Formation of Fatty Acid Salt-Induced Gel of Ovalbumin and the Mechanism for Gelation

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Gelation of ovalbumin (OVA) was induced by the addition of fatty acid salts (FAS), such as sodium caprate ($NaC_{10:0}$), to the protein solution at ambient temperature. The rheological and structural properties of the fatty acid salt-induced gels (FAS-IG) were studied using two kinds of FAS. Ten percent OVA in the presence of 2% $NaC_{10:0}$ formed a transparent and soft gel above 15 °C. At 3% sodium oleate ($NaC_{18:1}$), a transparent and softer gel was also formed above 25 °C. When the FAS-IG was heated, the storage modulus G' increased to 3 times as high as that of the gel before heat treatment. Electron microscopy showed that the FAS-IG has a more homogeneous network with larger pore size than do the heat-induced FAS gel and the heated FAS-IG. Analyses by circular dichroism spectroscopy indicated that native OVA changed into molten globule state by the addition of FAS.

Keywords: *Ovalbumin; dynamic rheological properties; electron microscopy; CD spectroscopy; gelation; molten globule state*

INTRODUCTION

Gelation is one of the most important functional properties of food protein. Gelation of proteins can be induced under various conditions, namely, heating (Hegg et al., 1979; Egelandsdal, 1980; Van Kleef, 1986), cooling (Kobayashi and Nakahama, 1986), high pressure (Hayashi, 1987a,b), addition of chemical compounds, which induces aggregation of proteins such as SH compounds, particular salts, and some enzymes, etc. (Hirose et al., 1986; Xiong and Kinsella, 1990; Nio et al., 1986). A number of methods have been employed for the gelation of proteins in food processing, and the gels formed by each technique have different textures, flavors, colors, etc. Creation of new techniques for making protein gels are, therefore, useful to make new foods that have unique properties even from quite popular food materials.

In ovalbumin (OVA), there are numerous studies on the physicochemical properties and the mechanism for formation of the gels, which are mainly induced by heat treatment. Nakamura et al. (1978) and Hegg et al. (1979) have studied the denaturation and aggregation of OVA by heating treatment under various conditions of pH or ionic strength and discussed the process for gel formation. Egelandsdal (1980), Doi et al. (1987), and Kitabatake et al. (1988) reported that hardness and turbidity of OVA gels could be changed by control of the protein concentration, ionic strength, and pH. Hayakawa et al. (1992) reported that high pressure could induce gelation of OVA, and the gel properties were different from those of heat-induced gels. We have reported the formation of transparent and high water-holding gels of several food proteins (sesame, rice globulins, and OVA) (Yuno-Ohta et al., 1992, 1994, 1996) by the addition of fatty acid salts (FAS) and heat treatment. Particularly in OVA, desirable gels with different gel hardnesses, water-holding abilities, and transparencies could be formed by changing the concentration of protein and FAS. Although heating is very convenient and sanitary and, thus, a popular method for gelation, the heating step results in significant loss of flavor, producing unexpected tastes in food materials. Recently, we found that OVA is able to form a transparent gel by the addition of FAS at room temperature. The FAS-induced gelation has many advantages in food processing because the method produces a unique gel texture and the original flavor of food materials can be kept after the gelation. Our objective was to determine the characteristics of this FAS-induced gel. Furthermore, we investigated the mechanism for the formation of this FAS-induced gel of OVA.

MATERIALS AND METHODS

Materials. OVA was purchased from Taiyo Chemicals Ltd., Mie, Japan, or purified from egg white as described (Takahashi and Hirose, 1992). Sodium caprate ($NaC_{10:0}$) and sodium oleate ($NaC_{18:1}$) were purchased from Sigma Chemical Co., St. Louis, MO. Other reagents were obtained from Wako Pure Chemicals, Osaka, Japan.

Preparation of FAS-Induced OVA Gel. Ten percent ovalbumin dissolved in water was mixed with 2−3% FAS solution (stock solutions, ex. 10−20% sodium caprate or sodium oleate in water was used), and then sodium chloride was added to the mixture to adjust the ionic strength to 0.2−

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10.1021/jf980165f CCC: $15.00
Published on Web 10/08/1998

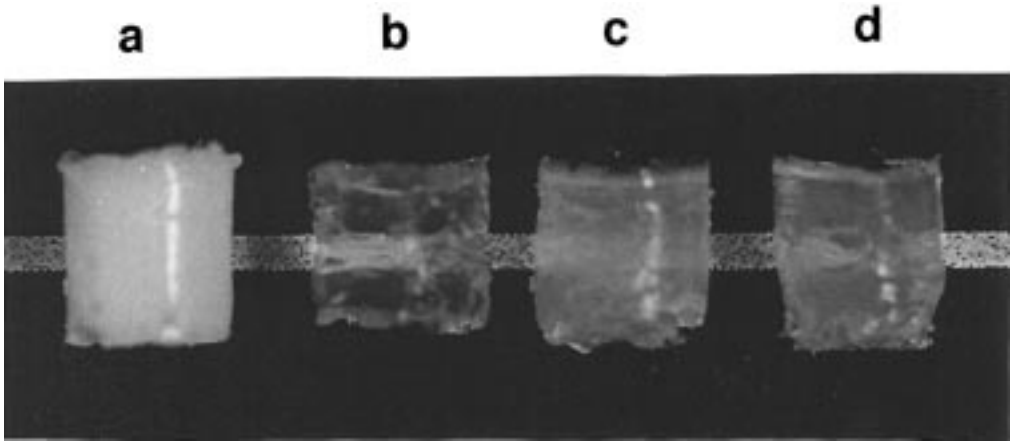

Figure 1. Effects of temperature on the transparency of OVA gels with $NaC_{10:0}$: (a) control gel in the absence of $NaC_{10:0}$ (95 °C, 10 min); (b) a gel in the presence of 2% $NaC_{10:0}$ (25 °C, 200 min, FAS-induced gel); (c) heated FAS-induced gel (25 °C, 200 min/95 °C, 10 min); (d) a heat-induced gel in the presence of 2% $NaC_{10:0}$ (95 °C, 10 min).

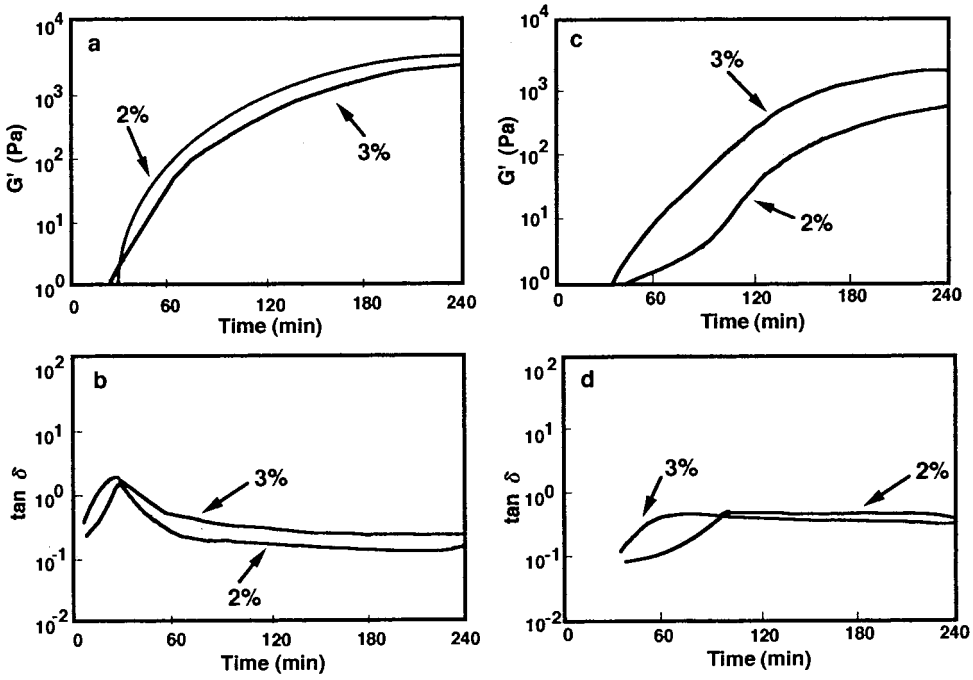

Figure 2. Storage modulus G' and loss tangent tan δ of 10% OVA over time by the addition of $NaC_{10:0}$ (a, b) or $NaC_{18:1}$ (c, d).

0.3. The pH of the mixture was 7−8. Aliquots (100 μL) of the mixture homogenized with a glass stick were immediately transferred into glass tubes (60 mm × 5 mm i.d.), which was sealed at the bottom end and kept at room temperature (~25 °C) for 90−180 min. After gelation, the gels were removed from the glass tubes and then used for experiments.

Rheological Measurements. The storage modulus G' and the loss modulus G'' were determined by a Rheolograph Sol (Toyo-seiki Ltd., Tokyo, Japan). The sample solution was placed between parallel plates, and the gap between the blade and both sides of the plates was set to 1 mm. The sample solution was subjected to a shear oscillation of 3 Hz frequency.

Scanning Electron Microscopy (SEM). The OVA gels were sliced into small pieces (1 mm × 1 mm × 3 mm) with a razor blade. To prevent solubilization of the gel in the fixative, each sample was treated with diluted solutions. That is, each sample was fixed in 0.5% glutaraldehyde in 0.05 M sodium phosphate buffer (pH 7.1) for 40 h at room temperature and then washed with phosphate buffer. Furthermore, the sample was fixed in 0.5% osmium tetraoxide in 0.05 M sodium phosphate buffer (pH 7.1) for 24 h at 4 °C. After exhaustive washing in phosphate buffer, the sample was washed with water. The fixed samples were dehydrated through exposure to a series of increasing ethanol concentrations [50%, 70%, 80%, 90%, 99.5%, 100% (I), 100% (II)], for 20 min at each stage. Preparations were next soaked in isoamyl acetate for 30 min and critical point dried using liquid CO_2 without deformation of the gels (Hitachi Kouki HCP-2). The dehydrated gel samples were broken and mounted on an aluminum specimen plate with adhesive agent and then coated with 50 Å of an alloy of platinum and palladium with ion sputter (Hitachi E-1030). Micrographs were taken with a Hitachi 4500 scanning electron microscope at an accelerating voltage of 1.5 kV and an emission current of 10 μA.

Transmission Electron Microscopy (TEM). After dehydration, TEM samples were transferred to propylene oxide before they were embedded in a synthetic polymer, Luveak 812, (Nakalai Tesque, Kyoto) using the method of Luft (1961). After being trimmed, the sample was sectioned with a microtome. Ultrathin sections (60−90 nm) were cut by glass knives set on an ultramicrotome (Reichert), put on support mesh, and then double-stained with uranyl acetate (4% w/v) using the method Watson (1958) and lead citrate (4% w/v). The sections were examined in a transmission electron microscope, H-700H (Hitachi Kouki, Japan), at acceleration voltage of 100 kV.

Analyses by Circular Dichroism (CD) Spectroscopy. CD spectra of 0.1% OVA containing $NaC_{10:0}$ at various concentrations (0, 0.02, 0.1, 0.5, and 2.0%) in 0.05 M sodium

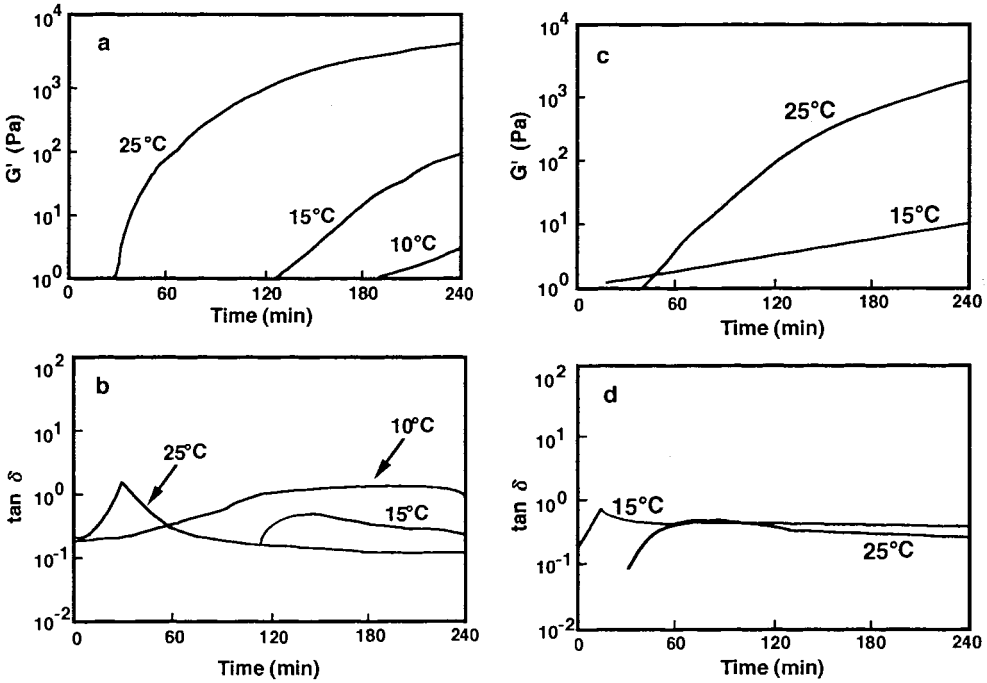

Figure 3. Storage modulus G' and loss tangent tan δ of 10% OVA over time with $NaC_{10:0}$ or $NaC_{18:1}$ at various temperatures (a, b) in the presence of 2% $NaC_{10:0}$; (c, d) in the presence of 3% $NaC_{18:1}$.

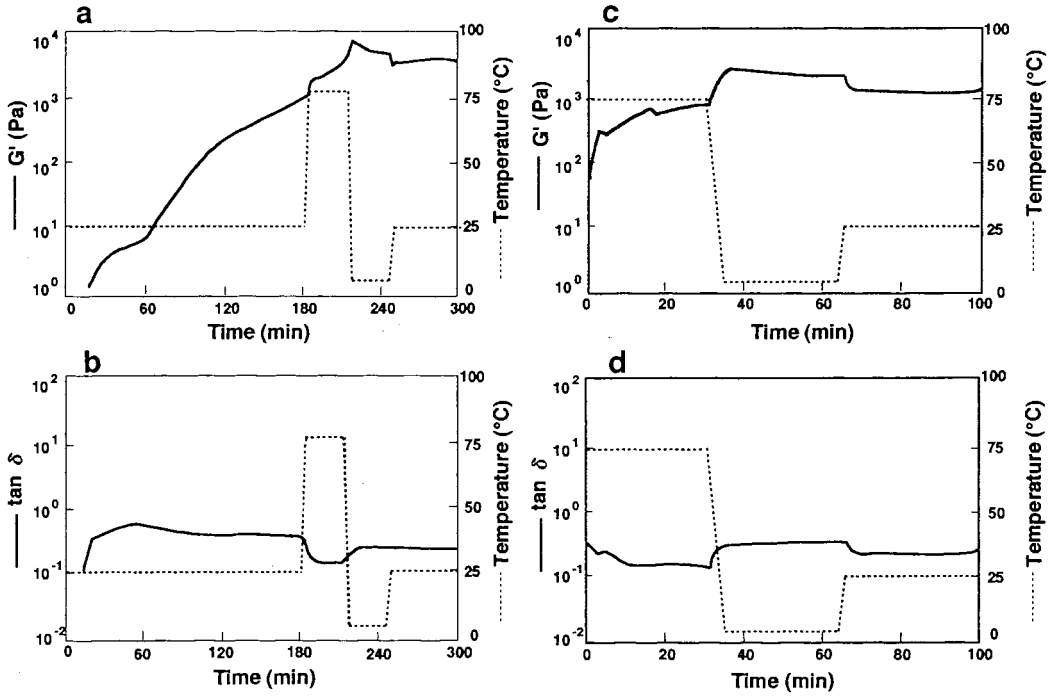

Figure 4. Comparison of storage modulus G' and loss tangent tan δ under different conditions for gelation: (a, b) effect of heating of $NaC_{10:0}$-induced gels on the changes in the viscoelastic properties (FAS-IG/H); (c, d) changes in the viscoelastic properties during the formation of heat-induced gel in the presence of $NaC_{10:0}$ (FAS·H-IG).

phosphate buffer (pH 7) at 25 °C were measured. After 0.1% OVA in the buffer was mixed with $NaC_{10:0}$, the mixture was incubated for 30 min at 25 °C. CD spectra were measured at the same temperature with a Jasco J-720 spectropolarimeter, using a 0.1 cm cuvette (for far-UV) and a 1 cm cuvette (for near-UV). The CD spectra were determined at three times, and data for triplicate measurements were averaged.

RESULTS AND DISCUSSION

FAS-Induced Gelation of OVA. Figure 1 shows the appearance of a control OVA gel without FAS, an FAS-induced gel (FAS-IG), a heated FAS-IG (FAS-IG/H), and a heat-induced gel in the presence of 2% $NaC_{10:0}$ (FAS·H-IG). In the absence of FAS, OVA does not form a gel at 25 °C. However, OVA containing 2% $NaC_{10:0}$ formed a transparent gel at 25 °C within 3 h. The FAS-IG was more transparent than heat-induced gels containing FAS reported previously (Yuno-Ohta et al., 1996).

We have already reported that FASs having >10 carbon atoms per chain are able to form transparent gels of OVA with heat treatment (Yuno-Ohta et al., 1996). The viscoelastic properties of these gels are well-

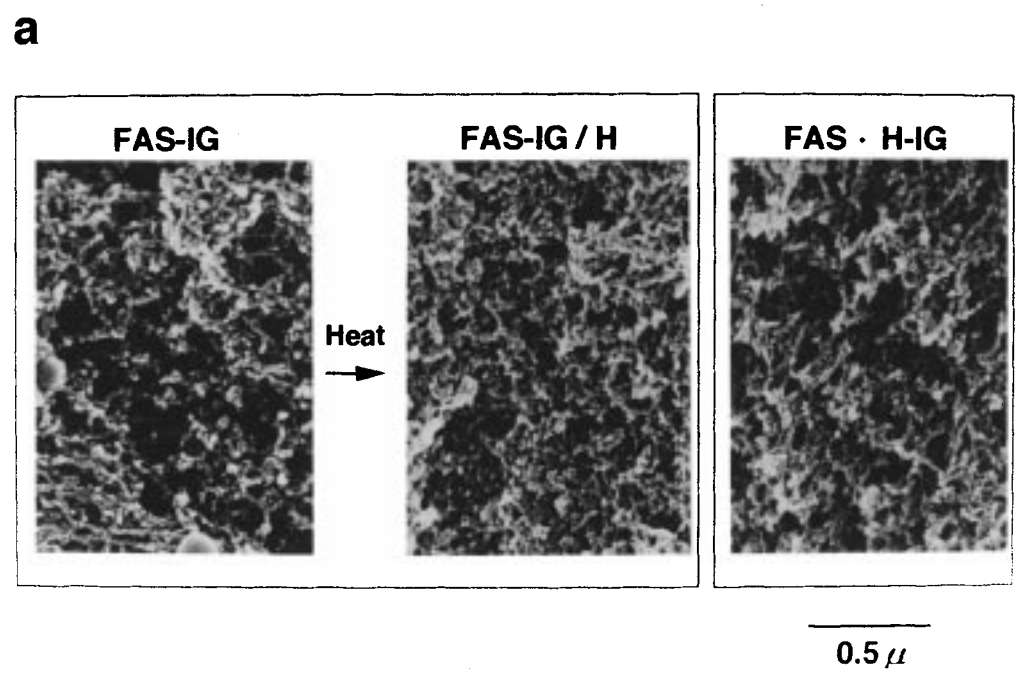

Figure 5. Scanning and transmission electron micrographs of OVA gels: (a) SEM; (b) TEM; FAS-IG, fatty acid salt-induced gel; FAS-IG/H, heated fatty acid salt-induced gel; FAS·H-IG, heat-induced gel with fatty acid salt.

defined by storage modulus G', loss modulus G'', and loss tangent tan δ. Changes of G', G'', and tan δ in the gelation process of 10% OVA by the addition of $NaC_{10:0}$ were determined by dynamic viscoelastic measurements (Figure 2a,b). The incubation temperature was kept at 25 °C. The mixture did not form a gel at 1% $NaC_{10:0}$. In contrast, in the presence of 2 or 3% of $NaC_{10:0}$, the storage modulus G' increased with incubation time. The G' and G'' of the mixture with 2 or 3% FAS began to increase gradually and reached a maximum at 200 min (G'' values are not shown), although dynamic modulus values are measured with slight vibration by sine strain in the Rheolograph Sol. Usually, when the mixture is settled at 25 °C, the gelation takes place within 120 min. The vibration for the measurement probably delayed the time that is needed to form gels. The decrease in tan δ of OVA containing 2% $NaC_{10:0}$ was greater than that of OVA containing 3% $NaC_{10:0}$. The increase of the storage modulus G' and the decrease of tan δ indicate that the system tends to be a more solidlike state in gel formation; a concentration of 2% $NaC_{10:0}$ was considered adequate for gel formation at 25 °C. The effect of $NaC_{18:1}$ on the gel formation was also examined. At 1% of $NaC_{18:1}$, the mixture of OVA and FAS did not form a gel, similar to the case of $NaC_{10:0}$. Parts c and d of Figure 2 show the changes of G' and tan δ with incubation time at 2 or 3% $NaC_{18:1}$. These results indicate that $NaC_{18:1}$ was also available for formation of FAS-IG without heating. When $NaC_{18:1}$ was used, the dynamic modulus began to increase more slowly than that of $NaC_{10:0}$. In contrast to the case of $NaC_{10:0}$, the addition of 3% of $NaC_{18:1}$ was more effective in forming a more elastic gel. These results suggested that a molar ratio of FAS to OVA molecule was important

to form an elastic and transparent gel rather than the absolute concentration of FAS (both 2% $NaC_{10:0}$ and 3% $NaC_{18:1}$ correspond to ~45 molar ratio for 10% OVA). The gel containing 3% $NaC_{18:1}$ has a texture similar to that of tapioca.

Effects of Temperature for the Formation of FAS-Induced Gel. To determine the factor for the formation of FAS-IG, the effects of incubation temperature were investigated. The storage modulus G' at 25 °C increased much more rapidly than at 15 °C in $NaC_{10:0}$-induced gel. Moreover, at 10 °C the gel was not formed even after 240 min (Figure 3a). These results suggest that, unlike gelatin, the molecular force which mainly contributes to the formation of FAS-IG probably is not a hydrogen bond. FAS-IG was formed even in the presence of 40 mM *N*-ethylmaleimide (NEM) (data not shown). This result suggests that the contribution of disulfide bonds to the formation of FAS-IG is also small. We presume that hydrophobic interactions are more important for FAS-IG.

Figure 3c shows the effect of temperature on the formation of $NaC_{18:1}$-induced gel. After 240 min, G' was ~10 Pa at 15 °C; the G' was about one-tenth that of $NaC_{10:0}$-induced gel at the same temperature. This indicates that $NaC_{10:0}$ was more effective in gel formation than $NaC_{18:1}$ at 15 °C.

Effects of Heating of Gels on the Changes in the Viscoelastic Properties. OVA containing FAS could form a soft and transparent gel without heat treatment as mentioned above. We further investigated how the dynamic modulus of viscoelasticity of FAS-IG was changed by the following heat treatment after gelation. Parts a and b of Figure 4 show changes in the G' and tan δ of the gel by heating. After the FAS-IG was formed, the gel was heated for 20 min at 75 °C and quickly cooled. After the heating and cooling treatment, the G' increased to 3 times as high as that before the treatment. A decrease in tan δ with increase in the temperature was also seen. The heated FAS-IG (FAS-IG/H) was more elastic than the heat-induced gel of OVA with FAS (FAS·H-IG) (Figure 4c).

Relationship between the Viscoelastic Properties of FAS-IG and Their Microstructures. Our results showed that different conditions for gelation gave characteristic viscoelastic properties even at the same concentration of FAS. Because this difference seemed to result from the difference in fine structures of the gels, microstructural differences in the gel were investigated using electron microscopy. Three kinds of gels, FAS-IG, FAS-IG/H, and FAS·H-IG, as shown in Figure 5a, were examined using SEM. Figure 5b shows the microstructure of the gels by TEM. Although microstructures of both FAS-IG and FAS·H-IG showed filamentous network, the internetwork space (pore size) of FAS-IG is larger than that of FAS·H-IG. The larger pore sizes of FAS-IG probably have advantages in the water-holding capacity of the gels. On the other hand, in the microstructure of FAS-IG/H, the formation of aggregates was observed at several places in addition to the increase in the density of the network. The thickness of each filament observed in FAS-IG was ~80 Å. The results of SEM of these gels were supported well by TEM. Furthermore, the SEM and TEM indicate that the network structure of FAS-IG/H was finer than that of FAS·H-IG. It probably explains the slight difference in hardness between the two gel types. These results also indicate that the physicochemical properties of the gels are closely connected with their microstructures. As a consequence, the much softer gel texture of FAS-IG compared with that of FAS·H-IG is probably attributable to the thinner filaments and larger pore size of the network.

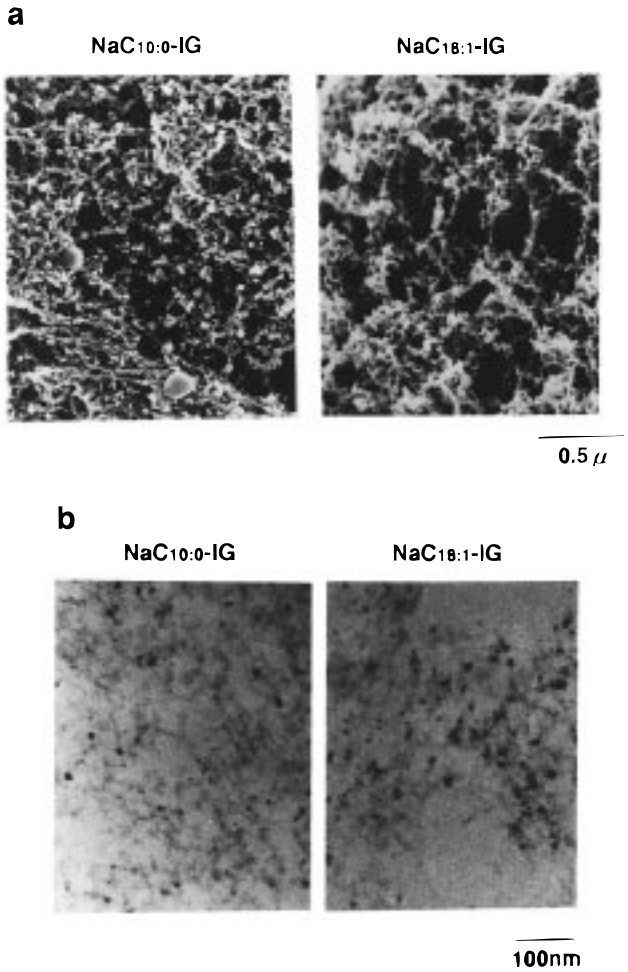

Figure 6. Microstructure of $NaC_{10:0}$ and $NaC_{18:1}$-induced gels: (a) SEM; (b) TEM.

Our results also showed that when the suitable amount of FAS with different carbon chain lengths was added to OVA, each gel formed had characteristic physicochemical properties. To define this point, the microstructures of $NaC_{10:0}$- and $NaC_{18:1}$-induced gels were compared using SEM and TEM (Figure 6). Both of these gels showed more homogeneous and filamentous microstructures than control (without FAS) gel with heat treatment (Yuno-Ohta et al., 1996). When we compared the microstructures, the $NaC_{10:0}$-induced gel showed finer network but thicker flaments than $NaC_{18:1}$- induced gel. That is, $NaC_{18:1}$-induced gel has larger pores in the network. On the basis of the findings, again, it is obvious that the physicochemical properties of the gels are closely associated with the microstructure; the $NaC_{10:0}$-induced gel with a finer and more homogeneous network structure is harder. In contrast, the $NaC_{18:1}$-induced gel has larger pores in its network, which gives, therefore, a very soft gel texture.

Changes in CD Spectra of OVA by the Addition of FAS. Gelation of OVA only by the addition of FAS strongly suggests that structural changes of OVA are caused by FAS. Because FAS is not a strong denaturant, it is of interest that the addition of FAS actually induced structural changes of OVA. CD spectra of OVA

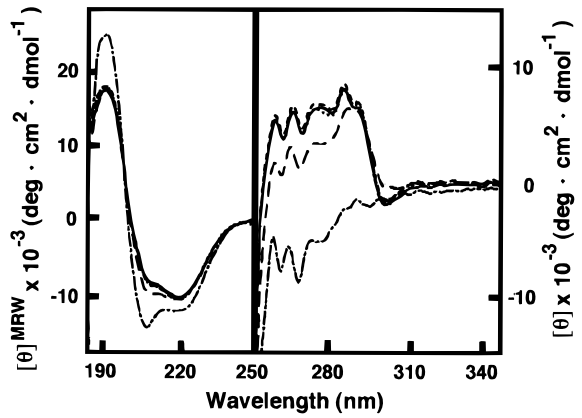

Figure 7. Changes in CD spectrum of OVA by $NaC_{10:0}$: (—) 0%; (· · ·) 0.02%; (- - -) 0.1%; (— — —) 0.5%; (— - —) 2%.

by FAS were observed (Figure 7). The spectrum in the far-UV area was slightly changed by the addition of 2% FAS, indicating that the secondary structure of OVA is basically maintained even after the addition of 2% FAS. However, in the near-UV area, the spectra showed great differences by the addition of 2% FAS, indicating that the tertiary structure was completely collapsed under the condition. Koseki et al. (1988) and Tatsumi and Hirose (1997) investigated conformational changes in OVA at acidic pH and stated that OVA molecules kept their native globular conformation but that the flexibility of their side chain increased and they were very susceptible to denaturation. The structure of FAS-IG in our study is similar to the so-called "molten-globule state" (Kuwajima, 1989), which was suggested by them in OVA. The molten-globule state is a unique state in which the protein molecule has a native-like backbone secondary structure, whereas the side chain's environment undergoes a denaturation-like alteration; therefore, the protein molecule is somewhat expanded but retains a more compact conformation than the fully denatured state (Hirose, 1993). The molten globule state has been shown to be involved in the functional properties of food proteins (Hirose, 1993).

In conclusion, our results indicated that by the addition of FAS, OVA forms transparent and high water-holding gels without heating. The addition of FAS is a very conventional method for gelation. Furthermore, the new transparent gels with FAS, which have adequately oily and soft textures despite low content of fat, will be available as a substitute fat in foods. We should further examine this technique for other kinds of food protein to produce other new good food materials in addition to investigating the mechanism for the gel formation.

ACKNOWLEDGMENT

We thank Dr. Hiroyuki Ohta for his helpful discussions and critical reading of the manuscript.

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Received for review February 19, 1998. Revised manuscript received July 8, 1998. Accepted August 19, 1998. We thank the Skylark Food Science Institute for its financial support.

JF980165F