Structural Characteristics and Foaming Properties of β-Lactoglobulin: Effects of Shear Rate and Temperature

L. G. Phillips,*,† S. E. Hawks,† and J. B. German‡

Department of Food Science, Stocking Hall, Cornell University, Ithaca, New York 14853, and Department of Food Science and Technology, University of California, Davis, California 95616-8598

The main purpose of this study was to determine the extent to which the process of foaming changes the structure ob β-lactoglobulin (β-Lg). β-Lg unfolded while existing as a foam, but changes in conformation were reversible upon collapse of the foam. A plot of the overrun (a measure of air incorporation in the foam) for β-Lg foams (pH 7) versus temperature (3–45 °C) was a sigmoidal curve that resembled a two-state denaturation curve. Foaming properties were influenced by temperature and pH, which was attributed to differences in protein structure, in diffusion rate and in the number of molecules in solution for β-Lg at pH 9 as compared to pH 7. There was a strong correlation ($r > 0.9$) between the secondary structure of β-Lg in solution and the observed foaming properties. A direct link between the structure of β-Lg in solution and its foaming properties was established.

Keywords: *Foam; β-Lg structure; foaming properties; β-Lg functionality*

INTRODUCTION

Proteins comprise a major class of functional ingredients that are used in a variety of foods to enhance foaming, emulsifying, gelling, and water binding properties (Damodaran, 1989; Song and Damodaran, 1987; German and Phillips, 1989). Understanding the protein structure–function relationship responsible for these phenomena is a goal of many protein chemists (Li-Chan and Nakai, 1989). Proteins can stabilize foams which is an important attribute for a number of foods. Their capacity to form a foam reflects the amphipathic nature of proteins and their ability to form interfacial films (Graham and Phillips, 1976; German and Phillips, 1989; MacRitchie, 1986).

The extent of protein interaction with the air–water interface is dependent on the ability of the protein molecule to move to an interface, unfold at the interfaces and form viscous films (Graham and Phillips, 1976). The ability to unfold is influenced by all the forces that contribute to maintaining the structure of the protein, including electrostatic, hydrophobic, hydrogen bonding, and disulfide bonds. It has been proposed that molecular flexibility and hydrophobic forces are most responsible for proteins adsorbing at the interface, just as transferring hydrophobic amino acids from water into a more apolar environment is the predominant criterion responsible for initiating protein folding (Li-Chan and Nakai, 1989; Damodaran, 1989).

Adsorbing hydrophobic residues to the air–water interface should have a similar effect as burying them inside the apolar interior of the protein unless unfolding at the interface leads to a greater number of hydrophobic residues being exposed to the water (Damodaran, 1989; Phillips et al., 1991). In this regard, the shearing forces encountered during the whipping process may provide a means of altering protein flexibility by transferring proteins to an interface, and thus exposing the protein molecules to a large air–water surface area such that adsorption and unfolding can occur (Prins, 1988).

Damodaran (1989) has suggested that interfacial adsorption is not merely dependent on diffusing to the interface and providing sufficient concentrations for interfacial adsorption. He suggested that the interfacial energy must be large enough to overcome the activation energy for protein unfolding and penetration of hydrophobic residues into the air–water interface.

If interfacial film formation and ultimately foaming formation are predominantly dependent on the protein surface hydrophobicity and flexibility, then any changes that alter either of these factors should result in a change in the foaming properties of the system. The magnitude of hydrophobic interactions is temperature dependent (e.g., dissociation of oligomeric food proteins at low temperature), thus reducing the temperature should reduce the driving force for interfacial adsorption (Damodaran, 1989).

The new interfacial surface area available to the protein should be somewhat dependent on the shear rate that the protein solution is exposed to during foam formation (Phillips, 1992). A change in shear rate should alter the interfacial energy of the system and may ultimately unfold the protein further, thus increasing its flexibility.

A structurally well-defined protein such as β-lactoglobulin (β-Lg) is required to monitor the reversible and irreversible changes in protein structure that occur concomitant with changes in foaming properties. β-Lg is unique in that at pH 9 it exists as a flexible monomer with an exposed free thiol, whereas at pH 7 it is a more rigid dimer with its free thiol buried (Papiz et al., 1986; Kella and Kinsella, 1988a; Sawyer and Papiz, 1985; Tanford et al., 1959).

The objectives of this study were (1) to determine the extent to which the process of foaming changes the structure of β-Lg; (2) to ascertain the effect of shear rate, pH; and temperature during foam formation on the foaming properties of β-Lg; (3) to quantify the relationship between the structure of β-Lg in solution and its effect on the foaming properties; and (4) to ascertain if β-Lg unfolds when it exists as part of a foam.

† Cornell University.
‡ University of California, Davis.

0021-8561/95/1443-0613$09.00/0

MATERIALS AND METHODS

Protein. β-Lg was prepared from raw milk using the method of Ebler et al. (1990). The casein was removed from the milk at pH 4.6 by adding HCl (50% v/v). Trichloroacetic acid (TCA) (3 g/10 mL) was added slowly to the whey supernatant to precipitate all whey proteins except β-Lg. The supernatant was dialyzed until all salt (constant conductivity) and lactose (lactose/D-galactose kit, Boehringer-Mannheim, Mannheim, Germany) were removed from the β-Lg solution. The dialyzed β-Lg solution was freeze-dried, and the protein solutions (0.02% azide and 5 mg/mL β-Lg) were microfiltered through a 0.22 μm filter and then adjusted to the appropriate pH with dilute HCL or NaOH as needed. The protein concentration was determined by absorbance at 278 nm using an extinction coefficient for β-Lg of 0.96 mL/(mg × cm) (Kella and Kinsella, 1988b).

Overrun. Overrun was measured using the method of Phillips et al. (1990). The various β-Lg samples (pH 7 at 3, 10, 20, 25, 30, or 45 °C and pH 9 at 3 or 25 °C, 5 mg/mL, 75 mL total volume) were whipped at a speed of either 275 or 390 rpm using a Sunbeam Deluxe 235 W Mixmaster for 5 min intervals (Sunbeam Corp.,Oak Brook, IL). These speeds were selected because they corresponded to half speed and full speed respectively on the mixer. The mixer speed was calibrated using a strobemeter (Strobotac 1538-A, General Radio Co., Concord, MA). All foaming studies were done in a controlled environment room maintained at 85% relative humidity and the appropriate temperature.

Foam Stability. Foam stability was determined by the method of Phillips et al. (1990). The weight of the liquid separating from the foam was continuously recorded using a Sartorius balance (model 1212MP Brinkman Instruments Co., Westbury, NY) connected to an Apple IIe computer using an interface board (IMI, State College, PA). The time required for half the original weight of the foam after whipping to drain as liquid was reported as 50% drainage, an index of instability. The drained liquid was collected in a tared container on the balance pan.

Foam Collapse. β-Lg foams formed after whipping 5, 10, 15, or 20 min were collapsed by placing 1 g of foam in a centrifuge tube and then centrifuging at 5000*g* for 10 min.

Solubility. The solubility of β-Lg before and after foaming was measured according to the standard solubilization procedure (Morr et al., 1985). Solubility was measured as the percentage change in β-Lg concentration for the original solution versus the collapsed foam.

Electrophoresis. β-Lg samples were analyzed before and after whipping by the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) method of Fling and Gregerson (1986). Electrophoresis was done to establish the extent of covalent polymerization via free thiol–disulfide interchange brought about by foaming β-Lg solutions. A Hoefer SE200 miniature slab gel electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA) was used. The running gel was an 8–17% acrylamide gradient gel.

Aliquots of β-Lg before and after whipping were obtained (5 mg/mL) and prepared for SDS-PAGE. This included reacting the β-Lg samples with *N*-methylmaleidmide (NEM) solution to block any free thiols and prevent any polymerization artifacts from occurring during electrophoresis.

Turbidity. The extent of aggregation of the protein dispersions was determined by transmittance at 550 nm using a Spectronic 700 spectrophotometer (Bausch and Lomb, Rochester, NY). The values were expressed as T_{550}.

Surface Hydrophobicity (H_o). H_o was determined by the hydrophobic probe binding method of Creamer et al. (1982) using 8-anilino-1-naphthalenesulfonic acid, hemimagnesium salt (ANS) (Sigma, St. Louis, MO) as a probe. Samples of β-Lg whipped for 0, 5, 10, 15, and 20 min were used in the concentration range from 0.01 to 1.1 mg/mL. The H_o of β-Lg was calculated from the slope of the relative fluorescence (R) vs % (w/v) protein concentration (C_p). The relative fluorescence was defined by $R = (F - F_o)/F_o$ where F is the fluorescence reading of the protein–ANS conjugate and F_o is the fluorescence reading of the ANS solution of the same concentration without β-Lg. The H_o of β-Lg before and after whipping was reported as the change in H_o or ΔH_o.

Free Thiol Content. Free thiol content was measured in triplicate using 5′-dithiobis(2-nitrobenzoic acid) (DTNB) (Aldrich, Milwaukee, WI) (Kella and Kinsella, 1988b). One milliliter of β-Lg solution was transferred to a test tube, the exact volume of protein solution was determined by taring the weight of the test tube and weighing the protein solution. To β-Lg samples, 40 μL of solution (10 mg DTNB /mL in 0.5 M phosphate buffer, pH 7) was added to each tube and then 3.4 mL of buffer solution (9 M urea and 0.5 M Tris/HCl buffer, pH 8.15) was added. Blanks were prepared using water instead of protein solution. After 5 min, the absorbance at 412 nm was determined.

Circular Dichroism (CD). CD spectra were obtained using an Aviv model 61 DS CD spectrometer (Aviv Assoc. Inc., Lakewood, NJ) equipped with a temperature-controlled circulating water bath. Wavelength calibration was checked using benzene vapor at 266.71 nm. Wavelength amplitude was calibrated using (1*S*)-(+)-10-camphorsulfonic acid (CSA) (Sigma, St. Louis, MO). The ratio of CSA peaks at 290 and 192.5 nm was between 1.9 and 2.2. Xenon lamp energy and noise were checked prior to experiments. Conditions for analysis of protein solutions (0.65 mg of β-Lg /mL) were as follows: wavelength range, 180–240 nm; 1 nm wavelength stepsize; 0.4 s averaging time; 1.5 nm bandwidth; and 1 mm path length.

Each sample was scanned seven times and the resulting spectra were averaged and smoothed using a third-order polynomial operation (Aviv Software, Aviv Assoc. Inc.). The mean residue elipticity (Θ) was calculated using 162 as the number of amino acids in the sequence and a molecular weight for β-Lg of 18250 Da. The path length was 1 mm. The data were analyzed using a protein secondary structure program (PROSEC, Aviv Assoc. Inc.) that was modified according to Phillips (1992).

Gel Filtration Chromatography. A Sephacryl S-100 (Pharmacia, Uppsala, Sweden) gel filtration column was used to measure the molecular weight of β-Lg. The column buffer was 50 mM phosphate buffer (pH 7) with 100 mM KCl and 0.02% sodium azide. Glycerol (5% w/w) was added to all samples prior to loading on the column. The molecular weight standards were: β-lactoglobulin, α-lactalbumin, cytochrome *c*, and bovine serum albumin (Sigma). Blue dextran (Sigma) was used to determine the void volume, V_o. The column was run with 62 cm pressure and a flow rate of 12 mL/h. The bed volume was 124.66 mL (column height, 63 cm; column diameter, 1.6 cm). A standard curve was

Table 1. Effects of Shear Rate and Temperature on the Foaming Properties and Structure of β-Lactoglobulin Solutions (0.5% Concentration)

shear rate (rpm)	temp (°C)	pH	overrun (%) 5 min whip	10 min whip	15 min whip	20 min whip	foam stability (min)	solubility (%) after whipping	turbidity ($\%T_{550}$) after whipping	free thiol content (no. molecule) after whipping	SDS-PAGE no. bands after whipping	ΔH_o (%)
275	3	7	384	368	391	406	8.1	98	97.0	1.06	1	9.5
275	25	7	498	525	535	547	8.8	100	97.2	0.95	1	3.4
390	3	7	207	213	196	185	1.2	100	97.3	0.96	1	4.8
390	25	7	441	475	498	525	7.2	100	96.3	0.97	1	28.6
275	3	9	528	559	581	584	26.0	100	97.5	0.95	1	8.1
275	25	9	503	553	575	611	12.0	100	97.4	1.08	1	5.5
390	3	9	582	561	527	506	21.5	99	96.0	1.01	1	4.6
390	25	9	748	746	724	728	11.8	100	96.8	0.96	1	-5.5
95% confidence interval			45	45	45	45	45	1.2	2.4	0.11		

developed by plotting log molecular weight versus elution volume (V_e)/void volume (V_o).

Enzyme Hydrolysis. The extent of unfolding of β-Lg solutions and foams was assessed by measuring the susceptibility to Pronase digestion. β-Lg solutions (pH 7.0, 0.1 M phosphate buffer, or pH 7.0 no buffer) and foams collected after whipping for 5 or 20 min were hydrolyzed with Pronase. Pronase digests protein to free amino acids without significant decomposition of the Pronase (Calbiochem, San Diego, CA).

Proteolysis was initiated by adding Pronase solution (2 mg/mL) to the foam and a control, β-Lg solution (5 mg/mL), until a final concentration of 0.014 mg of Pronase/mL was obtained. The volume of foam was determined using the following equation: foam volume = initial volume whipped (75 mL) × (% overrun/100). The samples were incubated at 25 °C and 85% relative humidity. The extent of proteolysis was followed by a TCA precipitation method (Phillips, 1992). In a centrifuge tube, 1 g aliquots of the enzyme–foam mixture were mixed with an equal volume of 20% TCA solution. The samples were centrifuged for 15 min at 16000*g* and the absorbance of the supernatant at 280 nm was measured. Samples were taken at several time intervals throughout the proteolysis. The change in absorbance with time ($\Delta A_{280}/\Delta$min) was determined.

Statistics. Data were analyzed using the statistical analysis package SAS (SAS, 1985). The mean square error term from the analysis of variance was used to calculate 95% confidence intervals. The Pearson correlation coefficient (r) was recorded as a measure of linearity.

RESULTS AND DISCUSSION

Conformational Changes Caused by Foaming. *Protein Aggregation.* The changes in the structure of β-Lg brought about by foam formation were investigated. The solubility and turbidity of the different β-Lg solutions were reported as a measure of the effects of the pH, temperature, and shear rate during foaming on the extent of protein aggregation. Prior to foaming, the solubility ranged from 98% to 100% and the turbidity of the protein solutions ranged from 98% to 99% transmittance at 550 nm ($\%T_{550}$). Following foam formation and collapse at a variety of pHs, shear rates, and temperatures, the solubility ranged from 98% to 100% and the turbidity ranged from 96.3 to 97.0 $\%T_{550}$ (Table 1). Hence, there were no significant changes ($p > 0.05$) in solubility or turbidity measured after foam formation and collapse (Table 1). These results established that there was not any lasting change in solubility or visible aggregation occurring after foam formation and subsequent collapse.

Further studies were initiated to detect structural changes in the β-Lg molecule caused by the foaming process. The free thiol present in β-Lg can react with an adjacent free thiol via oxidation to produce a covalent bond between two monomers or it can react with a disulfide bond to form interchange products (Kella et al., 1989). The free thiol content of β-Lg before and after foam formation was measured to determine if any oxidation had occurred. Moreover, SDS-PAGE was done to determine the extent of polymerization induced by foaming especially that originating from free thiol oxidation or free thiol–disulfide interchange.

The free thiol content of β-Lg before foaming was not significantly different ($p > 0.05$) from the free thiol content after foaming the protein solution for 20 min, i.e. the free thiol content after foaming was roughly one per β-Lg monomer (Table 1). Tested under conditions where any disulfide bonds formed during foaming would be intact during electrophoresis, only one band was present on the gel that corresponded to β-Lg monomer (Table 1). The SDS-PAGE results confirmed that no polymerization was induced by foam formation or collapse. Therefore, no noticeable free thiol–disulfide interchange or free thiol oxidation was induced by foaming β-Lg solutions under the conditions tested.

Surface Hydrophobicity. Further studies were done to determine if foaming caused any significant changes in β-Lg structure. The change in surface hydrophobicity (ΔH_o) caused by foaming was measured to determine the extent of unfolding and exposure of hydrophobic groups. The ΔH_o after whipping β-Lg solutions at pH 7 using shear rate of 275 and 390 rpm was 3.4% and 28.6%, respectively (Table 1). Furthermore, whipping the pH 9 solution at 275 rpm resulted in a ΔH_o of 5.5% (Table 1) whereas whipping at the higher speed, 390 rpm, reduced H_o to −5.5%.

For the pH 7, 3 °C samples, ΔH_o was higher at 275 rpm than at 390 rpm (Table 1). These results parallel the higher overrun and foam stability at 275 rpm for the pH 7, 3 °C β-Lg solutions (Table 1). For the pH 9 samples, ΔH_o was higher at 3 °C than at 25 °C. The pH 9, 3 °C samples had positive ΔH_o values (Table 1). This coincided with the higher foam stability values at pH 9 for 3 °C versus 25 °C (Table 1). The ΔH_o results suggested that subtle changes in protein structure were induced by foaming.

Secondary Structure. Circular dichroism (CD) was used to measure the amount of change in secondary structure caused by foaming. For β-Lg foams whipped at pH 7, 25 °C, an increase in α-helix, a decrease in β-sheet and an increase in random coil were observed

Table 2. Effects of Shear Rate and Temperature on the Secondary Structure of β-Lactoglobulin[a]

shear rate (rpm)	temp (°C)	pH	α-helix (%)	β-sheet (%)	β-turn (%)	random coil (%)
0	25	7	12	51	11	26
275	25	7	15	44	14	28
390	25	7	16	36	17	31
0	3	7	9	63	7	21
275	3	7	13	53	11	24
390	3	7	13	50	13	25
0	25	9	13	42	12	32
275	25	9	16	38	14	33
390	25	9	17	34	15	34
0	3	9	12	44	11	32
275	3	9	13	46	12	28
390	3	9	11	52	7	29

[a] Samples were whipped for 20 min.

with increasing shear rate for the protein solutions collected from the foams (Table 2). These findings corresponded to the ΔH_o results and signify that slight conformational changes remained after the foams were collapsed for analysis (pH 7, 25 °C).

A slight increase in α-helix, a decrease in β-sheet, and an increase in random coil content was observed at pH 9 as compared to pH 7 prior to foaming (Table 2). The differences corresponded to the conformational differences observed for the β-Lg monomer (pH 9) as compared to the β-Lg dimer (pH 7) (Papiz et al., 1986; Phillips, 1992; Sawyer and Papiz, 1985).

The secondary structure of the β-Lg solutions (pH 9, 25 °C) following foaming increased in α-helix, decreased in β-sheet, and increased in random coil content (Table 2). The same trends in secondary structure were observed for foams whipped at pH 7 (Table 2). The α-helix and random coil content increased while the β-sheet decreased for samples whipped at pH 7, 3 °C (Table 2). The secondary structure trends for whipping at pH 7 and 3 °C or 25 °C were similar (Table 2). In general, slight changes in secondary structure were observed after foaming.

Structural Integrity of the Dimer Region. β-Lg exists as a dimer in solution at pH 7. The secondary structure of β-Lg must be intact for the β-Lg subunits to associate as a dimer (Tanford et al., 1959). Gel filtration chromatography was done at pH 7 after foaming the samples at either pH 7 or pH 9 to determine if the conformation of β-Lg was affected by foaming to an extent that the dimer could not form (Table 3).

From elution volumes (V_e) for the molecular weight standards, the following equation of a line ($r^2 = 0.999$) was developed: log(molecular weight) = $[-1.459 \times V_e/V_o] + 6.439$. The gel filtration analysis was carried out at 3 °C. The β-Lg standard eluted as a dimer under these conditions, which provided further evidence that electrostatic interactions play an important role toward the stabilization of β-Lg dimers at low temperatures where hydrophobic interactions are reduced (Table 3).

Foam samples whipped at 275 or 390 rpm and at pH 7 or 9 were collapsed and adjusted to pH 7 with buffer. All eluted as dimers with apparent molecular weights of approximately 37000 when placed in pH 7 buffer and passed through the column (Table 3). The results as established by ΔH_o, CD, and gel filtration studies demonstrated that although slight changes in secondary structure were observed following foaming, these changes were reversible. The protein refolded to an extent that the native dimer could form at pH 7 regardless of the pH at which it was whipped.

Table 3. Effects of Shear Rate and Temperature on the Elution of β-Lactoglobulin Using a 100 000 Molecular Weight Cutoff, Sephacryl Gel Filtration Column[a]

shear rate (rpm)	pH during foaming	temp (°C)	V_e (mL)	V_e/V_o	apparent molecular weight (Da)
0	7	25	172.0	1.28	37 300
275	7	25	171.0	1.27	38 600
390	7	25	171.0	1.27	38 600
0	7	3	171.8	1.28	37 300
275	7	3	171.0	1.27	38 600
390	7	3	171.8	1.28	37 300
0	9	25	172.0	1.28	37 300
275	9	25	170.8	1.28	37 300
390	9	25	173.5	1.29	36 000
275	9	3	170.1	1.26	39 900
390	9	3	170.5	1.27	38 600
blue dextran			134.6	1.00	

[a] All samples were adjusted to pH 7 prior to loading. All samples were eluted in 50 mM phosphate buffer (pH 7) and 100 mM KCl. Samples were whipped for 20 min.

Slight alterations in the CD spectra and ΔH_o of β-Lg were observed immediately after the foams made with the various β-Lg solutions were collapsed, however foaming did not cause any irreversible changes in the conformation of β-Lg. This was established because the observed structural changes did not diminish the typical association behavior of β-Lg in solution. β-Lg had enough structural integrity to associate as a dimer in solution at pH 7 following foaming (Table 3). This, along with the complete lack of free thiol oxidation or free thiol–disulfide interchange products, was considered ample evidence that the unfolding occurring during foam formation was for the most part reversible.

Foaming Properties. *Shear Rate and Temperature.* The parameters, pH, temperature during foam formation, and shear rate, had a substantial influence on the foaming properties of β-Lg. The largest overrun values (748%) were obtained at pH 9 and 25 °C when the foams were made using the faster shear rate (390 rpm) (Table 1). The magnitude of these overrun values was quite remarkable when one considers that only 0.5% protein was used for these experiments. By contrast, the lowest foam stability (1.2 min) and overrun values (185%) were obtained at pH 7 and 3 °C when whipping at the faster shear rate (390 rpm) (Table 1). Furthermore, the highest foam stability values (26 min) were measured at pH 9 after whipping at the slower shear rate (275 rpm) and 3 °C (Table 1). Therefore, the foaming properties of β-Lg were affected by pH, temperature, and shear rate collectively (Table 1). The results suggested that two major factors were influencing the observed foaming properties that were hydrophobic and hydrodynamic in nature. The protein–protein hydrophobic interactions were reduced as the temperature was decreased (O'Keefe et al., 1991) from 3 to 25 °C. Hence, this caused a reduction in overrun and foam stability. The hydrodynamic factor was the effect of temperature and pH on the diffusion rate of β-Lg (Cantor and Schimmel, 1980). The diffusion rate of a protein is typically decreased as the temperature is reduced (Cantor and Schimmel, 1980). This would have an adverse effect on the overrun and foam stability if a high enough shear rate were used during foam formation because the new surfaces would be formed too quickly. There would not be sufficient time for the protein to diffuse to the new surface and stabilize it (Graham and Phillips, 1976; Phillips, 1981).

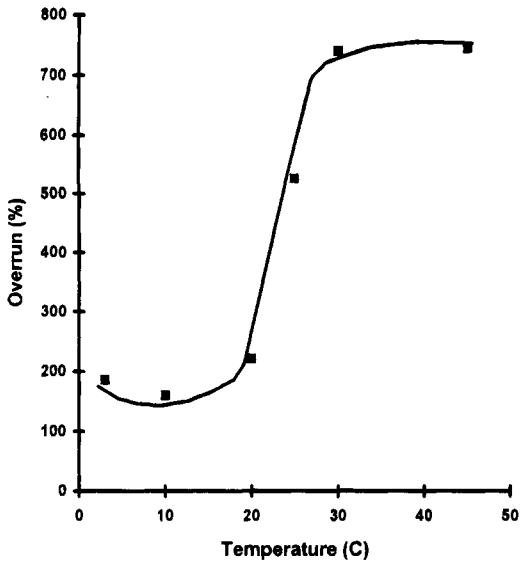

Figure 1. Effects of temperature on the overrun of β-lactoglobulin solutions (0.5% concentration, pH 7) after whipping at 390 rpm for 20 min (with 95% confidence intervals).

An example of reduced hydrophobic interaction coinciding with a reduced diffusion rate was the overrun and foam stability values observed with β-Lg at pH 7 and 3 °C (Table 1). The foams formed at 275 rpm had foam stability and overrun values that were slightly lower at 3 °C as compared to 25 °C but the values obtained at 3 °C and the faster shear rate (390 rpm) were extremely low (Table 1). The foams formed when whipping at 390 rpm were being destroyed by the foaming process because the protein did not have sufficient time to diffuse to the interface and did not have sufficient hydrophobic interactions to form a cohesive film.

A different situation was observed at pH 9, i.e. whipping at 390 rpm and 3 °C did not destroy the foams and the foam stability was higher at 3 °C as compared to 25 °C (Table 1). These results suggested that the dissociation of the dimer to a monomer and the subsequent unfolding of β-Lg at pH 9 resulted in improved foaming properties that were not adversely influenced by reduced temperature. The dissociation of the dimer at pH 9 would have doubled the number of molecules in solution that would roughly double the diffusion rate (Cantor and Schimmel, 1980). Hence, the higher diffusion rate, increased number of molecules in solution, and more unfolded β-Lg molecules at pH 9 resulted in a higher overrun (Table 1). Once the foam was formed, the higher viscosity of water at the lower temperature may have stabilized the foams against collapse (Cantor and Schimmel, 1980).

Temperature. Overrun measurements for β-Lg solutions (pH 7) at 390 rpm and 3, 10, 20, 25, 30, and 45 °C were obtained to study further the effect of temperature on the foaming properties of β-Lg (Figure 1). The plot of overrun after 20 min whipping versus temperature was a sigmoidal curve, which resembled a two-state denaturation curve (Cantor and Schimmel, 1980) (Figure 1). Overrun was much higher when the temperature was increased above 20 °C with overrun reaching a maximum of approximately 740% between 30 °C and 45 °C (Figure 1).

The foaming properties of β-Lg were influenced by temperature (Figure 1). The plot of the overrun for β-Lg solutions (pH 7) whipped at 390 rpm for 20 min versus temperature (3–45 °C) was a sigmoidal curve that resembled a two state denaturation curve (Cantor and Schimmel, 1980). The temperatures used for the foaming studies were not high enough to alter the conformation of β-Lg in solution significantly (Kella and Kinsella, 1988a). The temperature required to unfold 50% of the β-Lg molecules in solution at pH 7 is 69.2 °C according to Kella and Kinsella (1988a). This is a higher temperature than the temperature range (3–45 °C) used for the foaming experiments (Figure 1). The influence of temperature on the foaming properties of β-Lg suggested that the disrupting forces imposed on the protein during the foaming process reduced the temperature necessary for 50% unfolding to roughly 22 °C (Figure 1). This was concluded from the substantial rate of increase in overrun observed for the β-Lg solutions (pH 7) between 20 and 30 °C (Figure 1). Kella and co-workers (1989) have demonstrated that the foaming properties of β-Lg and other whey proteins are dependent on the extent of protein unfolding; i.e., increased unfolding resulted in large increases in the observed overrun, which would be consistent with the current findings.

Table 4. Correlation of Foaming Data for β-Lactoglobulin with the Corresponding Structural Data

structural parameters[a]	overrun (%) 5 min whip	10 min whip	15 min whip	20 min whip	foam stability (min)
	Shear Rate = 275 rpm[c]				
H_o[b]	0.168	0.084	0.126	0.029	0.876
α-helix	0.922	0.965	0.961	0.986	0.349
β-sheet	−0.932	−0.963	−0.977	−0.989	−0.580
β-turn	0.941	0.976	0.970	0.986	0.374
random coil	0.897	0.921	0.946	0.950	0.686
	Shear Rate = 390 rpm[d]				
H_o[b]	0.017	−0.069	−0.156	−0.234	0.657
α-helix	0.938	0.960	0.979	0.982	0.653
β-sheet	−0.973	−0.966	−0.952	−0.928	−0.823
β-turn	0.921	0.943	0.965	0.969	0.672
random coil	0.954	0.930	0.898	0.860	0.885

[a] Structural parameters for β-Lg were measured in solution before foaming. [b] H_o, surface hydrophobicity before foaming the protein sample. [c] 275 rpm equals half speed for the mixer. [d] 390 rpm equals full speed for the mixer.

Correlations between Structure and Foaming Properties. The correlations between the foaming data under the various conditions tested and the corresponding conformational data were calculated (Table 4). The correlation between the surface hydrophobicity and the overrun values obtained at 275 rpm was not very strong (Table 4). The foam stability for the β-Lg solutions whipped at 275 rpm did correlate well with the surface hydrophobicity of the corresponding β-Lg solutions prior to foaming ($r = 0.876$). However, the highest correlation coefficients were obtained when comparing the overrun values obtained at 275 rpm with the corresponding fractions of secondary structure for β-Lg in solution prior to foaming (Table 2). The r values ranged from 0.897 to −0.989 (Table 4).

Similar results were obtained when comparing the foaming data obtained for β-Lg solutions whipped at 390 rpm with the corresponding structural data. Surface hydrophobicity correlated best with foam stability ($r = 0.657$) (Table 4). The strongest correlations were between the overrun data and the corresponding fractions of secondary structure for β-Lg, i.e., r values ranged from 0.860 to 0.982 (Table 4).

The relationship between the fractions of secondary structure and the overrun obtained after whipping for 20 min at 275 rpm was investigated further (Figure 2).

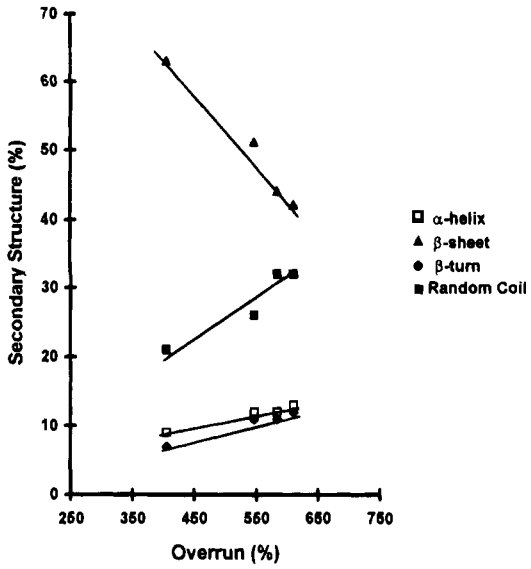

Figure 2. Relationship between the fractions of secondary structure for β-lactoglobulin prior to whipping and the observed overrun values obtained at 275 rpm.

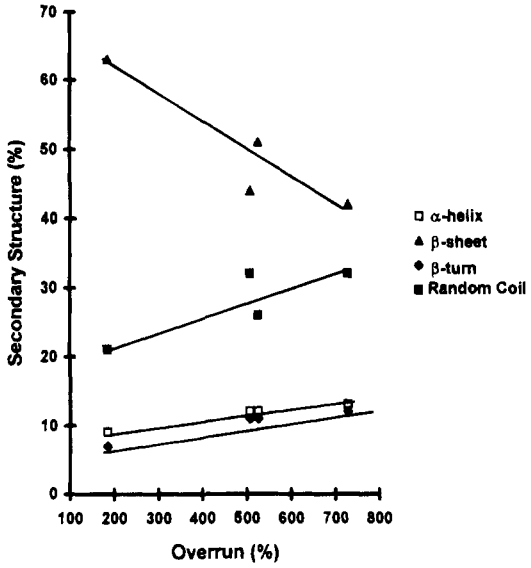

Figure 3. Relationship between the fractions of secondary structure for β-lactoglobulin prior to whipping and the observed overrun values obtained at 390 rpm.

The overrun values increased as the β-sheet content in solution was reduced. Furthermore, the overrun for β-Lg solutions formed at 390 rpm followed the same trends, i.e. an increase in random coil, α-helix, and β-turn content corresponded to higher overrun values (Figure 3).

The high correlations between the structure of β-Lg in solution prior to foaming and the observed foaming properties demonstrated that ultimately the foaming properties of β-Lg were directly influenced by the structural perturbations of temperature and pH (Table 4). The overrun values increased as the β-sheet and the random coil content in solution were reduced and increased, respectively (Figures 2 and 3). The results demonstrated a direct link between the structure of β-Lg in solution prior to whipping and the observed overrun values.

Extent of Protein Unfolding in a Foam. Very little, if any, oxidation, free thiol–disulfide interchange, aggregation, or denaturation was observed for the β-Lg solutions whipped under various conditions of shear, pH, and temperature (Tables 1–3). This study did not address the possibility for reversible unfolding of β-Lg during foaming. Enzyme hydrolysis was done to determine the accessibility to proteolytic action of the β-Lg molecules while in a foamed state.

Table 5. Effects of Shear Rate on the Hydrolysis of β-Lactoglobulin (pH 7) Whipped in Buffer or without Buffer

shear rate (rpm)	buffer added	whip time (min)	extent of hydrolysis (ΔA_{280}/min)	overrun (%)
0	yes	0	1.0	
275	yes	5	4.8	534
275	yes	20	7.3	636
390	yes	5	14.6	763
390	yes	20	14.8	755
0	no	0	2.5	
275	no	5	5.7	498
275	no	20	8.6	547
390	no	5	11.4	441
390	no	20	9.1	525

The β-Lg solutions were either dissolved in a 0.1 M phosphate buffer (pH 7) or in water adjusted to pH 7. This allowed comparisons to be made on the effect of the added phosphate salts on foaming as well as the performance of the enzyme. The β-Lg solutions with buffer had a higher overrun than the samples whipped without buffer (Table 5). The maximum overrun was 763% for β-Lg solutions whipped for 5 min at 390 rpm with buffer and 547% for samples (pH 7) whipped for 20 min at 275 rpm without buffer (Table 5).

The rate of β-Lg hydrolysis by Pronase increased by as much as 15-fold following foaming (Table 5). These results were comparable to those of Shimizu et al. (1985), who observed an increase in the rate of hydrolysis of whey protein adsorbed to an oil–water interface. The rate of hydrolysis was higher for the samples whipped at 390 rpm by as much as 3-fold as compared to the samples whipped at 275 rpm (Table 5). This was the case regardless of whether or not buffer was present (Table 5). Samples whipped at the same shear rate had similar rates of hydrolysis regardless of whether buffer was present or not (Table 5). β-Lg did unfold while existing as a foam because the results for enzyme hydrolysis studies demonstrated that the β-Lg molecule was more accessible to proteolytic action when it was in the foamed state. Thus, whipping the β-Lg solutions resulted in unfolding but adding phosphate buffer resulted in a higher overrun, i.e. enhancing film formation and air entrapment (Table 5).

CONCLUSIONS

In conclusion, the new and significant findings of this study are that although β-Lg did unfold substantially during foaming, this denaturation was reversible. Both hydrophobic and hydrodynamic effects determined the magnitude of foaming observed for β-Lg under the conditions tested. Furthermore, a link between the structure of β-Lg in solution and the subsequent foaming properties observed was established.

LITERATURE CITED

Cantor, C.; Schimmel, P. Biophysical Chemistry, Part III. *The Behavior of Biological Macromolecules*; Freeman and Company: New York, 1980.

Creamer, K., Zoerb, H., Olson, N. and Richardson, T. Surface hydrophobicity of α_{s1}-I, α_{s2}-Casein A and B and its implication in cheese structure. *J. Dairy Sci.* **1982**, *65*, 902.

Damodaran, S. Interrelationship of molecular and functional properties of food proteins. In *Food Proteins: Structure and Functional Relationships*; Kinsella, J. E., Soucie, W., Eds.; Am. Oil Chem. Soc.: Champaign, IL, 1989. pp 21–51.

Ebler, S.; Phillips, L. G.; Kinsella, J. E. Purification of β-lactoglobulin: Isolation of genetic variants and influence of purification method on secondary structure. *Milchwissenschaft* **1990**, *45*, 694.

Fling, S.; Gregerson, D. Peptide and protein molecular weight determination by electrophoresis using a high-molarity Tris buffer system without urea. *Anal. Biochem.* **1986**, *155*, 83.

German, J. B.; Phillips, L. G. Structures of proteins important in foaming. In *Food Proteins: Structure and Functional Relationships*; Kinsella, J. E., Soucie, W., Eds.; Am. Oil Chem. Soc.: Champaign, IL, 1989.

Graham, D.; Phillips, M. The conformation of proteins at interfaces and their role in stabilizing emulsions. In *Theory and Practice of Emulsion Technology*; Smith, A., Ed.; Academic Press: New York, 1976; Chapter 5, pp 75-78.

Kella, N. K.; Kinsella, J. E. Enhanced thermodynamic stability of β-lactoglobulin at low pH: A possible mechanism. *Biochem. J.* **1988a**, *255*, 113.

Kella, N. K.; Kinsella, J. E. Structural Stability of β-Lactoglobulin in the Presence of Kosmotropic Salts. *Int. J. Pept. Protein Res.* **1988b**, *32*, 396.

Kella, N. K.; Yang, T.; Kinsella, J. E. Effects of disulfide bond cleavage on structural and interfacial properties of whey proteins. *J. Agric. Food Chem.* **1989**, *37*, 1203.

Li-Chan, E.; Nakai, S. Effects of molecular changes (SH groups and hydrophobicity) of food proteins on their functionality. In *Food Proteins: Structure and Functional Relationships*; Kinsella, J. E., Soucie, W., Eds.; Am. Oil Chem. Soc.: Champaign, IL, 1989; pp 232–251.

MacRitchie, F. Spread monolayers of proteins. *Adv. Colloid Interface Sci.* **1986**, *25*, 341.

Morr, C.; German, B.; Kinsella, J. E.; Regenstein, J.; Van Buren, J.; Kilara, A.; Lewis, B.; Mangino, M. A collaborative study to develop a standardized food protein solubility procedure. *J. Food Sci.* **1985**, *50*, 1715.

O'Keefe, S. F.; Resurreccion, A. P.; Wilson, L. A.; Murphy, P. A. Temperature effect on binding of volatile flavor compounds to soy protein in aqueous model systems. *J. Food Sci.* **1991**, *56*, 802.

Papiz, M. Z.; Sawyer, L.; Eliopoulos, E. E.; North, A. C.; Findlay, J. B.; Sivaprasadarao, R.; Jones, T. A.; Newcomer, M. E.; Kraulis, P. J. The Structure of β-lactoglobulin and Its Similarity to Plasma Retinol-Binding Protein. *Nature* **1986**, *324*, 383.

Phillips, L. G. The relationship between the structure, interfacial and foaming properties of β-Lactoglobulin. Dissertation, Cornell University, 1992.

Phillips, M. Protein conformation at liquid interfaces and its role in stabilizing emulsions and foams. *Food Technol.* **1981**, *35*, 50.

Phillips, L. G.; German, J. B.; O'Neill, T. E.; Foegeding, E. A.; Harwalkar, V. R.; Kilara, A.; Lewis, B. A.; Mangino, M. E.; Morr, C. V.; Regenstein, J. M.; Smith, D. M.; Kinsella. J. E. Development of a standardized method for the measurement of the foaming properties of food proteins: A collaborative study. *J. Food Sci.* **1990**, *55*, 1441.

Phillips, L. G.; Yang, S. T.; Kinsella, J. E. Effects of Neutral Salts on the stability of whey protein isolate foams. *J. Food Sci.* **1991**, *55*, 588.

Prins, A. Principles of foam stability. In *Advances in Food Emulsions and Foams*; Dickinson, E., Stainsby, G., Eds.; Elsevier Applied Science: New York, 1988.

SAS. *SAS Users Guide*; SAS Institute: Cary, NC, 1985.

Sawyer, L.; Papiz, M. Structure and Function of Bovine β-Lactoglobulin. *Biochem. Soc. Trans.* **1985**, *13*, 265.

Shimizu, M.; Saito, M.; Yamauchi, K. Hydrophobicity and emulsfying activity of milk proteins. *Agric. Biol. Chem.* **1986**, *50*, 791.

Song, K.; Damodaran, S. Structure-function relationship of proteins: adsorption of structural intermediates of bovine serum albumin at the air–water interface. *J. Agric. Food Chem.* **1987**, *35*, 236.

Tanford, C.; Bunville, L. G.; Yasuhiko, N. The reversible transformation of β-Lactoglobulin at pH 7.5. *J. Am. Chem. Soc.* **1959**, *81*, 4032.

Received for review August 8, 1994. Revised manuscript received November 15, 1994. Accepted November 30, 1994.[⊗]

JF940453L

[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1995.