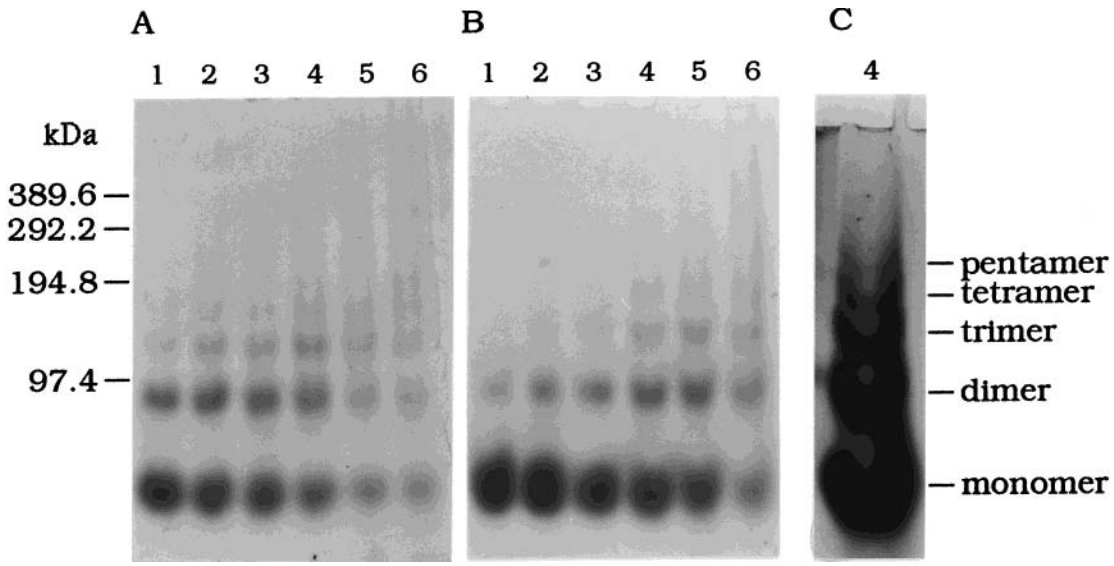

Figure 2. SDS−PAGE patterns of various DHOVAs in the absence (A and C) and the presence (B) of 2-ME using 3.5% acrylamide separating gel. FDOVA: (1). DHOVA (120 °C): (2) 10 and (3) 30 min, and (4) 1.5, (5) 3, and (6) 6 h. Parts A and B (40 μg of protein/lane): staining with Coomassie brilliant blue R-250. Part C (10 μg of protein/lane, sample in Figure 2A, lane 4): staining with silver-staining kit.

sodium phosphate buffer (pH 7.4) after reheating at 60 °C for 3.5 min and centrifuging (2000*g*, 5 min), respectively. Their determined values in every DHOVA solution were not different from those of FDOVA without dry heating (data not shown). Eventually, it was found that dry heating at 120 °C up to 6 h for FDOVA did not apparently induce changes in solubility or turbidity when reheated in the used condition.

Next, we determined the heat aggregation and coagulation of mixed solutions of DHOVA and FDOT. In a previous paper (Watanabe et al., 1999), it was suggested that DHEW (6 h) inhibited the heat coagulation of OT in mixed fresh egg white at a level of more than equivalent weight. Therefore, in this paper, DHOVA/FDOT mixtures at a ratio of 1:1 in weight were arbitrarily selected to examine the inhibiting effects of DHOVA on the aggregation and coagulation of the mixed OT.

Each of the 5% (w/w) solutions of FDOVA and DHOVAs (30 min−6 h) solubilized with 10 mM sodium phosphate buffer (pH 7.4) was mixed at a volume of 1:1 with 5% (w/w) FDOT solution solubilized with the same buffer. They were then reheated at 60 °C for 3.5 min and centrifuged (2000*g*, 5 min). Their photographs were shown in Figure 3, and the turbidities and coagulated protein ratios in their samples were also indicated in Figure 4. Coagula were formed in the sample mixed with DHOVA (30 min) and FDOT, as was formed in that mixed with FDOVA and FDOT, whereas they were not found in mixtures with DHOVAs (for times longer than around 2 h) and FDOT; i.e., the coagulated protein ratios greatly decreased with an increase in dry-heating time up to 2 h. On the other hand, the supernatants from the mixed solutions of DHOVA treated for 30 min and FDOT were almost transparent upon the formation of coagula, whereas the turbidities increased with an increase in dry-heating time up to 2 h, and then decreased with a continuation of dry heating to 6 h. When FDOT solution was separately heated at 60 °C for 3.5 min as described above, almost all OT in the solution coagulated. Thus, solutions with low turbidity values without coagula could be prepared when the mixtures of DHOVAs treated for longer than around 2 h and FDOT were reheated at 60 °C for 3.5 min. In other words, the coagulation of OT was greatly inhibited by the presence of DHOVAs treated for longer than 2 h, when their mixtures were reheated.

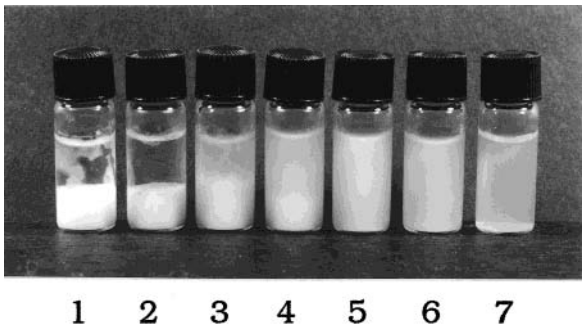

Figure 3. Photographs of the mixed solutions of DHOVA−FDOT after reheating (60 °C, 3.5 min) and centrifugation (20*g*, 5 min). The various DHOVA solutions (5% w/w) solubilized with 10 mM sodium phosphate buffer (pH 7.4) were mixed at a volume of 1:1 with FDOT solution (5% w/w) solubilized with the same buffer and reheated. FDOVA−FDOT (1). DHOVA [(120 °C): (2) 30 min, and (3) 1, (4) 1.5, (5) 2, (6) 3, and (7) 6 h]−FDOT.

The SDS−PAGE patterns of the supernatants separated in Figure 4 were shown in Figure 5, in the absence (Figure 5A) and presence (Figure 5B) of 2-ME, respectively. Figure 5A showed the presence of monomer types of OVA and OT together with soluble aggregates in the stacking and running gels. It was found that with the increase in dry-heating time, the intensities of stained monomer bands of OVA decreased and those of OT were kept almost constant. On the other hand, Figure 5B showed that the intensities of monomer bands of OVA decreased with dry-heating time and those of OT increased abruptly at the dry-heating time of 2 h. Moreover, the soluble aggregates in the stacking gel disappeared in every sample, although they were partly found in the running gel with increasing dry-heating time. The results suggested that soluble aggregates in the supernatants obtained from the mixtures of DHOVAs (treated for longer than around 2 h) and FDOT consisted of complexes of DHOVAs (probably monomers

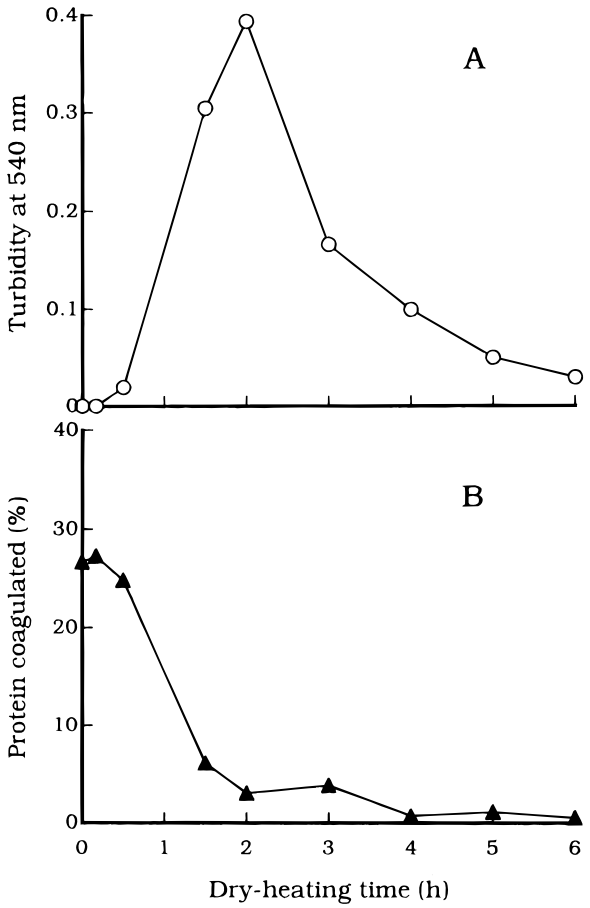

Figure 4. Turbidities (40 times diluted supernatant, 540 nm, A) and coagulated protein ratios (%, B) in the supernatants from mixed solutions of DHOVAs (120 °C, up to 6 h) and FDOT after reheating (60 °C, 3.5 min) and centrifugation (2000*g*).

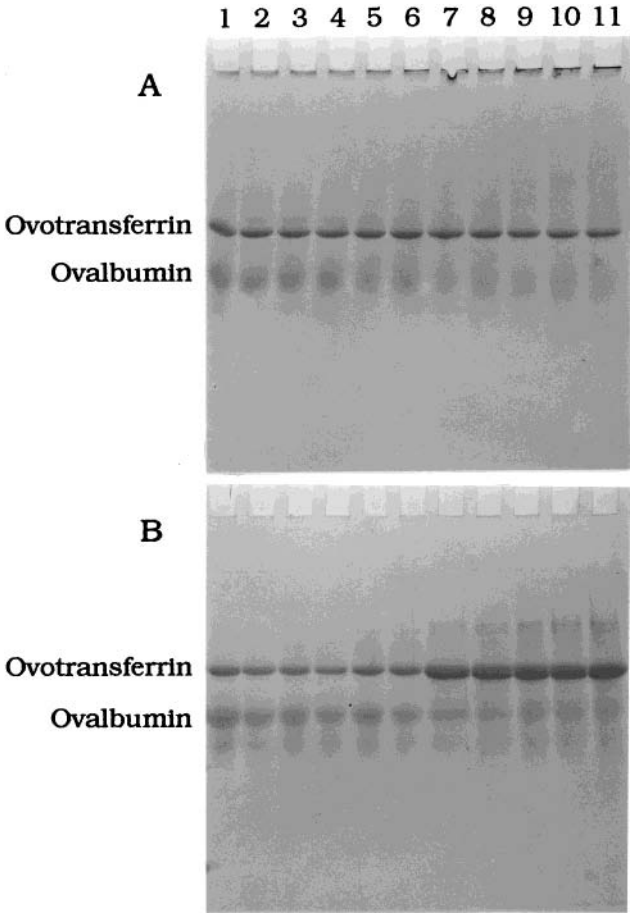

Figure 5. SDS−PAGE patterns supernatants from mixed solutions of DHOVAs (120 °C, up to 6 h) and FDOT after reheating (60 °C, 3.5 min) and centrifugation (2000*g*) in the absence (A) and presence (B) of 2-ME using 12.5% acrylamide separating gel. FDOVA−FDOT: (1). DHOVA [(120 °C): (2) 10, (3) 20, (4) 30, and (5) 45 min, and (6) 1, (7) 2, (8) 3, (9) 4, (10) 5, and (11) 6 h]−FDOT. A and B (40 μg of protein/lane): staining with Coomassie brilliant blue R-250.

and aggregates of OVA) and OT, and that interactions between DHOVAs and OT were mainly attributable to a disulfide bond with noncovalent cross-linkages such as electrostatic interaction.

To examine the components in coagula and the binding forces between the components, soluble proteins were extracted with each of seven solvents (A−G) from coagula, which were formed in a DHOVA (30 min)−FDOT mixture when reheated at 60 °C for 3.5 min and applied to SDS−PAGE (Figure 6). The proteins in every extract were OVA and OT. Most of the proteins extracted with solvents B, C, E, and F should be regarded as noncoagula which were incorporated into the coagulum, because the patterns in the SDS−PAGE of their extracts were almost the same as those of proteins extracted with solvent A (distilled water). Ratios in intensities of the stained bands of OVA to OT in the extracts with solvents D and G were different from those in the other extracts. That is, the larger quantities of OT were solubilized with solvents containing SDS, and almost all coagula were apparently dissolved with solvent G. These results showed that the main protein in coagula was OT, and the cooperative actions of NaCl, urea, and 2-ME in the presence of SDS as a main effective component were important for the solubilization of coagula.

The effects of reheating times up to 1 h at 60 °C were shown in Figure 7 on the turbidities (Figure 7A) and coagulated protein ratios (Figure 7B) of the mixtures of FDOVA−FDOT solution and DHOVAs (30 min and 6 h)−FDOT solutions comparing with those of FDOT solution. In the FDOT and FDOVA−FDOT solutions, the formations of coagulum with transparent supernatant occurred both abruptly (∼15 min) and gradually (15−60 min) with heating times, and in DHOVA (30 min)−FDOT solutions, such formations occurred with the development of turbidity due to the soluble aggregates in less than 10 min of reheating and transparent supernatant in more than 10 min. In the DHOVA (6 h)−FDOT solution, no formations of coagulum occurred with the gradual developments of turbidity.

Figure 8 showed the effects of reheating temperatures for 30 s on the turbidities and coagulated protein ratios in the mixed solutions of FDOVA−FDOT (Figure 8A) and DHOVA (6 h)−FDOT (Figure 8B), for comparisons with controls of the corresponding unheated and reheated (60 °C, 3.5 min) samples. In FDOVA−FDOT mixtures, the coagulated protein ratios increased greatly at 75 °C and then only slightly at 80 and 85 °C, and the supernatants from heated solutions as well as those from unheated ones were almost transparent, depending on the formations of some coagula. On the other hand, in DHOVA (6 h)−FDOT mixtures, the coagulated protein ratios increased slightly at temperatures from 65 to 80 °C with the gradual developments of turbidity but greatly at 85 °C with higher transparency. Thus, the differences in the inhibiting effect between FDOVA

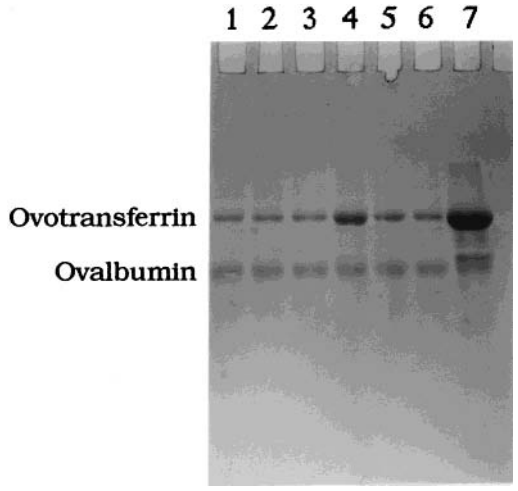

Figure 6. SDS−PAGE patterns of soluble fractions extracted with various solvents from coagula in the presence of 2-ME using 12.5% acrylamide separating gel: (1) solvent A, distilled water, pH adjusted to 7.4; (2) solvent B, 20 mM phosphate buffer, pH 7.4; (3) solvent C, solvent B + 0.6% NaCl; (4) solvent D, solvent C + 0.5% SDS; (5) solvent E, solvent C + 1.5 M urea; (6) solvent F, solvent C + 0.01 M 2-ME; and (7) solvent G, solvent C + 0.5% SDS + 1.5 M urea + 0.01 M 2-ME. The coagula formed in the mixture of DHOVA (dry-heating conditions, 120 °C, 30 min)−FDOT after reheating (60 °C, 3.5 min) and centrifugation (2000*g*) were used.

and DHOVA (6 h) were clearly observed except for the case of 85 °C and 30 s. The apparent loss of the inhibiting effect of DHOVA (6 h) on the coagulation of OT under the reheating condition of 85 °C and 30 s might be due to the fact that DHOVA itself began to aggregate with the other DHOVA and OT, and to gel (coagulate) to the insoluble form.

We determined the effects of ionic strength (Figure 9) and pH (Figure 10) of solvents used for the solubilization of samples on the turbidities and coagulated protein ratios in the mixed solutions of FDOVA−FDOT and DHOVAs (up to 6 h)−FDOT when reheated (60 °C, 3.5 min).

The effects of ionic strength of the buffer on turbidity development (Figure 9A) and coagulum formation (Figure 9B) were remarkable. That is, with increasing ionic strength, almost clear solutions without coagulum could be obtained in DHOVAs prepared even in shorter dry-heating times. In distilled water and 20 mM sodium phosphate buffer, dry-heating times of more than 3 and 1 h were required for the inhibition of OT−coagulum formation, respectively. In a 100 mM phosphate buffer system, no DHOVAs−FDOT mixtures showed the formation of coagulum when reheated. Even in the samples of FDOVA, ionic strength affected coagulum formation, which was inhibited with an increase in ionic strength. Thus, coagulum formation and turbidity development were greatly inhibited with increased ionic strength by suppressing the interactions through electrostatic forces between OT and OT. However, with increased dry-heating time, the effects of ionic strength were weakened and turbidity development and coagulum formation were also inhibited in the solution solubilized with distilled water.

With a decrease from pH 9.0 to 5.5 (10 mM phosphate buffer), longer dry-heating times were required to obtain nonturbid solutions (Figure 10A) without coagulum (Figure 10B). That is, dry-heating times of more than 1.5 h at pH 9.0, 2 h at pH 7.4, and 3 h at pH 6.5 were

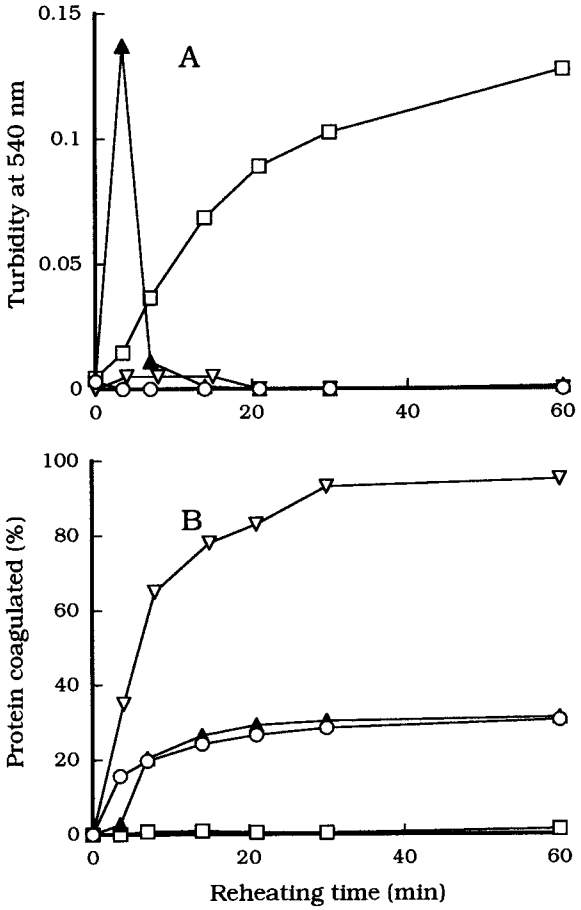

Figure 7. Turbidities (40 times diluted supernatant, 540 nm, A) and coagulated protein ratios (%, B) in the supernatants from mixed solutions: ○, FDOVA−FDOT; ▲, DHOVA (dry-heating conditions, 120 °C, 30 min)−FDOT; □, DHOVA (dry-heating conditions, 120 °C, 6 h)−FDOT, and ▼, the FDOT solution after reheating at 60 °C for various times up to 60 min and centrifugation (2000*g*).

required. At pH 5.5, a dry-heating time of 6 h induced a coagulum-free solution with turbidity.

DISCUSSION

The results obtained with dry-heated egg white (DHEW, 120 °C) in a previous study (Xu et al., 1998) showed that the coagulum formation of DHEW proteins was inhibited remarkably with an increase in dry-heating time when solubilized at 10% (w/w) concentration (pH 7.4) and reheated at 60 °C for 3.5 min, and that DHEWs treated for more than around 2 h suppressed the heat coagulation of fresh egg white when they were mixed and reheated. Thus, heat pasteurization of egg white without the formation of any coagula and with less development of turbidity was found to be possible by the addition of DHEW. Furthermore, it was suggested that soluble linear aggregates of OVA formed during dry heating for >2 h inhibited the formation of OT coagulum by the noncovalent bonds and sulfhydryl−disulfide interchange reaction in the pasteurization process (60 °C, 3.5 min). The results reported in this study on a molecular basis of OVA and OT confirmed those findings: i.e., DHOVAs prepared by dry heating at 120 °C exhibited much higher inhibiting effects on the formation of OT coagulum depending on the longer dry-heating time, when mixed solutions of DHOVA and OT were reheated at 60 °C for 3.5 min. It is interesting

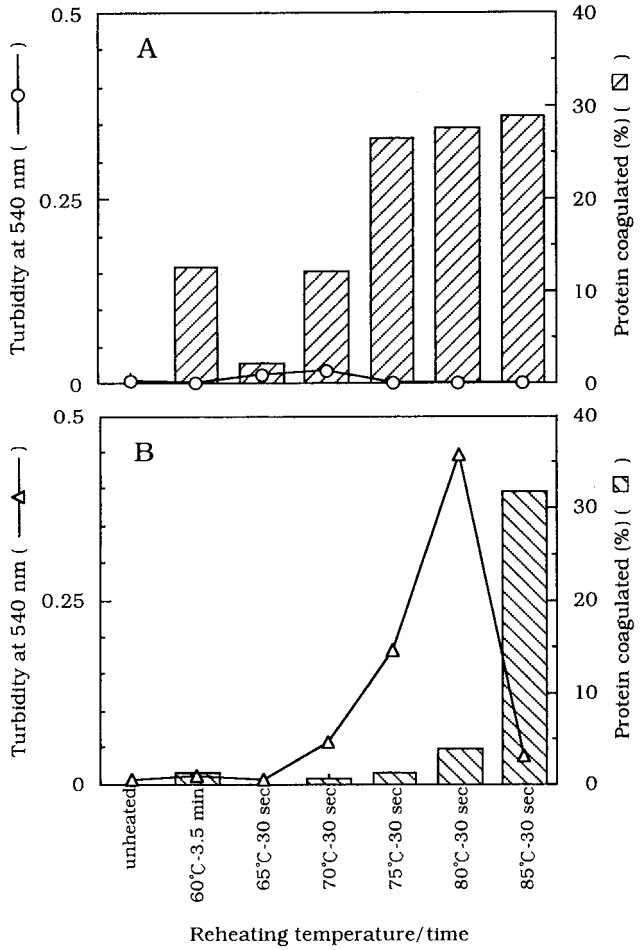

Figure 8. Turbidities (40 times diluted supernatant, 540 nm) and coagulated protein ratios (%) in the supernatants from the mixed solutions of FDOVA−FDOT (A) and DHOVA (dry-heating conditions, 120 °C, 6 h)−FDOT and (B) solubilized with 20 mM phosphate buffer (pH 7.4) after the treatments for 30 s at various temperatures (65−85 °C) and centrifugation (2000*g*), for comparison with unheated and heated (60 °C, 3.5 min) samples.

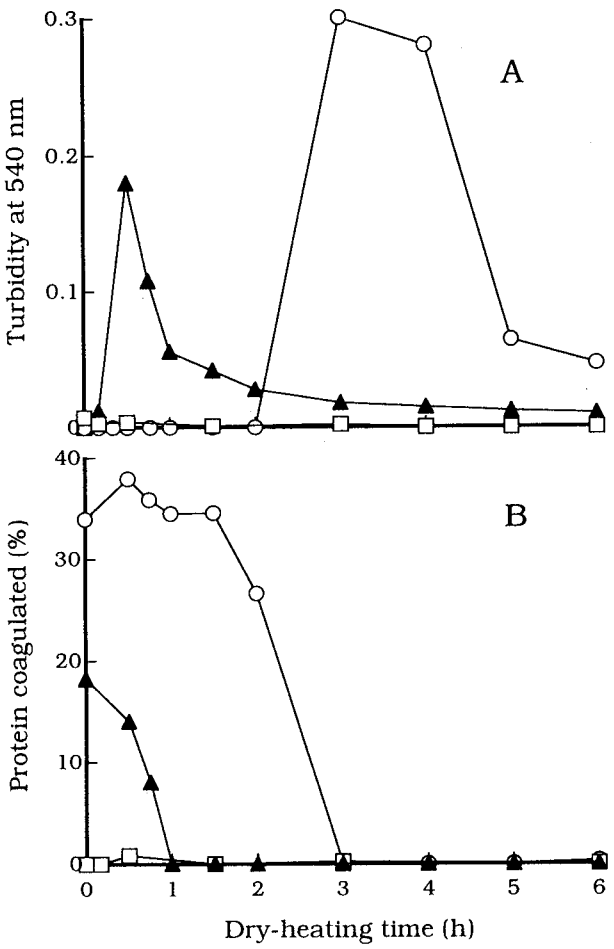

Figure 9. Turbidities (40 times diluted supernatant, 540 nm, A) and coagulated protein ratios (%, B) in the supernatants from the mixed solutions of FDOVA−FDOT and DHOVAs (dry-heating conditions, 120 °C, up to 6 h)−FDOT solubilized with solvents with various ionic strengths (○, distilled water; ▲, 20 mM phosphate buffer, pH 7.4; □, 100 mM phosphate buffer, pH 7.4) after reheating (60 °C, 3.5 min) and centrifugation (2000*g*).

to note that DHOVA treated for 6 h inhibited the coagulum formation of OT on reheating at 65−80 °C for 30 s and at 60 °C for up to 1 h, although the turbidity in those solutions developed gradually with increases in reheating temperature and time. Whether the DHOVAs denatured and aggregated further during reheating treatments was not determined in this study. It was indicated that OVA in egg white did not denature and aggregate upon heating below 70 °C (Matsuda et al., 1981), and that purified OVA aggregated at the temperature range between 70 and 75 °C in 170 mM NaCl solution (pH 7 and 9) (Hegg et al., 1979). The differences in heat stability of aggregates and monomers of DHOVAs from those of OVA and FDOVA will be revealed in further studies. Clarification is also needed as to whether the aggregates of DHOVA having higher molecular weights as seen in the dry-heated sample for 6 h exhibited much higher inhibiting effects on OT coagulation than dimer and monomer of DHOVA.

The facts that transparent solutions without any coagulum were prepared with an increase in ionic strength in this study differed from the results observed by others (Hatta et al., 1986). Some conformational changes in the OVA that occurred during heating in the dry state were attributed to the lower sensitivity to NaCl (Matsudomi et al., 1991). The effective action of DHOVAs in the inhibition of coagulum formation was different from all DHEWs: i.e., with an increase in dry-heating time the effect of ionic strength was weakened, and transparent solutions without coagula were prepared in solvents with lower ionic strength. However, such characteristics of the various DHOVAs and the mechanisms underlying such phenomena were not made clear in this study. The inhibition of coagulum formation at higher ionic strength might be dependent on the fact that formations of soluble aggregates between DHOVA and OT were induced by the shielding of ionic charges on proteins. On the other hand, with increases in ionic strength and pH (Figures 9 and 10), coagulum formation in the mixtures of DHOVA and FDOT was gradually inhibited. These results suggested that an electrostatic attraction between OT and OT was important for the formation of coagulum.

The interactions between various aggregates of DHOVA and OT were suggested to be the main factor contributing to the inhibition of coagulation. The analyses of degrees of denaturation and aggregation of DHOVA during dry heating should help to elucidate the interactions between DHOVA and OT in the subsequent reheating process. In the results previously described by us (Xu et al., 1998) as well as by others (Kato et al., 1990; Mine, 1997), the mild conformational changes and

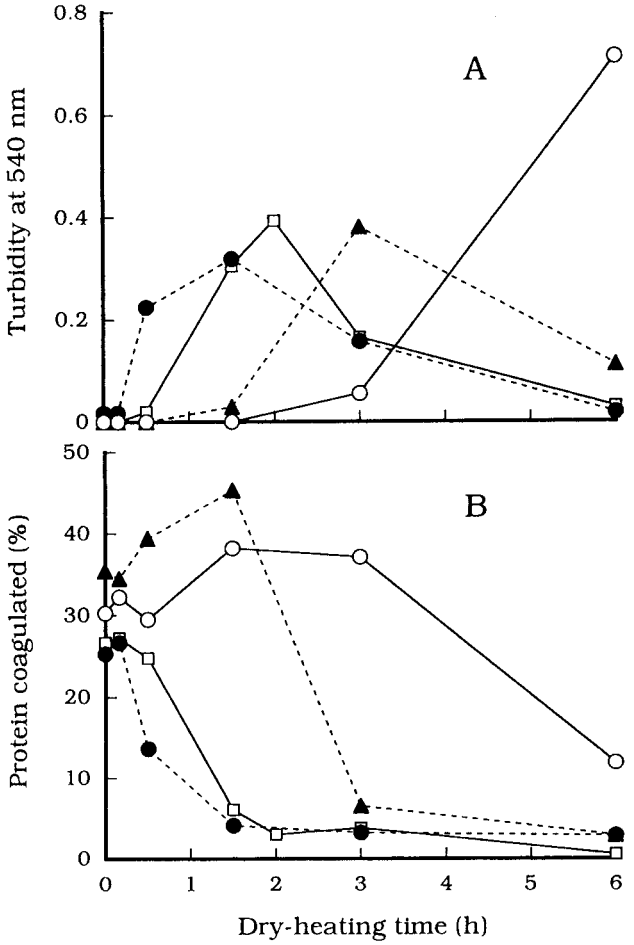

Figure 10. Turbidities (40 times diluted supernatant, 540 nm, A) and coagulated protein ratios (%, B) in the supernatants from the mixed solutions of FDOVA−FDOT and DHOVAs (dry-heating condition, 120 °C, up to 6 h)−FDOT solubilized with solvents with various pHs (○, 10 mM phosphate buffers, pH 5.5; ▲, pH 6.5; □, pH 7.4; and ●, pH 9.0) after reheating (60 °C, 3.5 min) and centrifugation (2000*g*).

formations of water-soluble aggregates in DHEW proteins during the dry-heating process were recognized, as shown by the significant increase in surface hydrophobicity and by the surface sulfhydryl group with only a slight change in the CD spectrum, and SDS−PAGE patterns. Mine (1996) reported that the DHEW proteins denatured and formed linear soluble aggregates with molecular weights of 200−375 kDa upon dry heating. In this study, aggregates having apparent molecular weights higher than 669 kDa in the DHOVA (6 h) were found in the gel-filtration patterns (Figure 1), although the main components were roughly less than trimer (molecular weight: 135 kDa). It will be necessary, as described above, to clarify the relationships between the dimensions of such linear soluble aggregates and the levels of the inhibiting effects on OT coagulation.

The isoelectric points of OVA and OT have been reported to be 4.5 and 6.1, respectively. OVA has a tendency to aggregate by heat on the acidic side depending on the ionic strength. OT in egg white was also noted to be more stable under heat treatment at pH 9 that at pH 7, and to be more unstable at pH 4 than at pH 7, OVA and OT in egg white aggregated at 82 and 62 °C, respectively, at pH 5.5 for 30 s (Watanabe et al., 1985). It is interesting to note that the coagulation of OT was greatly inhibited by the presence of DHOVA (120 °C, 6 h) at pH 5.5 in this study.

At present, there is no explanation as to the detailed mechanisms behind how OVA partially denatured and aggregated stepwise during dry heating in the presence of trace amounts of water, or how the partially denatured monomer and aggregates of DHOVA interacted with OT when the mixtures of DHOVA and OT were reheated. Studies are underway to answer those questions.

CONCLUSIONS

We can conclude the following from this study:

OVAs dry-heated at 120 °C inhibited the heat aggregation and coagulation of OT when its solutions were mixed with DHOVAs treated for more than around 2.0 h and heated at the usual pasteurization temperature (60 °C, 3.5 min). It was suggested that the water-soluble aggregates and probably the monomers in DHOVA interacted with OT via disulfide cross-linkages and noncovalent bonds, and suppressed the OT−OT interactions that led to coagulation. DHOVA (6 h) inhibited the coagulum formation of OT on heating at 65−80 °C for 30 s and 60 °C for up to 1 h, although the turbidity in the mixed solutions developed gradually with increases in heating temperature and time. With increases in ionic strength and pH, coagulum formation in the mixtures of DHOVA (6 h) and OT was gradually inhibited. Additional research, however, will be needed to elucidate the detailed mechanism behind this coagulation inhibition.

LITERATURE CITED

Cunningham, F. E.; Lineweaver, H. Stabilization of egg white proteins to pasteurization temperatures above 60 °C. *Food Technol.* **1965**, *19*, 1442−1447.

Davies, G. E.; Stark, G. R. Use of dimethyl suberimidate, a cross-linking reagent, in studying the subunit structure of oligometric proteins. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *66*, 651−656.

Hatta, H.; Kitabatake, N.; Doi, E. Turbidity and hardness of heat-induced gel of hen ovalbumin. *Agric. Biol. Chem*. **1986**, *50*, 2083−2089

Hegg, P. O.; Martens, H.; Lofqvist, B. Effects of pH and neutral salts on the formation and quality of thermal aggregates of ovalbumin. A study on thermal aggregation and denaturation. *J. Sci. Food Agric.* **1979**, *30*, 981−993

Kato, A.; Ibrahim, H. R.; Watanabe, H.; Honma, K.; Kobayashi, K. Structural and gelling properties of dry-heating egg white proteins. *J. Agric. Food Chem*. **1990**, *38*, 32−37.

Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. **1951**, *193*, 265−275.

Matsuda, T.; Watanabe, K.; Sato, Y. Interaction between ovomucoid and lysozyme. *J. Food Sci*. **1982**, *47*, 637−641.

Matsudomi, N.; Ishimura, Y.; Kato, A. Improvement of gelling properties of ovalbumin by heating in dry states. *Agric. Biol. Chem*. **1991**, *55*, 879−881.

Mine, Y. Laser light scattering study on the heat-induced ovalbumin aggregates related to its gelling property. *J. Agric. Food Chem*. **1996**, *44*, 2086−2090.

Mine, Y. Effect of dry heat and mild alkaline treatment on functional properties of egg white proteins. *J. Agric. Food Chem*. **1997**, *45*, 2924−2928.

Watanabe, K.; Matsuda, T.; Nakamura, R. Heat-induced aggregation and denaturation of egg white proteins in acid media. *J. Food Sci*. **1985**, *50*, 507−510.

Watanabe, K.; Xu, J. Q.; Shimoyamada, M. Inhibiting effect of egg white dry-heated at 120 °C on heat aggregation and

coagulation of egg white and characteristics of dry-heated egg white. *J. Agric. Food Chem*. **1999**, *47*, 4083−4088.

Weber, K.; Osborn, M. The reliability of molecular weight determinations by dodecyl sulfate−polyacrylamide gel electrophoresis. *J. Biol. Chem*. **1969**. *244*, 4404−4412.

Xu, J. Q.; Shimoyamada, M.; Watanabe, K. Gelation of egg white proteins as affected by combined heating and freezing. *J. Food Sci*. **1997**, *62*, 963−966.

Xu, J. Q.; Shimoyamada, M.; Watanabe, K. Heat aggregation of dry-heated egg white and its inhibiting effect on heat coagulation of fresh egg white. *J. Agric. Food Chem*. **1998**, *46*, 3027−3032.

Yamashita, H.; Ishibashi, J.; Hong, Y.-H.; Hirose, M. Involvement of ovotransferrin in the thermally induced gelation of egg white at around 65 °C. *Biosci. Biotechnol. Biochem*. **1998**, *62*, 593−595.

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