Raman Spectroscopic Study of Thermally Induced Gelation of Whey Proteins

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α-Lactalbumin and β-lactoglobulin solutions [15 % (w/v) in D_2O, pD 6.8, 20 mM NaCl] were heated at 50, 70, or 90 °C for 30, 60, or 90 min. Only the samples heated at 90 °C formed transparent gels. The amide I′ and amide III′ bands of the Raman spectra implicated an increase of β-sheet structure with a simultaneous decrease of helical structure in heated α-lactalbumin, while an increase of β-sheet with a simultaneous decrease of turn structure was suggested in heated β-lactoglobulin. Changes in other regions of the Raman spectra could be interpreted as changes in disulfide conformation as well as in microenvironment around several amino acid residues, i.e., Trp, Tyr, and His. These changes were observed after heating at 70 and 90 °C but were more intense in the gelled (90 °C) than in the ungelled samples and in β-lactoglobulin than in α-lactalbumin. These observations are in accord with the fact that β-lactoglobulin forms gels much more easily than α-lactalbumin.

INTRODUCTION

Gelation is one of the most fundamental phenomena observed during food processing. Thermally induced gelation of whey proteins has been recently reviewed (Mulvihill and Kinsella, 1987). Although various studies based on rheological, calorimetric, and solubility measurements have been reported [e.g., Paulsson et al. (1986, 1990), Foegeding et al. (1992), Hines and Foegeding (1993), Ruegg et al. (1977), Dannenberg and Kessler (1988), Mulvihill and Kinsella (1988), and Kuhn and Foegeding (1991)], structural changes of the whey protein molecules during thermally induced gelation have not been clearly elucidated. In a review on gelation of proteins, Ziegler and Foegeding (1990) stated that, surprisingly, gelation of isolated β-lactoglobulin has not been the subject of many investigators. This may be due to the difficulty in analyzing the solid-like structure at high protein concentration, which may be highly turbid in some cases.

Raman spectroscopy provides information on the vibrational motions of molecules, which can be used in prediction or identification of chemical structure of molecules. An advantage of Raman spectroscopy over other spectroscopies or chemical analyses is its versatility in application to aqueous solutions, nonaqueous liquids, fibers, films, powders, gels and crystals without destruction of the samples. Painter (1984) pointed out that Raman spectroscopy has a clear but as yet unrealized potential for characterizing the individual components of food systems and should be sensitive to structural changes during processing such as mixing, aeration, heating, fiber formation, and gelation. Raman spectra of proteins can give information regarding peptide backbone conformation as well as microenvironment around some side chains. Li-Chan and Nakai (1991) used Raman spectroscopy in the study of thermally induced or dithiothreitol-induced gelation of lysozyme to show structural differences among the gels prepared under the different conditions.

The objective of this study was to demonstrate the capability of Raman spectroscopy to detect structural changes in thermally induced α-lactalbumin and β-lactoglobulin gels.

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MATERIALS AND METHODS

α-Lactalbumin (Product L5385), β-lactoglobulin (Product L0130), and deuterium chloride (Product D4376) were purchased from Sigma Chemical Co. (St. Louis, MO), while deuterium oxide (Product MD-175, minimum 99.9 atom % D) was from MSD Isotope (Montreal, Canada). Deuterium oxide solution of each protein was prepared by dissolving the protein in deuterium oxide containing 20 mM NaCl, and the apparent pD (direct reading on the pH meter) was adjusted to 6.8 by using 1.0 N DCl. After centrifugation to remove air bubbles, the protein concentration of each solution was adjusted to 15% (w/v), calculated from absorbance at 280 nm of diluted aliquots of the protein solutions by using $A_{280}^{1\%}$ values of 20.1 and 9.6 for α-lactalbumin and β-lactoglobulin, respectively (Eigel et al., 1984). After the protein solution was gently introduced into a hematocrit capillary tube (Nichiden-Rika Glass Co., Ltd.), both ends of the tube were heat-sealed with care not to heat the protein sample. Tubes containing the protein solution were then heated in a hot water bath at 50, 70, or 90 °C for 30, 60, or 90 min. After cooling in an ice water bath for 5 min, the tubes were kept in a cold room (5 °C) overnight. Occurrence of gelation was detected by visual observation of immobilization of small air bubbles in the protein solution.

Raman spectra were recorded on a JASCO Model NR-1100 laser Raman spectrophotometer with excitation from the 488-nm line of a Spectra-Physics Model 168B argon ion laser. The Raman scattering of protein solutions or gels in a transverse/transverse arrangement (incident laser beam directed perpendicular to the capillary axis held horizontally) was measured at ambient temperature under the following conditions: laser power, 200 mW; slit height, 4 mm; spectral resolution, 5.0 cm^{-1} at 19 000 cm^{-1}; sampling speed, 120 cm^{-1} min^{-1} with data collected at every cm^{-1}; 8 scans per replicate. All computations on the recorded spectra were performed by using LabCalc (Galactic Industries Corp., Salem, NH) with Square Tools (Spectrum Square Associates, Ithaca, NY) on an IBM compatible personal computer. Original spectra in the 450–1750-cm^{-1} region were normalized to the intensity of the H–C–H deformation mode at 1453–1458 cm^{-1}, after baseline correction. All analyses were performed three times, and the results were reported as the average of these replicates. Assignment of the major bands in the spectra to vibrational motions of various side chains or peptide backbone was based on comparison to Raman spectra reported or summarized in the literature or monographs of Raman spectra of proteins (Frushour and Koenig, 1975; Kitagawa et al., 1979; Williams et al., 1980; Carey, 1982; Harada et al., 1982; Harada and Takeuchi, 1986; Tu, 1986; Byler and Susi, 1988).

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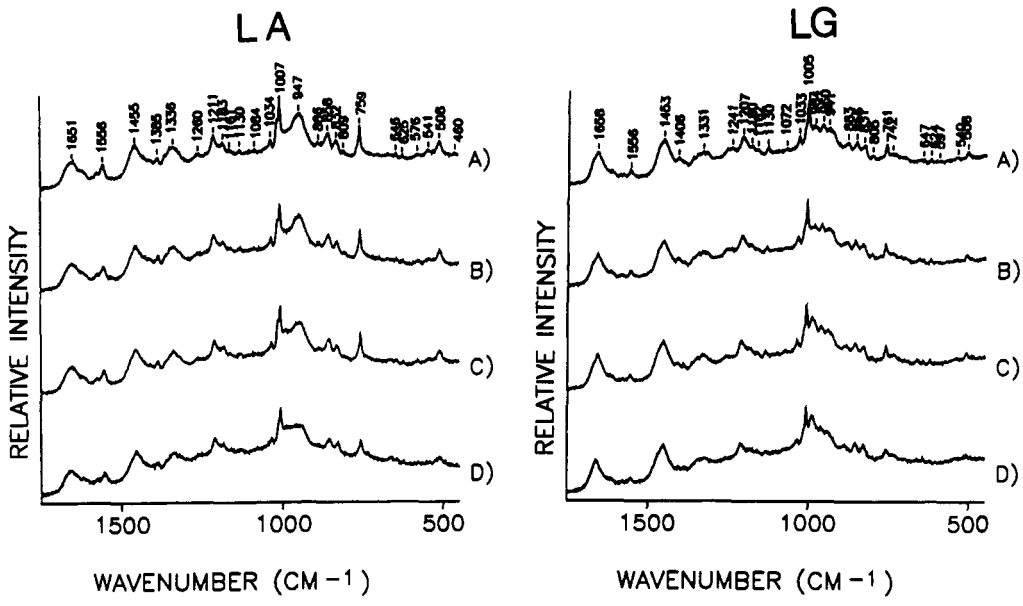

Figure 1. Raman spectra (450–1750 cm^{-1}) of whey protein solutions (or gels). LA, α-lactalbumin; LG, β-lactoglobulin; A, unheated; B, heated at 50 °C for 90 min; C, heated at 70 °C for 90 min; D, heated at 90 °C for 90 min.

Table I. Tentative Assignment of Major Bands in the Raman Spectra of α-Lactalbumin and β-Lactoglobulin (20 mM NaCl, D_2O, pD 6.8)

α-lactalbumin		β-lactoglobulin	
wavenumber,[a] cm^{-1}	assignment[b]	wavenumber,[a] cm^{-1}	assignment[b]
508	ν S–S	508	ν S–S
541, 576	Trp	540	Trp
625	–	597	–
646	–	624	–
759	Trp	647	–
809	–	742	–
832, 858	Tyr	761	Trp
886	Trp	805	–
947	amide III′ (α-helix)	831, 856	Tyr
1007	Phe	883	Trp
1014 (sh)	Trp	941	amide III′ (α-helix)
1034	Phe	960	amide III′
1084, 1130, 1161	ν C–N	984	amide III′ (β-sheet)
1183	Tyr	1005	Phe
1211	Tyr, Phe	1033	Phe
1260	–	1072, 1130, 1162	ν C–N
1336, 1385	Trp	1180	Tyr
1455	δ CH_2	1207	Tyr, Phe
1556	Trp	1241	–
1651	amide I′	1333	Trp
		1406	His
		1453	δ CH_2
		1556	Trp
		1658	amide I′

[a] Standard deviation (three replicates, eight scans per replicate) of ±2–3 cm^{-1}. [b] ν, stretching mode; δ, deformation mode; sh, shoulder; –, not assigned clearly.

RESULTS AND DISCUSSION

α-Lactalbumin and β-lactoglobulin solutions were gelled upon heating at 90 °C, but no gelation was detected at other temperatures. Figure 1 shows typical Raman spectra of whey protein solutions before and after heating at 50, 70, or 90 °C for 90 min. Tentative assignment of some major bands in the spectra of unheated whey proteins in D_2O is summarized in Table I.

Figure 2 shows the difference spectra calculated from each heated sample and corresponding unheated sample shown in Figure 1 over the conformation-sensitive amide I′ region. In the case of α-lactalbumin, a decrease in the intensity around 1640 cm^{-1} where the contribution of α-helix is dominant and an increase of the intensity over the range 1660–1690 cm^{-1} where the contribution of β-sheet and turns are dominant are observed concomitantly for the samples heated for 90 min at temperatures above 70 °C. The degree of intensity changes is greatest for the sample heated at 90 °C. In the case of β-lactoglobulin, an increase in the intensity in the β-sheet-dominant region (around 1660 cm^{-1}) and a decrease of the intensity in the turns-dominant region (1670–1700 cm^{-1}) are observed after 90 min at either 70 or 90 °C.

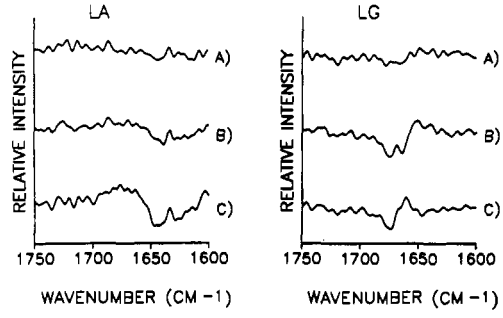

Figure 2. Difference spectra of whey protein solutions (or gels) over the amide I′ region. LA, α-lactalbumin; LG, β-lactoglobulin; A, [50 °C, 90 min] – [unheated]; B, [70 °C, 90 min] – [unheated]; C, [90 °C, 90 min] – [unheated].

Figure 3 shows the intensity changes over the amide III′ region, which is another conformation-sensitive area, for both whey proteins heated under different conditions. The heat treatment at 50 °C does not appear to cause significant change in peptide backbone conformations of the whey proteins even after 90 min. The heat treatments at 70 °C, which did not gel the whey proteins regardless of the heating time, increased the peak intensity of the band assigned to β-sheet for both proteins and decreased the peak intensity assigned to α-helix for α-lactalbumin. The heat treatments at 90 °C, which caused gelation of the whey proteins at all heating times employed, considerably increased the peak intensity of the band representing β-sheet in both proteins. The decrease of α-helix portion is also recognized in α-lactalbumin. These changes in conformation were more pronounced at 90 °C than at 70 °C and were observed within the first 30 min of heating

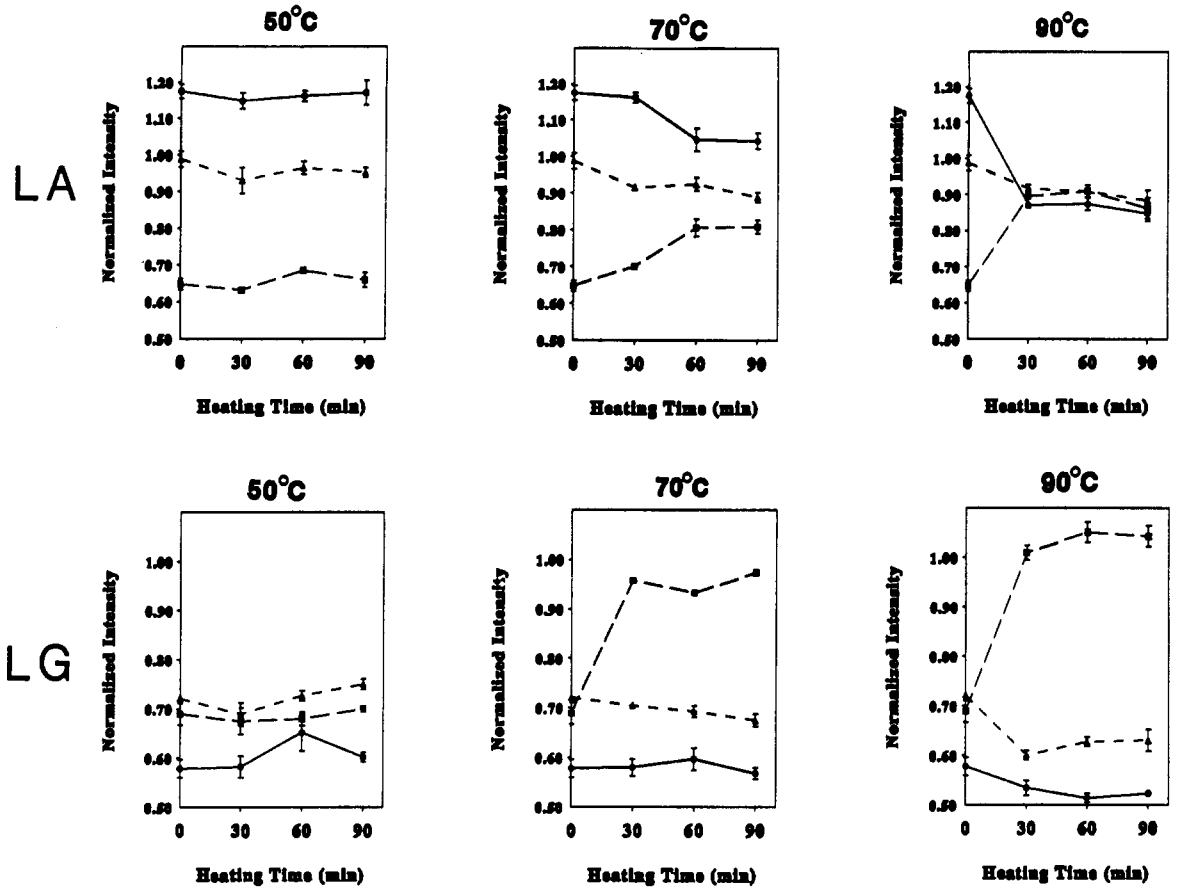

Figure 3. Normalized intensities of three major peaks in the amide III′ region as a function of heating temperature and time. LA, α-lactalbumin; LG, β-lactoglobulin; ●, peak around 945 cm^{-1} (α-helix); ▲, peak around 960 cm^{-1} (not defined); ■, peak around 985 cm^{-1} (β-sheet).

at 90 °C for both proteins; at 70 °C the changes were observed after 30 min for β-lactoglobulin and after 60 min for α-lactalbumin.

According to Wang and Damodaran (1991), the transformation of α-helix and aperiodic structure into β-structure to bring the amount of β-sheet structure up to a critical level is required for gel formation, on the basis of their circular dichroism study of protein fluid expressed from gels of globular proteins, e.g., BSA and soy proteins. Our results are in accord with their hypothesis. A decrease of β-turns was also observed during heat treatment of ovalbumin (Kato and Takagi, 1988).

Ziegler and Foegeding (1990) stated that denaturation is often considered to be a prerequisite to gel formation for globular proteins. The transformations of α-helix to β-sheet in α-lactalbumin and of turns to β-sheet in β-lactoglobulin are parallel to trends observed on heat-induced gelation of lysozyme with and without reduction as reported previously (Li-Chan and Nakai, 1991). It is reasonable to postulate that the SH–SS relationships in α-lactalbumin and β-lactoglobulin resemble lysozyme before and after reduction, respectively. α-Lactalbumin, similar to lysozyme, contains only SS groups and thus maintains molecular structure more rigidly. On the other hand, β-lactoglobulin, similar to reduced lysozyme, contains SH groups and thus is able to initiate SH–SS interchange with adequate flexibility in the structure which may allow unwinding of turns upon heating. According to Legowo et al. (1993), mixtures of α-lactalbumin and β-lactoglobulin in a ratio of 2:8 gelled at a concentration of 8% by heating at 80 °C for 15 min; however, mixtures at ratios of 5:5 and 8:2 required the addition of reduced glutathione for gelation.

Figures 4 and 5 illustrate the changes in peak intensities of bands assigned to vibrational motions of several protein side chains in α-lactalbumin and β-lactoglobulin, respectively, which reflect the microenvironment around the side chains. The band around 508 cm^{-1} in the spectra of both proteins could be assigned as disulfide bonds in gauche–gauche–gauche conformation (Kitagawa et al., 1979). Minor bands near 525 and 540 cm^{-1} have been suggested to be caused by disulfide bands in gauche–gauche–trans and trans–gauche–trans conformations, respectively (Kitagawa et al., 1979; Nakanishi et al., 1974), although tryptophan residues may contribute to the band at 540 cm^{-1} (Byler et al., 1983). A decrease in intensity and a broadening of these bands, especially the one near 508 cm^{-1}, were clearly seen in the samples heated at 90 °C (Figure 6). According to a survey of X-ray evidence, the great majority of cystine linkages in proteins are in a gauche–gauche–gauche or a trans–gauche–trans extended conformation (Richardson, 1981). An effect of heating on the conformation of disulfide bonds was reported by Kitagawa et al. (1979). The 525-cm^{-1} line observed at 8 °C in Bence–Jones protein, which corresponds to trans–gauche–gauche conformation, disappeared at 33 °C and the 512-cm^{-1} line assigned to the gauche–gauche–gauche conformation appeared instead. Similarly, a change in disulfide conformation upon shift in the dimer–monomer equilibrium of acid phosphatase was reported by Twardowski (1982). The disulfide bridge peak shifted from 550 and 525 cm^{-1} in dimer to 500 cm^{-1} in the monomer. However, in our case the relative intensity of the 508-cm^{-1} peaks which represent S–S stretch in gauche–gauche–gauche conformation decreased, with slight change in the 645-cm^{-1} peaks assigned as C–S stretching in gauche–

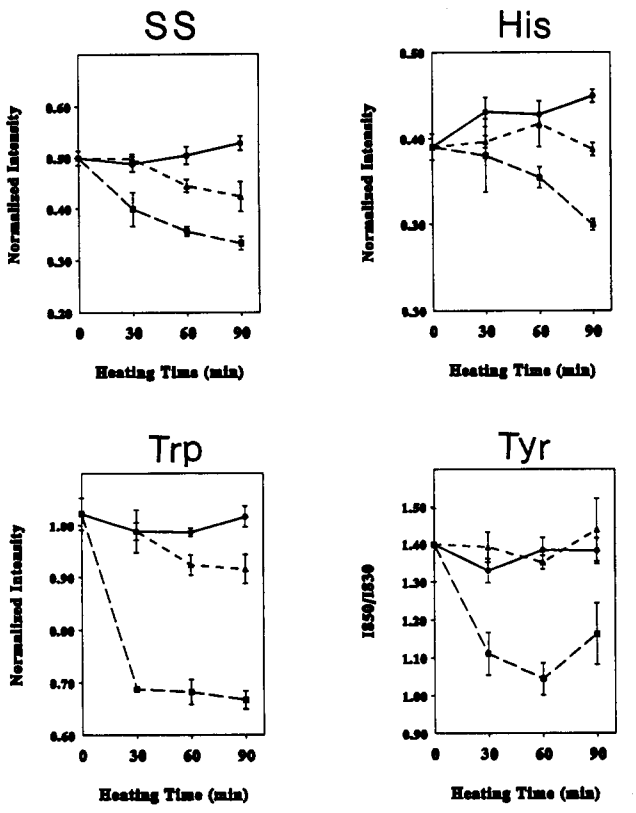

Figure 4. Normalized intensities of several peaks reflecting the microenvironment of α-lactalbumin side chains. SS, peak around 508 cm^{-1} (disulfide bond in gauche–gauche–gauche conformation); His, peak around 1408 cm^{-1} (imidazolium ring of histidine); Trp, peak around 760 cm^{-1} (tryptophan); Tyr, ratio of peak around 830 cm^{-1} to peak around 850 cm^{-1} (tyrosine); ●, heated at 50 °C; ▲, heated at 70 °C; ■, heated at 90 °C.

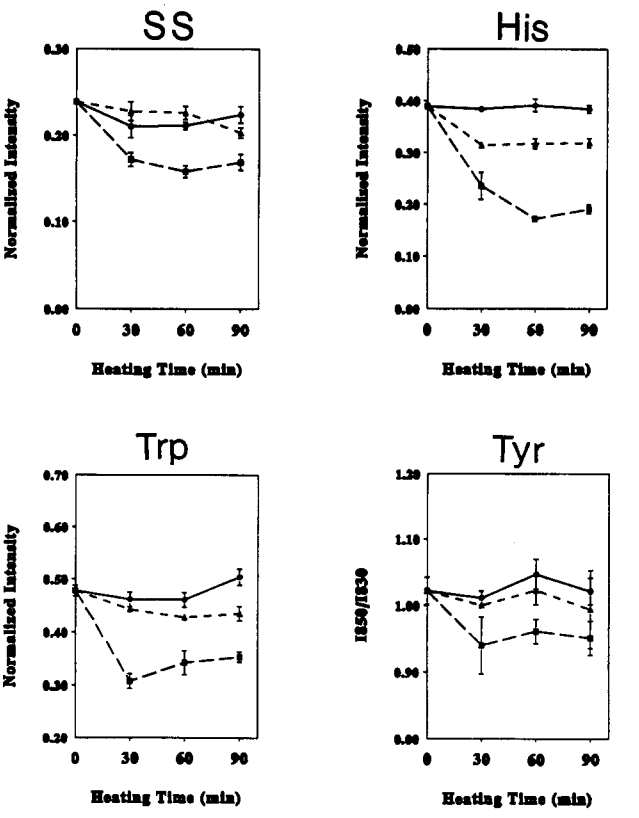

Figure 5. Normalized intensities of several peaks reflecting the microenvironment of β-lactoglobulin side chains. (Abbreviation and symbols are as described in the caption to Figure 4.)

gauche–gauche conformation by Byler et al. (1983). This may suggest that conformation of disulfide bonds in native α-lactalbumin as well as β-lactoglobulin was altered upon heating at 90 °C (Figures 4 and 5). The same but less intensive changes are also observed in the spectra of α-lactalbumin samples heated at 70 °C for 60 and 90 min (Figure 4). According to Ziegler and Foegeding (1990), the role of disulfide bonds and sulfhydryl groups in gelation is always clouded by the possibility of intra- and intermolecular sulfhydryl/disulfide interchange producing a variety of cross-links without a net change in concentration of sulfhydryl or disulfides. Therefore, the decrease observed in 508 cm^{-1} could be evidence of losing the native form of disulfide bonds as a result of gelation, although no evidence has been found in the literature to support this hypothesis. Although possible shadowing of the 508-cm^{-1} peak due to burying of SS bonds inside the gel matrix should not be ignored, it is not likely since there is no similar change of the 645-cm^{-1} peak upon gelation. Further work is required to support this hypothesis.

Exposure of buried tryptophan residues in proteins is observed by the decrease of the peak intensity around 760 cm^{-1} (Kitagawa et al., 1979). The samples heated at 90 °C, which were gels, had much lower peak intensities around the above wavenumber than the controls for both proteins. The exposed tryptophan may play a role in hydrophobic interaction in the gels. The ratio of the tyrosyl doublet around 850 and 830 cm^{-1} is known as a good indicator of the hydrogen bonding of the phenolic hydroxyl group. Standard values for normal, strongly hydrogen-bonded, and ionized tyrosines have been proposed (Siamwiza et al., 1975). Although the effect of O-deuteration on the standard values has not been reported, the observed trend in which the doublet ratio decreases in all of the gelled samples suggests that some tyrosine residues in α-lactalbumin as well as β-lactoglobulin become more involved as strong hydrogen bond donors through thermally induced gelation at 90 °C. Upon reduction of disulfide bonds, ribonuclease A showed a substantial decrease in α-helical content and conversion to a weakly hydrogen-bonded form (Lord and Relyea, 1981). A decrease in the 850/830 ratio has also been reported to reflect an increase in "buriedness" (Tu, 1986), suggesting possible involvement of tyrosyl residues in intermolecular interactions. The importance of the intermolecular hydrogen-bonding formation between β-sheets acting as junction zones in stabilizing the gel network was suggested by Wang and Damodaran (1991).

A peak around 1408 cm^{-1} assigned as the N-deuterated imidazolium ring of histidine has been reported to be sensitive to the ionization state of histidyl residues (Harada et al., 1982). This peak was visible only as a small hump in the spectra of α-lactalbumin but appeared clearly in the spectra of β-lactoglobulin in its native state (Figure 1). The peak intensities at 1408 cm^{-1} of the β-lactoglobulin samples heated at 70 °C as well as 90 °C and of the α-lactalbumin samples heated at 90 °C were lower than for the corresponding unheated control proteins. The results indicate dedeuteration which may result from heating, especially at 90 °C. The environmental changes around histidine residues which are conferred by this change in imidazole ionization state through gelation have not been reported for whey proteins in the literature. However, Fisher et al. (1990) observed the behavior on carbon-13 NMR of histidine side chains in soy glycinin which was similar to that of hydrophobic amino acid side chains; i.e., peaks became sharper, consistent with increased motion and protein unfolding at high temperatures. Upon gelation, the peaks were broader, consistent

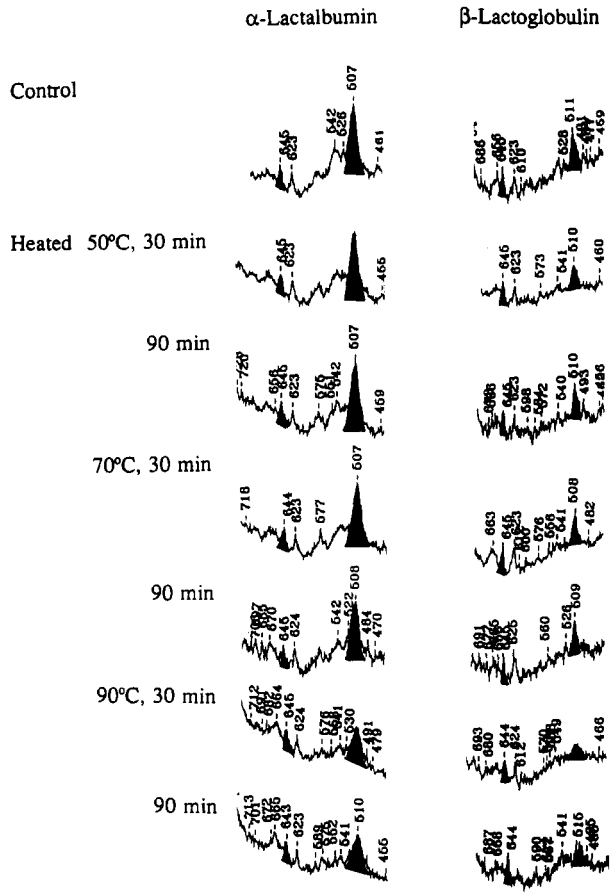

Figure 6. Effects of heating of whey protein solutions (or gels) on Raman spectra /(450–700 cm^{-1}) representing disulfide conformation.

with involvement in gel structure. These changes were more evident in β-conglycinin, which contains many fewer SS bonds than glycinin. Whether this change in histidine side chains contributes to the gelation mechanism or is simply an effect of gelation is not known.

Kim et al. (1987) reported that β-lactoglobulin content determined by reversed-phase HPLC was highly correlated with the gel strength and other functional properties of whey protein concentrate, whereas no significant relationship was found between α-lactalbumin content and gel strength. Although α-lactalbumin is readily heat-denatured, it has a much greater tendency to renature than to form aggregates (Ruegg et al., 1977). Paulsson et al. (1986) reported that at pH 6.6 and 1% NaCl α-lactalbumin did not gel even at 20% protein concentration, whereas a 5% solution of β-lactoglobulin heated to 70–80 °C gelled readily. Using 7% protein solutions buffered at pH 7 and containing 100 mM NaCl, Hines and Foegeding (1993) reported that a weak α-lactalbumin gel formed after 3 h of heating at 80 °C, while a strong gel formed within the first 60 min of heating β-lactoglobulin at 80 °C. Addition of a minimum level (25–30 mM) of NaCl is required for gelation of either whey protein isolate at 80 °C (Kuhn and Foegeding, 1991) or β-lactoglobulin at 90 °C (Mulvihill and Kinsella, 1988). Our study used 15% protein solutions in deuterium oxide at apparent pD of 6.8 and in the presence of 20 mM NaCl; under these conditions transparent gels were formed only at 90 °C. The Raman spectra indicated that conformational changes in α-lactalbumin were observed only after prolonged heating (60 or 90 min) at 70 °C, whereas changes were observed for β-lactoglobulin even after 30 min at 70 °C. Both proteins demonstrated more pronounced changes in secondary structure and in conformation and microenvironment around amino acid residue side chains after heating at 90 °C than at 70 °C, and these changes were observed within the first 30 min of heating at 90 °C.

In conclusion, Raman spectroscopy enabled direct detection of structural changes among the whey protein samples treated under different heating conditions. Generally, substantial increase of β-sheet structure, conformational variation of disulfide bonds, dedeuteration of imidazolium ring of histidine, exposure of tryptophan, and increase of hydrogen bonding of tyrosine were caused through the gelation of both α-lactalbumin and β-lactoglobulin. The information obtained from this study will be useful for correlating with physical properties, e.g., gel strength, molecular size, and viscoelasticity of the gels, in the future.

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LITERATURE CITED

Byler, D. M.; Susi, H. Application of computerized infrared and Raman spectroscopy to conformation studies of casein and other food proteins. *J. Ind. Microbiol.* **1988**, *3*, 73–88.

Byler, D. M.; Susi, H.; Farrell, H. M., Jr. Laser-Raman spectra, sulfhydryl groups, and conformation of the cysteine linkages of β-lactoglobulin. *Biopolymers* **1983**, *22*, 2507–2511.

Carey, P. R. *Biochemical Applications of Raman and Resonance Raman Spectroscopies*; Academic Press: New York, 1982.

Dannenberg, F.; Kessler, H.-G. Reaction kinetics of the denaturation of whey proteins in milk. *J. Food Sci.* **1988**, *53*, 258–263.

Eigel, W. N.; Butler, J. E.; Ernstrom, C. A.; Farrell, H. M., Jr.; Harwalkar, V. R.; Jenness, R.; Whitney, R. McL. Nomenclature of proteins of cow's milk: Fifth revision. *J. Dairy Sci.* **1984**, *67*, 1599–1631.

Fisher, M. S.; Marshall, W.; Marshall, H. F., Jr. Carbon-13 NMR studies of the effect of gelation and heat and chemical denaturation at neutral pH on soy glycinin and β-conglycinin. *J. Agric. Food Chem.* **1990**, *38*, 957–966.

Foegeding, E. A.; Kuhn, P. R.; Hardin, C. C. Specific divalent cation-induced changes during gelation of β-lactoglobulin. *J. Agric. Food Chem.* **1992**, *40*, 2092–2097.

Frushour, B. G.; Koenig, J. L. Raman spectroscopy of protein. In *Advances in infrared & Raman spectroscopy*; Clark, R. J. H., Hester, R. E., Eds.; Heyden: London, 1975; Vol. 1, pp 35–97.

Harada, I.; Takeuchi, H. Raman and ultraviolet resonance Raman spectra of protein and related compounds. In *Spectroscopy of Biological Systems*; Clark, R. J. H., Hester, R. E., Eds.; Wiley: London, 1986; pp 113–175.

Harada, I.; Takamatsu, T.; Tatsumi, M.; Lord, R. C. Raman spectroscopic study of the interaction between sulfate anion and an imidazolium ring in ribonuclease A. *Biochemistry* **1982**, *21*, 3674–3677.

Hines, M. E.; Foegeding, E. A. Interactions of α-lactalbumin and bovine serum albumin with β-lactoglobulin in thermally induced gelation. *J. Agric. Food Chem.* **1993**, *41*, 341–346.

Kato, A.; Takagi, T. Formation of intermolecular β-sheet structure during heat denaturation of ovalbumin. *J. Agric. Food Chem.* **1988**, *36*, 1156–1159.

Kim, Y. A.; Chism, G. W.; Mangino, M. E. Determination of beta-lactoglobulin, alpha-lactalbumin and bovine serum albumin of whey protein concentrate and their relationship to protein functionality. *J. Food Sci.* **1987**, *52*, 124–127.

Kitagawa, T.; Azuma, T.; Hamaguchi, K. The Raman spectra of Bence-Jones proteins. Disulfide stretching frequencies and dependence of Raman intensity of tryptophan residues on their environments. *Biopolymers* **1979**, *18*, 451–465.

Kuhn, P. R.; Foegeding, E. A. Mineral salt effects on whey protein gelation. *J. Agric. Food Chem.* **1991**, *39*, 1013–1016.

Legowo, A. M.; Imade, T.; Hayakawa, S. Heat-induced gelation of the mixture of α-lactalbumin and β-lactoglobulin in the presence of glutathione. *Food Res. Int.* **1993**, *26*, 103–108.

Li-Chan, E.; Nakai, S. Raman spectroscopic study of thermally and/or dithiothreitol induced gelation of lysozyme. *J. Agric. Food Chem.* **1991**, *39*, 1238–1245.

Lord, R. C.; Relyea, N. M. Laser Raman spectroscopy of biomolecules. 15. Conformational study of partially reduced, fully reduced and cyanogen bromide-treated ribonuclease A. *J. Raman Spectrosc.* **1981**, *11* (5), 315–319.

Mulvihill, D. M.; Kinsella, J. E. Gelation characteristics of whey proteins and β-lactoglobulin. *Food Technol.* **1987**, *41* (9), 102–111.

Mulvihill, D. M.; Kinsella, J. E. Gelation of β-lactoglobulin: Effects of sodium chloride and calcium chloride on the rheological and structural properties of gels. *J. Food Sci.* **1988**, *53*, 231–236.

Nakanishi, M.; Takesada, H.; Tsuboi, M. Conformation of the cystine linkages in bovine α-lactalbumin as revealed by its Raman effect. *J. Mol. Biol.* **1974**, *89*, 241–243.

Painter, P. C. The application of Raman spectroscopy to the characterization of food. In *Food analysis. Principles & Techniques. Volume 2. Physicochemical Techniques*; Gruenwedel, D. W., Whitaker, J. R., Eds.; Dekker: New York, 1984; pp 511–545.

Paulsson, M.; Hegg, P.; Castberg, H. B. Heat-induced gelation of individual whey proteins. A dynamic rheological study. *J. Food Sci.* **1986**, *510*, 87–90.

Paulsson, M.; Dejmek, P.; van Vliet, T. Rheological properties of heat-induced β-lactoglobulin gels. *J. Dairy Sci.* **1990**, *73*, 45–73.

Richardson, J. S. The anatomy and taxonomy of protein structure. *Adv. Protein Chem.* **1981**, *34*, 167–339.

Ruegg, M.; Moor, U.; Blanc, B. A calorimetric study of the thermal denaturation of whey proteins in simulated milk ultrafiltrate. *J. Dairy Res.* **1977**, *44*, 509–520.

Siamwiza, M. N.; Lord, R. C.; Chen, M. C.; Takamatsu, T.; Harada, I.; Matsuura, H.; Shimanouchi, T. Interpretation of the doublet at 850 and 830 cm^{-1} in the Raman spectra of tyrosyl residues in proteins and certain model compounds. *Biochemistry* **1975**, *14*, 4870–4876.

Tu, A. T. Peptide backbone conformation and microenvironment of protein side chains. In *Spectroscopy of Biological Systems*; Clark, R. J. H., Hester, R. E., Eds.; Wiley: London, 1986; pp 47–112.

Twardowski, J. Laser Raman spectroscopy of postsynthetic modification forms of acid phosphatase from rat liver. In *Raman Spectroscopy*, Proceedings of the 8th International Conference; Lascombe, J., Huong, P. V., Eds.; Wiley: Chichester, U.K., 1982; pp 775–776.

Wang, C.-H.; Damodaran, S. Thermal gelation of globular proteins: Influence of protein conformation on gel strength. *J. Agric. Food Chem.* **1991**, *39*, 433–438.

Williams, R. W.; Cutrera, T.; Dunker, A. K.; Peticolas, W. L. The estimation of protein secondary structure by laser Raman spectroscopy from the amide III′ intensity distribution. *FEBS Lett.* **1980**, *115*, 306–308.

Ziegler, G. R.; Foegeding, E. A. The gelation of proteins. *Adv. Food Nutr. Res.* **1990**, *34*, 203–298.

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