JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Heat-Induced Gels of Egg White/Ovalbumins from Five Avian Species: Thermal Aggregation, Molecular Forces Involved, and Rheological Properties

YUANXIA SUN AND SHIGERU HAYAKAWA*

Department of Biochemistry and Food Science, Kagawa University, Ikenobe, Miki, Kagawa 761 0795, Japan

Product processing (heating, pH change, etc.) usually alters protein structure, improves rheological properties, and gives a unique texture to foods. The thermal aggregation and structural properties of ovalbumins from five avian species were studied at different pH values by polyacrylamide gel electrophoresis (PAGE) and determinations of sulfhydryl group content and surface hydrophobicity. The results showed that sulfhydryl group content changed insignificantly in heat-denatured ovalbumins other than hen ovalbumin (pH-independent), and surface hydrophobicity markedly increased (pH-dependent) after heating, with a significant difference among species. Furthermore, it was demonstrated that the hydrophobic interaction and sulfhydryl−disulfide interchange reaction were necessary in the aggregation and cross-linking of gel networks. Creep tests were also used to characterize the gel network structures of various egg white/ovalbumins upon heating. The viscoelastic behavior of the ovalbumins of all species was dependent on pH values, and changed significantly with the phylogeny of these species. With increases in pH value (7.0−8.5), the heat-induced gels of ovalbumins gradually changed from turbid to translucent, the instantaneous modulus (E_0) increased slightly and reached a nearly constant value, and the Newtonian modulus (η_N) increased significantly in each sample. The heated egg white from these five avian species also formed highly viscoelastic gels, with a good correlation of viscoelastic behavior between ovalbumin and egg white in corresponding species.

KEYWORDS: Gelation; viscoelastic properties; ovalbumin; egg white; hydrophobicity; sulfhydryl group; thermal aggregation

INTRODUCTION

Egg white protein (EW) has several excellent functional properties, one of the most important of which is to improve the consistency of foods by forming thermally induced gels. These heat-induced gels provide a medium for delivering nutrients and flavors, and give a unique texture to foods. Thus, EW constitutes a mixture of materials of great importance to the food industry. Ovalbumin (OVA) is a globular protein with a molecular weight of 45.5 kDa. Because OVA is the major protein of EW, its behavior dominantly affects the gelation of EW. Although there have been numerous studies on the effect of heat treatment on the gel formation of hen EW/OVA (*1*−*4*), little research has been conducted on the heat-induced gel formation of EW/OVA from other avian species. To understand whether the gelling mechanism of hen OVA is shared by related avian species, the aggregation process, the intermolecular interactions, and the rheological behavior from different species' OVAs were investigated. Moreover, the information on heat-induced gelation of other species OVAs can better be applied for processing high-quality foods.

In general, several intrinsic factors that affect the functional properties of food proteins include their amino acid sequence and composition, structure (secondary, tertiary), surface hydrophilic/hydrophobic character, and net charge and distribution. In previous investigations (*5*), it was indicated that the number of free cysteine sulfhydryl (SH) groups of OVA differed among species, and that the variations in amino acid sequences are closely related to the phylogeny. It was also shown that the nature of the aggregation process from heated OVAs is highly dependent on the avian species, with different numbers of free SH groups (*6*). In addition, environmental conditions (pH, ionic strength, NaCl, and temperature) also influence gel network structure and rheological characteristics by affecting the availability of covalent and noncovalent bonding groups for stabilization of tertiary structure and intermolecular interactions (*3*, *7*−*9*).

Intermolecular interactions of denatured molecules are commonly involved in heat-induced gelation of EW/OVA. Many investigators have worked to elucidate the mechanism of thermal

* Corresponding author. Fax: +81-87-891-3021. E-mail: Hayakawa@ag.kagawa-u.ac.jp.

10.1021/jf0109975 CCC: $22.00
Published on Web 02/08/2002

aggregation (*2, 10−12*) or gelation (*1, 3, 4, 13−15*) of hen EW/OVA. The overall gelation process requires that the proteins unfold or undergo some conformational changes initially and that the second aggregation step proceeds relatively more slowly than the first to allow the denatured protein molecules to orient themselves and interact at specific points, thus forming a three-dimensional network (*11*). Arntfied et al. (*16*) investigated the role of disulfide (SS) bonds in heat-induced networks from OVA and vicilin, and found that SS bonds may contribute to the elasticity and strength of protein networks. The nature of cross-links in protein gels has been discussed by several authors (*17, 18*). The consensus view is that with the exception of SS bonds in some protein gels, the molecules are held together by a combination of weak intermolecular forces, i.e., hydrogen bonds, electrostatic forces, van der Waals forces, and hydrophobic interactions. These results implicate essentially all of the potential interaction sites or functional groups of the molecules in the gelation process, including SH groups.

Rheological analysis gives useful information on the gel structure (*19*). Hickson et al. (*7, 8*) measured changes in heat-induced gel characteristics during cold storage of egg albumen. Viscosity, elasticity, and gel strength increased during storage of eggs, and correlated closely with pH value increases during storage. OVA gels formed at a pH between 4 and 6 are turbid, whereas gels made at a pH above or below this range are clear (*3*). Holt et al. (*9*) observed maximum viscosity, maximum elasticity, and gel strength as functions of temperature, pH, and concentration of NaCl using a composite treatment design. Arntfield et al. (*20*) studied the microstructure and rheological characteristics of heat-induced networks for hen OVA and vicilin, and indicated that at pH 8.5 both vicilin and OVA formed reasonably well-cross-linked networks. However, as for OVA gels from different avian species, a precise rheological study has not been done. Consequently, it should be of great interest to elucidate the relationship between rheological properties and intermolecular interaction of OVAs from different avian species at different pH values upon heat-induced gelation.

The objectives of this study were to clarify the effects of intermolecular interactions involving SS bonds and hydrophobic interactions on aggregation as well as gelation, and the effects of pH value on viscoelastic behavior, of heat-induced OVA gels formed from five avian species. Furthermore, this study aimed to elucidate the relationship between OVA gel formation and structural change with a simple and purified protein using rheological analysis of the gels. We also evaluated some rheological properties of EW from different avian species using creep tests, so that food scientists can better manipulate these properties to obtain desirable foods.

MATERIALS AND METHODS

Materials. The EW solutions (isolated from eggs laid within 3 days; pH, 8.95 ± 0.21) were from three galliform species (hen, H; guinea fowl, G; turkey, T) and two anseriform species (Pekin duck, D; mallard duck, M). OVAs were purified from different avian species EW by crystallization in an ammonium sulfate solution and recrystallization four times. All chemicals were of analytical grade.

Preparation of OVA Solutions. OVAs were dissolved in distilled water containing 86 mM NaCl at the concentration of 10% (w/v) and dialyzed in the same solution overnight at 4 °C. Samples were adjusted with 1 N NaOH to different values of pH ranging from 7.0 to 8.5 with 0.5 intervals. The protein concentration used for all experiments was 8% (w/v). The protein content was determined according to the Lowry procedure (*21*). For hydrophobicity and SH group measurement, NaCl in the samples was omitted.

Gel Electrophoresis. Native-polyacrylamide gel electrophoresis (Native-PAGE) was performed using 7.5% gels, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 12.5% gels under both reducing and nonreducing conditions according to the method of Laemmli (*22*). The gels were stained with Coomassie Brilliant Blue R-250 (CBB).

Total Sulfhydryl Groups. The SH group content of OVAs from the five avian species was determined at least nine times from three replications, according to the method of Beveridge et al. (*23*). The OVA solutions (8%, w/v) at pH 7.0 or 8.0 described above were heated at 80 °C for 15 min, diluted to 1% (w/v) with buffer A (86 mM Tris−glycine buffer containing 4 mM Na_2−EDTA, pH 8.0), and then an aliquot was diluted 30-fold with buffer B (1% SDS, 8 M urea in buffer A). To a 1-mL sample was added 0.01 mL of Ellman's reagent (10 mM DTNB). After 15 min, the absorbance at 412 nm was measured, and an unheated sample and reagent were also included for each determination as control and blank values, respectively. The content of SH groups was calculated using a molar absorbance of 13 600 M^{-1} cm^{-1}.

Surface Hydrophobicity (H_0). The surface H_0 of native and heated OVAs was determined by the fluorescence probe (1-anilino-8-naphthalene sulfonate, ANS) method of Hayakawa and Nakai (*24*). Solutions containing 8% (w/v) protein (pH 7.0 or pH 8.0) were heated and diluted with 10 mM phosphate buffer saline (PBS) at pH 7.0 or pH 8.0 for a series of five concentrations between 0.01 and 0.05%. Then, 15 μL of ANS (8.0 mM in 10 mM PBS) was added to 3 mL of sample solution. Fluorescence intensity (FI) was measured with a JASCO spectrofluorometer FP770 at an excitation wavelength of 390 nm and emission wavelength of 470 nm. For each determination, a 0.04 mM ANS methanolic solution was used to adjust the relative FI. The initial slope of the FI versus protein concentration (%) plot, which was calculated by linear regression analysis, was used as an index of the protein surface H_0.

Preparation of Gel. The solutions containing 8% (w/v) protein and 86 mM NaCl at different pH values (pH 7.0−8.5 with 0.5 intervals) were degassed under vacuum for 1 h, poured into a sausage cellulose casing (diameter 25 mm), and then heated in a water bath at 80 °C for 30 min. The gels were immediately cooled to room temperature by immersion in tap water for 30 min, and allowed to stand at room temperature for 1 h. The fresh EWs from the five avian species (pH 8.95 ± 0.21) were homogenized at 1000 rpm and 4 °C to form a fine dispersion, then degassed and heated as described above. The gels from EW/OVAs were cut into cylindrical blocks (20-mm height and 25-mm diameter) and carefully removed from the casing for a determination of creep characteristics.

Creep Measurement. A Rheoner II Creep Meter (RE2-3305, Yamaden, Co., Japan) was used for creep measurements. The measurements were carried out under uniaxial compression at 5 mm/sec. Eight gel cylinders were tested for each replication. Data represent the mean values of 16 analyses from two replications, and are compared using the *t* test. Significance of differences is defined at 0.05. So that the creep was applied validly, the linearity between stress and strain was maintained for each measurement. Based on preliminary tests (in the linearity region), all measurements were performed within the region of constant strain (12% ± 3). The creep curves were drawn on the display and analyzed with software developed for creep analysis (Ver. 2.1, Yamaden, Co., Japan), where these gels conformed to a six-element mechanical model consisting of one Hookean body (one spring), two Voigt bodies (two dashpots and two springs), and one Newtonian body (one dashpot) (*25*). This six-element model is described by the following eq 1 based on the relationship between stress and strain, and the compliance data of EW/OVAs gels from creep experiments are analyzed by eqs (2) and (3) (*26*):

$$\epsilon(t) = S/E_0 + \sum_{i=1}^{2} S/E_i\,(1 - e^{(-t/\tau_i)}) + (S/\eta_N)t \quad (1)$$

$$J(t) = \epsilon(t)/S \quad (2)$$

$$J(t) = J_0 + \sum_{i=1}^{2} J_i\,(1 - e^{(-t/\tau_i)}) + t/\eta_N \quad (3)$$

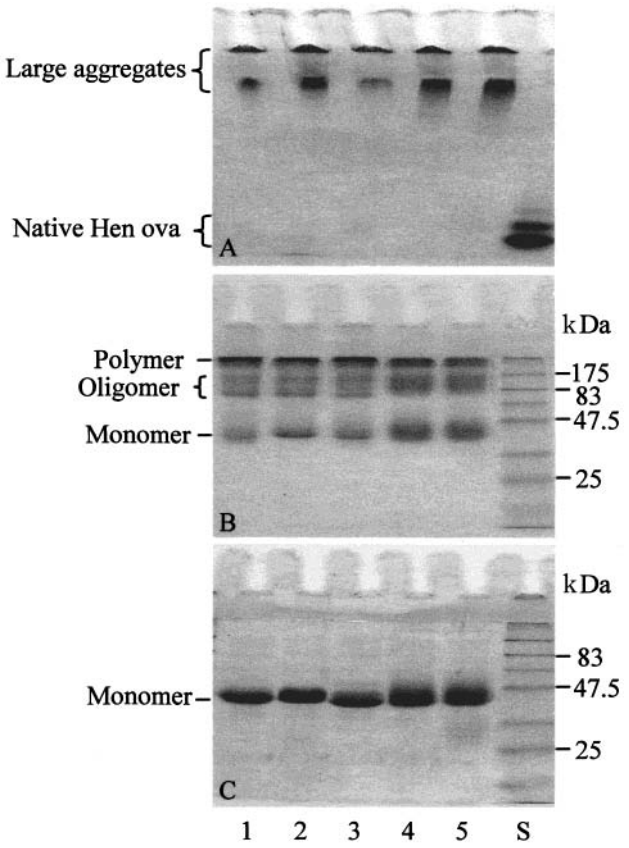

Figure 1. Heat-induced polymerization of five species OVAs. (A) Native-PAGE patterns of heated OVAs. (B) SDS-PAGE patterns of heated OVAs without mercaptoethanol, and (C) with mercaptoethanol. OVA dispersions (8%) from five species were heated at 80 °C for 15 min, respectively. Lanes: S, native hen OVA and molecular weight markers; 1, hen; 2, guinea fowl; 3, turkey; 4, mallard duck; 5, Pekin duck.

where $\epsilon(t)$ = strain (dimensionless), S = stress (N/m^2), E_0 = elastic modulus of Hookean body (instantaneous modulus) (N/m^2), E_i = elastic modulus of Voigt body (N/m^2), τ_i = retardation time (sec), η_N = Newtonian viscosity (Pa s), $J(t)$ = creep compliance (m^2/N), J_0 = instantaneous compliance (m^2/N), J_i = retarded compliance (m^2/N), and t = time (sec).

RESULTS

Gel Electrophoresis. The electrophoretic patterns from native-PAGE and SDS-PAGE showed no significant difference between heating at pH 7.0 and 8.0; therefore, we chose to analyze OVA polymers formed by heating at pH 7.0 only.

The electrophoretic patterns of OVAs (8%) heated at 80 °C for 15 min were analyzed by native-PAGE. The result revealed that OVA monomers completely disappeared (**Figure 1A**). Bands of high-molecular-weight polymers appeared on top of separating and stacking gel, suggesting that all monomers were involved in polymer formation. SDS-PAGE patterns were analyzed under reducing and nonreducing conditions to determine the significance of intermolecular interactions. Electrophoresis of the heated OVAs under nonreducing conditions gave many bands involving monomers, oligomers, and higher molecular weight polymers (**Figure 1B**). When heated samples were treated with 2-ME, the polymeric and oligomeric bands completely disappeared; a single band appeared in all species at a position close to that of the native OVAs (**Figure 1C**).

There was a marked difference in the aggregation process between galliform and anseriform OVAs. Protein bands labeled

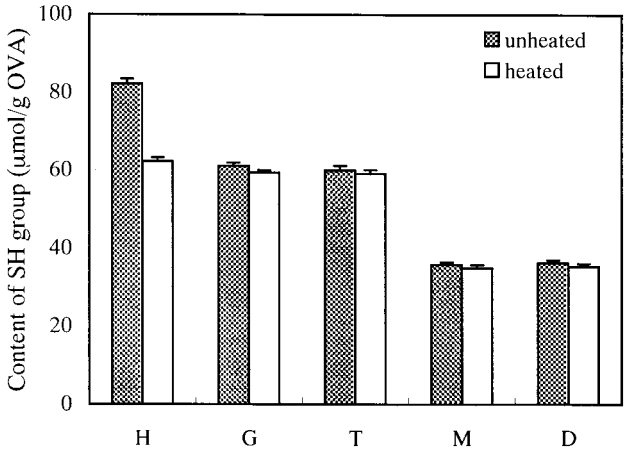

Figure 2. Comparison of SH group contents between native and heat-denatured OVAs from five avian species at pH 7.0. H, hen; G, guinea fowl; T, turkey; M, mallard duck; D, Pekin duck. Each value represents the mean ± standard deviation (n = 9).

polymer with low electrophoretic mobilities were clearly observed near the top of the separating gel (**Figure 1B**), and the intensities of the bands from galliform OVAs were higher than those of the bands from anseriform OVAs. On the contrary, the monomeric bands with the higher staining intensities were observed in electrophoretic patterns of Pekin duck and mallard duck OVAs. Furthermore, the differences of the polymers among five species on SDS-PAGE have also been analyzed by quantitating peak areas in elution profiles of gel filtration chromatography (*6*). The results showed that the amounts of polymer and monomer of heated OVAs from galliform species were higher and lower than those from anseriform species, respectively. Therefore, it was suggested that the aggregation process was quantitatively affected by different avian OVAs from five species, possessing different numbers of free SH groups.

Change in Sulfhydryl Groups. The content of total SH groups in heated and unheated OVAs at pH 7.0 from the five avian species was measured in the presence of 8 M urea and 1% SDS (**Figure 2**). The SH group content decreased drastically in heat-denatured hen OVA, whereas the change was insignificant in other species OVAs upon heating. A similar result was also found when heating OVAs at pH 8.0, suggesting that change in the SH group content was pH-independent under this condition (data not shown). Because of the significant decrease in SH groups from hen OVA ($p < 0.01$) (**Figure 2**), a three-dimensional network structure might be formed by extra SS bonds upon heating. However, the SH group content of the other species OVAs decreased slightly or changed insignificantly upon heating, indicating that the heat treatment did not cause marked oxidation of the SH groups. In this case, intermolecular SS bonds might be caused by SH/SS interchange reactions, and not by SH oxidation.

Surface Hydrophobicity (H_0). The surface H_0 of heat-denatured proteins was measured using a fluorescence probe, ANS. The probe tests whether normally buried hydrophobic regions are exposed to the solvent environment after the denaturation of the protein upon heating. As shown in **Figure 3**, none of the five species OVAs exhibited florescence in a native state at either pH 7.0 or 8.0, indicating no extensive unfolding or rearrangement of the protein molecule to expose buried hydrophobic groups. However, the surface H_0 of all OVAs increased significantly upon heating ($p < 0.001$), with a species dependence. In addition, it was also shown that a marked

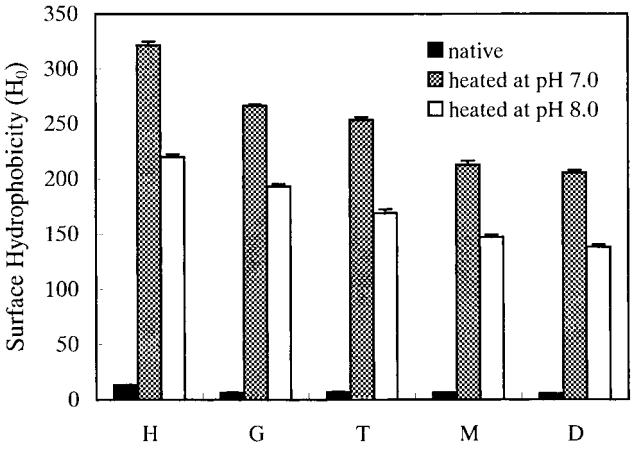

Figure 3. Comparison of surface hydrophobicities of five species OVAs at pH 7.0 and pH 8.0 with heating at 80 °C for 15 min by the fluorescence probe, ANS. Each value represents the mean ± standard deviation of triplicates.

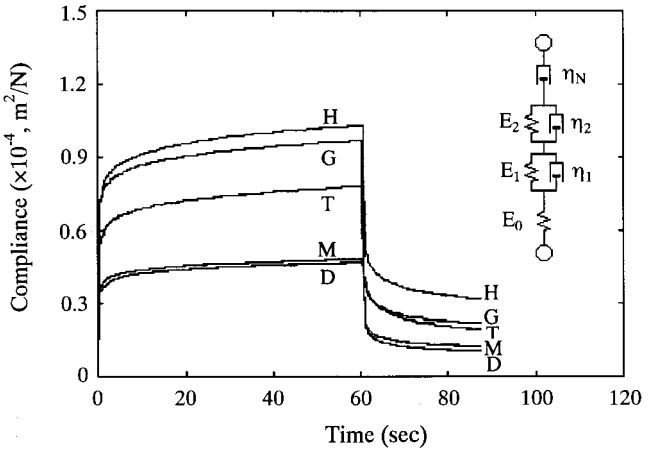

Figure 4. Creep-compliance curves of OVA gels from five avian species by a six-element mechanical model (inset). Gels were prepared by heating the 8% OVAs solutions at pH 8.5, 80 °C for 30 min, followed by rapid cooling to room temperature by immersion in tap water.

fall in surface H_0 of each species OVA was accompanied by an increase in pH from 7.0 to 8.0 ($p < 0.01$) (**Figure 3**), but this was still significantly higher than that of the unheated sample. The results indicated that the heating of protein molecules of OVAs could create extensive unfolding and expose buried hydrophobic groups, and the surface H_0 was pH-dependent in this condition.

Creep Test of OVA/EW Gels. Creep tests provide useful information on the viscoelastic properties of heat-induced 8% OVA gels from different avian species formed at 80 °C for 30 min. The typical creep curves from the five species OVA gels and the corresponding mechanical model obtained following compression of the gel for 60 s are shown in **Figure 4**. Upon analysis of these creep curves according to eqs 1 and 3, a six-element mechanical model provided a good approximation of the observed behavior. This was consistent with models for other gels such as whey protein isolates (*25*), hen egg white (*27*), and soy protein (*28*). In this study, the OVA gels were tested for only 60 s, because longer time measurements may involve great chemical and physical changes (*29*).

Creep compliance, the reciprocal of elastic modulus, was studied by applying eq 3 because it yields more quantitative information concerning molecular structures of the material. As shown in **Figure 4**, the creep compliance curves were determined with different stresses (shown in **Table 1**) in order to remain within a constant strain (12% ± 3). The result showed that the creep compliance of the OVA gels from galliform species was higher than that of the gels from anseriform species, suggesting the former was softer and more deformable, as higher values in the creep-compliance curve result in lower viscoelastic constants. For the apparent characteristic of gels, the five avian species OVAs formed translucent gels, and anseriform OVA gels showed a higher translucency than galliform OVA gels at pH 8.5. According to Lynch and Mulvihill (*30*), instantaneous compliance (J_0) may be related to the undisturbed protein network structure, and a higher gel rigidity exhibits a lower J_0. As shown in **Table 1**, the smaller J_0 of OVA gels fromanseriform OVAs also indicated that these gels were more rigid and elastic than those from galliform OVAs. In addition, Newtonian viscosity (η_N) may be attributed to the breakdown of protein network structure (*30*). The larger Newtonian viscosity of OVA gels from anseriform OVAs suggested a greater resistance to flow (**Table 1**).

These observations were in agreement with the viscoelastic behavior determined from the EW of five avian species. The five avian species EW formed turbid gels when heated at 80 °C for 30 min, and the viscoelastic parameters are summarized in **Table 1**. The data showed that the E_0 and η_N values of EW were about 1.5−2.0 times that of OVAs in each species, respectively, and viscoelastic character followed in the order hen < guinea fowl and turkey < mallard duck and Pekin duck (shown in **Table 1**). The correlation coefficients (R^2) between OVA and EW gels were 0.95 and 0.99 for E_0 and η_N, respectively, indicating a good correlation of viscoelastic behavior between OVA and EW gels upon heating.

Effect of pH on Viscoelastic Properties. To understand the mechanism of OVA gel formation from the five avian species, the creep behavior of OVA gels made with 8% protein in a pH range of 7.0−8.5 was measured. Using a constant strain (with different stress), the creep compliance curves decreased with increasing pH value for each species OVA (not shown), indicating that all gels from the five OVAs became more rigid with increasing pH value (7.0−8.5). Viscoelastic parameters (E_0 and η_N) were calculated for five avian OVAs at different pH values as shown in **Figure 5**, and data are expressed as mean ± standard deviation. The E_0 increased slightly from pH 7.0 to 8.0, and reached an almost constant value from 8.0 to 8.5 (**Figure 5A**), whereas the η_N increased significantly with pH, particularly in OVAs from mallard duck and Pekin duck (**Figure 5B**). These results show the viscoelastic parameters of all OVA gels increased as pH increased, but the magnitudes of viscoelastic parameters were different in each species OVA, and these differences are closely related to the phylogeny of the species. The apparent characteristic of the gels was that OVAs formed turbid and less viscoelastic gels during heating at pH 7.0, then became translucent with increasing pH from 7.0 to 8.5. These results suggest that the viscoelastic behavior of all species OVAs was dependent on pH values, and showed that there was a significant difference in viscoelasticity among avian species.

DISCUSSION

As demonstrated by native-PAGE, the OVAs immediately aggregate at neutral pH when denatured by heating, and huge polymers of OVA are formed. The soluble aggregates can be partially dissociated into monomers and oligomers in the presence of SDS, and further reversed into complete monomers in the presence of 2-ME, suggesting the involvement of SS bonds and intermolecular hydrophobic interaction. Shimada and

Table 1. Comparison of Viscoelastic Parameters for Egg White/OVA Gels from Five Avian Species[a]

	OVAs at pH 8.5				EW at pH 8.95 ± 0.21			
	$S(\times 10^3)$	$J_0(\times 10^{-4})$	$E_0(\times 10^4)$	$\eta_N(\times 10^6)$	$S(\times 10^3)$	$J_0(\times 10^{-4})$	$E_0(\times 10^4)$	$\eta_N(\times 10^6)$
hen	1.20	0.75	$1.33^a \pm 0.04$	$6.70^a \pm 0.07$	1.93	0.43	$2.32^a \pm 0.06$	$12.82^a \pm 0.08$
guinea fowl	1.40	0.71	$1.41^a \pm 0.08$	$7.72^b \pm 0.03$	2.90	0.29	$3.41^b \pm 0.03$	$18.87^b \pm 0.04$
turkey	1.40	0.52	$1.93^b \pm 0.25$	$8.45^c \pm 0.15$	2.90	0.23	$4.45^c \pm 0.03$	$18.39^b \pm 0.05$
mallard duck	2.60	0.33	$3.01^c \pm 0.23$	$18.22^d \pm 0.34$	3.87	0.18	$5.65^d \pm 0.04$	$34.65^c \pm 0.91$
Pekin duck	2.60	0.32	$3.14^c \pm 0.10$	$18.35^d \pm 0.10$	3.87	0.17	$6.00^d \pm 0.12$	$33.76^c \pm 1.30$

[a] S, stress (N/m^2); J_0, instantaneous compliance (m^2/N); E_0, instantaneous modulus (N/m^2); η_N, Newtonian modulus (Pa s). Correlation coefficients of elasticity (E_0) and viscocity (η_N) between OVA and egg white in corresponding species: $R^2 = 0.95$ and $R^2 = 0.99$, respectively. Each value of E_0 and η_N represents the mean ± standard deviation (n =16), means at same column with different letters are significantly different ($p < 0.05$).

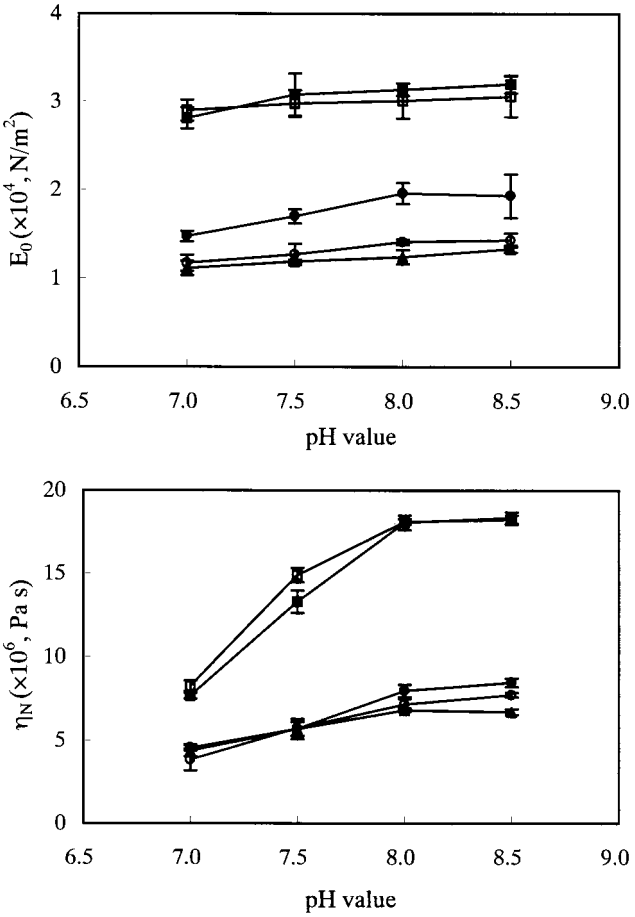

Figure 5. Variation of viscoelastic parameters with pH value for OVA gels from five avian species. E_0, instantaneous modulus; η_N, Newtonian modulus. ▲, Hen; ○, guinea fowl; ●, turkey; □, mallard duck; ■, Pekin duck. Data are expressed as mean ± standard deviation (n = 16). Gels were made as described in **Figure 4**.

Matsushita (*31*) reported that the first step of hen OVA coagulation involves the formation of SS bonds and the exposure of hydrophobic groups. Mine et al. (*32*) also proposed that the finite size soluble egg albumen aggregates which are formed from partially unfolded protein molecules with a considerable secondary structure, were constructed mainly from SS bonds and β-sheet hydrophobic forces.

As demonstrated by the change in SH group contents after heating, intermolecular SS bonds in the network of protein gels depended on not only numerous SH/SS interchange reactions but also the oxidation of SH into additional SS bonds in avian species. OVA is one of the relatively few proteins containing both SH groups and SS bonds in the molecule. It has been indicated that native OVAs contain one SS bond ($Cys^{73}-Cys^{120}$) in each species, but contain different SH groups according to the avian species: 4 for hen (Cys^{11}, Cys^{30}, Cys^{367}, and Cys^{382}), 3 for guinea fowl and turkey (Cys^{11}, Cys^{367}, and Cys^{382}), and 2 for Pekin duck and mallard duck (Cys^{11} and Cys^{331}) (*5*). Hayakawa and Nakai (*24*) suggested that only one SH radical in the total 4 SH groups of hen OVA molecule might be readily available for aggregation when heat-denatured. Onda et al. (*33*) investigated the refolding process of OVA from a urea-denatured state produced by the nucleophillic attacks of 4 cysteines on the native SS bond. The result shows that the most rapid reaction, forming a mispaired SS bond, depends on an attack by Cys^{30} forming $Cys^{30}-Cys^{73}$ and $Cys^{30}-Cys^{120}$. These results not only reflect the difference of SH group contents among the five species OVAs, but also suggest that the reactivity of SH groups is different in OVA molecules.

Determinations of hydrophobicity revealed a dramatic increase in surface H_0 in various OVAs upon heating. This is consistent with the data of Kato and Nakai (*34*), indicating an exposure of hydrophobic groups in the unfolded protein molecules. Kato and Takagi (*35*) further studied the change in the secondary structural elements upon heat denaturation of hen OVA by using CD analysis, interpreting as the result of an increase in β-sheet structure with a concomitant decrease of the helix content of the molecule. The critical effect of intermolecular hydrophobic interactions on the promoted β-sheet contents was emphasized. In the present study, the surface H_0 of all heated OVAs at pH 8.0 was found to be much lower than that at pH 7.0. Hayakawa and Nakai (*24*) also indicated that ANS H_0 decreased with increases of pH value in the range of 5.5 to 8.0, and suggested that thermal coagulation of OVA was in part due to hydrophobic interactions, but that excessive surface H_0 of proteins decreased gel strength. Because the hydrophobic nature of polypeptide side chain can be influenced by pH, the importance of this observation deserves further elucidation. In addition, there were significant variations in the surface H_0 of OVAs from different avian species, which might have a close relationship with primary structure, e.g., the hydrophobic amino acid content of proteins. In the previous study, in a comparison with hen OVA the variations in amino acid sequences of the peptides containing cysteine residues for turkey and guinea fowl OVAs were about 10%, and for Pekin and mallard duck OVAs about 20%, indicating a close relationship among avian species (data not published).

The presence of different amino acids in the polypeptide chain with different charges and hydrophobicities and of SS bonds implies that different protein−protein bonds are involved (*11*). From the above description, it was suggested that the degree of aggregation from galliform OVA was higher than that from anseriform OVA molecules. This was due to the difference in the content of SH groups and surface H_0 between anseriform and galliform OVAs, which might induce a difference in the

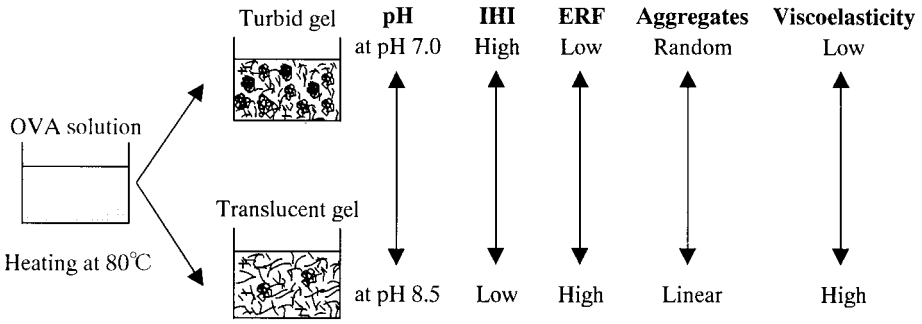

Figure 6. A schematic representation of turbid and translucent gels of denatured OVA by heating under different pH values. IHI, intermolecular hydrophobic interaction; ERF, electrostatic repulsive force.

contribution of aggregated interaction forces and generate a stronger intermolecular attraction with SS bonds and intermolecular hydrophobic interaction (IHI) in galliform OVA gels. It was also reported that the aggregation behavior of denatured proteins appears to play a crucial role in the subsequent gelling process of proteins (*12*), and further, a particular network of proteins was formed when partially unfolded OVA aggregate randomly into grapelike clusters (turbid gel) or linearly to form a "string of head" (transparent gel) (*14*), whereas the gel properties of the translucent gels were closer to those of transparent gel than the turbid gel, and might be formed by coexisting random and linear aggregates. As described physical characteristic, the anseriform OVA gels had the higher viscoelastic values (E_0 and η_N) than those of galliform OVAs gels; particularly, E_0 and η_N values of anseriform OVA gels were more than two or three times those of galliform OVAs gels at pH 8.5, respectively (**Table 1** and **Figure 5**). The apparent characteristics of the gels were that galliform OVA gels had a higher turbidity at pH 7.0 and a lower translucency at pH 8.5 than anseriform OVA gels. The results suggested that a galliform OVA molecule might undergo a conformational transition from an aggregated state to more random multiple cross-linking structures upon heating. Therefore, galliform OVA gels were less viscoelastic than anseriform OVA gels.

When the pH value was increased from 7.0 to 8.5, the product on heating changed from a turbid gel to a translucent gel, and the parameters E_0 and η_N also altered with this change in the gel state (**Figure 5**). Changes in E_0 and η_N as a function of thermal OVA gels might reflect changes in molecular structure of the network, and these changes might have resulted from rearrangement and/or rupture of intermolecular hydrophobic interaction (IHI) and electrostatic repulsive forces (ERF) at different pH values (*36*). In a vicinity around the isoelectric point (pI, 4.6) of OVA, the net charge of OVA molecules is close to zero, so that IHI becomes much stronger than ERF of heat-denatured OVA. In this case, the heat-denatured OVA become tangled strongly to produce a turbid suspension. On the contrary, at pH values away from pI, the heat-denatured OVA generates soluble polymers without gelation, because ERF becomes much stronger and overwhelms IHI. Therefore, gelation of OVA occurs at pH regions not so far from the pI and at appropriate concentrations of NaCl, where IHI and ERF were well balanced (*36*−*40*). A speculative model of the change in the gel structure is depicted in **Figure 6**. In this study, all OVAs from five avian species can form gels at the pH range from 7.0 to 8.5 upon heating, and the parameters increased with increase at pH and almost reached constant values from pH 8.0 to 8.5 (**Figure 5**). At this stage, the OVA gels have a higher viscoelastic and translucent behavior, because of the fine structure of the network of the gels with linear aggregates and the balance of IHI and ERF.

The structural changes in protein molecules between viscoelastic and less viscoelastic gels are of great interest in food systems. From the above discussion, it is suggested that the heat-induced fine network structure might result from a good balance between the IHI and the ERF. However, the detailed manner in which the gels formed is unclear. Additional research will be needed to elucidate the mechanism of formation at the molecular level, and so expand the utilization of different EW/OVAs in the food industry.

CONCLUSION

Heat-denatured OVAs from all avian species can form large aggregates by intermolecular hydrophobic interaction and SS bonds, involving SH/SS exchange reaction and SH oxidation into SS bonds. The contributions of surface H_0 (pH-dependent) and SH groups (pH-independent) to aggregation and rheological properties appear to depend on avian species; furthermore, the physical characteristics of heat-induced OVA gels are controlled by the balance of IHI and ERF. The rheological properties of the OVAs of all species in the present study are pH-dependent and change significantly with the phylogeny of these species, and the OVAs correlate closely with the corresponding species EW in the viscoelastic parameters of heat-induced gels.

LITERATURE CITED

(1) Egelandsdal, B. Heat-induced gelling in solutions of ovalbumin. *J. Food Sci.* **1980**, *45*, 570−573, 581.
(2) Ma, C. Y.; Holme, Y. Effect of chemical modifications on some physicochemical properties and heat coagulation of egg albumen. *J. Food Sci.* **1982**, *47*, 1454−1459.
(3) Hatta, H.; Kitabatake, N.; Doi, E. Turbidity and hardness of a heat-induced gel of hen egg ovalbumin. *Agric. Biol. Chem.* **1986**, *50*, 2083−2089.
(4) Kitabatake, N.; Tani, Y.; Doi, E. Rheological properties of heat-induced ovalbumin gels preparared by two-step and one-step heating methods. *J. Food Sci.* **1989**, *54*, 1632−1638.
(5) Sun, Y.; Hayakawa, S. Sequential comparison of peptides containing half-cystine residues from ovalbumin of six avian species. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 2589−2596.
(6) Sun, Y.; Hayakawa, S. Thermally induced aggregates in mixture of α-lactalbumin with ovalbumins from different avian species. *J. Agric. Food Chem.* **2001**, *49*, 2511−2517.
(7) Hickson, D. W.; Dill, C. W.; Morgan, R. G.; Sweat, V. E.; Suter, D. A.; Carpenter, Z. L. Rheological properties of two heat-induced protein gels. *J. Food Sci.* **1982**, *47*, 783−785.
(8) Hickson, D. W.; Alford, E. S.; Gardner, F. A.; Diehl, K.; Sanders, J. O.; Dill, C. W. Changes in heat-induced rheological properties during cold storage of egg albumen. *J. Food Sci.* **1982**, *47*, 1908−1911.
(9) Holt, D. L.; Watson, M. A.; Dill, C. W.; Alford, E. S.; Edwards, R. L.; Diehl, K. C.; Gardner, F. A. Correlation of the rheological behavior of egg albumen to temperature, pH, and NaCl concentration. *J. Food Sci.* **1984**, *49*, 137−141.

(10) Kato, A.; Takagi, T. Estimation of the molecular weight distribution of heat-induced ovalbumin aggregates by the low-angle laser light scattering technique combined with high-performance gel chromatography. *J. Agric. Food Chem.* **1987**, *35*, 633−637.
(11) Kato, A.; Ibrahim, H.; Takagi, T.; Kobayashi, K. Excellent gelation of egg white preheated in the dry state is due to the decreasing degree of aggregation. *J. Agric. Food Chem.* **1990**, *38*, 1868−1872.
(12) 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.
(13) Hegg, P.-O. Conditions for the formation of heat-induced gels of some globular food proteins. *J. Food Sci.* **1982**, *47*, 1241−1244.
(14) Doi, E. Gels and gelling of globular proteins. *Trends Food Sci. Technol.* **1993**, *4*, 1−5.
(15) Hickson, D. W.; Dill, C. W.; Morgan, R. G.; Suter, D. A.; Carpenter, Z. L. A comparison of heat induced gel strengths of bovine plasma and egg albumen proteins. *J. Anim. Sci.* **1980**, *51*, 69−73.
(16) Arntfield, S.; Murray, D.; Isomond, M. A. H. Role of disulfide bonds in determining rheological and microstructural properties of heat-induced protein networks from ovalbumin and vicilin. *J. Agric. Food Chem.* **1991**, *39*, 1378−1385.
(17) Ziegler, G. R.; Foegeding, E. A. The gelation of proteins. In *Advances in Food and Nutrition Research;* Kinsella, J. E., Ed.; Academic Press: New York, 1990; Vol. 34.
(18) Clark, A. K. Gels and gelling. In *Physical Chemistry of Foods;* Schwartzberg, H. G., Hartel, R. W., Eds.; Dekker: New York, 1992.
(19) Kamada, Y.; Rector, D.; Kinsella, J. E. Influence of temperature of measurement on creep phenomena in glycinin gels. *J. Food Sci*. **1988**, *53*, 589−591.
(20) Arntfield, S. D.; Murray, E. D.; Ismond, M. A. H. Influence of protein charge on the thermal properties as well as microstructure and rheology of heat induced networks for ovalbumin and vicilin. *J. Texture Stud.* **1990**, *21*, 295−322.
(21) 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.
(22) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970,** *227*, 680−685.
(23) Beveridge, T.; Toma, S. J.; Nakai, S. Determination of SH- and SS-groups in some food proteins using Ellman's reagent. *J. Food Sci*. **1974**, *39*, 49−51.
(24) Hayakawa, S.; Nakai, S. Contribution of hydrophobicity, net charge and sulfhydryl groups to thermal properties of ovalbumin. *Can. Inst. Food Sci. Technol. J.* **1985,** 18, 290−295.
(25) Katsuta, K.; Rector, D.; Kinsella, J. E. Viscoelastic properties of whey protein gels: mechanical model and effects of protein concentration on creep. *J. Food Sci.* **1990**, *55*, 516−521.
(26) Peleg, K. Linearization of relaxtion and creep curves of solid biological materials. *J. Rheol.* **1980**, *24*, 451−455.
(27) Isozaki, H.; Akabane, H.; Nakahama, N. Viscoelasticity of hydrogels of agar-agar analysis of creep and stress relaxation. *Nippon Nogeikagaku Kaishi* **1976**, *50*, 265−272.
(28) Chronakis, I. S. Network formation and viscoelastic properties of commercial soy protein dispersions: effect of heat treatment, pH and calcium ions. *Can. Inst. Food Sci. Technol. J.* **1996**, *29*, 123−134.
(29) Mitchell, J. R. The rheology of gels. *J. Texture Stud.* **1980**, *11*, 315−317.
(30) Lynch, M. G.; Mulvihill, D. M. The influence of caseins on the rheology of iota-carrageenan gels. *Food Hydrocolloids* **1994**, *8*, 317−329.
(31) Shimada, K.; Matsushita, S. Thermal coagulation of egg albumen. *J. Agric. Food Chem.* **1980**, *28*, 409−412.
(32) Mine, Y.; Noutomi, T.; Haga, N. Thermally induced changes in egg white proteins. *J. Agric. Food Chem.* **1990**, *38*, 2122−2125.
(33) Onda, M.; Tatsumi, E.; Takahashi, N.; Hirose, M. Refolding process of ovalbumin from urea-denatured state. *J. Biol. Chem.* **1997**, *272*, 3973−3979.
(34) Kato, A.; Nakai, S. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochim. Biophys. Acta* **1980**, *624*, 13−20.
(35) Kato, A.; Takagi, T. Formation of intermolecular α-sheet structure during heat denaturation of ovalbumin. *J. Agric. Food Chem.* **1988**, *36*, 1156−1159.
(36) Hatta, H.; Hagi, T.; Hirano, K. Chemical and physicochemical properties of hen eggs and their application in foods. In *Hen Eggs: Their Basic and Applied Science;* Yamamoto, T., Juneja, L. R., Hatta, H, Kim, M, Eds.; CRC Press: Boca Raton, FL, 1996; pp 124−125.
(37) Kitabatake, N.; Hatta, H.; Doi, E. Heat-induced and transparent gel prepared from hen egg ovalbumin in the presence of salt by a two-step heating method. *Agric. Biol. Chem.* **1987**, *51*, 771−778.
(38) Kitabatake, N.; Shimizu, A.; Doi, E. Preparation of transparent egg white gel with salt by a two-step heating method. *J. Food Sci.* **1988**, *53*, 735−738.
(39) Doi, E.; Koseki, T.; Kitabatake, N. Effects of limited proteolysis on functional properties of ovalbumin. *J. Am. Oil Chem. Soc.* **1987**, *64*, 1697−1703.
(40) Matsudomi, N.; Ishimura, Y.; Kato, A. Improvement of gelling properties of ovalbumin by heating in dry state. *Agric. Biol. Chem.* **1991**, *55*, 876−881.

Received for review July 30, 2001. Revised manuscript received November 12, 2001. Accepted December 4, 2001.

JF0109975