Phospholipid/Fatty Acid-Induced Secondary Structural Change in *β*-Lactoglobulin during Heat-Induced Gelation

Shinya Ikeda,[†] E. Allen Foegeding,*[,†] and Charles C. Hardin[‡]

Departments of Food Science and Biochemistry, North Carolina State University, Raleigh, North Carolina 27695

Effects of phosphatidylcholine (PC) and the predominant fatty acids (FAs) in milk, butyrate, oleate, and palmitate, on secondary structural changes in *β*-lactoglobulin (*β*-LG) during heat-induced gelation were analyzed on the basis of circular dichroism (CD) spectra. Small-strain oscillatory measurements were carried out to characterize viscoelastic properties of the heat-induced gels. In the absence of added salt, PC and FAs induced helix formation of *β*-LG on heating to 80 °C and increased the storage moduli (*G*′) of heat-induced gels. In the presence of 500 mM NaCl, PC did not change the CD spectrum of *β*-LG but decreased *G*′. In contrast, butyrate substantially unfolded *β*-LG in 500 mM NaCl on heating, forming very elastic gels with increased *G*′ values. Palmitate and oleate induced *β*-LG gel formation at 25 °C without heating; heating to 80 °C almost completely unfolded *β*-LG in 500 mM NaCl.

Keywords: *β-Lactoglobulin; phosphatidylcholine; fatty acid; gelation; secondary structure; viscoelasticity*

INTRODUCTION

Heat-induced gelation is one of the most important functional properties that whey protein isolates (WPIs) or concentrates (WPCs) exhibit since gelation accounts for appearance, texture, and water holding properties of many food products (Ziegler and Foegeding, 1990). The four major proteins in bovine whey-based ingredients are *β*-lactoglobulin (*β*-LG), α-lactalbumin, bovine serum albumin, and immunoglobulins; *β*-LG dominates their gelation properties (Hines and Foegeding, 1993). When heat is applied to an aqueous WPI or WPC dispersion, partial unfolding of proteins begins, accompanied by exposure of interior reactive regions and sulfhydryl groups. Denatured proteins can aggregate via intermolecular interactions, namely, hydrophobic forces, disulfide bonds, hydrogen bonds, electrostatic interactions, etc. Under appropriate conditions, these adhesive effects produce a macroscopically continuous three-dimensional gel network of protein and entrapped solvent (Ziegler and Foegeding, 1990).

Whey protein ingredients also contain mineral salts, lipids, and lactose. The relative amounts of these components vary, depending on whey source and ingredient processing. Since interactions among components strongly affect gelation properties of proteins, the properties of whey protein ingredients also vary due to the inherent variability in composition (Morr and Foegeding, 1990).

Our previous studies suggested that the gelation properties of whey protein ingredients are not the result of a simple summation of contributions from each component; they act synergistically. When phospholipids were added to whey protein dispersions up to the same levels of proteins, the mechanical strength of heat-induced gels became either stronger or weaker, depending on the NaCl concentration (Ikeda and Foegeding, 1999a,b). At pH 7 and low ionic strength, proteins tend to form linear aggregates due to electrostatic repulsive forces among negatively charged protein molecules, leading to a fine-stranded transparent network (Clark et al., 1981a; Foegeding et al., 1995). Adding crude phospholipid (lecithin) to WPI suspensions under these conditions substantially increased the storage modulus (*G*′) and fracture modulus (G_f) of heat-induced gels. Results suggested that lecithin interacted with the protein gel network (Ikeda and Foegeding, 1999a,b). Brown et al. (1983) reported that phosphatidylcholine (PC), the major component of lecithin, was capable of binding to denatured *β*-LG at low ionic concentration but did not bind to native *β*-LG. Since *β*-LG exposes internal hydrophobic regions at high temperatures, heat-induced structural changes in *β*-LG would trigger interactions between the hydrocarbon chains of PC and *β*-LG. On the other hand, at high ionic concentrations, a random aggregation of protein occurs due to the absence of electrostatic repulsive forces among protein molecules, leading to a formation of an opaque, particulate network (Clark et al., 1981a; Foegeding et al., 1995). Lecithin interacted with proteins differently under these conditions, resulting in lower *G*′ and G_f (Ikeda and Foegeding, 1999a,b). In addition, increasing the ion concentration in the presence of phospholipid may create a poorer solvent system, which interferes with gel network formation through demixing.

It is well-known that mineral salts cause transitions in protein gel networks from fine-stranded to particulate networks with increasing ionic concentration and drastically change rheological properties of the gels (Clark et al., 1981a; Foegeding et al., 1995). These transitions

* To whom correspondence should be addressed. Telephone: (919) 513-2244. Fax: (919) 515-7124. E-mail: allen_foegeding@ncsu.edu.

[†] Department of Food Science.

[‡] Department of Biochemistry.

10.1021/jf990434h CCC: $19.00
Published on Web 02/24/2000

are not associated with changes in protein secondary structures (Clark et al., 1981b; Foegeding et al., 1992). In contrast, interactions between PC and β-LG seem to cause changes in the secondary structure of β-LG (Brown et al., 1983). Therefore, it is of interest to determine whether PC-induced secondary structure changes affect the rheological properties of heat-induced gels. The objective of this study was to investigate the effects of PC on structural changes during heat-induced gelation of β-LG. Effects of three predominant fatty acids in milk and whey protein ingredients, i.e., butyrate, oleate, and palmitate (Creamer and MacGibbon, 1996; Vaghela and Kilara, 1996), were also investigated. The far UV circular dichroism (CD) was employed to study structural changes in β-LG since it is dominated by contributions due to chiral interactions between the peptide chromophore and residues in the immediate environment and is particularly suitable for secondary structural analyses in the presence of lipids or fatty acids.

MATERIALS AND METHODS

Materials. β-Lactoglobulin (L-3908, Lot 48H7018, a mixture of variants A and B), l-β-phosphatidylcholine (P-7318, Lot 68H8006, purified from egg-yolk), sodium butyrate (B-5887, Lot 115H5021), sodium oleate (O-7501, Lot 117H5180), and sodium palmitate (P-9767, Lot 68H5189), were purchased from Sigma Chemicals (St Louis, MO). All other chemicals were of reagent grade quality.

Circular Dichroism (CD). β-LG solutions were prepared in 10 mM Tris−HCl buffer (pH 7) containing either 0 or 500 mM NaCl to compare effects of ionic concentration on interactions between β-LG and phosphatidylcholine (PC) or fatty acids (FAs). β-LG was first hydrated to approximately 2.5 mg/mL in the buffer. Once thoroughly mixed, the solution was adjusted to pH 7 with 0.1 M NaOH and filtered through a 0.45 μm syringe filter (Gelman Sciences, Ann Arbor, MI). Following degassing under vacuum to remove all visible air bubbles, the protein concentration was determined spectrophotometrically at 278 nm using $\epsilon_{278} = 0.955$ cm^2/mg (Bell and McKenzie, 1967) and diluted to 2 mg/mL protein. PC or FAs were suspended in 10 mM Tris−HCl buffer (pH 7) containing either 0 or 500 mM NaCl to approximately 4.5 mg/mL. The PC dispersions were sonicated (120 W) at 70 °C for about 30 min until the suspensions became sufficiently clear for spectrophotometry (Brown et al., 1983). The β-LG solution was mixed in a ca. 1:1 weight ratio either with the buffers or the PC/FAs dispersions and then diluted to a final protein concentration of 1 mg/mL and a PC/FAs concentration of 2 mg/mL with the appropriate buffers.

CD spectra were recorded on a JASCO J-600 spectropolarimeter (JASCO, Japan). The samples were analyzed at 25 and 80 °C in a 0.1 mm path length jacketed quartz cell connected to a temperature-controlled circulating water bath. At each temperature, the samples were allowed to equilibrate for 10 min before data acquisition. The quartz cell was washed with nitric acid and double-distilled water sequentially and dried between each measurement. The spectra were collected in 0.2 nm steps at a rate of 50 nm/min over the wavelength range 180−260 nm and truncated at a wavelength where the photomultiplier tube voltage went more negative than −800 mV, which corresponds to a transmittance of less than ca. 1%. Baseline acquisitions for each sample (the dispersions only without β-LG) were measured as soon after the corresponding protein acquisitions as possible. Each spectrum was acquired five times, and the results were averaged. The reported spectra are the smoothed average of two experimental results performed independently. Molar circular dichroism, $\Delta\epsilon$, corresponds to $[\theta]/3298$, where $[\theta]$ is the residue-average molar ellipticity in units of (deg·cm^2)/dmol (obtained by dividing the measured ellipticity by the residue-average molecular weight of the protein).

Secondary structure contents were estimated by fitting the CD spectra in the 184−260 nm range to a combination of reference spectra of proteins using the Fortran program Varselec supplied by Compton and Johnson (1986) and Johnson (1988, 1990). The reference set of proteins consisted of β-chymotrypsin, cytochrome *c*, elastase, lysozyme, myoglobin, papain, ribonuclease A, subtilisin BPN′, flavodoxin, prealbumin, subtilisin *Novo*, carboxypeptidase A, Bence-Jones protein, rubredoxin, *Eco*R1, tumor necrosis factor-α, and γ-crystallin.

Dynamic Rheological Measurement. β-LG and PC or FAs were hydrated together to approximately 12% w/w protein and 12% w/w PC or FAs by stirring for 1 h in distilled water or 500 mM NaCl. The suspensions were degassed in a vacuum chamber connected to an aspirator until all visible air bubbles disappeared. The mixture was adjusted to pH 7 with 1 M NaOH or 1 M HCl and diluted to 12% w/v protein and 12% w/v PC/FAs with distilled water or 500 mM NaCl. The mixed suspensions were then degassed for 1 h.

A Bohlin VOR rheometer (Bohlin Instruments Inc, Cranbury, NJ) was used to determine rheological transitions that occurred during in situ thermal processing of the WPI suspensions. The Bohlin C14 concentric cylinder measuring system was used in all experiments. The measuring system consisted of an oscillating cup and a fixed bob attached to a 1.82 g·cm or 13.2 g·cm torque bar. The WPI suspensions were carefully poured into the cup at 25 °C and covered with a thin layer of mineral oil to prevent water evaporation during experiments. Gels were formed by heating the samples from 25 to 80 °C at a constant rate of 2.5 °C/min, holding at 80 °C for 30 min, cooling to 25 °C at a constant rate of 1 °C/min, and then holding at 25 °C for 30 min. To minimize gel network damage and ensure linear viscoelastic behavior, testing frequency and strain during the gelation process were selected on the basis of preliminary experimental results from frequency and strain sweeps of thermally formed gels. A frequency of 0.05 Hz and a maximum strain of 0.01 were used to measure G', loss moduli (G''), and phase angles of samples during the entire thermal treatment. However, both G' and G'' developed similarly and the G' values were considerably greater in magnitude than G'' values, suggesting predominantly elastic gels were formed. Therefore, only G' values are reported in this study. Frequency sweep measurements were done at the end of the entire thermal processing at 0.01−10 Hz at a maximum strain of 0.01. The G' showed power law relationships with frequency f:

$$G' = mf^n \quad (1)$$

Values of the two constants, m and n, were determined by least-squares fitting the above equation to twice replicated data.

RESULTS AND DISCUSSION

Secondary Structure. Figure 1 shows the time dependence of the ellipticity at 205 nm (θ_{205}) of β-LG when the temperature was raised from 25 to 80 °C. Changes in θ_{205} due to heat denaturation of β-LG were complete within a few minutes, consistent with previous NMR experiments which suggested that conformational transitions in the β-LG structure occur within several minutes and are followed by a slow gelation step that continues for several hours (Li et al., 1994). Therefore, all the samples were equilibrated for 10 min at the experimental temperature before obtaining the CD spectrum.

CD spectra of β-LG at 25 °C are shown in Figure 2. For the systems without added NaCl, spectra were obtained in the 184−260 nm range. The shape of the native β-LG spectrum was similar to reported spectra (Foegeding et al., 1992; Chen et al., 1993; Griffin et al., 1993; Qi et al., 1997), and the estimated secondary structure contents (Table 1) were similar to those

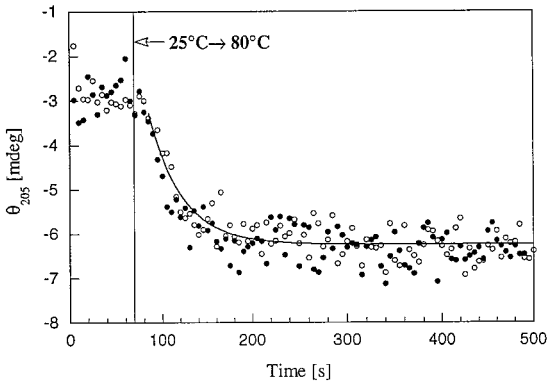

Figure 1. Time dependence of ellipticity of 1 mg/mL β-lactoglobulin (pH 7, no salt added) at 205 nm. The vertical line indicates the time when the temperature was instantaneously raised from 25 to 80 °C. Solid and open circles represent results of two replicates.

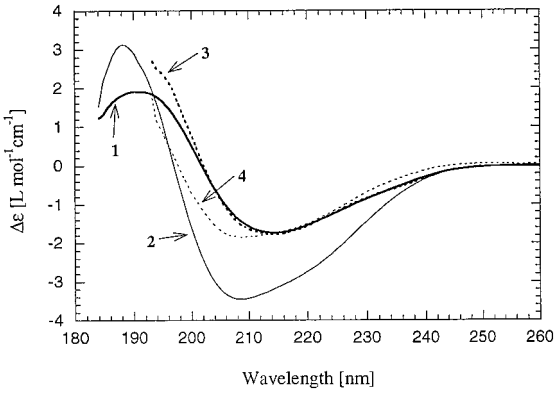

Figure 2. Circular dichroism spectra of 1 mg/mL β-lactoglobulin (pH 7) at 25 °C in the presence of: no additives (1); 2 mg/mL oleate (2); 500 mM NaCl (3); 2 mg/mL oleate and 500 mM NaCl (4).

Table 1. Effects of Temperature and Phosphatidylcholine and Fatty Acids on Secondary Structure Contents of β-Lactoglobulin

temp, °C	additive	α-helix	β-sheet	turn	other
25	–	0.15	0.47	0.14	0.23
	oleate	0.27	0.22	0.15	0.34
25	–	0.15[a]	0.43[a]	0.12[a]	0.31[a]
	–	0.17[a]	0.45[b]	0.12[b]	0.26[b]
	–	0.10[c]	0.50[c]	0.08[c]	0.35[c]
80	–	0.11	0.38	0.15	0.34
	phosphatidylcholine	0.13	0.37	0.17	0.34
	butyrate	0.19	0.23	0.23	0.34
	oleate	0.22	0.24	0.24	0.30
	palmitate	0.23	0.27	0.22	0.28
80	–	0.18[b]	0.48[b]	0.12[b]	0.22[b]
	–	0.02[c]	0.42[c]	0.06[c]	0.50[c]
	–	0.07[d]	0.51[d]	0.11[d]	0.32[d]

[a] Taken from Chen et al. (1993). [b] Taken from Griffin et al. (1993). [c] Taken from Qi et al. (1997). [d] Calculated on the basis of the X-ray crystal structure analysis (Papiz et al., 1986).

estimated using data truncated at 190 nm (Chen et al., 1993) or 200 nm (Griffin et al., 1993). Adding PC, butyrate, or palmitate at 25 °C in the absence of added salt did not change the spectrum of β-LG (results are not shown). Only oleate produced a significant change in the CD spectrum (Figure 2). β-Lactoglobulin is known to bind palmitate at low concentrations (Spector and Fletcher, 1970; Frapin et al., 1993); however, the solubility of palmitate is substantially lower than that of oleate in water at 25 °C (McBain and Sierichs, 1948). Therefore, the absence of changes in the CD spectrum in the presence of palmitate may result from its very

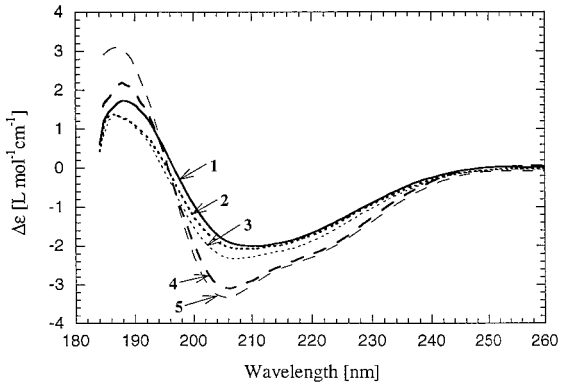

Figure 3. Circular dichroism spectra of 1 mg/mL β-lactoglobulin (pH 7) with no added salt at 80 °C in the presence of: no additives (1, solid line); 2 mg/mL phosphatidylcholine (2, thick dotted line); 2 mg/mL butyrate (3, thin dotted line); 2 mg/mL oleate (4, thick dashed line); 2 mg/mL palmitate (5, thin dashed line).

low availability. The estimated secondary structure contents suggested that adding oleate induced a significant increase in β-helix content and a decrease in β-sheet content (Table 1).

A similar trend in effects of PC/FAs on the spectrum at 25 °C was observed in the presence of 500 mM NaCl (Figure 2). Adding PC, butyrate, or palmitate did not change the spectrum of β-LG (data not shown). Adding oleate changed the shape of the spectrum less than in the absence of NaCl (Figure 2), most likely due to decreased solubility in the presence of added salt (McBain et al., 1943). Secondary structure analyses were not carried out for systems with 500 mM NaCl because the data were truncated at around 195 nm due to low transmittance.

The CD spectra at 80 °C reflect changes in the secondary structure in β-LG due to heat-induced partial unfolding (Figure 3). In the case of β-LG without PC/FAs, raising the temperature from 25 to 80 °C decreased the α-helix and β-sheet contents (Table 1). While earlier studies based on CD analyses indicated increases in the α-helix or β-sheet structure content at 80 °C (Griffin et al., 1993; Matsuura and Manning, 1994), a recent study showed that most of the α-helical structure and a slight amount of β-sheet structure were lost at around 60 °C (Qi et al., 1997), consistent with recent results of deuterium exchange NMR experiments (Belloque and Smith, 1998). The results of Qi et al. (1997) are based on CD spectra extending to 170 nm using a Synchrotron Radiation Source and the estimated secondary structure contents of native β-LG are close to those predicted on the basis of the crystal structure (Table 1). Spectra obtained here extended to 184 nm, so the results of secondary structure estimates were in line with those of Qi et al. (1997).

All of the FAs and PC induced changes in β-LG secondary structure at 80 °C (Figure 3), while transitions were only observed for oleate at 25 °C (Figure 2). The solubility of palmitate at 80 °C is sufficiently high (McBain and Sierichs, 1948) that it should not limit interactions with β-LG. All of the FAs and PC increased the α-helix content and decreased the amount of β-sheet (Table 1). Incerased chain length was correlated with larger secondary structure changes (palmitate ∼ oleate > butyrate), suggesting that FAs interact with β-LG mainly through hydrophobic interactions. β-LG is believed to have one hydrophobic binding site in the calyx

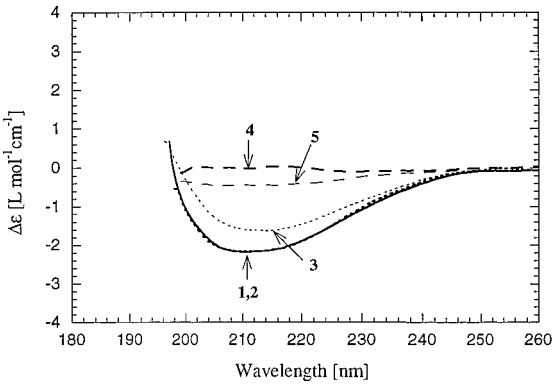

Figure 4. Circular dichroism spectra of 1 mg/mL β-lactoglobulin (pH 7) in 500 mM NaCl at 80 °C in the presence of: no additives (1, solid line); 2 mg/mL phosphatidylcholine (2, thick dotted line); 2 mg/mL butyrate (3, thin dotted line); 2 mg/mL oleate (4, thick dashed line); 2 mg/mL palmitate (5, thin dashed line).

formed by the β-barrel (Papiz et al., 1986) but is also capable of binding a large number of FAs weakly at the hydrophobic surface site in the region of the α-helix (residues 130−140) and β-strands A (16−27) and I (145−150) (Wang et al., 1998). Shimizu and Saito (1996) pointed out that residues 125−143 form an amphiphilic helix, and that was maintained even in surface-denatured β-LG produced at the interfaces between oil and aqueous phases formed by emulsification. Therefore, it is possible that binding of FAs and PC prevents heat-induced helix unfolding or even induces helix reformation from unfolded regions.

In 500 mM NaCl at 80 °C, PC did not change the β-LG spectrum down to 198 nm while the featureless shape of the spectrum suggests that FAs substantially destroy the secondary structure (Figure 4). Larger molecular weight FAs did so more effectively (palmitate ∼ oleate > butyrate), again suggesting that hydrophobic interactions occur between FAs and β-LG. Since both FAs and β-LG are negatively charged at pH 7, electrostatic repulsive forces between [β-LG·FA] complexes and unbound FAs increase with an increased number of bound FAs per protein molecule. Therefore, FA binding would be limited to a certain degree at lower ionic strength. In the presence of 500 mM NaCl, the electrostatic repulsive forces should be sufficiently shielded to almost completely unfold β-LG. The mechanism of interaction between PC and β-LG is less clear. Brown et al. (1983) reported that PC was capable of binding to β-LG at low ionic strength but did not bind at high ionic concentration. Furthermore, experimental and theoretical studies generally indicate that electrostatic interactions between lipids and proteins are required in the early stages of lipid binding to proteins (Pedersen et al., 1995; Ben-Tal et al., 1997; Chen et al., 1997; Terzi et al., 1997; Denisov et al., 1998). While PC is isoelectric (zwitterionic) at pH 7, the electropositive choline group, whose charge would be shielded in 500 mM NaCl, may play a critical role in binding to negatively charged β-LG. It is also possible that a protein- and lipid-rich phase were demixed by the poor solvent produced in the presence of both PC and NaCl (Ikeda and Foegeding, 1999b). The absence of changes in the CD spectrum can be explained if PC does not bind to β-LG in 500 mM NaCl.

Gelation. Our previous studies of the effects of lecithin (crude PC) on WPI gelation suggested that lecithin acts as a filler, interacting with the gel network

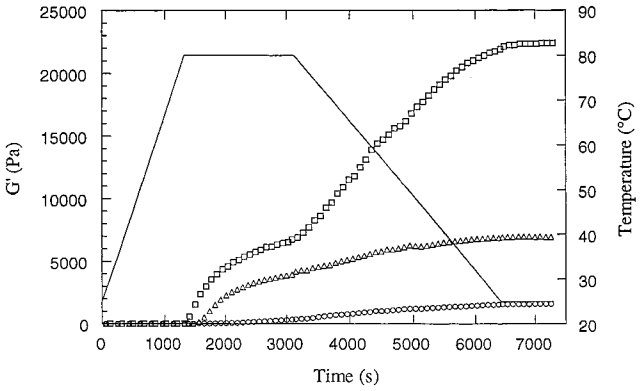

Figure 5. G' development in 12% w/v β-lactoglobulin (pH 7) with no added salt in the presence of: no additives (circle); 12% w/v phosphatidylcholine (triangle); 12% w/v butyrate (square). Solid line represents the temperature history.

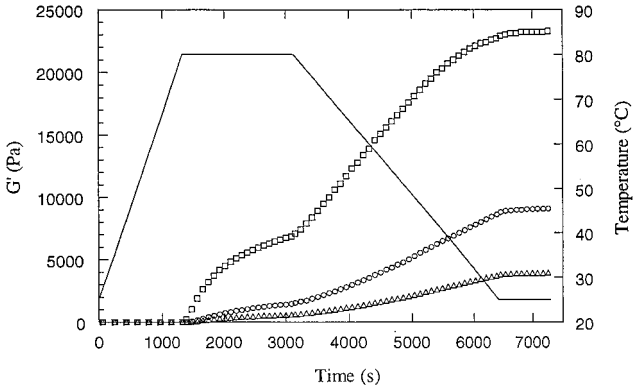

Figure 6. G' development in 12% w/v β-lactoglobulin (pH 7) in 500 mM NaCl in the presence of: no additives (circle); 12% w/v phosphatidylcholine (triangle); 12% w/v butyrate (square). Solid line represents the temperature history.

at low NaCl concentration, reinforcing the mechanical strength of the gels, while, at high NaCl concentration, lecithin interfered with the gel network formation (Ikeda and Foegeding, 1999a,b).

Adding oleate and palmitate induced gelation during hydration of β-LG at room temperature (data not shown). The gel state remained during heating to 80 °C (data not shown). Yuno-Ohta et al. (1998) investigated FA-induced gelation of ovalbumin and concluded that the FA-induced gelation is mainly driven by hydrophobic interactions since (1) the gelation rate appeared to decrease with decreasing temperature from 25 to 10 °C and (2) the gelation occurred even in the presence of *N*-ethylmaleimide, a reducing agent that breaks disulfide bonds. It is likely that adding oleate and palmitate induced hydrophobic interactions among β-LG molecules, leading to gelation even at room temperature. However, no changes in the secondary structure of β-LG were observed when palmitate was added at 25 °C (Figure 2). Therefore, changes in the secondary structure do not seem to be a requirement for gelation of β-LG. Effects of PC and butyrate on heat-induced gelation of β-LG are discussed in detail hereafter.

Figures 5 and 6 show examples of gelation curves obtained under various conditions. In all cases, G' values started to increase during the temperature holding processing at 80 °C. The time dependence of this process was used to predict the gelation time (t_g) (Table 2). A dramatic increase in G' occurred during cooling to 25 °C but G' values remained similar when samples were held at 25 °C. These results were similar to G' develop-

Table 2. Effects of Phosphatidylcholine and Butyrate on Rheological Properties of β-Lactoglobulin Gels[a]

NaCl (mM)	additives	G'_{80} (Pa)	G'_{25} (Pa)	$\Delta G'_{heat}/\Delta G'_{cool}$	t_g (s)[b]	n
0	−	320	1520	0.27	645	0.033
	phosphatidylcholine	3890	6910	1.29	270	0.020
	butyrate	6570	22400	0.42	45	<0.001
500	−	1440	8730	0.20	270	0.033
	phosphatidylcholine	620	4030	0.18	375	0.047
	butyrate	6770	23100	0.42	60	<0.001

[a] Average of replications. [b] Time after the temperature reached 80 °C.

ment patterns of WPI gelation obtained in our previous study (Ikeda and Foegeding, 1999b). Values of G' at the end of the holding at 80 °C (G'_{80}) and following the entire thermal treatment (G'_{25}) are summarized in Table 2. Adding PC increased G' when no salt was added and decreased G' in the presence of 500 mM NaCl. Butyrate substantially increased G' regardless of NaCl concentration.

The increase in G' during heating, $\Delta G'_{heat}$ ($=G'_{80}$), was attributed mainly to disulfide bond formation and hydrophobic interactions (Bowland et al., 1995). The increase in G' during cooling, $\Delta G'_{cool}$ ($=G'_{25} - G'_{80}$), was attributed to hydrogen bond formation (Bowland et al., 1995). Although the $\Delta G'_{cool}$ values are not considered to be independent of conversion of proteins during heat-induced gelation or on the structure of protein aggregates, and thus depend on $\Delta G'_{heat}$, the $\Delta G'_{heat}/\Delta G'_{cool}$ ratio reflects the relative contributions of those intermolecular interactions to the final G'. Adding PC in the absence of NaCl substantially increased $\Delta G'_{heat}/\Delta G'_{cool}$ (Table 2), the same effect produced when lecithin was added during WPI gelation in our previous study (Ikeda and Foegeding, 1999b). Therefore, both PC and lecithin appear to facilitate disulfide bonding and/or hydrophobic interactions between protein molecules during heating. The shorter gelation time observed in the presence of PC (Table 2) also suggests that PC accelerates protein aggregation during heating. At pH 7 and low ionic strength, electrostatic repulsive forces exist among negatively charged protein molecules. The probability of forming an aggregate on a collision between protein molecules is much less than unity (reaction limited aggregation) due to the high energy barrier for interaction produced by these repulsions (Hagiwara et al., 1996; Ikeda et al., 1999). Additives that interact with proteins would increase the overall probability of aggregate formation. PC interacts with β-LG at low ionic concentration (Figure 2), and electrostatic repulsive forces are essentially absent between PC and β-LG since PC is isoelectric across a wide pH range (Cornell and Patterson, 1989). Therefore, it is reasonable that adding PC accelerates aggregation by increasing the aggregation probability at low ionic strength. In addition, active fillers that interact with gel networks are known to increase the mechanical strengths of gels (van Vliet, 1988).

Negative effects of PC addition on rheological properties of gels were observed in the presence of 500 mM NaCl (Table 2). These results can be explained if PC does not bind to β-LG at 500 mM NaCl since inactive fillers are known to decrease gel moduli (van Vliet, 1988). At high ionic concentration, repulsive forces between whey proteins should not limit aggregation since the negative charges on β-LG molecules are shielded (diffusion-limited aggregation process) (Hagiwara et al., 1996; Ikeda et al., 1999). The presence of inactive fillers is expected to decrease the probability of collisions between proteins and thus delay the gel formation. Furthermore, the presence of phospholipid at high ionic concentrations might create a poorer solvent. If demixing takes place due to a decrease in solubility, the formation of a continuous protein network may be disrupted, leading to weaker gels.

Sodium chloride addition did not influence the effects of butyrate on the rheological properties of heat-induced β-LG gels (Table 2). Adding butyrate significantly increased G' and $\Delta G'_{heat}/\Delta G'_{cool}$ and decreased t_g at all NaCl concentrations tested, suggesting that unfolded β-LG in 500 mM NaCl (Figure 4) is capable of forming mechanically stronger gels. While adding 12% w/v sodium butyrate is associated with about 1 M sodium ions, adding sodium chloride further than 500 mM is known to reduce gel strength (Foegeding et al., 1995).

Frequency dependencies of G' are summarized in Table 2. The power law exponent n is regarded to be an indication of the viscoelastic nature of the gel: n is zero for purely elastic gels and increases with larger contributions by the viscous component. All n values are less than 0.05, consistent with our previous results (Ikeda and Foegeding, 1999b) and ensuring that all of the gels are predominantly elastic in nature. Adding PC in the absence of NaCl reduced the n values of the resulting gels, indicating the formation of more elastic networks. Meanwhile, gels with added PC at 500 mM NaCl were less elastic. Adding butyrate produced very elastic gels characterized by almost frequency independent G' values at all NaCl concentrations tested.

CONCLUSION

Interactions between β-LG and FAs or PC depended upon the NaCl concentration. Phosphatidylcholine in low ionic strength β-LG solutions prevented heat-induced loss of α-helix structure at 80 °C, accelerated heat-induced gelation, and reinforced the mechanical strength of gels. In the presence of 500 mM NaCl, PC did not change the CD spectrum of β-LG (extending to 198 nm) yet interfered with gel network formation. These results support our previous hypothesis that phospholipids act as an active filler at low ionic strength but do not interact with β-LG at higher ion concentrations. On the other hand, FAs induced substantial α-helix formation at low ionic strength and unfolding at higher ionic strength. Butyrate was less effective in inducing secondary structure changes than FAs with longer carbon chains, i.e., palmitate or oleate, suggesting that hydrophobic interactions between FAs and β-LG dominate. While denaturation is regarded as one of the prerequisites for β-LG gelation, a specific secondary structure is not critical since both the α-helix-induced β-LG obtained at low ionic strength (Table 1) and unfolded β-LG produced at higher ionic strength (Figure 4) were capable of forming gels with higher G' values than form in the absence of FAs. Additionally, palmitate-induced gels form at 25 °C without changing the secondary structure of β-LG. Correlations between structural alternations of protein and their gelation mechanisms will require further investigation, especially with attention to tertiary structural details. Effects of lipids on gelation are expected to depend not only on the type of lipids but also on the levels in the system. We are currently investigating effects of endogenous lipids in whey protein ingredients on gelation.

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Received for review April 28, 1999. Revised manuscript received December 23, 1999. Accepted December 30, 1999. Paper number FSR99-15 of the journal series of the Department of Food Science at North Carolina State University, Raleigh, NC 27695. This work was supported by a grant from the Southeast Dairy Foods Research Center and Dairy Management, Inc.

JF990434H