Effect of Contaminant on Adsorption of Whey Proteins at the Air−Water Interface

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The dynamics of adsorption of commercial samples of α-lactalbumin and β-lactoglobulin was investigated through the measurement of dynamic surface pressure and surface concentration via a radiotracer method. An unusual two-step adsorption behavior was observed for both proteins at low concentration which was believed to be due to the presence of a surface active contaminant. This hypothesis was tested by comparing the native whey protein samples with those purified with charcoal extraction. Analysis of the charcoal extract by thin-layer chromatography revealed the presence of contaminating free fatty acid and triglycerides in the commercial samples. β-Lactoglobulin was found to contain a higher concentration of these contaminants than α-lactalbumin. Extraction by charcoal was found not to modify the protein structure with respect to their near- and far-UV circular dichroism spectra. The presence of contaminant resulted in a higher steady-state surface pressure for β-lactoglobulin, but the protein was not displaced from the interface. On the other hand, the presence of a low concentration of surfactant was sufficient to decrease the steady-state surface concentration of α-lactalbumin due to the displacement of the protein from the interface by the lipids.

Keywords: *β-Lactoglobulin; α-lactalbumin; lipid contaminant; adsorption; air−water interface*

INTRODUCTION

The amphipathic nature of proteins, resulting from the presence of both polar and nonpolar amino acids, causes them to be preferentially adsorbed at various interfaces such as gas−liquid, liquid−liquid, and solid−liquid interfaces. The surface activity of proteins is of importance in a wide range of food and biologically based consumer products and is exploited in numerous industrial applications. In the food industry, proteins are used to stabilize foams and emulsions with prolonged shelf life (Halling, 1981; Dickinson and Stainsby, 1982; Dickinson, 1989; Dickinson and McClements, 1995). Knowledge of their kinetics of adsorption is important for (i) proper understanding of their role in stabilizing foams and emulsions and (ii) for developing new stabilizers. Protein adsorption is believed to occur in three steps (Van Aken and Merks, 1996). A protein molecule first diffuses from the bulk to the subphase just below the interface. After overcoming electrostastic and surface pressure energy barriers, the protein molecule adsorbs at the interface. Upon adsorption, the protein molecule will partially unfold at the interface (Graham and Philips, 1979; Maramatsu and Saboka, 1963) and form a condensed viscoelastic film of immobile, highly self-interacting molecules, which can stretch and deform, resisting disturbance and reducing film rupture (Clark et al., 1995). In systems containing both proteins and surfactants, competitive adsorption between the two classes of molecules for the interface depends on several factors such as their relative concentrations and surface activities and the nature and the strength of the protein−surfactant interactions (Dickinson and Woskett, 1989; Walstra and De Roos, 1993; Murray and Dickinson, 1996). The interactions between an ionic surfactant with gelatin have been found to produce a thicker and stronger adsorbed layer thus enhancing the stability of the system (Wüstneck and Müller, 1986). However, numerous work has shown that the presence of a competing low-molecular-weight surfactant in a protein system alters the functional properties of the solution and has a detrimental effect on the stability of foams and emulsions (De Feijter et al., 1987; Chen et al., 1993; Courthaudon et al., 1991a−d; Dickinson and Tanai, 1992; Dickinson et al., 1989; Coke et al., 1990; Clark et al., 1991, 1992, 1993, 1994, 1995; Cornec et al., 1996, 1998; Sarker et al., 1995). This is because surfactants stabilize foams and emulsions by forming a very fluid adsorbed layer. These molecules can migrate rapidly to reduce any interfacial tension gradient, which may arise from local perturbations of the interface. This migration drags the interlamellar liquid into the affected area and contributes to the restoration of the equilibrium film thickness resulting in the Marangoni effect (Ewers and Sutherland, 1952; Walstra, 1989). In case of proteins, because of their much smaller surface concentration (Walstra, 1996) and surface diffusion coefficient (Bos et al., 1997), stabilization by the Gibbs−Marangoni mechanism is less important. However, proteins impart stability by forming a viscoelastic adsorbed interfacial layer (Clark, 1995; Bos et al., 1997). Competitive adsorption of low-molecular-weight surfactants disrupts these protein−protein interactions in the adsorbed layer, thus compromising the surface mechanical properties. On the other hand, the presence of "protein islands" in the

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adsorbed layer results in impeded surface diffusion of the surfactant.

Many proteins used in foods have a physiological role that involves interactions with low-molecular-weight surface active species such as lipids. For example, bovine serum albumin (BSA) is a carrier of free fatty acids in serum (Spector, 1975). Recent report of a homology in the primary and three-dimensional structure between β-lactoglobulin and human retinol-binding protein (Papiz et al., 1986; Godovac-Zimmerman and Braunitzer, 1987) has led to the speculation that β-lactoglobulin might be involved in the transport of retinol in milk. The presence of a hydrophobic binding pocket at the surface of the protein could lead to a contamination of protein preparation with extraneous surface active species. Recently, Clark et al. (1995) reported that the presence of a surface active contaminant in commercial β-lactoglobulin preparations significantly altered their surface rheological properties. Paulson and Dejmeck (1992) suggested that contamination of BSA by bound fatty acids may account for the differences in film pressure reported in the literature, pointing out that this kind of measurement is very sensitive to sample impurities at low concentrations. The aim of this work is to investigate the effect of such contamination on the surface activity of major whey proteins, β-lactoglobulin, and α-lactalbumin. A radiotracer method is employed to compare the dynamics of surface pressure and surface concentration of native nonextracted β-lactoglobulin and α-lactalbumin with their charcoal-extracted counterparts.

EXPERIMENTAL PROCEDURES

Materials. β-Lactoglobulin (product L0130, lot 114H7055) and α-lactalbumin (type III, product L6010, lot 128F8140) were purchased from Sigma Chemical Inc. Charcoal was from Norit Co. Inc. (Atlanta, GA). Isotope ^{14}C-formaldehyde (37.3%) was purchased from Sigma Chemical Inc., and sodium cyanoborohydride, from Aldrich Chemical Inc. All other chemicals were purchased from Sigma and used without further purification. All the experiments were carried out at pH 7.4 using 10 mM commercial sodium phosphate buffer containing 0.9% NaCl. In all the experiments, ultrapure deionized water was used.

Methods. *Protein Purification by Charcoal Treatment.* β-Lactoglobulin and α-lactalbumin were purified according to the method described by Clark et al. (1995). Charcoal was added to a phosphate-buffered-protein solution using a ratio of 6 mg of charcoal/mg of protein. The sample was allowed to equilibrate for 20 min at room temperature. Then, the charcoal was removed by centrifugation (13 000 rpm for 15 min).

Radiolabeling of Proteins. Proteins were radiolabeled using the method described by Hunter et al. (1991). Proteins were dissolved in 0.05 M phosphate buffer (pH 7) and mixed with 0.1 M sodium cyanoborohydride and ^{14}C-formaldehyde (102 μCi) and allowed to react for 2 h at room temperature. After the reaction, the mixture was dialyzed against a 0.05 M phosphate buffer for 30 h at 4 °C for complete removal of unreacted species. Commercial, charcoal-treated β-lactoglobulin and α-lactalbumin were found to have between 1 and 1.4 amide groups labeled per molecule (2.52, 2.82, 4.82, and 3.72 μCi/mg of protein, respectively) as analyzed with a scintillation counter (Tri-carb 4000, from Packard instruments). Protein concentration was determined using the BCA assay (Smith et al., 1985). It is to be noted that the degree of modification due to radiolabeling is small and therefore does not significantly affect the surface properties of the protein. Moreover, comparison of the spread monolayer isotherm of native and radiolabeled bovine serum albumin as reported by Cho et al. (1997a) indicated no significant differences in the surface activity between the two because of radiolabeling. In addition, Graham and Phillips (1979) showed that radiolabeling did not affect the surface activity of different proteins by comparing the adsorption data at air−water interfaces using radiotracer and ellipsometry. It is important to note that all the experiments (with unpurified and charcoal-treated proteins) were done using radiolabeled proteins so that only the effect of charcoal treatment on the surface activity of proteins is examined.

Adsorption from Solution. The rate of change of surface pressure of protein solutions was monitored by the Wilhemy plate method using a computer-controlled Langmuir minitrough (KSV, Helsinki, Finland). A Teflon trough of 330 × 75 mm was used. In a typical experiment, a protein solution (with concentration ranging from 0.5 to 2×10^{-4} wt %) was prepared in a 0.01 M phosphate buffer (pH 7, containing 0.9% NaCl). First the trough was filled with the buffer solution without any protein (surface tension $= \gamma_0$) and the surface was carefully aspired to remove surface impurities before the surface pressure was adjusted to zero ($\pi = \gamma_0 - \gamma$). Then, the protein solution was gently poured into the trough, and the surface pressure was monitored. The data were automatically taken and stored through a computer interface. Adsorption experiments were carried until the surface pressure was steady, which usually took around 20 h. All experiments were done at room temperature.

At the same time, the surface concentration of ^{14}C-labeled proteins at the air−water interface was monitored by the surface radioactivity method (Cho, 1996; Cho et al., 1997b) using a gas proportional detector with a Mylar window (2 × 2 in.) (Ludlum Instruments Inc., Sweetwater, TX). The distance between the Mylar window and the liquid surface in the trough was about 3 mm. The carrier gas was 10% methane and 90% argon. The counts per minute (cpm) were integrated using a ratemeter (Ludlum model 520).

Calibration of Ludlum Gas Proportional Detector. To convert cpms to surface concentrations, the Ludlum gas proportional detector was calibrated with radioactive samples of known surface or bulk concentrations. The bulk radioactivity calibration procedure of Hunter et al. (1991) was employed for background correction. $^{14}CH_3COONa$ was employed for the calibration of the bulk radioactivity. Different amounts (25, 45, 80, and 120 μL) of 50 μCi/mL sodium acetate solutions in ethanol were added to 175 mL of phosphate buffer to give solutions of bulk radioactivities of 7.14×10^3, 1.285×10^4, 2.285×10^4, and 3.4×10^4 μCi/m^3.

For each protein studied (commercial and charcoal-treated β-lactoglobulin or α-lactalbumin), [^{14}C]proteins, rather than [^{14}C]stearic acid, were used for calibration of the surface radioactivity, since by using [^{14}C]stearic acid one tends to underestimate the surface concentration because of its much smaller molecular size compared to β-lactoglobulin or α-lactalbumin (Xu and Damodaran, 1993; Cho et al., 1997b). A total of 175 mL of 0.01 M phosphate buffer (pH 7, containing 0.9% NaCl) was poured into the Langmuir minitrough, and a spread monolayer of [^{14}C]protein was formed using Trurnit's method (Trurnit, 1960). Trurnit's spreading method has been widely used for soluble proteins. A 50 μL aliquot of aqueous [^{14}C]protein solution of 0.0247 wt % concentration was dripped from the top of a glass rod (5 mm diameter and 5 cm height) positioned above the air−water interface so that the solution spread uniformly on top of the interface (MacRitchie, 1990). It was shown by Cho et al. (1996) that, under these conditions, all the protein molecules are at the interface. The detector was placed at a distance of 3 mm above the air−water interface, and the steady-state cpm was measured. The area of the air−water interface was compressed in stages to provide several surface radioactivities which were used for the detector calibration.

Circular Dichroism Measurements. Far-UV CD spectra (260−190 nm) and near-UV CD spectra (320−260 nm) were measured using a Jasco J600 spectropolarimeter, and data were recorded on-line using a computer. The solution spectra presented were an average of four scans, recorded at 10 nm min^{-1}. An instrument sensitivity of ±20 mdeg full scale was

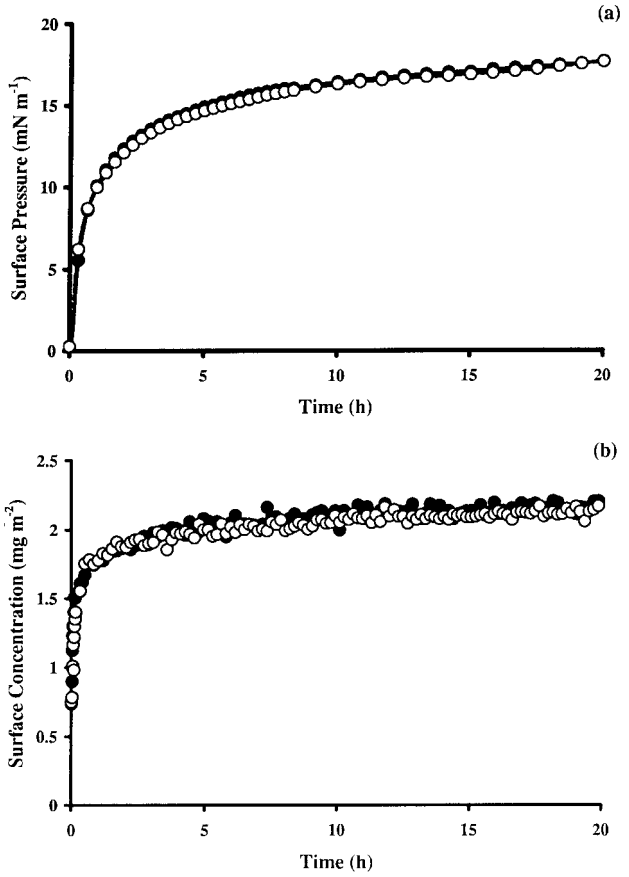

Figure 1. Reproducibility of (a) π vs time and (b) Γ vs time for ^{14}C-labeled β-lactoglobulin. ○ and ● are the data point for two repeated experiments.

routinely used, along with a 4 s time constant. The far-UV CD spectra of protein solution (1 mg mL^{-1}) were measured using a Suprasil-quartz-demountable cell of 0.1 mm path length (from Hellma, NJ), and the near-UV CD spectra of protein solution (0.5 mg mL^{-1}) were measured using a Suprasil-quartz cell of 1 cm path length (from Hellma, NJ).

Results were expressed in terms of specific ellipticity:

$$[\psi]_\lambda = \frac{\theta_\lambda}{c \times l}$$

$$\theta_\lambda = \text{ellipticity (deg)}$$

$$c = \text{concentration (g/mL)}$$

$$l = \text{path-length (dm)}$$

Thin-Layer Chromatography (TLC). The contaminant was removed from the protein by charcoal that was equilibrated with the protein solution. The charcoal was then separated by centrifugation. The contaminant was subsequently extracted for TLC analysis with a chloroform−methanol mixture (1:2 v/v). The extracts were dried under nitrogen and resuspended in a small volume of chloroform and then applied as a 2 cm streak to silica gel plate (Whatman) activated prior to use by incubation at 110 °C for 2 h. The thin-layer plates were then developed in either a polar or a nonpolar solvent system. The polar solvent system used was chloroform−methanol−water (65:25:4, by volume), and the nonpolar solvent system was hexane−diethyl ether−formic acid (80:20:2, by volume). After development and drying, the separation of samples was visualized by either exposure to iodine vapor (polar solvent system) or by spreading of a 2′,7′-dichlorofluorescein solution (nonpolar solvent system).

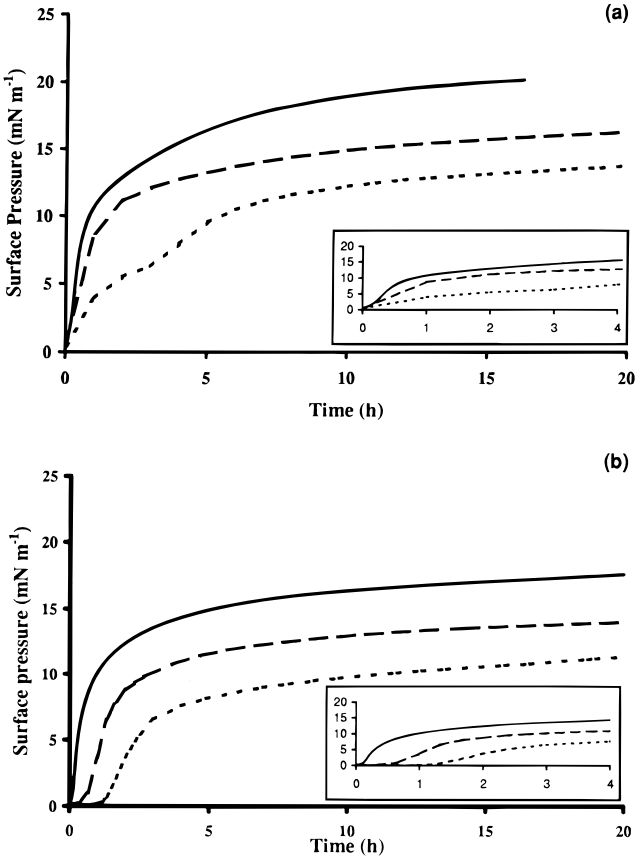

Figure 2. Effect of the bulk concentration of (a) ^{14}C-β-lactoglobulin and (b) ^{14}C-charcoal-treated β-lactoglobulin on its dynamic surface pressure $\pi(t)$, (- - -) 0.5×10^{-4} wt %; (— —) 1×10^{-4} wt %; (—) 2×10^{-4} wt %.

Surface Hydrophobicity Measurements. Protein surface hydrophobicity was determined from the fluorescence of protein bound with *cis*-parinaric acid (Kato and Nakai, 1980). Ethanolic solutions of *cis*-parinaric acid (3.6×10^{-3} M) were purged with nitrogen, and equimolar butylated hydroxytoluene was added as an antioxidant. A 10 μL volume of this solution was added to 2 mL of protein solution in 0.01 M potassium phosphate buffer (pH 7.4) containing 0.02 wt % sodium dodecyl sulfate (SDS). Fluorescence intensities of the protein−fatty acid conjugate were measured at 420 nm with an excitation at 325 nm. The initial slope of the fluorescence intensity versus protein concentration (w/v %) was used as a surface hydrophobicity index of the protein. The total hydrophobicity of native BSA (bovine serum albumin) as reported in the literature (Bigelow, 1967) was used as a calibration for the conversion of the above surface hydrophobicity index to surface hydrophobicity.

RESULTS

Adsorption of Whey Proteins at the A/W Interface. To verify the effect of convection as a result of pouring in the protein solution into the trough at the start of an experiment, the adsorption experiments were repeated. A typical reproducibility of the dynamics of surface pressure and surface concentration for purified β-lactoglobulin is shown in Figure 1a,b, respectively. As can be seen from the figure, the results were found to be reproducible with a 2% maximum deviation for π and a 6% maximum deviation for Γ.

The effect of bulk concentration on the evolution of the surface pressure with time is shown in Figure 2a for β-lactoglobulin and in Figure 3a for α-lactalbumin. At intermediate times, the surface pressure reached a

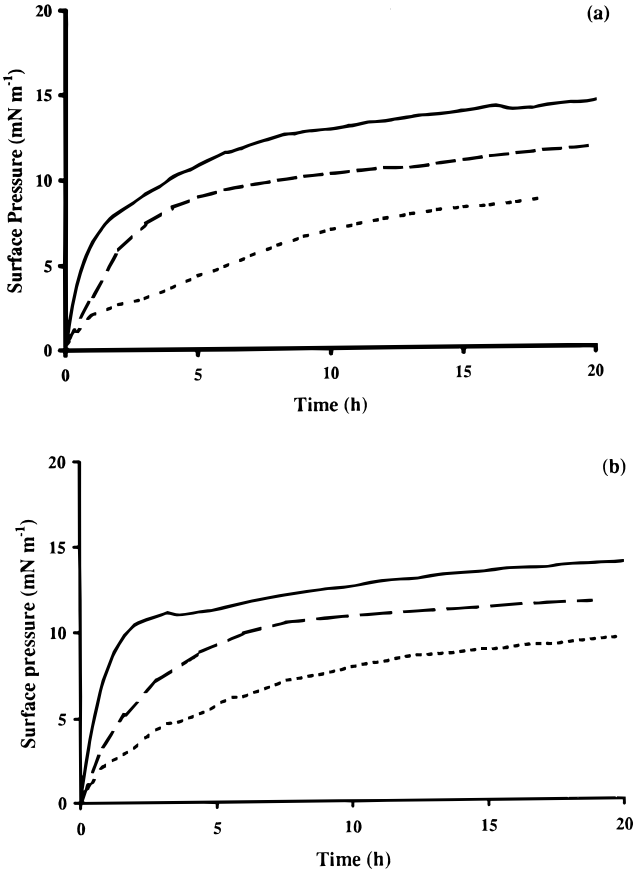

Figure 3. Effect of the bulk concentration of (a) ^{14}C-α-lactalbumin and (b) ^{14}C-charcoal-treated α-lactalbumin on its dynamic surface pressure $\pi(t)$: (- - -) 0.5×10^{-4} wt %; (— —) 1×10^{-4} wt %; (—) 2×10^{-4} wt %.

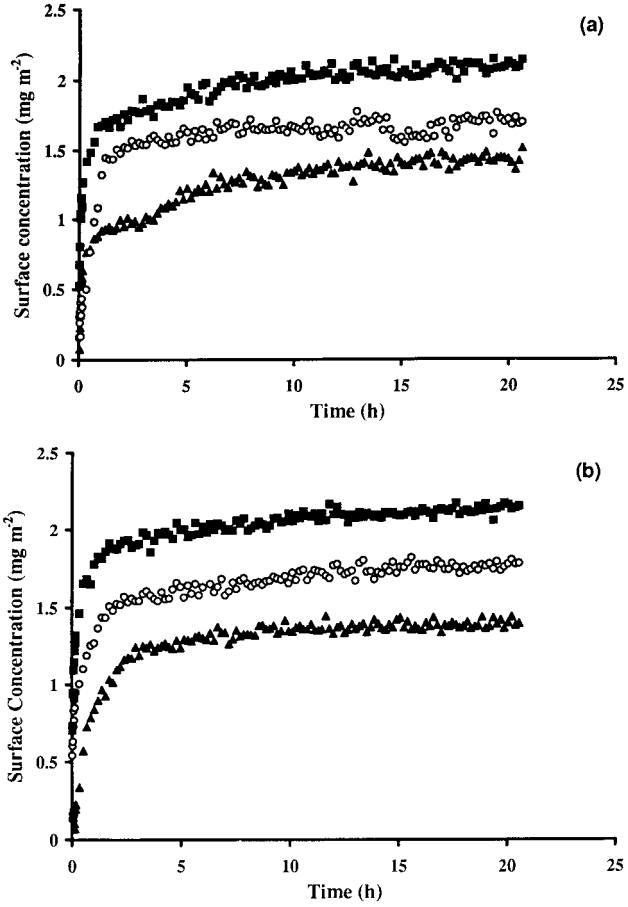

Figure 4. Effect of the bulk concentration of (a) ^{14}C-β-lactoglobulin and (b) ^{14}C-charcoal-treated β-lactoglobulin on its dynamic surface concentration $\Gamma(t)$: (▲) 0.5×10^{-4} wt %; (○) 1×10^{-4} wt %; (■) 2×10^{-4} wt %.

plateau (or inflection point) after about 2.5 h and then gradually increased up to a steady state value which was dependent on the bulk concentration. No plateau was observed at higher concentrations. Presence of a plateau may be interpreted as resulting from the presence of low concentrations of low-molecular-weight, highly surface-active contaminant in the protein solution.

Effect of Charcoal Treatment. Evolution of surface pressure of charcoal-treated-protein preparations at equivalent protein concentration are given for β-lactoglobulin and α-lactalbumin in Figures 2b and 3b, respectively. Charcoal extraction of the protein resulted in the absence of a plateau at all concentrations. The higher was the bulk concentration, the faster was the increase in surface pressure, especially at small times. For β-lactoglobulin, an apparent time lag was observed for lowest bulk concentration during which π was negligible. This time lag was found to decrease as the bulk protein concentration was increased. Indeed, the time lag lasted up to 30 min at $c_b = 1 \times 10^{-4}$ wt % and 1 h at $c_b = 0.5 \times 10^{-4}$ wt %, after which the surface pressure was found to increase rapidly up to a steady-state value. No time lag was observed for α-lactalbumin at any bulk concentration examined. The surface pressure of the solution containing commercial β-lactoglobulin (i.e. not treated with charcoal) was observed to reach a higher steady-state value than that for the charcoal-treated β-lactoglobulin. This was consistent with the presence of a surface active contaminant in the native sample which adsorbed at early stages. However, π was found to increase more rapidly at smaller times for charcoal-treated α-lactalbumin even though the steady-state values were comparable for commercial and charcoal-treated proteins. The reason for this discrepancy at short times is not clear.

Evolution of Surface Concentration. Effect of charcoal treatment on the evolution of the surface concentration was also investigated. Results are shown in Figures 4 and 5. At every bulk protein concentration studied, the surface concentration reached the same steady-state value for both native and charcoal-treated β-lactoglobulin thus indicating that the surface active contaminant does not prevent the protein from adsorbing or does not displace the protein from the interface and, consequently, after 20 h of adsorption, the same amount of protein is absorbed at the interface. However, closer examination of the data revealed that the steady-state surface concentration is reached after a longer time for nontreated β-lactoglobulin suggesting that the presence of contaminant delayed adsorption of the protein. For α-lactalbumin, however, the steady-state surface concentration was observed to be lower for nontreated protein than for charcoal-treated protein thereby suggesting that competitive adsorption between the surface active contaminant and the protein does occur resulting in the displacement of the protein from the interface.

Circular Dichroism Study. Effect of purification by charcoal treatment on the protein structure was investigated by far-UV (260−190 nm) and near-UV (340−255 nm) circular dichroism (Figure 6). Negative near-UV circular dichroism minima at 267, 277, 284,

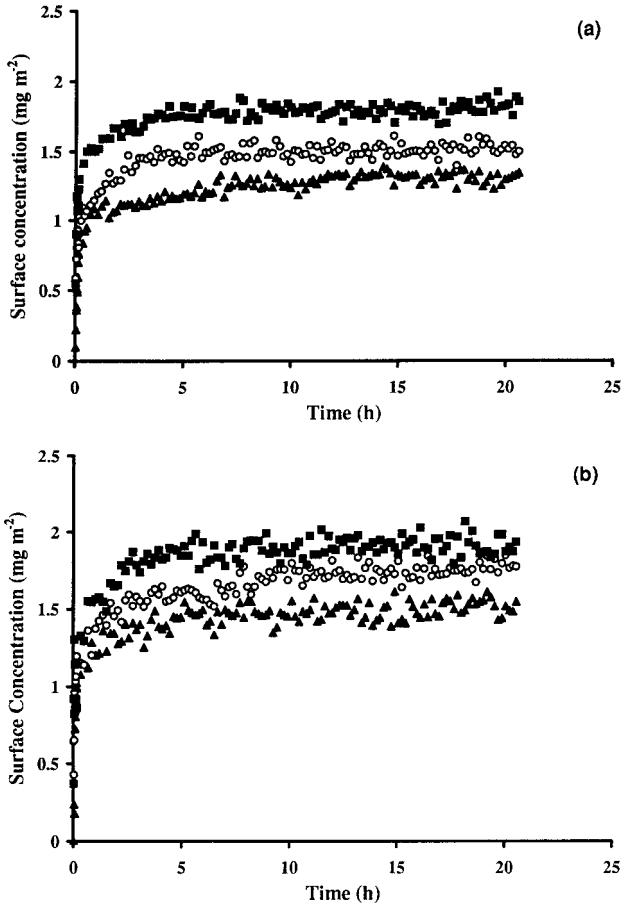

Figure 5. Effect of the bulk concentration of (a) ^{14}C-α-lactalbumin and (b) ^{14}C-charcoal-treated α-lactalbumin on its dynamic surface concentration $\Gamma(t)$: (▲) 0.5×10^{-4} wt %; (○) 1×10^{-4} wt %; (■) 2×10^{-4} wt %.

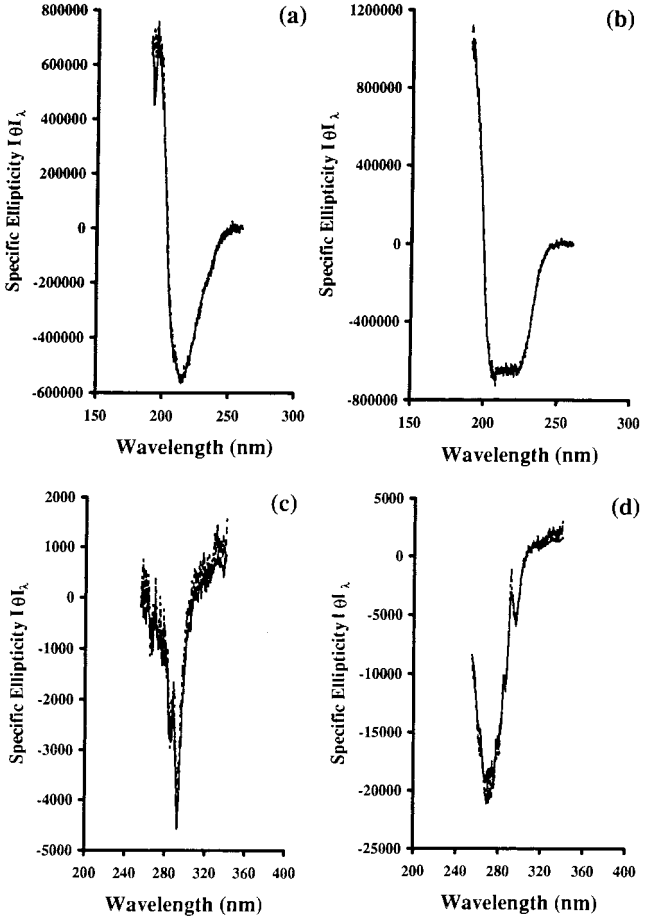

Figure 6. Effect of charcoal treatment on the far-UV circular dichroism spectrum of (a) β-lactoglobulin and (b) α-lactalbumin and of the near-UV circular dichroism of (c) β-lactoglobulin and (d) α-lactalbumin [(—) native protein; (- - -) charcoal-treated protein].

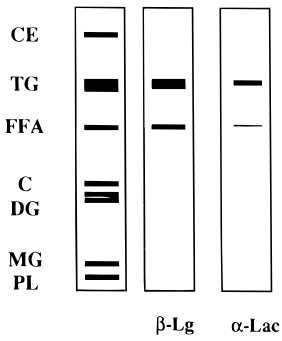

Figure 7. Analysis of the material extracted by the charcoal: CE, cholesterol ester; TG, triglycerides; FFA, free fatty acids; C, cholesterol; DG, diglycerides; MG, monoglycerides; PL, phospholipids; β-Lg, β-lactoglobulin; α-Lac, α-lactalbumin.

and 293 nm were found to be the same for both native and charcoal-treated β-lactoglobulin. These minima were assigned respectively to cystine, tyrosine, and tryptophan residues by Townend et al. (1967), and from these results, it can be inferred that extraction by charcoal does not lead to a change in folding in the proximity of these residues. CD spectra recorded in the far-UV and near-UV for native and charcoal-treated protein were quite similar showing that the extraction by charcoal does not lead to a change in the secondary or tertiary structure of either β-lactoglobulin and α-lactalbumin.

Thin-Layer Chromatography. The plate developed in a polar solvent system revealed a component which migrated with the solvent front in all the samples. Such a result was also reported by Clark et al. (1995). The plate developed in a nonpolar solvent showed the presence of two components which moved the same way as a tristearin and an oleic acid standard (Figure 7). This result suggested that the surface active contaminants present in β-lactoglobulin and α-lactalbumin solutions are fatty acid and triglyceride. However, the intensity of the fluorescence of bands corresponding to lipids was lower in α-lactalbumin than in β-lactoglobulin preparations suggesting that β-lactoglobulin solutions may contain more surface active contaminants than α-lactalbumin.

Surface Hydrophobicity Measurement. Surface hydrophobicity of native and charcoal-treated protein solutions was determined by measuring the fluorescence intensity of a protein−*cis*-parinaric acid conjugate (Kato and Nakai, 1980). The presence of lipid contaminant did not affect the surface hydrophobicity index, determined from the slope of fluorescence intensity, which was found to be 581 for both native as well as charcoal-treated α-lactalbumin and 1086 for both native as well as charcoal-treated β-lactoglobulin, corresponding to a surface hydrophobicity of 6.04×10^{-21} and 4.27×10^{-21} J/residue, respectively.

DISCUSSION

Evolution of surface pressure was observed to occur in two steps during the dynamic adsorption of β-lacto-

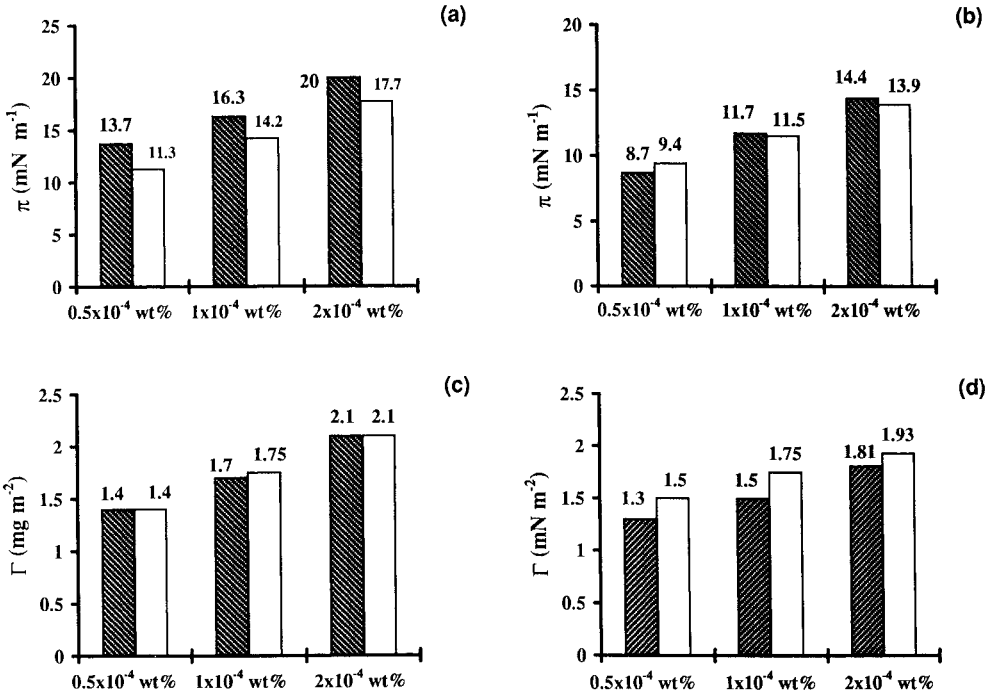

Figure 8. Effect of the lipid contaminant on the steady-state surface pressure of (a) β-lactoglobulin and (b) α-lactalbumin and on the steady-state surface concentration of (c) β-lactoglobulin and (d) α-lactalbumin (filled, commercial protein; open, charcoal-treated protein).

globulin and α-lactalbumin at the air−water interface for lowest concentrations examined. Such a behavior was consistent with the presence of a small molecular weight surface active contaminant in the protein solutions. Presence of lipid contaminants in commercial β-lactoglobulin preparation has already been reported (Clark et al., 1995). Contamination of β-lactoglobulin by lipids is not unreasonable since β-lactoglobulin has been shown to interact with a wide range of hydrophobic compounds such as fatty acids, triglycerides, phospholipids, alkanes, and aliphatic ketones (Brown, 1984; Perez and Calvo, 1994). A recent crystal structure of β-lactoglobulin (Papiz et al., 1986) showed that its tertiary structure as well as its amino acid sequence is rather similar to those of a large number of proteins including fatty acid binding and retinol binding proteins suggesting that β-lactoglobulin could participate in binding, protecting, and facilitating the intestinal adsorption of retinol in newborn calves (Pervaiz and Brew, 1985). Furthermore, the fact that β-lactoglobulin binds long chain fatty acids quite strongly which appear to occupy and overlap the site with retinol (Creamer and McGibbon, 1996) could lead to contamination of β-lactoglobulin by such free fatty acids. It has also been shown that β-lactoglobulin increases the activity of ruminant pharyngeal lipase by removing free fatty acids that would otherwise inhibit the enzyme activity (Puyol et al., 1991) suggesting that the biological role of β-lactoglobulin could be to aid milk fat digestion in the newborn animal (Perez et al., 1991). On the other hand, the biological role of α-lactalbumin does not involve any binding activity with lipid compounds. α-Lactalbumin acts as a cofactor for galactosyltransferase in lactose synthesis. α-Lactalbumin interacts with the enzyme to promote the transfer of galactose from UDP-galactose to glucose to form lactose. This complex formation lowers the Michaelis constant K_m for glucose in this reaction from 1400 to 4 mM thus ensuring maximum synthesis of lactose at the low glucose concentration found in mammary tissue (Wong et al., 1996). α-Lactalbumin, unlike β-lactoglobulin, was found not to bind palmitic acid in vitro (Puyol et al., 1991). However, it was found to interact with retinol (Puyol et al., 1991) and also with nonpolar acidic and zwitterionic lipids (Brown, 1984). In addition, glycerol monostearate and glyceryl distearate expanded spread monolayers of α-lactalbumin at neutral pH (Rahman and Sherman, 1982) suggesting complexation of the protein with the lipid.

Analysis of charcoal extract by thin-layer chromatography revealed the presence of fatty acid and triglycerides in both β-lactoglobulin and α-lactalbumin preparations. However the fluorescence intensity was higher in β-lactoglobulin−charcoal extract than in α-lactalbumin−charcoal extract suggesting that β-lactoglobulin was more contaminated than α-lactalbumin. Measurement of the surface hydrophobicity revealed that the contaminant concentration was not high enough to modify significantly the surface hydrophobicity of the proteins. The procedure used was shown not to alter protein structure by analyzing charcoal-extracted protein by circular dichroism in the far- and near-UV.

Evolution of surface pressure and surface concentration of nontreated and charcoal-treated proteins were compared. The surface pressure of charcoal-treated protein did not exhibit a two-step evolution. Comparison of the steady-state values reached by surface pressure and surface concentration showed that the effect of the lipid contaminant on protein adsorption was different for both proteins (Figure 8). After 20 h of adsorption, the surface pressure of charcoal-treated β-lactoglobulin was found to be lower than that for nontreated protein whereas the surface concentration reached the same steady-state value. The lipid contaminant, therefore, did adsorb at the air−water interface, thus resulting in an increase in the surface pressure. However, its concentration was not high enough to displace the protein from the interface. For

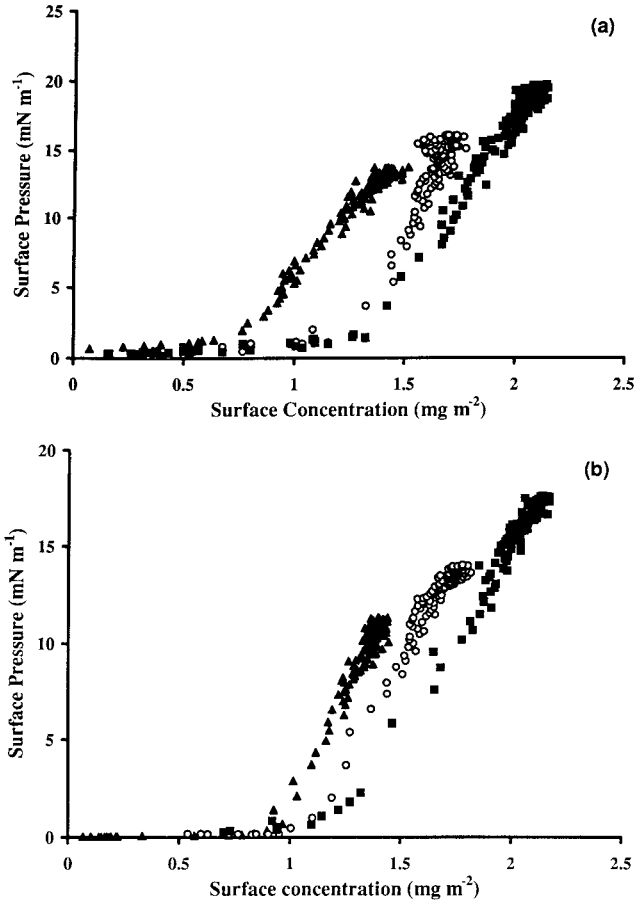

Figure 9. Effect of the lipid contaminant on the dynamic π-(t)-$\Gamma(t)$ relationships of β-lactoglobulin: Comparison between (a) the commercial and (b) the charcoal-treated β-lactoglobulin: (▲) 0.5×10^{-4} wt %; (○) 1×10^{-4} wt %; (■) 2×10^{-4} wt %.

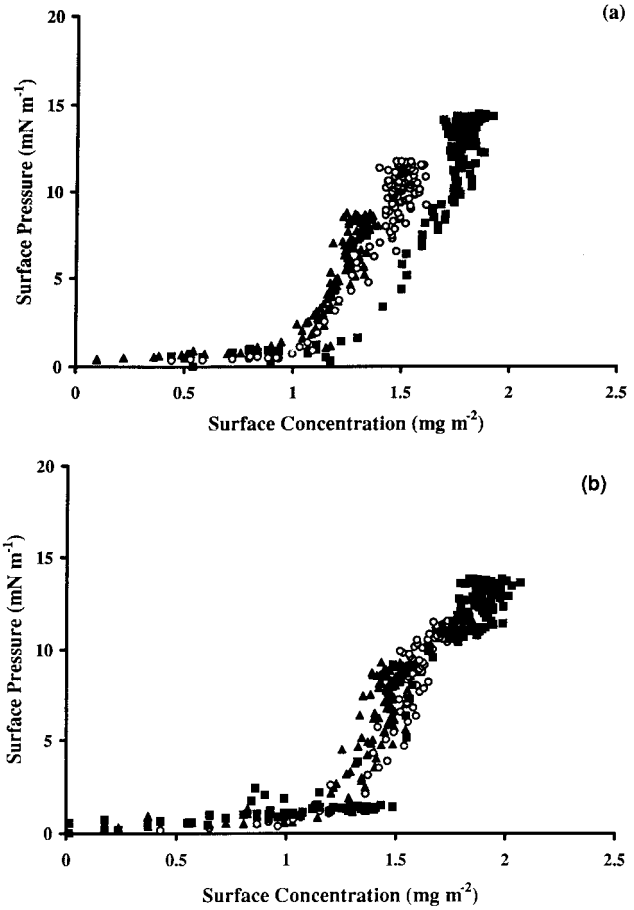

Figure 10. Effect of the lipid contaminant on the dynamic $\pi(t)$-$\Gamma(t)$ relationships of α-lactalbumin: Comparison between (a) the commercial and (b) the charcoal-treated α-lactalbumin: (▲) 0.5×10^{-4} wt %; (○) 1×10^{-4} wt %; (■) 2×10^{-4} wt %.

α-lactalbumin, however, the adsorption of the contaminant resulted in a lower surface concentration suggesting some displacement of the protein from the interface, but no difference was observed in the steady-state surface pressure. These results may be explained by the differences in the surface active properties between these two proteins. Castle et al. (1987) reported that the interfacial viscosity of α-lactalbumin was much lower than that for β-lactoglobulin, thus suggesting that α-lactalbumin exhibits a much weaker intermolecular interactions at the air−water interface than β-lactoglobulin (Clark, 1995). Clark and co-workers (Coke et al., 1990; Clark et al., 1991, 1994; Wilde and Clark, 1993) investigated the effect of the addition of Tween 20, a nonionic hydrophilic surfactant, on milk protein-stabilized foams. They observed that Tween 20 was considerably more effective at reducing the stability of α-lactalbumin-stabilized foam (instability did occur at a molar ratio of 0.05) than β-lactoglobulin stabilized foam (no instability was induced before the ratio molar reached 0.9). Using the fluorescence recovery after photobleaching (FRAP) technique, they also observed that less surfactant was required to induce surface diffusion in an adsorbed layer of α-lactalbumin than in the adsorbed layer of β-lactoglobulin suggesting that α-lactalbumin is displaced more easily by Tween 20 from the air−water interface than β-lactoglobulin. α-Lactalbumin was also found to be more easily displaced from the oil−water interface by β-casein than β-lactoglobulin (Dickinson et al., 1989). From these results, it may be proposed that the contaminant concentration in β-lactoglobulin preparation is not high enough to displace the protein from the interface. However its adsorption at the interface induces an increase in the surface pressure. Even though the contaminant was found in lower concentration in α-lactalbumin preparations, it was sufficient to displace the protein from the interface. It can be supposed that the effect of displacement of the protein from the interface on the surface pressure was compensated by adsorption of lipid contaminant, and consequently, no appreciable change was observed in the surface pressure. Clark et al. (1995) also reported that the presence of the contaminating surfactants was most clearly evident from their rheological measurement in the early stages of adsorption. This is consistent with our observation that the plateau observed during the surface pressure evolution took place in the first 2 h of adsorption.

From $\Gamma(t)$ and $\pi(t)$, $\pi-\Gamma$ plots have been constructed for different bulk concentrations which are shown in Figures 9 and 10. For β-lactoglobulin, $\pi(t)$-$\Gamma(t)$ plots do not fall into the same curve. The lower was the bulk concentration, the higher was the surface pressure for the same surface concentration. This indicated that β-lactoglobulin unfolds more easily as the protein concentration is lower since more area is available at the interface for unfolding. The same behavior was observed with charcoal-treated β-lactoglobulin. For untreated β-lactoglobulin, the surface pressure was found to be higher at the same surface concentration compared to the treated β-lactoglobulin at all bulk

concentrations possibly because of the adsorption of contaminant. This effect was more pronounced at the lowest concentration (0.5×10^{-4} wt %). This indicates that the contribution of the contaminant to the surface pressure is more evident at lower bulk concentration. For charcoal-treated β-lactoglobulin, the evolution of surface pressure was characterized by an induction period during which the surface pressure does not increase whereas protein does adsorb as shown by the evolution of the surface concentration. However, the time scale for adsorption and further molecular reorientation of small molecular weight surfactants such as fatty acids is much shorter than that for proteins (De Feijter and Benjamins, 1986). Consequently, no time lag was observed for nontreated β-lactoglobulin solution. Similar behavior was also observed for α-lactalbumin. However the difference between the $\pi-\Gamma$ plots of nontreated and treated proteins were less pronounced because of less contamination.

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