Rheological Properties of Sodium Caprate-Induced β-Lactoglobulin Gel and Changes in Secondary Structure During Gelation

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ABSTRACT: In this study, we found that transparent gels of β-lactoglobulin (β-LG) were formed by adding different concentrations of sodium caprate to protein solutions at ambient temperature. We investigated changes in the dynamic viscoelasticity of the mixture with time at 25°C and found that more than 12% β-LG induced the formation of a viscoelastic gel with a suitable amount of sodium caprate (for example, 12% β-LG and 3.6% sodium caprate). Furthermore, we analyzed the changes in the secondary structure of proteins during the gelation step by FTIR spectroscopy. Dissociation of the β-LG dimer was first observed just after mixing with sodium caprate. Furthermore, in the β-LG protein in which the original contents were predominantly β-sheets, intermolecular β-sheets attributable to aggregation increased with a decrease in the content of intramolecular β-sheets. Sodium caprate-induced gel was heated at 80°C for 30 min after the gel was formed, and a large increase in the intermolecular β-sheet bands was observed by heat treatment. These results suggest that the formation of sodium caprate-induced gels of β-LG was accompanied by less marked changes in the protein conformation than those in heat-induced gels.

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We previously reported the formation of transparent and high water-holding gels from several food proteins [sesame, rice globulins, and ovalbumin (OVA) (1–3)] by heat treatment in the presence of FA salts (FAS). Particularly for OVA, desirable gels with different gel hardnesses, water-holding abilities, and transparencies were formed by changing the concentration of protein or FAS (3). Furthermore, we reported that OVA is able to form a transparent gel by the addition of a FAS such as sodium caprate or sodium oleate at room temperature (4). In that paper, we stated that 10% OVA formed a soft, transparent gel by the addition of 2% sodium caprate at ambient temperature, that the molar ratio of FAS to OVA was an important factor in forming an elastic, transparent gel, and that a molar ratio of about 45 was suitable.

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FAS-induced OVA gels without heat treatment have a unique gel texture that differs from those of heat-induced gels containing FAS, since heating treatment clearly affects the gel texture. This method is simple and safe; therefore, the gels will be useful materials in food processing. It is also interesting from this point of view to investigate the effects of FAS on other kinds of food proteins in addition to OVA protein.

β-Lactoglobulin (β-LG) is as important a food protein as OVA; although it is a globular protein like OVA, it contains numerous β-sheets in its structure. Many reports are available on the functional properties of β-LG (5–7). Mulvihill and Kinsella (5) reported that although heating a β-LG solution at pH 8 caused an increase in viscosity, self-supporting gels were not formed unless salts such as sodium chloride or calcium chloride were added, indicating that the rheological and textural properties of the gels were markedly affected by salt concentration. On the other hand, β-LG has a FA binding site (6), and Puyol *et al.* (7) investigated the effects of the binding of palmitic acid to β-LG. However, the formation of FAS-induced gels at ambient temperature has not been reported.

Elucidation of the structural character of the partially folded and denatured states during the gelation of proteins is very important for understanding the mechanisms involved in gelation. Painter and Koenig (8) suggested that an intermolecular β-sheet structure forms during thermal denaturation of various egg-white proteins. Analyses by circular dichroism (CD) spectroscopy indicated that native OVA changes into the molten globule state before gelation induced by the addition of FAS (4).

Although CD spectroscopy is highly sensitive for measuring the secondary and tertiary structures of proteins in solution, a very dilute protein solution (less than 0.1% protein, wt/vol) must be used for the analysis; therefore, CD analysis is difficult to use when studying structural changes during protein gelation. On the other hand, FTIR spectroscopy is one of the most useful methods for determining the secondary structure of globular proteins, since FTIR spectroscopy is suitable for any state (from gas to solid) or concentration of sample, and can be used at protein concentrations high enough for gelling.

In this paper, we investigated the formation of a transparent β-LG gel with sodium caprate without heat treatment to

clarify the characteristics of gels containing FAS at 25°C. Namely, we monitored the changes in the rheological properties with time of β-LG samples containing different amounts of sodium caprate by using a strain-controlled rheometer. Furthermore, we investigated the mechanisms involved in the formation of sodium caprate-induced gels of β-LG by using FTIR spectroscopy. Our findings demonstrate that FAS-induced gelation of these proteins is an appropriate model to monitor the gelation process of proteins.

EXPERIMENTAL PROCEDURES

Materials. β-LG from bovine milk [number L3908, approx. 90% (PAGE), containing β-LG A and B, chromatography purified and lyophilized] and sodium caprate (capric acid sodium salt, number C-4151, 99–100%) were purchased from Sigma Chemical Co. (St. Louis, MO). Other reagents were obtained from Wako Pure Chemicals (Osaka, Japan).

Preparation of gels. A sodium caprate-induced β-LG gel was prepared as follows. Ten to 14% β-LG powder was dissolved in water to which 0.2 M sodium chloride had been added and then was mixed with 2.4 to 4.8% sodium caprate (stock solution; for example, 30% sodium caprate in water was used). The final pH of the mixture was 7.6. In the case of gel formation with a constant ratio of sodium caprate to β-LG, three kinds of conditions were used: (i) 10% β-LG and 3% sodium caprate, (ii) 12% β-LG and 3.6% sodium caprate, and (iii) 14% β-LG and 4.2% sodium caprate.

Rheological measurements. Rheological measurements were performed using a dynamic strain-controlled RFS-III rheometer (TA Instruments, New Castle, DE) equipped with parallel plates of 50 mm in diameter working in the oscillatory mode at 25°C. The gap between the parallel plates was adjusted to 1 mm. In this case (50-mm diameter and a 1-mm gap), the sample volume was about 2 mL. The exposed circumference of the sample was covered by silicon oil to prevent evaporation. The storage modulus (G′), loss modulus (G″), and complex modulus (G*) were recorded. A strain-sweep test and a time-sweep test were performed at a frequency of 6.28 rad/s. First, the linear viscoelastic region of the strain for each sample was determined in the sol state by the strain-sweep test. However, in our case, the viscoelasticity of each sample significantly increased during the time-sweep test due to gel formation. Thus, the strain determined first could not be used throughout the time-sweep test. The dynamic strain-controlled RFS-III rheometer that we used had an auto strain mode in which the strain changed in response to changes in the linear region of the sample. Therefore, the preliminary time-sweep testing was performed with strain under the linear region in the sol state. After the preliminary time-sweep test, the strain-sweep test was performed to determine the linear region of the strain and the torque at maximum strain under the linear region of the gel state. From these preliminary tests (time-sweep and secondary strain-sweep tests), the adequate strain required to keep measurements under the linear region from the sol to the gel state in the sample was determined. The option auto-strain adjustment contained maximum applied strain, maximum applied torque, and strain adjustment (% of current strain). When the auto-strain adjustment mode was used, the strain automatically changed during the time-sweep test. The maximum strain value was determined by the secondary strain-sweep test. Therefore, after these optional values were input, an exact time-sweep test was performed.

FTIR measurements. IR spectra were recorded by an FTIR-8300 spectrometer (Shimadzu, Ltd., Kyoto, Japan). Spectral measurements were carried out from 2000 to 1000 cm^{-1} at a resolution of 4 cm^{-1} and 25°C. Interferograms from 40 scans were averaged to obtain one spectrum. Experiments were performed in D_2O, as this resulted in less interference with the protein spectrum than did H_2O. β-LG, dissolved at 12% (wt/vol) in D_2O, was mixed with 3.6% sodium caprate and 0.2 M sodium chloride. The protein solution was placed between a pair of CaF_2 disks with a 15-μm Teflon spacer and sealed with Teflon tape around the disks to avoid evaporation of the sample solution. Spectral measurements of the sample were repeated throughout the experiment. To determine the effect of heat treatment, after the spectra were first measured at 25°C, the CaF_2 disks containing the sample were heated at 80°C for 30 min in a water bath and cooled for more than 1 h at 25°C. Spectral measurement was then performed again. Reference spectra were recorded in the CaF_2 window using D_2O solution instead of a sample solution. IR spectra for the sample dissolved in D_2O were obtained by digitally subtracting the reference spectrum from the spectrum of each sample solution. Fourier self-deconvolution was performed using the software provided with the spectrometer. For deconvolution, band narrowing was achieved with a full width at half-maximum of 19 cm^{-1} and a pathlength of 0.104 mm, and a power spectrum was calculated from the interferogram using the apodization of a triangle[2] (9,10). Furthermore, two kinds of difference spectra were obtained, namely, between the spectrum of the sodium caprate-induced gel or that of the heated sodium caprate-induced gel and the sol state.

RESULTS AND DISCUSSION

Changes in the rheological properties of β-LG gel by the addition of sodium caprate. Changes in the dynamic viscoelasticity of β-LG with time using different amounts for sodium caprate were investigated. Figure 1 shows a typical pattern of the viscoelastic behavior of 12% β-LG with 2.4 to 4.8% sodium caprate during incubation at 25°C. The dynamic viscoelasticity of the mixture with 2.4% sodium caprate gradually increased *via* the crossover point of G′ and G″ at 916 min, and G′ reached 853 Pa at 2400 min (Fig. 1A, Table 1). At 3.6% sodium caprate, the crossover point of G′ and G″ was 198 min, which was faster than that of the gel containing 2.4% sodium caprate. The time corresponding to the crossover point generally indicates a phase transition from the sol to gel state. Therefore, this result showed that gelation of β-LG containing 3.6% sodium caprate occurred much faster than in the case of 2.4% sodium caprate. Furthermore, G′ reached 2926 Pa at 2400 min (Fig. 1B, Table 1), indi-

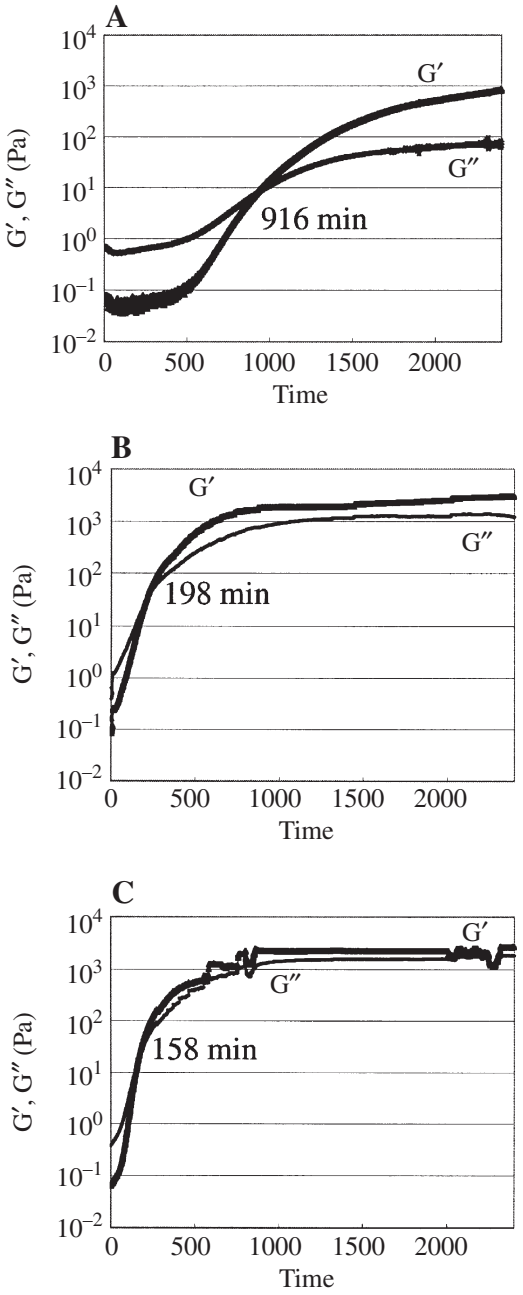

FIG. 1. Storage modulus (G′) and loss modulus (G″) of 12% β-lactoglobulin (β-LG) with different concentrations of sodium caprate with time. Measurements were performed at 6.28 rad/s and 25°C. (A) 2.4% sodium caprate; (B) 3.6% sodium caprate; (C) 4.8% sodium caprate. Thick line: G′; thin line, G″. Inserted time represents the crossover point of G′ and G″.

cating that the gel containing 3.6% sodium caprate was harder than the one with 2.4% sodium caprate. At 4.8% sodium caprate, the crossover point of G′ and G″ was 158 min, and the G′ reached 2225 Pa at 1200 min and 2710 Pa at 2400 min (Fig. 1C, Table 1), respectively. It seems that the curves for 3.6 and 4.8% sodium caprate nearly reached a plateau during this time sweep, showing that gelation was complete at these points. Table 1 shows G′ and G″ values at 1200 and 2400 min. Although there are few differences between Figures 1B and 1C, the difference between G′ and G″ was larger for 3.6% sodium caprate than for 4.8% sodium caprate. A difference such as this generally reflects the solidity of the gels. These data suggest that a more viscoelastic gel was formed in the presence of 3.6% sodium caprate.

Figure 2 shows the effects of protein concentration on the complex modulus of β-LG containing sodium caprate. In this experiment, a constant ratio of sodium caprate to β-LG was used. In 14% β-LG with 4.2% sodium caprate, the increase in the complex modulus was fastest among the three conditions, followed by 12% β-LG with 3.6% sodium caprate and 10% β-LG with 3% sodium caprate. This figure, together with the results in Figure 1, indicates that the absolute protein concentration is more important for increasing the complex modulus of samples than the ratio of sodium caprate to protein. Furthermore, it suggests that desirable gels that have different viscoelasticities can be formed by controlling the protein concentration in this system.

Puyol *et al.* (7) investigated the difference between gel characteristics with and without FA, and stated that a heat-induced gel with palmitic acid (1:1 molar ratio of protein and FA) had a lower storage modulus than that of FA-free β-LG. In that case, the gelation time was about 20 min for FA-free β-LG heated at 70°C, whereas no gelation was observed for the protein with bound palmitic acid after 60 min. We presume that the difference between our results and theirs may be due to the different proportions (molar ratios) of FA to protein used.

Changes in the secondary structures of β-LG during the formation of sodium caprate-induced gel. To clarify how changes in the secondary structure occurred during the formation of sodium caprate-induced β-LG gels, we analyzed changes in the FTIR spectra in the amide I′ region. Figure 3 shows the spectrum of 12% β-LG with or without 3.6% sodium caprate. In the case of β-LG alone (Fig. 3a), it shows six components: 1622, 1634, 1647, 1663, 1678, and 1691 cm^{-1} in the amide I′ region. On the other hand, in the presence of sodium caprate (Fig. 3b), four components were observed in the sol state just after

TABLE 1
Storage Modulus (G′) and Loss Modulus (G″) of 12% β-Lactoglobulin with Different Concentrations of Sodium Caprate

Sodium caprate (%)	G′ at 1200 min (Pa)	G″ at 1200 min (Pa)	G′ at 2400 min (Pa)	G″ at 2400 min (Pa)
2.4	50	23	853	68
3.6	1865	1058	2926	1183
4.8	2225	1505	2710	1827

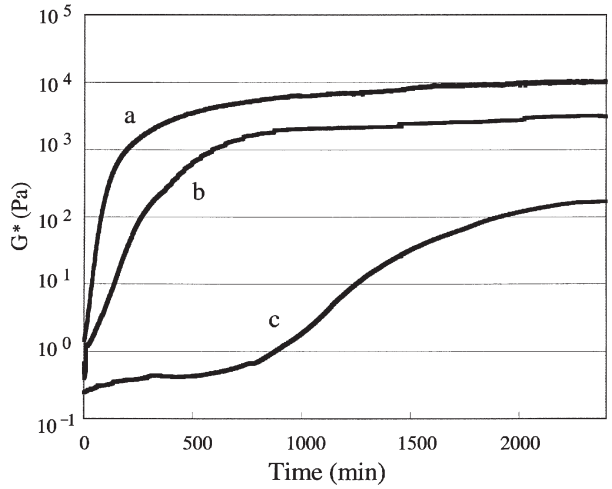

FIG. 2. Effects of protein concentration on the complex modulus (G*) of β-LG containing sodium caprate at a constant ratio. Measurements were performed at 6.28 rad/s and 25°C. (a) 14% β-LG and 4.2% sodium caprate; (b) 12% β-LG and 3.6% sodium caprate; (c) 10% β-LG and 3% sodium caprate. For abbreviation see Figure 1.

mixing: 1628, 1645, 1678, and 1693 cm^{-1}. In particular, it was characteristic that the shoulder at 1622 cm^{-1} was not found in the sol state. We confirmed that the spectrum, which originated from sodium caprate alone, was weak in that region.

A previous study by Boye *et al.* (11) showed that six major components occur in the amide I bands in β-LG. The six components were 1624, 1636, 1648, 1663, 1676, and 1692 cm^{-1}, and they assigned these components as follows: β-sheet; β-sheet; overlap of α-helix and random coils; turns; β-sheet/turns; and turns/β-sheet, respectively. Lefevre and Subirade (12) reported that β-LG shows two components around 1634 and 1623 cm^{-1} as a dimeric form at 30°C in addition to components at 1647 and 1677 cm^{-1}. The component at 1623 cm^{-1} disappeared up to 70°C, and a single component at 1630 cm^{-1} as a monomeric form was observed upon heating. Furthermore, Cairoli *et al.* (13) studied the aggregation process of β-LG by titration with 1,8-anilinonaphthalensulfonate and demonstrated that dimer dissociation was a necessary step in the sequential polymerization mechanism. Qi *et al.* (14) also showed the temperature- and concentration-dependent dissociation of the β-LG dimer and an associated conformational transition at low protein concentration by using Perkin-Elmer DSC7 and Microcal MC-2 calorimeters. From our data (Fig. 3), together with those of the above-mentioned studies, it seems that the dissociation of β-LG to a monomer occurred only with the addition of FAS.

Figure 4 shows the FTIR spectra of 12% β-LG with 3.6% sodium caprate (pD 7.6) at three different states: (i) The sol state 20 min after mixing the protein and sodium caprate is shown in Figure 4a. (ii) The sodium caprate-induced-gel that formed 30 h after mixing β-LG and sodium caprate is shown in Figure 4b. (iii) The spectra of deconvolution analyses clearly showed that a new peak at 1614 cm^{-1} appeared with a decrease in a major peak

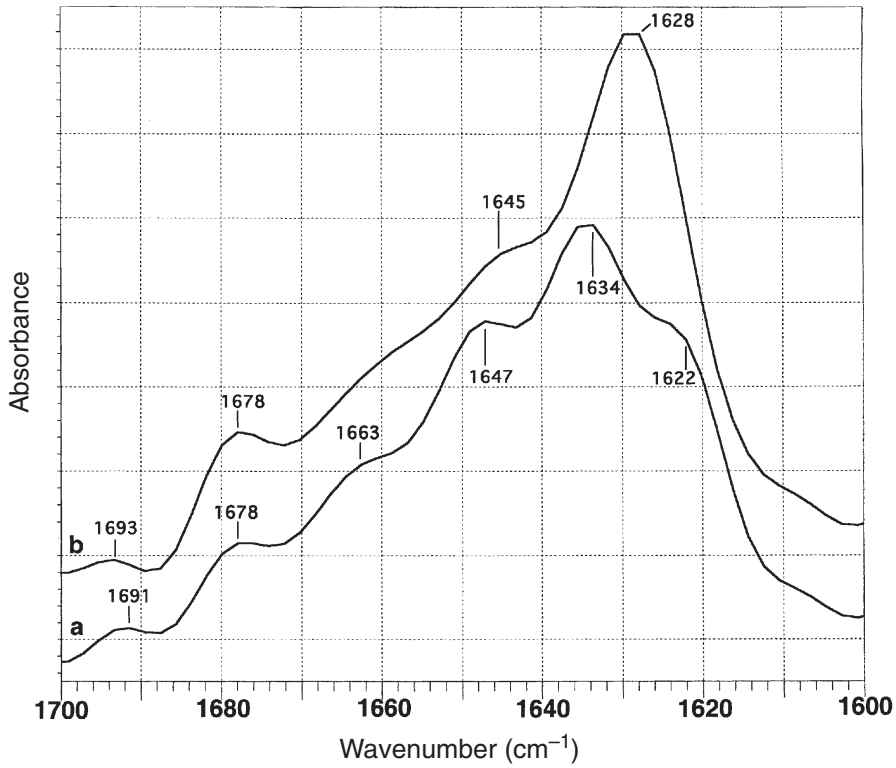

FIG. 3. Deconvoluted spectra in the amide I′ region of 12% β-LG with or without 3.6% sodium caprate. (a) 12% β-LG solution without sodium caprate; (b) β-LG solution with 3.6% sodium caprate. The pD of the solution was adjusted with NaOD. For abbreviation see Figure 1.

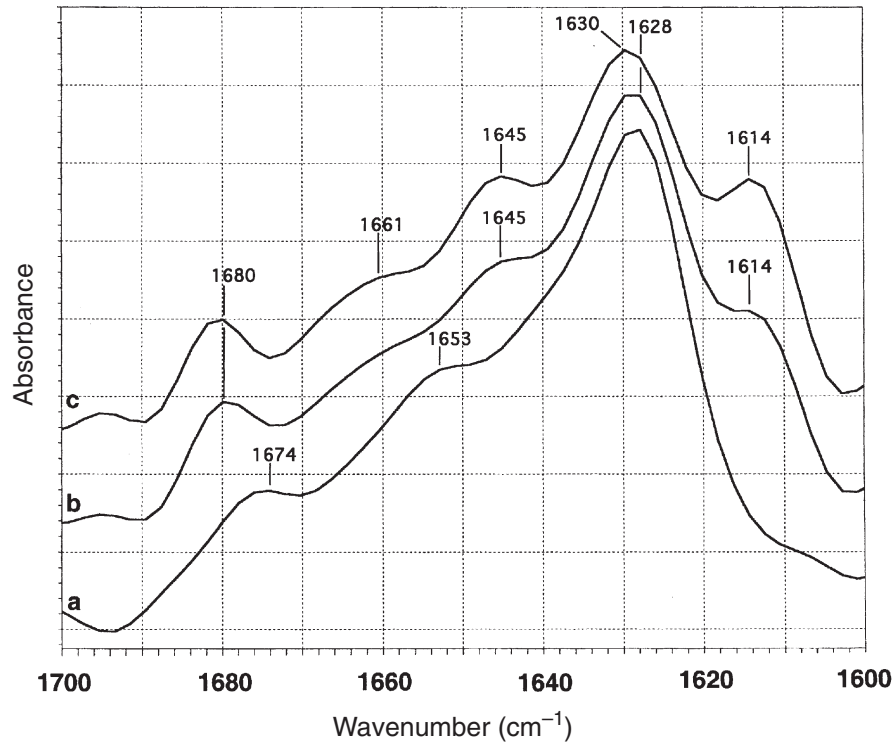

FIG. 4. Deconvoluted spectra in the amide I′ region of 12% β-LG in the presence of 3.6% sodium caprate. (a) β-LG solution just after mixing with 3.6% sodium caprate; (b) sodium caprate-induced β-LG gel; (c) heated sodium caprate-induced β-LG gel. For abbreviation see Figure 1.

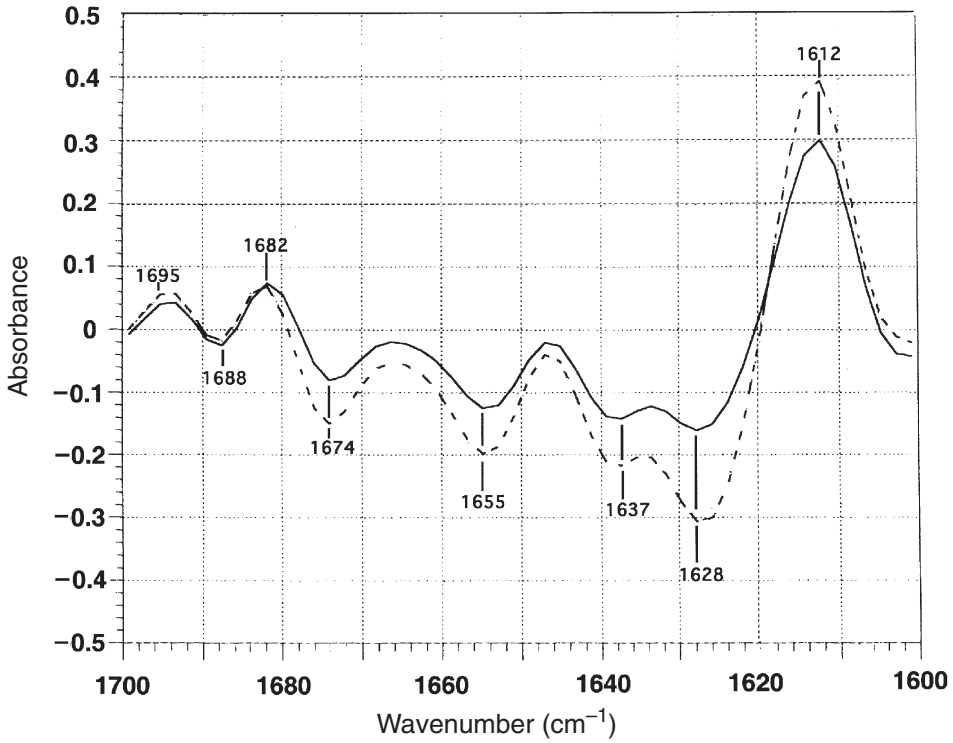

FIG. 5. Difference spectra of sodium caprate-induced gel or heated sodium caprate-induced gel and the sol state. The concentration of the sample was the same as that in Figure 3. (——) Difference spectrum obtained by subtracting sol from that of sodium caprate-induced gel; (------), difference spectrum obtained by subtracting sol from that of heated sodium caprate-induced β-LG gel. For abbreviation see Figure 1.

at 1628 cm^{-1} by sodium caprate-induced gelation. The sodium caprate-induced gel was heated at 80°C for 30 min after the gel had formed, and a further increase in the 1614 cm^{-1} band was observed by heat treatment (Fig. 4c).

Lefevre and Subirade (12) observed that the components at 1634 and 1691 cm^{-1} in 10% β-LG solution were found at 30°C, and at around 80°C the 1618 cm^{-1} component appeared. They suggested that this 1618 cm^{-1} component is characteristic of intermolecular β-sheets resulting from aggregation. In our case, we also observed five components at around 1614, 1628, 1645, 1661, and 1680 cm^{-1} in sodium caprate-induced gel and heated sodium caprate-induced gel. The band at 1614 $^{-1}$ seemed to correspond to the aggregation band.

Figure 5 shows the difference in spectra of sodium caprate-induced gel or heated sodium caprate-induced gel and the sol state. This figure clearly indicates that a decrease in the intensity at 1628 $^{-1}$ and an increase at 1612 cm^{-1} occurred during gelation. These spectra indicate that, regardless of heat treatment, the intramolecular β-sheet (1628 cm^{-1}) was converted to an intermolecular β-sheet due to aggregation (1612 cm^{-1}) during gelation. When β-LG sol was heated immediately after the addition of sodium caprate, almost the same spectrum was observed as with the heated sodium caprate-induced gel (data not shown). These data also suggest that although sodium caprate-induced gel was formed with similar conformational changes in the protein with heat treatment, in the FAS-induced gel the increase in the intermolecular β-sheet was less than that with heat treatment. This idea was also supported by our previous data on the microstructure of OVA gel containing sodium caprate, in which the gel state containing sodium caprate without heat treatment had a slightly rough network compared with the heat-induced gel (4). The results of FTIR spectroscopy suggest that sodium caprate induced the dissociation of the β-LG dimer, followed by an increase in the intermolecular β-sheet with time, resulting in a sodium caprate-induced gel of β-LG. It is important to examine this technique for other kinds of food proteins to produce other good food materials, in addition to investigating the mechanism involved in the gel formation in more detail.

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