JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Dielectric Study of Heat-Denatured Ovalbumin in Aqueous Solution by Time Domain Reflectometry Method

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The dielectric behavior of native and heat-denatured ovalbumins (OVAs) from three avian species in aqueous solution was examined over a frequency range of 100 kHz to 20 GHz, using the time domain reflectometry (TDR) method. For the native OVA solutions, three kinds of relaxation processes were observed at around 10 MHz, 100 MHz, and 20 GHz, respectively; these could be assigned to the overall rotation of protein molecules, the reorientations of the bound water, and the free water molecules, respectively. For the heat-denatured samples, three relaxation processes were also observed. However, the relaxation process at ∼100 MHz originated via a different mechanism other than the reorientation of bound water, namely, the micro-Brownian motion of peptide chains of heat-denatured protein. From the observed relaxation process at ∼100 MHz, the relaxation strength of heat-denatured OVA solution for duck was higher than that of OVA solutions for hen and guinea fowl and showed the pH dependency from pH 7.0 to 8.0 for OVAs obtained from all three species. Furthermore, the results demonstrated that the relaxation strength was closely related to surface hydrophobicity of protein molecules and gel rheological properties. It was suggested that the difference in the surface hydrophobicity of protein influenced the dielectric behavior of water around denatured protein, whereas the dielectric behavior of denatured protein could be an indication of the gel rheological properties. Such studies can aid in the understanding of the different network structures of OVA gels from three avian species.

KEYWORDS: Ovalbumin; dielectric relaxation; gelation; rheological properties; time domain reflectometry

INTRODUCTION

Protein−water interactions are key to biological functions. They are also significant factors that affect the physicochemical properties of protein in food processing. A number of works have been devoted to the research of water properties and have indicated that water molecules associated with proteins were involved in a variety of functional roles, such as enzyme catalysis (*1*), conformational stability (*2*), protein dynamic behavior (*3*, *4*), and rheological properties of heat-induced gel (*5*). It has been recognized that water in proteins exists as both bound and free states. The water molecules attach to the oxygen, nitrogen, and polar groups on the globule protein surface through hydrogen bonding (*6*). This bound water is thought to have an important influence on the functions of protein. Therefore, it is interesting to investigate the properties of water around the protein.

Dielectric relaxation spectroscopy is one of the most useful methods for dynamical study and conveying information about water status of samples in aqueous solutions, and it is becoming increasingly important in the hydration analysis of food systems (*7*, *8*). Recently, dielectric measurement using the time domain reflectometry (TDR) method has been performed on solid and liquid foods, such as rice, milk, egg white, fish, agar aqueous gel, and gelatin aqueous gel (*9*). Miura et al. (*9*) indicated that the TDR method could be widely applied to evaluate the quality of food, monitor its freshness, and control processing. Harvey et al. (*10*) determined the dielectric behavior of lysozyme with absorbed water by the TDR method; two distinct peaks were suggested: one is found at a frequency of ∼10 GHz and another locates at ∼100 MHz. Similarly, the dielectric analysis of the DNA aqueous solution (*11*) and the moist collagen (*12*) had indicated that there were two relaxation processes in both cases. The high-frequency process at ∼10 GHz was due to the reorientation of free water molecules. The relaxation strength for the low-frequency process at ∼100 MHz was concluded to be related to reorientation of bound water attached to DNA or protein. Furthermore, the dielectric behaviors have also been investigated for globular protein in aqueous solutions, and subsidiary dispersion due to bound water was found at an intermediate-frequency region between two principal dispersions due to molecular motions of protein and free water molecules, respectively (*13*, *14*).

Mashimo et al. (*15*) reported that poly(α-amino acid)s showed two absorption peaks in aqueous solution. One observed at high frequency was due to the orientation of water molecules.

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10.1021/jf030538z CCC: $27.50
Published on Web 03/18/2004

Another at low frequency had a possibility of being due to the peptide chain motion, because the relaxation strength depended on the ratio of the coiled part to the helical part. However, there remained a possibility that the relaxation came from another mechanism such as side group motions or orientation of water molecules bound to the polymer. Afterward, dielectric relaxation processes reflecting micro-Brownian motions of polymer chains in aqueous solutions of poly(vinylpyrrolidone) (PVP) (*16*), poly(glutamic acid) (PGA) (*15*), and heat-denatured protein (*13*) were observed by dielectric relaxation measurements using TDR methods. Dielectric response functions obtained for the chain motions were described by the Köhlrausch−Williams−Watts (KWW) type (*17*, *18*).

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 the major protein of EW, and its behavior dominantly affects the gelation of EW. Many investigators have worked to elucidate the mechanism of thermal aggregation or gelation of OVA and EW (*19*−*23*). The overall gelation process requires that the proteins unfold or undergo some conformational change, and the denatured protein molecules are allowed to orient themselves and interact at specific points, thus forming a different conformation of the polymer.

In our previous study (*23*), the aggregation process, the intermolecular interactions in denatured OVA solutions, and the rheological behavior of OVA gels were investigated for different avian species, suggesting that these properties were different from those of related avian species with pH dependency. However, little research has been conducted on protein−water interactions during the heat-induced gel formation of OVA from different avian species. Furthermore, it has not been clarified whether and how the structures and properties of water and peptide chains in denatured OVA solution might be influenced by the difference in the surface functional groups of protein. Dynamical investigations of the properties of water and peptide chain could increase an understanding of such phenomena as interactions of protein molecules, rheological properties, and gel formation at the molecular scale.

In the present study, to clarify the effects of protein−water interactions on heat-induced OVA gel behavior, the dielectric behavior was analyzed on three avian species OVAs in aqueous solution by the TDR method. High-precision measurements could be performed over a wide frequency range from 100 kHz to 20 GHz to obtain information on the protein−water interaction or interaction of peptide chains on heat-denatured protein.

MATERIALS AND METHODS

Materials. OVAs were purified from different avian species EWs by crystallization in an ammonium sulfate solution and recrystallization three times and then finally purified by CM-cellulose chromatography as described by Sun and Hayakawa (*24*). The EWs were obtained from two galliform species (hen and guinea fowl) and one anseriform species (duck).

Heat Treatment of Samples. OVAs from several avian species were dissolved in distilled water and dialyzed overnight at 4 °C. The solution was adjusted to pH 7.0 and 8.0 with 1 N NaOH, respectively, and given a final protein concentration of 4% (w/w). An aliquot of the sample solutions was heated in a water bath at 80 °C for 15 min and then cooled by ice−water to obtain denatured OVA samples. The treated samples were immediately performed for dielectric analyses. The protein concentration was determined according to the method of Lowry et al. (*25*) using bovine serum albumin (BSA) as a standard.

Dielectric Measurement by TDR Method. The system of the apparatus and the procedure of the measurement were reported elsewhere (*26*−*29*). An incident pulse with a rise time of 30 ps generated by a pulse generator passes through a 50 Ω coaxial cable and is reflected from the top of the cable, where the sample cell is attached. The reflected pulse from the sample cell was recorded at a sampling head (HP54121A) and digitized at a sampling scope (HP54121B).

Fourier transform of reflected wave from the unknown sample, $r_x(\omega)$ (ω is angular frequency), in the frequency domain and that from the reference sample, $r_s(\omega)$, are related to the complex permittivity of the unkown sample, $\epsilon_x^*(\omega)$, which is given by

$$\epsilon_x^*(\omega) = \epsilon_s^*(\omega)\frac{1 + \{cf_s(\omega)/[\,j\omega\gamma_d\epsilon_s^*(\omega)]\}\rho(\omega)}{1 + \{[j\omega\gamma_d\epsilon_s^*(\omega)]/cf_s(\omega)\}\rho(\omega)}\frac{f_x(\omega)}{f_s(\omega)} \quad (1)$$

where and

$$\rho(\omega) = [r_s(\omega) - r_x(\omega)]/[r_s(\omega) + r_x(\omega)]$$

$$f_x(\omega) = Z_x(\omega)\cot Z_x(\omega) \qquad Z_x(\omega) = (\omega d/c)\epsilon_x^*(\omega)^{1/2}$$

$$f_s(\omega) = Z_s(\omega)\cot Z_s(\omega) \qquad Z_s(\omega) = (\omega d/c)\epsilon_s^*(\omega)^{1/2}$$

and where $\epsilon_s^*(\omega)$ is the complex permittivity of the reference sample with known permittivity, d is the geometric cell length, and γ_d is the effective cell length. j represents an imaginary unit, and c is the speed of light propagation in a vacuum.

If the direct current (dc) conductivity of the reference is adjusted to be the same as that of the unknown sample using a standard solution, the contribution of dc conductivity can be subtracted from the complex permittivity. This subtraction is very important in dielectric measurements on electrolytic solutions as mentioned in a previous paper (*29*). The dc conductivity can be estimated, using a nonelectrolytic solvent as a reference, from the measurements of the reflected wave over a significantly long time. We used $\epsilon_s^*(\omega) - \sigma/j\omega\epsilon_0$ instead of $\epsilon_s^*(\omega)$ in eq 1, where σ is the dc conductivity and ϵ_0 is the dielectric constant of vacuum. We chose the aqueous solution of sodium chloride as a reference, and two sample cells were used—the shorter cell with d = 0.01 mm and γ_d = 0.13 mm over a frequency range higher than 100 MHz and the longer cell with d = 2.0 mm and γ_d = 4.90 mm in a range below 1 GHz. The outer and inner conductors of the sample cell were made of gold to prevent oxidation, and a flexible cable was attached to the sample cell. The measurement was performed at 25 °C.

Surface Hydrophobicity. The surface hydrophobicity was determined by the fluorescence probe (1-anilino-8-naphthalenesulfonate, ANS) method of Sun and Hayakawa (*23*). Solutions containing 8% (w/v) protein (pH 7.0 or 8.0) were heated and diluted with 10 mM phosphate-buffered saline (PBS) at pH 7.0 or 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.

Rheological Properties. The rheological properties of OVA gel were analyzed by creep test and measured by using a Rheoner creep meter (RE2-3305, Yamaden, Co., Japan). The measurements were carried out under uniaxial compression at 5 mm/s. The creep curves were drawn on the display and analyzed with software developed for creep analysis (ver. 2.1, Yamaden Co.), where the gels conformed to a six-element mechanical model (*23*).

RESULTS AND DISCUSSION

Dielectric Relaxation of Native OVAs in Aqueous Solution. Three dielectric relaxation processes were found apparently in aqueous solution of native OVAs. As an example, dielectric

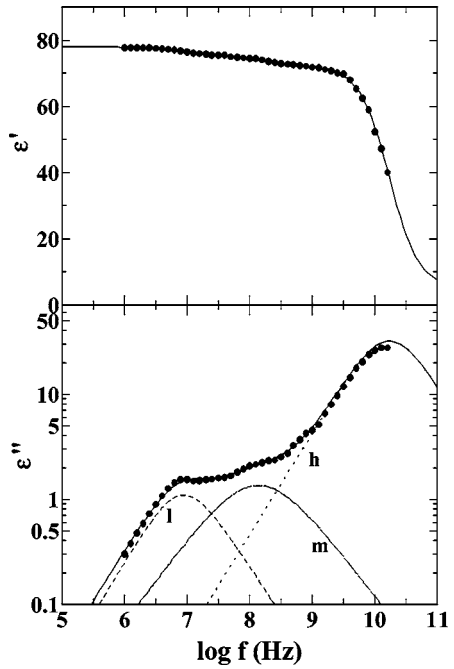

Figure 1. Dielectric dispersion and absorption of native hen OVA solution at pH 8.0. Solid, dotted, and broken curves are calculated from eq 1 by using relaxation parameters listed in **Table 1**. "h", "m", and "l" refer to the high-, intermediate-, and low-frequency processes, respectively.

dispersion and absorption curves for hen OVA solution at pH 8.0 are shown in **Figure 1**. These relaxation processes could be explained by the following mechanisms, from previous papers (*6*, *14*). The highest frequency relaxation process at ~20 GHz, denoted "h", was concluded to be due to the orientation of free water molecules, judging from the value of the relaxation time τ_h and relaxation strength $\Delta\epsilon_h$. The intermediate frequency relaxation process "m" at ~100 MHz might be due to the orientation of bound water, which attaches to the surface of the protein through hydrogen bonding. An overall rotation of protein molecules might be the mechanism responsible for the low-frequency relaxation process "l" observed at ~10 MHz.

The total complex permittivity $\epsilon^*(\omega)$ is assumed to be described by

$$\epsilon^*(\omega) = \epsilon_\infty + \epsilon_l^*(\omega) + \epsilon_m^*(\omega) + \epsilon_h^*(\omega) \qquad (2)$$

where ϵ_∞ is the constant extrapolated to $\omega = \infty$. $\epsilon_l^*(\omega)$, $\epsilon_m^*(\omega)$, and $\epsilon_h^*(\omega)$ are complex permittivities corresponding to the low-, intermediate-, and high-frequency processes, respectively, and are given as

$$\epsilon_l^*(\omega) = \frac{\Delta\epsilon_l}{(1 + j\omega\tau_l)^{\alpha_l}} \qquad (3)$$

$$\epsilon_m^*(\omega) = \frac{\Delta\epsilon_m}{\{1 + (j\omega\tau_m)^{\beta_m}\}^{\alpha_m}} \qquad (4)$$

$$\epsilon_h^*(\omega) = \frac{\Delta\epsilon_h}{1 + (j\omega\tau_h)^{\beta_h}} \qquad (5)$$

where $\Delta\epsilon$ is the relaxation strength, τ is the relaxation time, and α and β are parameters describing the distribution of relaxation times. α is the Cole−Davidson parameter (*30*), and β is the Cole−Cole parameter (*31*). For the intermediate relaxation process, the Havriliark−Negami (*32*) representation was applied, which can represent an extremely wide distribution of relaxation times.

Table 1. Dielectric Relaxation Parameter for Native OVAs from Three Avian Species

	pH	log τ_l	$\Delta\epsilon_l$	α_l	log τ_m	$\Delta\epsilon_m$	α_m	β_m	log τ_h	$\Delta\epsilon_h$	β_h	ϵ_∞
hen	7.0	−7.70	2.33	0.91	−8.96	2.81	0.98	0.92	−11.05	67.56	0.97	4.72
	8.0	−7.72	2.27	0.91	−8.92	3.73	0.97	0.81	−11.03	67.08	0.97	4.88
guinea fowl	7.0	−7.66	2.89	0.94	−8.89	3.07	0.93	0.85	−11.02	67.28	0.96	4.89
	8.0	−7.47	2.01	0.99	−8.86	3.90	0.97	0.82	−11.02	67.44	0.97	5.01
duck	7.0	−7.69	2.39	0.89	−8.79	3.87	0.98	0.85	−11.04	68.15	0.98	4.95
	8.0	−7.89	1.33	0.98	−8.87	4.95	0.97	0.81	−11.06	67.99	0.97	4.73

Those relaxation parameters were determined by a least-squares fitting procedure, and the values obtained from three species native OVAs are given in **Table 1**. The results indicated that there were similar dielectric behaviors for native OVAs from three avian species at either pH 7.0 or 8.0, except for the relaxation strength for the intermediate process. It might be suggested that the structures (secondary, tertiary) in different species OVAs were also similar, although there was some variability in the amino acid composition of different species OVAs (*24*). The water molecules can produce a short string or a small network on the protein surface around polar groups through hydrogen bonding; furthermore, the bound water formed a network that might stabilize the native protein structure. Thus, the dielectric behavior of the water around the various native OVAs surface might not cause a significant alteration. However, if the protein transformed its structure from a compact state to a random coiled state, the water molecules bound to polar groups of the protein surface might undergo a marked change by reductions of hydrophobic and hydrophilic interactions of denatured protein (*6*, *13*).

Dielectric Behavior of Heat-Denatured OVA Solutions. Similar representations to the native OVAs were applied to the lower and higher frequency relaxation processes. For the intermediate-frequency process, however, there might be two possible mechanisms that could explain such relaxation processes. Although it is possible for hydrophobic groups to hydrate according to unfolding of the polymer chain and the network of bound water can be formed, the hydration shows high-frequency relaxation at several gigahertz (*33*) and thus cannot explain the observed dielectric relaxation spectra. Another possible mechanism is the micro-Brownian motion of peptide chains (*13*).

It is well-known that in the native state OVA represents a reasonable rigid packed globule. However, the globular structure can be transformed into a partially unfolding state under heating and expose buried hydrophobic groups of protein. The conformational change of heat-denatured protein can bring about a transition from the ordered structure of bound water to disordered structure, in which the water molecules were difficultly bound to polar groups. In addition, it has been indicated that if the protein does not take a peculiar structure such as the tertiary structure of globular molecule, water molecules cannot be bound to it (*6*). Thus, it is suggested that the intermediate-frequency relaxation process could be caused by a micro-Brownian motion of peptide chains. Therefore, the KWW function (*17*, *18*) was used for the intermediate-frequency relaxation process and described by

$$\epsilon_m^*(\omega) = \int_0^\infty \Delta\epsilon_m \exp(-j\omega t)\left[\frac{d\Phi_k(t)}{dt}\right]dt \qquad (6)$$

and

$$\Phi_k(t) = \exp[-(t/\tau_m)^{\beta_{km}}] \qquad 0 < \beta_{km} \leq 1$$

Because $1 - \beta_{km}$ is a parameter representing a degree of coupling strength brought from the molecular environment (*34*, *35*), the lower value of β_{km} means the stronger coupling.

As shown in **Figure 2**, the complex permittivity of heat-denatured hen OVA solution at pH 8.0 as an example is satisfactorily explained by the three relaxation processes. The explanation implies that the relaxation process relating with reorientational motions of bound water cannot be detected. The regular structure of hydrogen bonding between water molecules and polymer might be indispensable for the formation of the network of bound water. However, the motion of polymer chains, which can potentially reorientate with the similar relaxation time to bound water, might not form the regular structure in aqueous solution, and therefore the network of bound structure disappeared due to inability to form the regular structure.

Compared with native OVA solution, $\Delta\epsilon_h$ and τ_h were scarcely changed (**Table 2**). In contrast, the relaxation strength of the intermediate- and low-frequency relaxation processes increased remarkably upon heating. It is suggested that unfolding of OVAs due to heating brings about long end-to-end distances and the large total dipole moment of the OVA. Therefore, it is considered that the effect of the unfolding of OVA by heating appeared in dielectric properties of the overall rotation in this work. Although it might be reasonable to assume that the relaxation time of the low-frequency process should increase when the volume of OVA increases by the unfolding of OVA, the relaxation time actually did not increase. As described above, the intermediate-frequency relaxation process in heat-denatured OVA solution might be dominantly caused by the chain motion on local parts of peptide chain. **Table 2** showed that the increase of $\Delta\epsilon_m$ in all heat-denatured OVAs depended on the avian species and followed the order hen < guinea fowl < duck. Furthermore, the relaxation strength for the intermediate process increased with increasing pH values (from 7.0 to 8.0) in aqueous solution for all species. This dependence of the relaxation strength of the intermediate process on kinds of avian species and pH values corresponded to the dependence of the relaxation strength $\Delta\epsilon_m$ of native OVAs. This correspondence might indicate that the chain motion of local parts of the heat-denatured OVA proteins was prescribed by the quantity or conformation of hydrophilic groups in the native OVA proteins.

Effect of Avian Species and pH on Dielectric Dehavior of OVA Solutions. To show the difference clearly among three avian species, the Cole−Cole plot is given in **Figure 3**. The plot for the heat-denatured OVA solutions showed a significant difference between duck and hen/guinea fowl avian species. This result implied that dielectric behaviors in heat-denatured OVA solutions from hen and guinea fowl (belonging to galliform species) were more similar to each other when compared with duck OVA (anseriform species). The result was consistent with our previous study (*23*, *24*) that hen and guinea fowl OVAs in the primary structure and surface hydrophobicity were considerably different from duck OVA. Sun and Hayakawa (*24*) indicated that the primary structure between galliform and anseriform species exhibited large variations by comparing the amino acid sequence of cysteine peptides from the six OVAs, particularly half-cystine, isoleucine, valine, and alanine. These variations can influence the hydrophobic nature of the polypeptide side chain. Henderson et al. (*36*) described the isolation of phosphoserine peptides from OVAs of eight related species and indicated that the variations in amino acid sequences on these peptides bore a close relationship with the phylogeny of these species. A phylogenetic tree has also been constructed from a comparison of the sequences of 248 residues from the eight OVAs. In the present study, the result indicated that the dielectric behavior of denatured OVAs had a dependence on the related avian species.

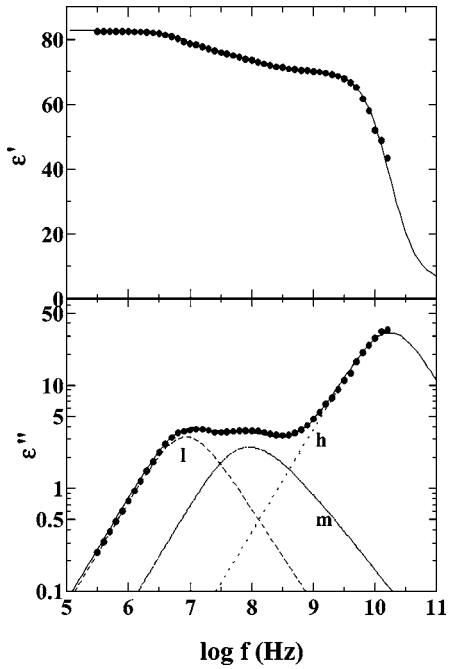

Figure 2. Dielectric dispersion and absorption of heat-denatured hen OVA solution at pH 8.0. Solid, dotted, and broken curves are calculated from eq 1 by using relaxation parameters listed in **Table 2**. "h", "m", and "l" refer to the high-, intermediate-, and low-frequency processes, respectively.

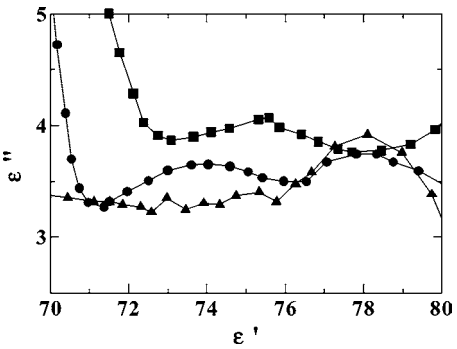

Figure 3. Cole–Cole plot of OVAs for duck (■), guinea fowl (●), and hen (▲) over the frequency range from $10^{7.2}$ to $10^{9.4}$ Hz.

Table 2. Dielectric Relaxation Parameter for Denatured OVAs from Three Avian Species upon Heating at 80 °C

	pH	log τ_l	$\Delta\epsilon_l$	α_l	log τ_m	$\Delta\epsilon_m$	β_{km}	log τ_h	$\Delta\epsilon_h$	β_h	ϵ_∞
hen	7.0	−7.63	6.58	0.92	-8.05	5.59	0.77	−11.01	64.68	1.00	4.79
	8.0	−7.71	6.56	0.92	−8.02	6.29	0.77	−11.00	65.05	1.00	4.93
guinea fowl	7.0	−7.63	7.21	0.96	−8.14	5.89	0.77	−11.02	64.09	1.00	4.71
	8.0	−7.60	6.97	0.92	−8.15	6.57	0.78	−11.06	63.69	0.97	5.00
duck	7.0	−7.64	7.50	0.96	−8.05	6.85	0.84	−11.06	64.58	0.99	5.00
	8.0	−7.62	6.92	0.94	−8.00	8.26	0.78	−11.05	66.26	0.99	4.83

On the other hand, the dielectric analysis indicated that $\Delta\epsilon_m$ for the intermediate-frequency relaxation process increased with pH transition from 7.0 to 8.0 in each sample (**Table 2**). The pH change in product processing usually alters the conformational structure of heat-denatured protein, improves rheological

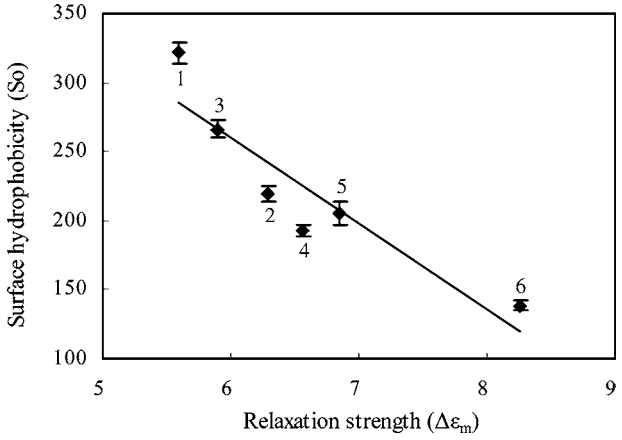

Figure 4. Plots of surface hydrophobicity for heat-denatured OVA solutions against relaxation strength: 1, hen OVA at pH 7.0; 2, hen OVA at pH 8.0; 3, guinea fowl OVA at pH 7.0; 4, guinea fowl OVA at pH 8.0; 5, duck OVA at pH 7.0; duck OVA at pH 8.0. The numbered plots of S_0 are reported in a previous work (*23*).

properties, and gives a unique texture to foods (*23*). It has been reported that surface hydrophobicity of denatured OVA solutions and rheological behavior of heat-induced OVA gels depended on the pH value and changed significantly with the phylogeny of these species (*23*).

Effect of Surface Hydrophobicity of Protein on Dielectric Properties. Many functional properties of food proteins are affected by the protein surface. OVA, a globular protein, tends to have the hydrophobic residues in the interior and the hydrophilic groups at the exterior of the protein molecule. However, heating causes large global conformational changes, which may generate molecular unfolding and expose buried hydrophobic groups. The relationship between $\Delta\epsilon_m$ and surface hydrophobicity of heat-denatured OVAs from three species at pH 7.0 and 8.0 was analyzed (**Figure 4**). The result showed that the surface hydrophobicity affected the dielectric behavior of heat-denatured OVAs, exhibiting a negative correlation ($R^2 = -0.84$). The surface hydrophobicity of OVAs was obtained from the previous work for OVAs from various species using a fluorescence probe, ANS (*23*). As expected, the hydrophobic groups were buried in the interior of OVA in the native state; thus, the surface hydrophobicity of protein molecules was near zero. However, the heat-denatured OVA occurred the change of hydrophobic and hydrophilic interactions through unfolding and exposing buried hydrophobic groups, subsequently resulting in alteration of the relaxation strength of the relaxation process corresponding to the micro-Brownian motion of peptide chains.

The hydrophobic regions packed inside the native OVA were exposed to the surface of the molecules through heat denaturing, thus generating intermolecular hydrophobic interaction of heat-denatured OVA. On the other hand, the electrostatic repulsive forces have been implicated by many workers as a major factor in the coagulation and gelation of OVA (*19*, *37*), but no direct evidence has been presented. However, it is clear that the pH of the solution affects the electrostatic charge on the surface of the OVA molecules. In the vicinity of the isoelectric point (p*I* 4.6) of OVA, the net charge of OVA molecules is zero. On the contrary, the net charge of protein and electrostatic repulsive forces increase at pH values away from the p*I* value. In addition, the change in pH value brought about the rearrangement and/or rupture of intermolecular hydrophobic interaction and electrostatic repulsive forces, further altering the dielectric behavior. When the intermolecular hydrophobic interaction was much stronger than the electrostatic repulsive forces between

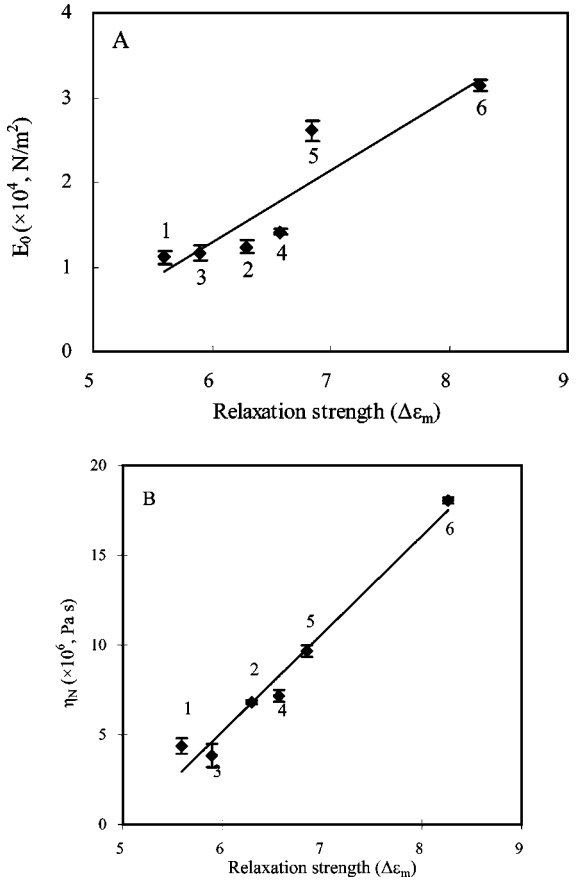

Figure 5. Plots of elasticity (E_0) and viscosity (η_N) for heat-induced OVA gels against relaxation strength, respectively: 1, hen OVA gel at pH 7.0; 2, hen OVA gel at pH 8.0; 3, guinea fowl OVA gel at pH 7.0; 4, guinea fowl OVA gel at pH 8.0; 5, duck OVA gel at pH 7.0; 6, duck OVA gel at pH 8.0. The numbered plots of E_0 and η_N are reported in a previous work (*23*).

denatured protein molecules, the heat-denatured molecules became strongly tangled to form random aggregate. This random aggregate might partially inhibit the micro-Brownian motion of peptide chains. When the intermolecular hydrophobic interaction decreased, while the electrostatic repulsive forces increased with pH increase from 7.0 to 8.0, each molecule might bind together at a few points to produce linear aggregates (*23*, *38*). This process could improve the mobility of peptide chains and enhance dielectric relaxation strength for the micro-Brownian motion of peptide chains. Thus, there was a negative relationship between the dielectric relaxation strength and surface hydrophobic interaction of denatured OVA at pH 7.0−8.0.

Dielectric Properties and Rheological Behavior of OVA Gels. To understand the relationship between the dielectric relaxation behavior and rheological properties of the heat-induced OVA gels, the dependence of the dielectric behavior on viscoelastic properties of heat-induced OVA gels was investigated at both pH 7.0 and 8.0 (**Figure 5**). The viscoelastic parameters for elasticity (E_0) and viscosity (η_N) were obtained in our previous work by creep test (*23*). The correlation coefficients (R^2) between $\Delta\epsilon_m$ and viscoelastic parameters for E_0 and η_N were 0.84 and 0.97 (panels **A** and **B**, respectively, of **Figure 5**), respectively, indicating a good correlation between relaxation strength and viscoelastic properties. **Table 2** showsthat the heat-denatured duck OVA solution had higher relaxation strength, and such higher relaxation strength could be due to the reasonable cross-linked networks and highly viscoelastic

gels formed by heating. It is generally accepted that whether a transparent or turbid gel occurs when the protein is heated depends on the aggregated types of proteins; that is, linear and random aggregates result in transparent and turbid gels, respectively (*22*, *39*). As described above, a linear aggregate in aqueous solution exhibited a more highly mobile peptide chain than the random aggregate and produced a transparent gel with a higher water-holding capacity and viscoelastic property. Consequently, it should be of great interest to elucidate the relationship between rheological properties and dielectric relaxation process in denatured OVAs upon heating. The dynamical investigations of the interaction of water and OVA protein could increase the understanding of gel formation and rheological properties in order to evaluate the quality of food and control food processing. However, the detailed structure and dynamic behavior in which the gels formed are unclear. Additional research will be needed to elucidate the mechanism of dynamic behavior at the molecular level; and so expand the utilization of different EW/OVA proteins in the food industry.

Conclusions. The dielectric relaxation of protein in aqueous solution for all OVA samples was interpreted as a sum of three relaxation processes. For the native OVA solutions, the dielectric relaxation process at ~100 MHz was due to the reorientation of bound water molecules. On the other hand, the relaxation process for the heat-denatured OVA solutions resulted mainly from the micro-Brownian motion of peptide chains. The results indicated that the reductions of hydrophobic and hydrophilic interactions of denatured OVA through unfolding and exposing buried hydrophobic groups could influence the dynamical property of local chains of denatured protein molecules, and the alteration of dynamical properties depended on the pH values for all denatured OVA samples. The dielectric relaxation strength $\Delta\epsilon_m$ for denatured OVA solutions was closely related to the rheological properties of OVA gels and exhibited a variation with the phylogeny of three species. It was shown that high relaxation strength in denatured OVA solution could result in the heat-induced fine network structure of the gel. These results suggested that the dielectric relaxation strength $\Delta\epsilon_m$ for the heat-denatured OVA might be considered to be an indication of gel properties. Such analysis enabled us to characterize more precisely the gel properties.

ABBREVIATIONS USED

OVA, ovalbumin; EW, egg white; ANS, 1-anilino-8-naphthalenesulfonate; TDR, time domain reflectometry; BSA, bovine serum albumin; KWW, Köhlrausch−Williams−Watts.

LITERATURE CITED

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Received for review July 29, 2003. Revised manuscript received January 15, 2004. Accepted January 29, 2004.

JF030538Z