Thermally Induced Aggregates in Mixtures of α-Lactalbumin with Ovalbumins from Different Avian Species

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Interactions between α-lactalbumin (α-La) and ovalbumin (OVA) in mixed systems (1:1 ratios; 2, 4, and 8% w/w total protein, respectively) heated at pH 7 and 80 °C for 15 min were studied using sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE), gel filtration chromatography (GFC), and competitive enzyme-linked immunosorbent assay (ELISA). Although α-La alone did not form aggregates upon heating, it formed large aggregates when heated with OVA. The aggregated molecules eluted at the void volume had a molecular mass >300 kDa. The aggregation process was quantitatively affected by different avian OVAs from five species, possessing different numbers of free sulfhydryl groups. The amount of aggregates (M_w > 300 kDa) increased in proportion to total protein concentration, and the amount of intermediate components (M_w < 300 kDa) and monomeric OVA and α-La also changed, correlating with total protein concentration during heating. The results also indicated that the aggregates and intermediates, which contained dimeric and trimeric α-La, were mainly formed by the intermolecular disulfide bonds. The different interactions observed in several avian OVAs may explain heat-induced gelation of various avian OVAs as well as the enhancement of heat-induced gelation of OVA by α-La.

Keywords: *Avian; aggregation; gelation; heating; α-lactalbumin; ovalbumin; disulfide bond; sulfhydryl group*

INTRODUCTION

A major functional property of ovalbumin (OVA) is heat-induced gelation. There have been many studies concerning the heat-induced gelation of OVA (*1*−*3*). Some interactions between different proteins have also been identified; for example, it has been shown that the addition of α-lactalbumin (α-La) to solutions of *β*-lactoglobulin (*β*-Lg) or OVA enhances the strength of the gels formed during heating (*4*−*7*). However, the mechanisms of the interactions between these proteins during gelation have not been fully elucidated. Mine (*8*) indicated that heat-induced OVA formed high molecular weight and polydisperse aggregates. Furthermore, the aggregation behavior of denatured OVA appeared to play a crucial role in the subsequent gelling process of the protein. It is known that soluble aggregates of polymerized whey proteins are formed during the early stages of heat-induced gelation and that subsequent polymerization results in the formation of a rigid gel network (*9*). Thus, the protein aggregation process is necessary to understand the gel formation and properties of these proteins upon heat-induced gelation.

In food proteins, sulfhydryl (SH) groups and disulfide (SS) bonds are very important because they determine protein functionality to a considerable extent. Dickinson and Matsumura (*10*) have shown that the SH−SS interchange reaction occurs in *β*-Lg-stabilized and a mixture of *β*-Lg- plus α-La-stabilized emulsions, but not in pure α-La-stabilized emulsion. Similar observations have also been made by Monahan et al. (*11*). In addition, SH−SS interchange reactions also have been implicated in the formation of albumin gels by numerous researchers. Arntfield et al. (*12*) investigated the role of SS bonds in heat-induced networks from OVA and vicilin, and the result indicates that SS bonds may contribute to the elasticity and strength of a protein network. The importance of covalent SS bonds in the gelation and coagulation of egg white has been documented (*13*, *14*). Examination of SH groups in protein networks has also produced a range of responses for different proteins. This has been reported by Beveridge et al. (*15*) for egg albumin and whey protein concentrate, by Hayakawa and Nakai (*16*) for egg albumin, by Yasuda et al. (*17*) for bovine serum albumin (BSA), and by Shimada and Cheftel (*18*) for whey protein isolate.

OVA is a main protein component of egg white. Hen OVA has one SS bond and four free SH groups within the intramolecular hydrophobic core in the native state (*19*). The amino acid compositions of nine avian OVAs were compared by Fothergill and Fothergill (*20*). It is apparent that some amino acids remained at a quite constant level, whereas others, such as half-cystine, showed considerable variability in the amino acid composition for the nine avian OVAs. In our previous paper, it was indicated that five avian OVAs possessed different numbers of free SH groups: three for guinea fowl and turkey and two for mallard duck and Pekin duck (*21*). The monomeric α-La is characterized by the presence of four SS bonds (*22*). It is generally believed that α-La does not polymerize readily, because it does not form gels on heating at neutral pH; however, the presence of other proteins, specifically those containing free SH groups (*4*−*7*, *23*), allows interactions to occur between α-La and added protein.

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The SS bonds in α-La, and one SS bond and free SH groups in different avian OVAs, would undergo a variety of reactions that may be important in the structural changes during the process of heat-induced aggregation. We applied different approaches to compare the interactions between α-La and OVAs from different avian species to gain insight into the thermal aggregation of this complex system. Understanding the impact of interaction between these proteins on network formation can facilitate the prediction of network characteristics in protein mixtures. Particularly, with OVAs from different species, desirable gels with different gel hardnesses, water-holding capacities, and transparencies could be formed by adding α-La at different concentrations so that food scientists can better manipulate the process to obtain high-quality foods.

To examine the correlation between the thermal aggregation and the free SH groups, the aggregation behavior of denatured OVAs from five avian species have been compared. The concentration-dependent aggregation processes in individual and mixed systems during heat-induced denaturation have also been investigated by using sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) and gel filtration chromatography (GFC). Furthermore, changes in immunochemical reactivity produced by those treatments were assessed by a competitive enzyme-linked immunosorbent assay (ELISA) with a monoclonal antibody produced against α-La.

MATERIALS AND METHODS

Materials. Whey protein isolates (WPI) were kindly provided by Meiji Dairy Products Co. (Higashimurayama, Japan). OVAs were purified from egg whites of three galliform species (hen, H; guinea fowl, G; turkey, T) and two anseriform species (mallard duck, M; Pekin duck, D). The monoclonal antibody Mab2 that can bind to α-La was prepared as in a previous study (*7*). Bovine serum albumin (BSA) was obtained from Sigma Chemical Co. All other chemicals used were of reagent grade.

Preparation of Proteins. α-La was fractionated from WPI according to the method of Legowo et al. (*6*). OVAs were prepared from egg whites of different avian species by crystallization in an ammonium sulfate solution, recrystallized three times, and finally purified by carboxymethylcellulose chromatography with acetic acid buffer (220 mM acetic acid and 12 mM sodium acetate buffer) at pH 4.0 with stepwise gradient elutions from 0 to 0.4 M NaCl. The purity of α-La and OVA was checked by SDS-PAGE.

Heat Treatment of Samples. Equal amounts of α-La and OVAs from several avian species were dissolved in distilled water. The solution of mixtures was adjusted to pH 7.0 with 0.1 N NaOH and given total final protein concentrations of 2, 4, and 8% (w/w), respectively. An aliquot of the mixed solutions was heated in a water bath at 80 °C for 15 min and then cooled by tap water to room temperature to obtain samples of the aggregates. The samples were immediately diluted to different concentrations for various analyses. Either α-La or OVA solution was also heated alone at various protein concentrations (1, 2, and 4%, w/w) under the same conditions as described above. The protein concentration was determined according to the method of Lowry et al. (*24*) using BSA as a standard.

SDS-PAGE and Amino Acid Sequencing. SDS-PAGE was performed using 12.5% gels under both reducing and nonreducing conditions according to the method of Laemmli (*25*). The heated samples were immediately diluted with 10 mM Tris-HCl buffer (pH 7.0) and given a final protein concentration of 0.12%. Ten microliter aliquots, which were prepared in SDS−sample treatment buffer with and without 2-mercaptoethanol (2-ME) and boiled at 100 °C for 2 min, were applied to each slot of gel and electrophoresed at a constant current of 12 mA. The gels were stained with Coomassie Brilliant Blue R-250 (CBB). The molecular mass of the complexes was estimated using standard protein markers.

After SDS-PAGE in the absence of 2-ME of samples, the separated proteins in the gel were transblotted to PVDF membranes (Immobilon-P, Millipore Co., MA). The transblotted PVDF membrane was directly stained with CBB and dried for analysis of amino acid sequence. The amino acid sequence was analyzed using a PE-ABI 491A protein sequencer.

GFC. The thermal aggregates were analyzed using a prepacked column (1 × 30 cm) of Superose 12 HR (Amersham Pharmacia Biotech, Uppsala, Sweden), attached to an HPLC system. The particular column packing was selected because it could separate the complexes in the molecular mass range from 1 to 300 kDa. An aliquot of the heated protein samples described above was diluted by a solution of phosphate buffer containing SDS to give a final protein concentration of 1 mg/mL in 50 mM phosphate at pH 7.0, 1% SDS. All solutions of sample were centrifuged at 10000*g* for 5 min to remove insoluble protein before they were loaded onto the column. The column was equilibrated with 50 mM phosphate buffer at pH 7.0 containing 0.1% SDS, and a 100 μL aliquot of the supernatant was eluted with the same buffer at a flow rate of 0.4 mL/min. The elution profile of the proteins was detected at 280 nm.

The peak area of each elution profile was estimated by an Auto-Recording Area Meter AAM-9 (Hayashi Denko Co. Ltd). These values were then used to calculate the relative amounts of aggregates and monomer of unreacted OVA or α-La for comparing the aggregation in heated OVAs alone and a mixture of α-La with OVAs from different avian species.

Competitive ELISA. The wells of the microplate (Greiner Co. Laboratory, 655001) were coated at 4 °C overnight with 100 μL/well of unheated 10 μg/mL α-La solution in PBS (pH 7.4). Wells were washed with PBS containing 0.05% (v/v) Tween 20 (PBS-T). Nonspecific binding sites were blocked by incubating with 200 μL/well of blocking reagent [PBS-T containing 0.2% (w/v) BSA] at 37 °C for 2 h. After each incubation step, the wells were washed three times with 200 μL of PBS-T solution.

The heated protein solutions were adjusted to an α-La concentration of 10 μg/mL with blocking reagent, and then 100 μL aliquots were serially diluted in eight steps ($\sim 3^{-7}$) with blocking reagent. The antibody Mab2 (dilution 1:500) was dissolved in blocking reagent. Equal volumes of antigen and antibody solutions were mixed and incubated for 1 h at 37 °C.

A 100 μL/well aliquot from the previous step was incubated at 37 °C for 1 h in wells coated with α-La (10 μg/mL) as described above. Goat anti-mouse IgG peroxidase conjugate, diluted 1:10000 in blocking reagent, was incubated (100 μL/well) for 1 h at 37 °C. The color reaction was developed by adding a substrate solution kit (ABTS Peroxidase Substrate System, Kirkegaard and Perry Laboratory Inc., Gaithersburg, MD). The absorbance was determined at 415 nm after incubation at a room temperature for 30 min.

For each sample, controls for either the presence or absence of coated antigen (α-La) were incorporated. The binding reactivity of protein with antibody was expressed as inhibition (percent), which was calculated by using the equation

$$\%\ \text{inhibition} = \frac{(A_p - A_a) - (A_s - A_a)}{A_p - A_a} \times 100$$

where A_p is absorbance for controls in the presence and A_a in the absence of coated antigen and A_s is absorbance of sample.

Statistical Analysis. The experiments for competitive ELISA were determined at least four times with individually prepared samples as each replicates. The concentration of α-La at 50% inhibition was calculated as a representative value at all replicates. The calculated values were compared using the *t* test. Significance of differences was defined at 0.05. The value of 50% inhibition was selected as a point that was highly sensitive to the binding reactivity. Means of triplicates and

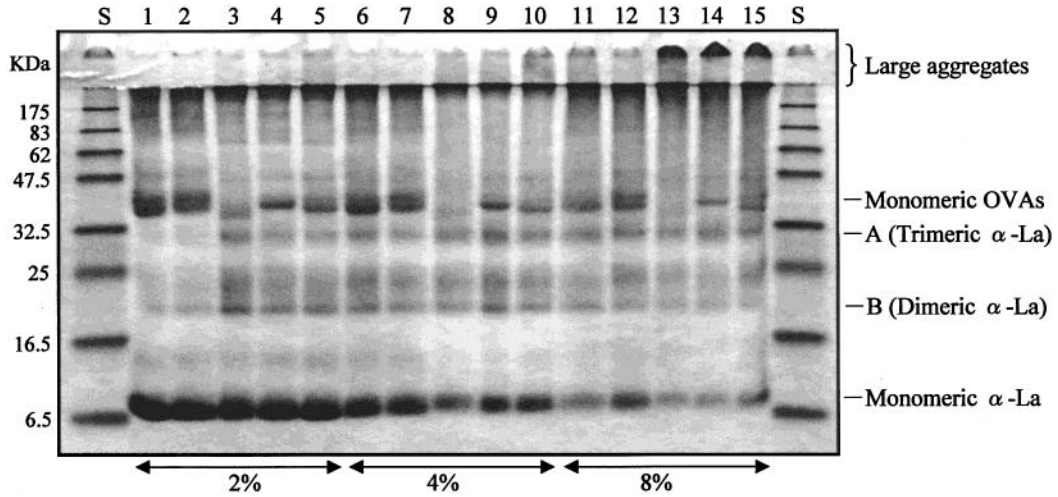

Figure 1. SDS-PAGE patterns of the mixtures of α-La and OVA from different avian species (ratios 1:1) heated at 80 °C for 15 min in the absence of 2-ME: (lane S) marker protein; (lanes 1−5) 2%; (lanes 6−10) 4%; (lanes 11−15) 8% protein concentration. For each protein concentration, lanes refer to (from left to right) Pekin duck, D; mallard duck, M; hen, H; guinea fowl, G; and turkey, T.

standard deviations for measured polymerization were compared, and differences ($p < 0.05$) among means also were detected using the *t* test.

RESULTS

SDS-PAGE Analysis. Comparison of the electrophoretic patterns in reducing and nonreducing conditions is widely used to identify the presence of disulfide bonds holding together aggregates of proteins. To estimate intermolecular covalent cross-linking, the mixtures of α-La with OVAs after heat treatment were analyzed by SDS-PAGE in both the absence and presence of 2-ME. The electrophoretic patterns under a nonreducing condition showed the bands of highly aggregated molecules (Figure 1). However, the patterns under a reducing condition showed no bands corresponding to aggregates (not shown). These results suggested that aggregates through SS bonding were formed in samples heated at 80 °C for 15 min. Furthermore, α-La and OVAs predominantly contributed to the heat-induced aggregation by intermolecular exchanges of SH−SS.

The effect of protein concentration of heated OVAs from five species on the extent of SS-mediated aggregation in the soluble protein fractions was determined by SDS-PAGE in the absence of 2-ME (Figure 1). Protein bands corresponding to high molecular size aggregates with low electrophoretic mobilities were clearly observed near the top of the separating gel and within or above the stacking gel. These were labeled "large aggregates" in Figure 1. The amount of large aggregates increased and that of monomeric α-La decreased with increasing protein concentration. The different aggregation patterns have been shown in five avian OVAs mixed with α-La. The monomeric hen OVA band rapidly diminished with increasing protein concentration, demonstrating that hen OVA engages readily in polymerization reactions. Compared to hen OVA, the monomeric bands in mallard duck and Pekin duck OVAs diminished less quickly. These results showed that there was a close relationship between SH group content in OVAs and aggregation.

A noticeable feature in the heat-treated mixtures was the appearance of the additional bands A and B in the absence of 2-ME (Figure 1). The N-terminal amino acid sequences of both bands showed a sequence of [^{1}EQLTKCEVFR^{10}], which is identical with the N-terminal sequence of α-La (*22*). Therefore, these bands corresponded to dimeric α-La (M_w = 29 kDa) and trimeric α-La (M_w = 43 kDa). Interestingly, the intensities of the dimeric and trimeric α-La bands decreased in α-La mixed with mallard duck and Pekin duck OVAs at a protein concentration of 2%.

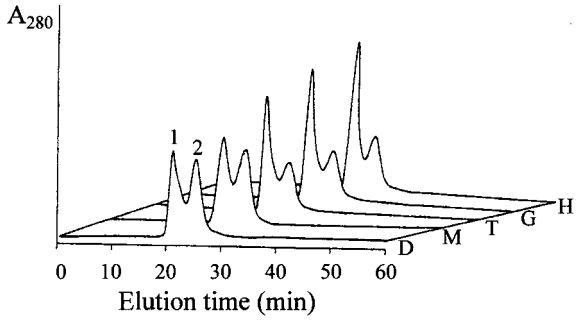

Figure 2. Comparison of the elution profiles of heated OVA from different avian species in GFC (Superose 12 HR 10/30) in the presence of 1% SDS; peak 1, high molecular weight aggregates; peak 2, monomeric OVA. OVAs were heated at 80 °C for 15 min at a protein concentration of 2%. All samples were diluted with PBS (pH 7) containing 1% SDS at a final concentration of 0.1%.

GFC. The elution profile showed a single peak, with no major difference between the unheated and heated samples of α-La (not shown). Similar results have been found by Matsudomi et al. (*4*), Legowo et al. (*6*, *7*), and Calvo et al. (*23*). On the other hand, the individual OVAs from five avian species showed two peaks after heating: one from OVA monomer with an elution time of 28.1 min (elution times are averaged for five species of OVA at different protein concentrations) and the high molecular weight aggregates, eluting at 21.7 min (Figure 2). The elution time of monomeric OVAs (28.1 min in native ovalbumin) and the elution profiles showed no distinct changes at different protein concentrations, whereas peak height changed upon heating. This agrees with the results of Kato et al. (*26*), who concluded that OVA molecules immediately aggregate when denatured at neutral pH by heating and huge polymers of OVA are formed.

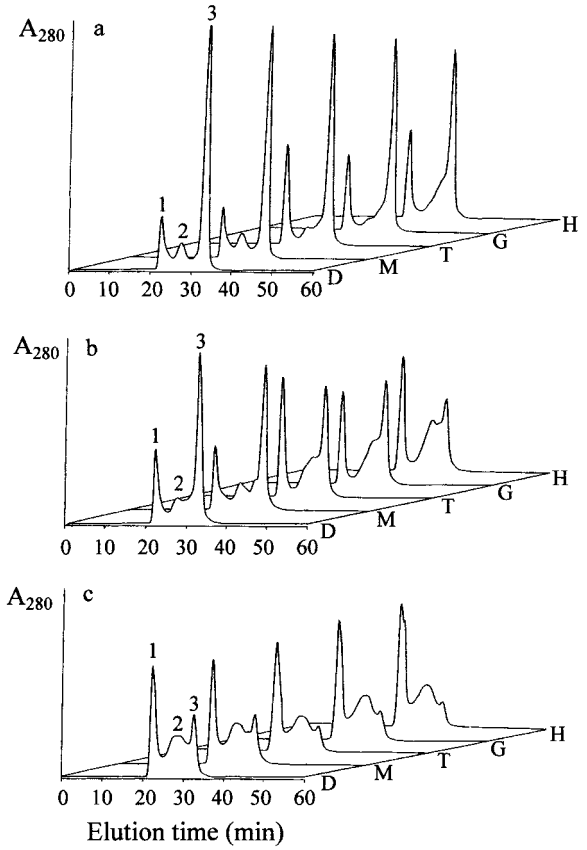

Figure 3. Comparison of the elution profiles of heated mixtures of α-La and OVA from different avian species in GFC (Superose 12 HR 10/30): peak 1, high molecular weight aggregates; peak 2, intermediates; peak 3, monomeric α-La. Mixtures (ratios 1:1) were heated at 80 °C for 15 min at total protein concentrations of 2% (a), 4% (b), and 8% (c), respectively. All samples were diluted with PBS (pH 7) containing 1% SDS at a final concentration of 0.1%.

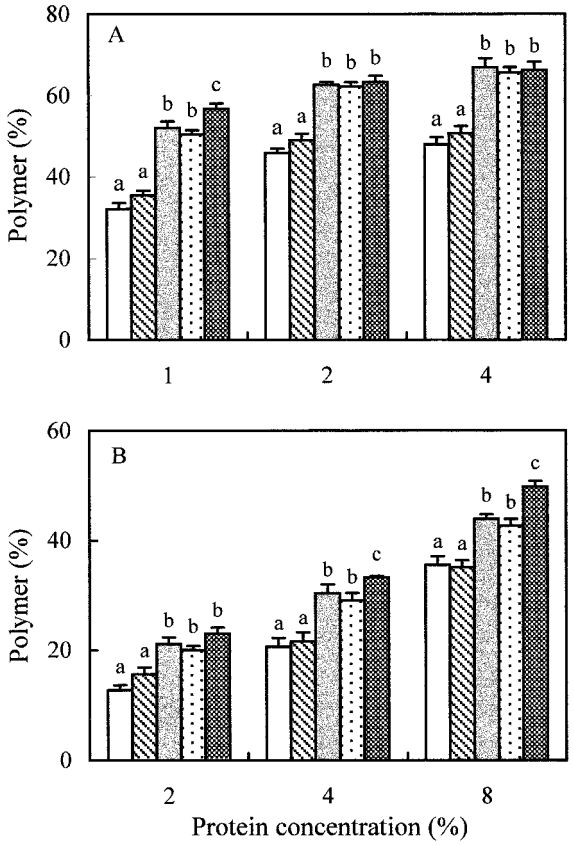

Figure 4. Thermally induced aggregation analyzed quantitatively upon heating of individual OVAs (A) or mixtures of α-La and OVAs (ratios 1:1) from different avian species (B) at different concentrations. The percentage of high molecular weight aggregates was calculated by comparing their distribution in GFC profiles (Figure 2 or 3) with peak area value given as percent of total peaks. For each protein concentration, columns refer to (from left to right) D, M, T, G, and H. Each value represents the mean ± SD of triplicates; means at the same concentration with different letters are significantly different ($p < 0.05$).

Hen OVA and α-La mixed at ratios of 1:1 (w/w) without heat treatment eluted at the retention times of 28.1 and 32.7 min, respectively. When OVA mixed with α-La was heated, the chromatogram showed another peak of large aggregates eluted at the void volume (retention time = 22.5 min) in addition to the peaks for monomeric OVA, dimeric/trimeric α-La, and monomeric α-La (Figure 3). The peak height of high molecular aggregates of mixed samples became gradually higher as the protein concentration increased. On the other hand, the peak height of monomeric α-La decreased as the protein concentration increased. The aggregates in heated mallard duck and Pekin duck OVAs mixed with α-La were produced less than those of other OVAs mixed with α-La. Little peaks of dimeric and trimeric α-La in heated mallard duck and Pekin duck OVAs mixed with α-La were observed at a concentration of 2% during heating (Figure 3a). The peak of intermediate was rather broad, large, and gradually shifted toward a higher molecular mass with increasing protein concentrations (Figure 3).

Quantitative Analysis of Aggregates. The profiles of heat-induced aggregation involved in individual OVAs (Figure 2) and a mixture of α-La with OVAs from different avian species (Figure 3) were investigated by GFC in the presence of 1% SDS. By quantitating peak areas in elution profiles of GFC, the percentage of high molecular weight aggregates being formed by disulfide bonds was calculated with respect to total protein (Figure 4).

The relative proportion of aggregates estimated from the peak area was compared to the aggregates of individual OVAs from different avian species (Figure 4A). The results showed that the amount of high molecular weight aggregates increased ($p < 0.05$) when the protein concentration increased from 1 to 2% during heating, but no further remarkable increases in the amount of aggregates were observed from 2 to 4%. In addition, the amounts of aggregates were different among avian species at a concentration of 1%, in the order hen > turkey, guinea fowl > mallard duck, Pekin duck. At 2 and 4% protein concentrations, the amount of aggregates in heated hen OVA was similar to that of heated guinea fowl and turkey OVAs. The apparent proportion of aggregates by heat treatment of OVAs mixed with α-La is shown in Figure 4B. Little difference in the proportion of aggregates for both mallard duck and Pekin duck OVAs, as well as both guinea fowl and turkey OVAs, was observed. The proportion of aggregates formed from the mixture of α-La and OVAs showed a remarkable concentration dependence compared to individual OVA. From these observations, the aggregation mechanism in mixed systems seems to be different from that of individual OVA.

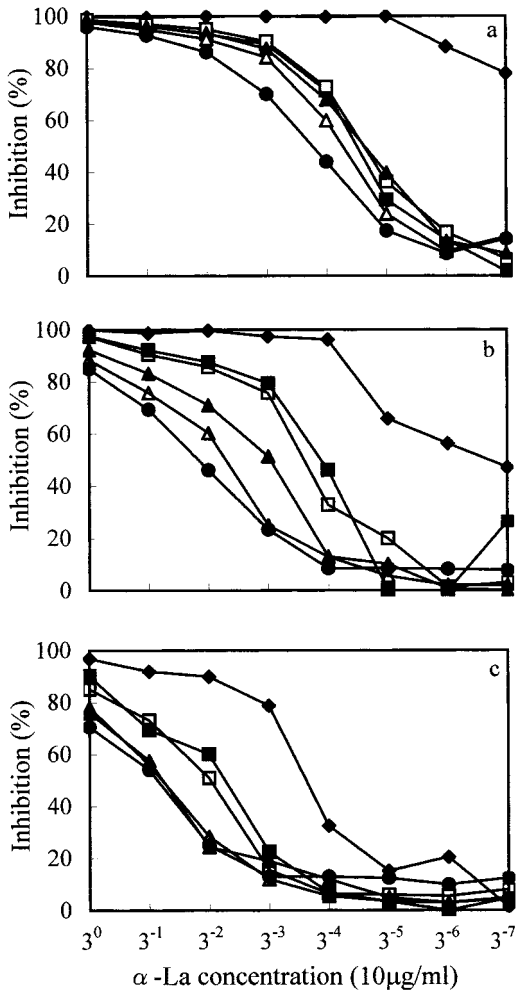

Figure 5. Competitive ELISA patterns of the mixtures of α-La and OVA from different avian species at total protein concentrations of 2% (a), 4% (b), and 8% (c), respectively: (◆) heated α-La alone; added OVAs were (■) D, (□) M, (▲) T, (△) G, and (●) H; respectively. Heating conditions were the same as in Figure 1. The heated protein solutions were adjusted to an α-La concentration of 10 μg/mL, and then aliquots were serially diluted at eight steps ($\sim 3^{-7}$), respectively. Each datum represents the mean of four times.

Binding Reactivity of α-La with Monoclonal Antibody. The binding of each heat-treated α-La mixed with different species of OVAs to antibody was evaluated by competitive ELISA. Binding reactivity is expressed as inhibition (percent), and each value given in Figure 5 represents the average of at least four separate assays. Monoclonal antibody Mab2 used in this study has high activities to bind either unheated or heated α-La, but it does not recognize α-La cleaved at at least one of four disulfide bonds (*7*). The heated α-La (1, 2, and 4%) showed high inhibition, reflecting its high binding reactivity with antibody. This result further suggested that α-La did not aggregate when heated alone. However, the reactivity of α-La in mixtures of α-La and OVAs decreased ($p < 0.05$) reactivity upon heating, and the reduction of binding showed concentration dependency (Figure 5).

The antibody-binding reactivity of α-La might be affected by the number of SH groups and protein concentration of added OVAs. Figure 5a showed no significant difference in reactivity for heated α-La mixed with OVAs of various avian species at a concentration of 2% except α-La mixed with hen OVA, in which a slight decrease ($p < 0.05$) in reactivity occurred. Figure 3a also showed that the reactivity of α-La with OVA was very low at a concentration of 2%. The loss of inhibition markedly occurred ($p < 0.05$) when α-La was mixed with avian OVAs at a protein concentration of 4% (Figure 5b). At a high concentration of 8%, the decrease ($p < 0.05$) in inhibition of α-La with hen, guinea fowl, and turkey OVAs was greater than that of α-La mixed with mallard duck and Pekin duck OVAs (Figure 5c). The decrease in inhibition indicates that aggregation tends to be high in heat-induced denaturation. The results obtained were in good agreement with those of the GFC analysis.

DISCUSSION

The results of SDS-PAGE and GFC analyses show that SS-mediated aggregation occurs during heat-induced denaturation either in OVAs or in α-La/OVAs over a wide protein concentration range. Although polymerization does not occur in pure α-La during heating, it was clearly demonstrated that α-La and OVAs were both present in the aggregates formed in the mixture of two proteins by SH−SS interchange reactions during heating. It is assumed that some aggregates are composed of heteropolymers, because α-La is not capable of forming polymers by itself under these experimental conditions. Monahan et al. (*11*) and Havea et al. (*27*) have shown that the SH−SS interchange reaction occurs in mixtures of α-La/β-Lg and α-La/BSA but not in pure α-La.

α-La contains four SS bonds and no free SH groups, and conformational changes in α-La can be induced by heat treatment. The resulting products can be considered to be similar to the molten globule state of α-La and can be called "molten globule-like" (MG) (*27*). Havea et al. (*27*) suggested that the adducts are formed by reaction of α-La with BSA, and once the reactive adduct is formed, there are at least two possible reactions that can occur, one forming the aggregates, another forming dimeric or trimeric α-La. From our results, the mixture of α-La/OVA behaves in a fashion similar to that of α-La/BSA, although in the case of OVA there is more than one free SH group. It is likely that the free SH group of ovalbumin could have created a free SH group in MG α-La molecules during heating via an SH−SS interchange reaction. Resulting SH groups can react with another α-La or OVA molecules. In the mixed α-La/OVA system it could be suggested that MG α-La probably formed some sort of adduct with the OVA polymers. Furthermore, the reactivity could be influenced by the amount of free SH groups in added OVAs, containing a different number of SH groups. The role of SH groups in the interactions between proteins during heating, such as β-Lg and BSA and β-Lg and α-La, has already been emphasized (*4*, *5*, *11*, *18*). The electrophoretic patterns (Figure 1) and the elution profiles of GFC (Figure 3) all indicated that the amount of large aggregates increased and that of monomers decreased with the increasing number of free SH groups in OVAs at the same concentration. Therefore, our results also indicated that the SH group content affected the conversion of reactive monomers to high molecular weight aggregates in the heat-induced aggregation process. The aggregation process of hen OVA was similar to that of guinea fowl and turkey OVAs when heated alone, particularly at high protein concentration. This is presumably because, as a result of thermal treatment,

the three free, highly reactive SH groups of hen OVA per molecule were exposed and may have had direct interchange with other SH groups when heated (*28*).

Immunological methods have become important analytical tools for studying food samples. Consequently, these methods have been applied to solve analytical problems in different systems (*29*, *30*). Interactions between α-La and OVAs with different numbers of SH groups were further investigated by competitive ELISA. The results showed that the immunochemical behavior also coincided with the taxonomic order to which the five species belonged. The structural relationships observed in these studies correlated well with the relationships indicated by immunochemical comparisons. It was indicated that the galliform OVAs (guinea fowl and turkey OVAs) showed fewer differences from hen OVA than did the anseriform OVAs (mallard duck and Pekin duck). Thus, there was little difference in immunochemical reactivity for both mallard duck and Pekin duck, despite the considerable difference compared with hen OVA.

As part of an exploration of the factors affecting the gelation of OVAs or α-La/OVAs, we have examined heat effects in the individual and mixed systems on protein aggregation. A better understanding of this heat-induced aggregation process should provide further basic information for the practical utilization of OVAs and α-La/OVAs gelation. Further research with respect to rheological properties is necessary to clarify the effects of the structure of different avian OVAs on gelation upon heating.

CONCLUSION

High molecular weight aggregates are formed in all heated samples, except α-La heated alone, but the proportions of aggregate are different depending on the protein concentration and character of OVAs from five avian species. When the mixture of α-La and OVAs forms aggregates in heat-induced denaturation, intermolecular SS bonding is likely to play a critical role. Furthermore, the nature of the aggregation process is highly dependent on OVAs from avian species, because they have different numbers of free SH groups. α-La interacts with OVA to form larger aggregates than those of OVA heated alone. Thus, this aggregation process is responsible for the enhancement of the heat-induced gelation of OVAs from different avian species by added α-La.

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