Adsorption of Whey Protein Isolate at the Oil−Water Interface as a Function of Processing Conditions: A Rheokinetic Study

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In this paper we present surface dynamic properties (interfacial tension and surface dilational properties) of a whey protein isolate with a high content of β-lactoglobulin (WPI) adsorbed on the oil−water interface as a function of adsorption time. The experiments were performed at constant temperature (20 °C), pH (5), and ionic strength (0.05 M). The surface rheological parameters and the interfacial tension were measured as a function of WPI concentration (ranging from 1×10^{-1} to 1×10^{-5}% w/w) and different processing factors (effect of convection and heat treatment). We found that the interfacial pressure, π, and surface dilational modulus, E, increase and the phase angle, ϕ, decreases with time, θ, which should be associated with WPI adsorption. These phenomena have been related to diffusion of the protein toward the interface (at short adsorption time) and to the protein unfolding and/or protein−protein interactions (at long-term adsorption) as a function of protein concentration in solution and processing conditions.

Keywords: *WPI; whey; tension; rheometer; rheological; protein; parameters; isolate; interfacial; β-lactoglobulin; adsorption*

INTRODUCTION

The surface dynamic properties and other physicochemical characteristics of proteins at fluid−fluid interfaces are of great importance for food formulations (Halling, 1981; Kinsella, 1976). The adsorption of protein at fluid−fluid interfaces is considered to play an important role in the formation and stabilization of food dispersions (Dickinson, 1992; Damodaran, 1990). In fact, during the formation of a dispersed system, the protein must be adsorbed at the interface to prevent the recoalescence of the initially formed bubbles or droplets. In addition, during the protein adsorption, the surface or interfacial tension of fluid−fluid interfaces decreases, which is an important factor both to optimize the input of energy involved in the emulsification or foaming process (Walstra, 1993) and, finally, to achieve smaller droplet and bubble size—which is an important factor for the stability of the dispersed system (Dickinson, 1992). The decrease in surface tension by proteins is caused by different processes (Graham and Phillips, 1979a; MacRitchie, 1978; Tornberg, 1978a): (i) the protein has to diffuse from the bulk phase to the subsurface (a layer immediately adjacent to the fluid interface) by diffusion and/or convection, (ii) this step is followed by the adsorption and unfolding of the protein at the interface, and (iii) the adsorbed protein segments rearrange at the fluid interface, a slow process caused by reorganization of the amino acid segments previously adsorbed on the interface. In addition to lowering the interfacial tension, protein can form continuous viscoelastic films around oil droplets or air bubbles via noncovalent intermolecular interactions and covalent disulfide cross-linking, which are important factors for the stability of colloidal dispersions (emulsions and foams) (Murray and Dickinson, 1996).

Although much is known about the physicochemical properties of proteins and their functional properties in model systems, predictions of their behavior in real food systems have not been successful (Damodaran and Paraf, 1997). The main reasons for this behavior is the denaturation of proteins during food processing as a consequence of extrinsic factors such as pH, ionic strength, temperature, shear, interactions with other food components (such as lipids, polysaccharides, sugars, salts, etc.), or a combination of these factors, including the effect of a mixture of proteins in the formulation (Bos et al., 1997; Damodaran and Paraf, 1997; Friberg and Larsson, 1997; Nylander and Ericsson, 1997; Sjöblom, 1996). Furthermore, other intrinsic molecular factors—such as size, charge distribution, hydrophobicity, hydrophilicity, molecular flexibility, conformational stability at fluid−fluid interfaces, rapid adaptability of the conformation to changes in its environment, steric properties, etc.—can affect the physicochemical and functional properties of proteins which govern the formation and stability of food colloids (emulsions, foams, gels, etc.) (Cayot and Lorient, 1997; Dalgleish, 1996, 1997).

This paper presents experimental information on surface dynamic properties (time-dependent interfacial pressure and viscoelastic characteristics) of whey protein isolate (WPI) adsorbed films at the oil−water interface, at 20 °C and pH 5. The concentration of protein in the bulk phase and different processing factors—such as the effect of convection at the interface and in the bulk phase and heat treatment of the protein before adsorption—were the variables studied. Whey protein is a mixture of proteins with numerous functional properties and is of great importance for the food industry. The main proteins are β-lactoglobulin and

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α-lactoglobulin, which represent ca. 70% of the total whey proteins and are responsible for the important functional properties of the whey protein ingredients (Cayot and Lorient, 1997). Whey protein isolates, which have protein concentrations in excess of 90%, have β-lactoglobulin as the major component. β-Lactoglobulin is a globular protein with a polypeptide chain of 162 residues stabilized by two disulfide cross-links and also contains an internal free sulfhydryl group which is sensitive to interfacial denaturation and heat treatment. The monomeric molecular mass is 18 300, but at pH 5−8, β-lactoglobulin exists as a dimer (Swaisgood, 1982). At the oil−water interface, β-lactoglobulin undergoes significant structural changes during film formation. In addition, β-lactoglobulin is sensitive to heat denaturation at temperatures close to 72 °C at neutral pH (Boye et al., 1997; Cayot and Lorient, 1997; Oakenfull et al., 1997).

MATERIALS AND METHODS

Chemicals. Whey protein isolate (WPI) is a native whey protein with a very high content of β-lactoglobulin (protein 92 ± 2%, β-lactoglobulin > 95%, α-lactoglobulin < 5%) obtained by fractionation and was supplied by Danisco Ingredients (Denmark). The sample was stored below 0 °C, and all work was done without further purification. Samples for interfacial characterization of WPI films were prepared using Milli-Q ultrapure water and buffered at pH 5.0. Trisun oil (fatty acid composition, C16, 4%; C18, 4%; C18:1, 80%; C18:2, 9%; C18:3, traces; C20, 0.5%; and C22, 1%), supplied by Danisco Ingredients, was Florisil 60−100 mesh (Aldrich)-treated to remove any surface-active impurities. Analytical-grade acetic acid and sodium acetate for buffered solutions were used as supplied by Sigma (>95%) without further purification. The absence of surface-active contaminants in the aqueous buffered solutions was checked by surface tension measurements before sample preparation. No aqueous solutions with a surface tension other than that accepted in the literature (72−73 mN/m at 20 °C) were used.

Methods. For interfacial tension and surface dilational properties measurements of adsorbed protein films at the oil−water interface, an automatic drop tensiometer developed by Labordenne et al. (1994) was utilized. The drop profile was processed according to the fundamental Laplace equation in order to obtain the interfacial tension (eq 1)

$$\frac{1}{x}\frac{\mathrm{d}}{\mathrm{d}x}(x \sin \Theta) = \frac{2}{b} - Cz \tag{1}$$

where x and z are the Cartesian coordinates at any point of the drop profile, b is the radius of curvature of the drop apex, Θ is the angle of the tangent to the drop profile, and C is the capillary constant, $C = g\rho/\sigma$, where σ is the interfacial tension, ρ is the difference between the densities of the two liquids, and g is the acceleration of gravity.

The computer calculates three characteristic parameters of the drop, namely, the area, A, volume, V, and interfacial or superficial tension, σ, up to a frequency of 25 times per second for about 5 s. The frequency of the measurements can be altered by selecting three different algorithms, to five measurements per second (fast mode) or lower (one measurement every 5 s) for high-precision requirements. The average standard accuracy of the interfacial tension is roughly 0.1 mN/m. However, the reproducibility of the results (for at least two measurements) was better than 0.5%.

The surface viscoelastic parameters—such as surface dilational modulus, E, and its elastic, E_d, and viscous, E_v, components—were measured as a function of time, θ. The amplitude, $\Delta A/A$, and angular frequency, ω, were maintained constant at 15% and 100 mHz, respectively. The percentage area change was determined (data not shown) to be in the linear region. The method involved a periodic automated-controlled, sinusoidal interfacial compression and expansion performed by decreasing and increasing the drop volume at the desire amplitude. The surface dilational modulus derived from the change in interfacial tension (dilational stress), σ (eq 2), resulting from a small change in surface area (dilational strain), A, (eq 3), may be described by eq 4 (Lucassen and van den Temple, 1972)

$$\sigma = \sigma_o \sin(\omega\theta + \phi) \tag{2}$$

$$A = A_o \sin(\omega\theta) \tag{3}$$

$$E = \frac{\mathrm{d}\sigma}{\mathrm{d}A/A} = -\frac{\mathrm{d}\pi}{\mathrm{d}\ln A} \tag{4}$$

where σ_o and A_o are the stress and strain amplitudes, respectively, ϕ is the phase angle between stress and strain, $\pi = \sigma^o - \sigma$ is the interfacial pressure, and σ^o is the interfacial tension in the absence of protein.

The dilational modulus is a complex quantity and composed of real and imaginary parts (eq 5). The real part of the

$$E = (\sigma_o/A_o)(\cos\phi + i \sin\phi) = E_d + i E_v \tag{5}$$

dilational modulus or storage component is the dilational elasticity, $E_d = |E| \cos\phi$. The imaginary part of the dilational modulus or loss component is the surface dilational viscosity, $E_v = |E| \sin\phi$. The ratio (σ_o/A_o) is the absolute modulus, $|E|$, a measure of the total unit material dilational resistance to deformation (elastic + viscous). For a perfectly elastic material, the stress and strain are in phase ($\phi = 0$) and the imaginary term is zero. In the case of a perfectly viscous material $\phi = 90°$ and the real part is zero. The loss-angle tangent can be defined by eq 6. If the film is purely elastic, the loss angle

$$\tan\phi = E_v/E_d \tag{6}$$

tangent is zero.

The experiments were carried out at 20 °C. The temperature of the system was maintained constant within ±0.1 °C by circulating water from a thermostat. The pH and the ionic strength were maintained constant at 5.0 and 0.05 M, respectively, by using an acetic-acetate buffer. Protein solutions ranging from 10^{-1} to 10^{-5}% w/w were freshly prepared by stirring for 30 min. The solution was placed in the syringe or cuvette and then in the compartment and was allowed to stand for 30 min to reach the desired constant temperature. Then either a drop of protein solution was delivered into the oil phase or a drop of oil was delivered in a solution of protein and allowed to stand for 120 min at 20 °C to achieve protein adsorption at the oil−water interface.

The effect of convection on protein adsorption at the oil−water interface was analyzed. In these experiments, the surface dynamic properties (surface tension and surface dilational properties) were monitored simultaneously during protein adsorption by the convection caused by the sinusoidal decreasing and increasing of the drop volume at a constant amplitude of 15%.

In another set of experiments, the effect of heat-treated protein on protein adsorption at the oil−water interface was analyzed. The protein solutions were left for 60 min at 80 °C and then cooled back to room temperature to allow protein gelation. Afterward, either a drop of heat-treated protein solution was delivered into the oil phase or a drop of oil was delivered in a solution of heat-treated protein and allowed to stand for 120 min at 20 °C to achieve protein adsorption at the oil−water interface. The interfacial tension and the viscoelastic properties were monitored simultaneously during the heat-treated protein adsorption.

The materials in contact with the oil phase and protein solution must be clean in order to prevent any contamination by surface-active compounds.

RESULTS AND DISCUSSION

1. Time Dependence of Surface Dilational Properties. Time-dependent interfacial pressure (π), surface

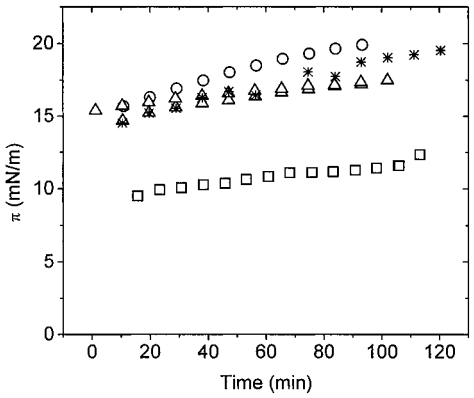

Figure 1. Time-dependent surface pressure for whey protein isolate adsorbed films at the oil−water interface at pH = 5, I = 0.05 M, and 20 °C. Protein concentration in the drop bulk phase (%, w/w): (○) 10^{-1}, (△) 10^{-2}, (□) 10^{-5}. Protein in the cuvette bulk phase (*) 10^{-2}%.

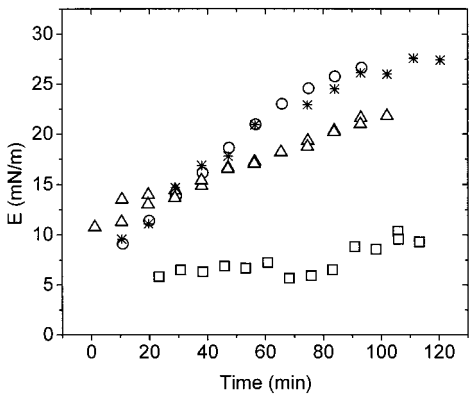

Figure 2. Time-dependent surface dilational modulus for whey protein isolate adsorbed films at the oil−water interface at pH = 5, I = 0.05 M, and 20 °C. Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. Protein concentration in the drop bulk phase (%, w/w): (○) 10^{-1}, (△) 10^{-2}, (□) 10^{-5}. Protein in the cuvette bulk phase (*) 10^{-2}%.

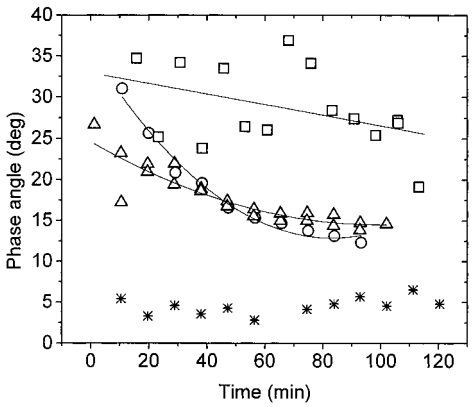

Figure 3. Time-dependent phase angle for whey protein isolate adsorbed films at the oil−water interface at pH = 5, I = 0.05 M, and 20 °C. Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. Protein concentration in the drop bulk phase (%, w/w): (○) 10^{-1}, (△) 10^{-2}, (□) 10^{-5}. Protein in the cuvette bulk phase (*) 10^{-2}%.

dilational modulus (E), and phase angle (ϕ) are plotted in Figures 1−3, respectively, for adsorbed films of whey protein isolate at the oil−water interface, as a function of the protein concentration in the aqueous bulk phase. In each experiment the temperature was maintained constant at 20 °C, and the pH and ionic strength of the aqueous phase were 5 and 0.05 M, respectively. The

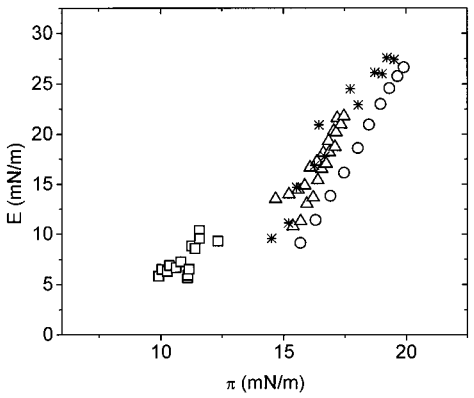

Figure 4. Surface dilational modulus as a function of surface pressure for whey protein isolate adsorbed films at the oil−water interface at pH = 5, I = 0.05 M, and 20 °C. Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. Protein concentration in the drop bulk phase (%, w/w): (○) 10^{-1}, (△) 10^{-2}, (□) 10^{-5}. Protein in the cuvette bulk phase (*) 10^{-2}%.

increase in the interfacial pressure (Figure 1) and in the surface rheological properties with time, especially the surface dilational modulus (Figure 2) and surface dilational elasticity, should be associated with WPI adsorption at the interface (MacRitchie and Alexander, 1963; Damodaran and Song, 1988; Graham and Phillips 1979c). This behavior was similar to that observed for BSA adsorption on water and aqueous solutions of ethanol (Rodríguez Niño et al., 1997a) and sucrose (Rodríguez Niño et al., 1997b) and for the apparent shear viscosity for proteins adsorbed at the tetradecane−water interface (Dickinson, 1998; Murray and Dickinson, 1996). Over the adsorption period studied here, the film behaved, from a rheological point of view, as viscoelastic with a phase angle higher than zero (Figure 3). However, ϕ decreased with time with more time dependence at the higher protein concentrations in the bulk phase. These results are consistent with the existence of higher protein−protein interactions, which are expected to be due to a higher protein concentration at the interface because both the adsorption time and the protein concentration in the bulk phase increase.

If the surface dilational modulus is due to the amount of protein adsorbed at the oil−water interface, all E data should be normalized in a single master curve of E versus π. Figure 4 shows that this normalization was possible. It can be seen that a line gave the combined results of the adsorption with different protein concentrations at different adsorption times. This master curve, characteristic for each protein, was similar to those obtained recently for Na-caseinate, ovalbumin, and BSA at the oil−water interface (Benjamins et al., 1996). As for other globular proteins, E increased with the interfacial pressure, and this dependence reflects the existence of interactions within the adsorbed protein residues. In agreement with the theory of Lucassen et al. (1975), the plot of Figure 4 suggests that interactions between adsorbed protein residues increase with surface pressure. In fact, at lower surface pressures (π < 12.5 mN/m), the slope of the $E-\pi$ plot was 1.6, close to the behavior of a ideal gas with low protein interactions. However, at higher surface pressures the slope changed suddenly to a value close to 4, which implies an important nonideal behavior with higher molecular interactions as the amount of protein at the interface increases. These results strengthen the hypothesis that

WPI and especially its major component β-lactoglobulin are adsorbed at the oil−water interface with two different structures (Graham and Phillips, 1979b and 1980). At lower interfacial pressures, β-lactoglobulin molecules could exist as trains with all amino acid segments located at the interface. As the surface pressure or the surface concentration exceed that of ca. 12.5 mN/m, a looping of the segments into the underlying oil and aqueous solutions could be produced. That is, in the most compact structure, a significant portion of the β-lactoglobulin amino acid residues could be extended into the underlying oil and aqueous solutions and what is most likely is that this structure adopts the form of more compact loops and tails, according to Graham and Phillips (1979b).

The looping of the amino acid residues of β-lactoglobulin molecules is more closely packed, and the surface density is higher as the adsorption time increases. The closer packing of protein at higher adsorption time is the consequence of the existence of a molecular rearrangement of the previously adsorbed protein molecules, as reflected by the significant increment in surface dilational modulus (Figure 2) and especially in its elastic component, as reflected by the decrease in the phase angle (Figure 3). This hypothesis will be discussed later from a rheokinetic point of view.

The time dependence during adsorption of WPI at the oil−water interface also depends on the conditions at which the adsorption takes place, either from a drop of protein solution delivered into the oil phase or from a drop of oil delivered into a solution of protein. It can be seen that the time dependence and the value of the interfacial pressure (Figure 1) and especially the dilational modulus (Figure 2) at higher adsorption time are higher as the protein is adsorbed from the aqueous solution in the cuvette than as the drop of protein solution is delivered into the oil phase. The conditions at which the protein adsorption take place also affect the viscoelasticity of the film (Figure 3). The film elasticity is higher as the protein is adsorbed from the cuvette to the oil drop delivered into the aqueous bulk phase. However, the present results indicate that the viscoelastic modulus is due mainly to the amount of adsorbed protein, as observed in the results included in Figure 4. It can be seen that the same master curve correlates the $E-\pi$ dependence no matter what the protein concentration in the bulk phase and the conditions at which the protein adsorption takes place.

2. Rheokinetics of Protein Adsorption at the Oil−Water Interface. The kinetics of protein adsorption at the oil−water interface can be monitored by measuring changes in interfacial pressure (π) (Dukhin et al., 1995; MacRitchie, 1978). The rate of change of surface concentration (Γ) can be expressed as (MacRitchie, 1978):

$$d\Gamma/d\theta = (d\Gamma/d\pi)(d\pi/d\theta) \quad (7)$$

If $(d\Gamma/d\pi)$ is constant, $d\pi/d\theta$ can be used to evaluate the rate of protein adsorption. During the first step, at relatively low pressures when diffusion is the rate-determining step, a modified form of the Ward and Torday equation (Ward and Torday, 1946) can be used to correlate the change in the interfacial pressure with time (eq 8)

$$\pi = 2C_oKT(D\theta/3.14)^{1/2} \quad (8)$$

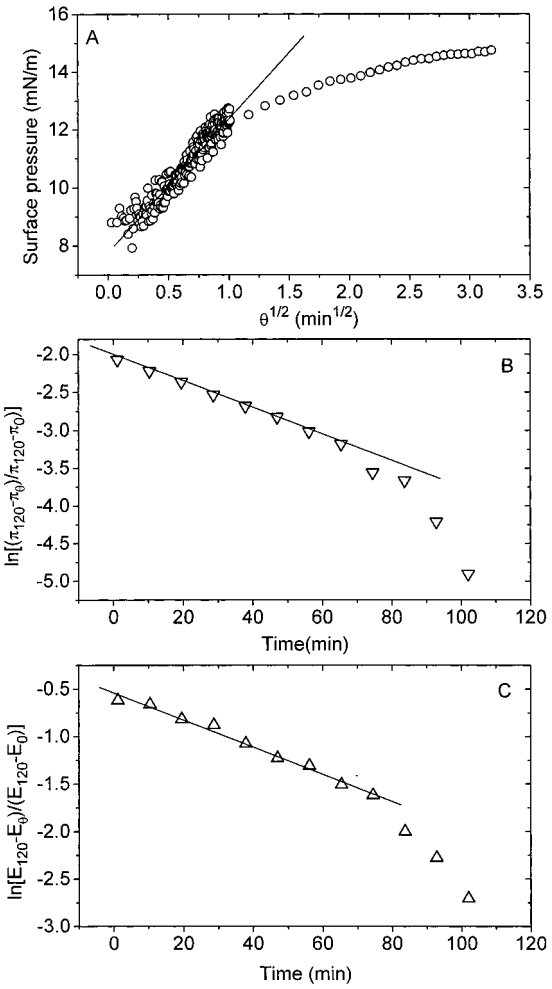

Figure 5. Example of the fit of rheokinetic parameters (surface pressure and surface dilational modulus) according to mechanisms of (A) diffusion of the protein to the oil−water interface and (B and C) adsorption and rearrangement of the adsorbed protein at the oil−water interface for whey protein isolate adsorbed films at the oil−water interface at pH = 5, I = 0.05 M, and 20 °C. Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. Protein concentration in the drop bulk phase 10^{-2}%, w/w.

where C_o is the concentration in the bulk phase, K is the Boltzmann constant, T is the absolute temperature, and D is the diffusion coefficient. If the diffusion of proteins at the interface controls the adsorption process, a plot of π against $\theta^{1/2}$ will then be linear (MacRitchie, 1978; de Feijter et al., 1987; Xu and Damodaran, 1994). An example of an application of eq 8 to WPI adsorption at the oil−water interface is given in Figure 5A and will be discussed in the next section.

To monitor unfolding at the interface and configurational rearrangements of adsorbed protein molecules, two different approaches can be used. The rate of these processes can be analyzed by a first-order equation (Graham and Phillips, 1979a; Tornberg, 1978a; Suttiprasit et al., 1992),

$$\ln \frac{\pi_{120} - \pi_\theta}{\pi_{120} - \pi_o} = -k_i\theta \quad (9)$$

where π_{120}, π_o, and π_θ are the interfacial pressures at 120 min of adsorption time, at time $\theta = 0$, and at any time θ, respectively, and k_i is the first-order rate constant. In practice, a plot of eq 9 usually yields two

Table 1. Characteristic Parameters for the Diffusion of WPI at the Oil−Water and Air−Water Interface at 20 °C and at pH 5

system	slope π vs $\theta^{1/2}$ (mN m^{-1} s$^{-0.5}$)	LR[c]
WPI native in drop (1×10^{-2}%)[a]	3.56	0.978
WPI native in drop (1×10^{-2}%)[b]	3.72	0.998
WPI native in cuvette (1×10^{-2}%)[a]	3.72	0.969
WPI heat-treated in drop (1×10^{-2}%)[a]	4.43	0.985
WPI native in drop (1×10^{-3}%)[a]	1.97	0.945
WPI native in drop (1×10^{-4}%)[a]	0.441	0.976
WPI native in drop (1×10^{-5}%)[a]	0.249	0.989

[a] Oil−water interface. [b] Air−water interface. [c] Linear regression coefficient.

or more linear regions. The initial slope is taken to correspond to a first-order rate constant of unfolding (k_1), while the second slope is taken to correspond to a first-order rate constant of rearrangement (k_2), occurring among a more or less constant number of adsorbed molecules.

The fact that the time dependence of the surface pressure follows the same trend as the protein surface concentration (MacRitchie, 1978; MacRitchie, 1989; Joos et al., 1992; Damodaran and Song, 1988) indicates that π and E depend on the surface coverage, which is expected to increase with time. Due to this similarity, we propose a first-order kinetic equation (eq 10) similar to eq 9 to monitor unfolding and configurational rearrangements of adsorbed protein molecules at the oil−water interface

$$\ln[(E_{120} - E_\theta)/(E_{120} - E_o)] = -k_i'\theta \qquad (10)$$

where E_{120}, E_0, and E_θ are the surface dilational modulus at $\theta = 120$ min of adsorption time, at time $\theta = 0$, and at any time θ, respectively, and k_i' are the first-order rate constants of penetration and further rearrangements of protein at the interface. The application of eqs 9 and 10 to WPI adsorption at the oil−water interface will be discussed in section 4.

3. Diffusion of Protein Molecules to the Oil−Water Interface. The adsorption kinetics of WPI at short adsorption times, up to approximately 60 s is controlled by the diffusion of the protein toward the interface, in agreement with the Ward and Torday model, eq 8. Figure 5A gives data of adsorption experiments carried out at a protein concentration in the drop bulk phase of 10^{-2}% w/w, as an example. Different protein concentrations in the bulk phase give similar results. The slope derived from the π vs $\theta^{1/2}$ line is shown in Table 1. The fit of experimental data by the model of Ward and Torday (eq 8) was made at an interval of time based on the best linear regression coefficient (LR), which is also included in Table 1. The results for WPI diffusion at a concentration in the bulk phase of (1×10^{-1})% w/w was not included in Table 1 because at higher protein concentrations the diffusion is too high to be measured with accuracy by the method used here. It can be seen that the slope of the π vs $\theta^{1/2}$ line increases with the WPI concentration in the bulk phase. This means that the diffusion of WPI from the bulk phase toward the subsurface follows a Fickinian model. Thus, it can be concluded that the diffusion of the protein is driven by the concentration gradient, in agreement with previous results by other authors (Benjamins et al., 1975; de Feijter and Benjamins, 1987; Graham and Phillips, 1979a; Tornberg, 1978b; Tornberg et al., 1982).

Table 2. Characteristic Parameters for Adsorption of WPI at the Oil−Water Interface at 20 °C and pH 5

system	$k_1 \times 10^3$, min^{-1} (LR)[a]	$k_1' \times 10^3$, min^{-1} (LR)[a]
WPI native in drop (1×10^{-1}%)	18.7 (0.997)	15.3 (0.998)
WPI heat-treated in drop (1×10^{-1}%)	29.5 (0.998)	29.7 (0.996)
WPI native in drop (1×10^{-2}%)	17.1 (0.999)	14.6 (0.993)
WPI native in cuvette (1×10^{-2}%)	15.2 (0.954)	42.8 (0.935)
WPI heat-treated in cuvette (1×10^{-2}%)	23.5 (0.967)	43.5 (0.987)
WPI native in drop (1×10^{-3}%)	7.93 (0.994)	5.08 (0.923)
WPI native in drop (1×10^{-4}%)	6.33 (0.996)	4.8 (0.910)
WPI native in drop (1×10^{-5}%)	7.8 (0.985)	6.7 (0.911)

[a] Linear regression coefficient.

The slope of the π vs $\theta^{1/2}$ line at 20 °C for a WPI concentration in the bulk phase of (1×10^{-2})% w/w (as an example) is essentially the same as for the air−water and oil−water interfaces (Table 1), which reinforces the idea that at short adsorption times the adsorption of WPI at the oil−water interface is diffusion-controlled. That is, the diffusion of WPI in the bulk phase does not depend on the fluid−fluid interface characteristics. However, the WPI diffusion is dependent on the protein conformation in the aqueous phase. It can be seen (Table 1) that the rate of WPI diffusion is higher for heat-treated WPI than for a native protein. This suggests that as a consequence of the protein denaturation, heat-treated WPI has more hydrophobic residues in the periphery of the whole molecule which act as a driving force for the diffusion, due to its higher incompatibility with the aqueous phase.

At higher adsorption times, in the period after that affected by the diffusion, an energy barrier for the WPI adsorption exists which could be attributed to the penetration, unfolding, and rearrangements of the protein at the interface (Figure 5A). Similar results were observed for BSA-adsorbed films at the air−water interface (Rodríguez Patino and Rodríguez Niño, 1995; Rodríguez Niño et al., 1997a and b).

4. Protein Penetration and Rearrangements at the Oil−Water Interface. The long-term adsorption of WPI at the oil−water interface is included in Figure 5. Figures 5B and 5C show complementary adsorption experiments carried out at a protein concentration in the drop bulk phase of 10^{-2}% w/w. Different protein concentrations in the bulk phase give similar results. We find, for all experiments of WPI adsorption, two linear regions in the plot of $\ln[(\pi_{120} - \pi_\theta)/(\pi_{120} - \pi_o)]$ vs θ (Figure 5B) or in the plot of $\ln[(E_{120} - E_\theta)/(E_{120} - E_o)]$ vs θ (Figure 5C). It is important to apply eqs 9 and eq 10 only in the period after that affected by diffusion.

To summarize the effect of protein concentration and processing conditions (adsorption of protein in the drop or in the cuvette, effect of convection, and effect of heat treatment) on the time dependence of interfacial pressure during protein adsorption at the oil−water interface, the first-order rate constants derived from eqs 9 and 10 are collected in Table 2. The fit of the experimental data to the mechanism was made at a time interval based on the best linear regression coefficient (LR), which is included in Table 2.

It must be emphasized that plots of both eqs 9 and 10 give complementary results for protein adsorption at the oil−water interface. As with eq 9, a plot of eq 10 usually yields two linear regions associated with the processes of penetration and unfolding (with a first-

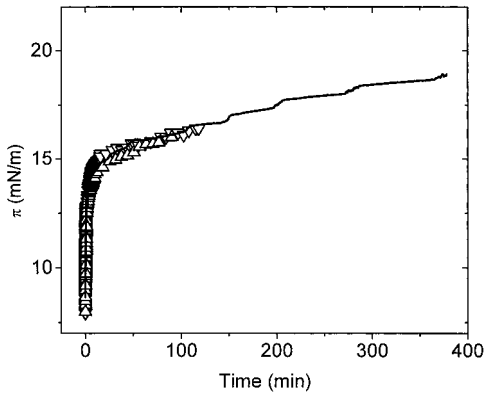

Figure 6. Time-dependent interfacial pressure for whey protein isolate adsorbed films at the oil−water interface at pH = 5, $I = 0.05$ M, and at 20 °C. Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. Protein concentration in the drop bulk phase: 10^{-2}%, w/w: (—) without convection, (Δ, ∇) with sinusoidal oscillation movement.

order rate constant k_1'), and rearrangement (with a first-order rate constant k_2'). The characteristic parameters derived from the application of eq 10 to results of WPI adsorption at the oil−water interface are included in Table 2. It was seen (data not shown) that the time intervals at which unfolding or molecular rearrangement is the mechanism that controls the adsorption are practically the same no matter which plot is used, that from either eq 9 or eq 10. However, because protein adsorption at fluid−fluid interfaces is very time-consuming (Rodríguez Patino and Rodríguez Niño, 1995), no attempt was made to discuss the experimental data for the second rearrangement step of previously adsorbed molecules.

5. Effect of Protein Concentration. To summarize the effect of protein concentration on the time dependence of π or E during WPI adsorption from the bulk phase, the first-order rate constants derived from eqs 9 and 10 are collected in Table 2. The following conclusions were drawn. The rate of WPI adsorption at the interface increased with WPI concentration in the aqueous phase. That is, at high WPI concentration in solution, the surface activity of WPI was high, which agrees with previous data in the literature for protein adsorption at the air−water interface (Rodríguez Patino and Rodríguez Niño, 1995; Rodríguez Niño et al., 1997a and b; Tornberg, 1978a; Phillips, 1981). Unfortunately, as far as we know, no measurements of surface coverage values are available for the systems studied here, but a qualitative agreement was observed recently for other globular proteins between air−water and oil−water interfaces in relation to their interfacial behavior (Benjamins et al., 1996). When an activation energy barrier to adsorption exists (as deduced previously from data in Figure 5A), the ability of the protein molecules to create space in the existing film and penetrate and rearrange at the interface is rate-determining. As can be seen in Table 2, k_1 and k_1' both increase with protein concentration. That is, penetration of protein at the interface is facilitated at higher protein concentrations in the bulk phase.

6. Effect of Interfacial Sinusoidal Oscillation. Figure 6 gives an example of the time dependence of the interfacial pressure for a concentration of WPI in the bulk phase of 1×10^{-2}%, w/w. It can be seen that the interfacial pressure and the time-dependent interfacial pressure for WPI adsorbed film in the presence

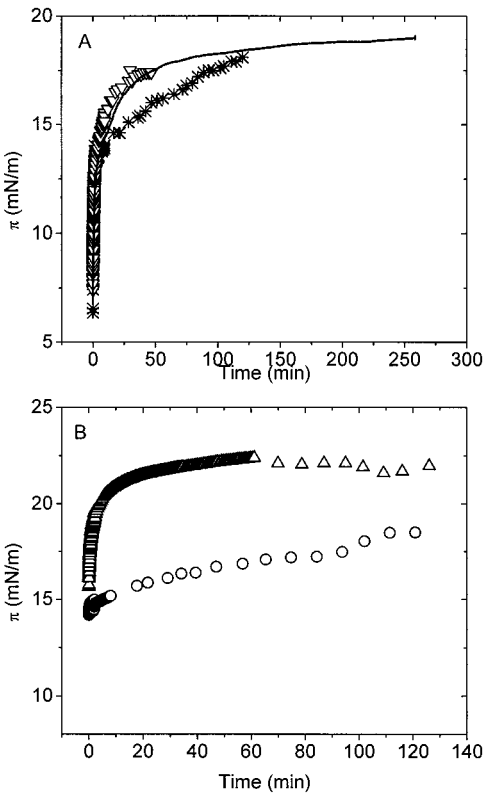

Figure 7. Time-dependent interfacial pressure for whey protein isolate adsorbed films at the oil−water interface at pH = 5, $I = 0.05$ M, and 20 °C. Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. (A) Protein concentration in the drop bulk phase: 10^{-2}%, w/w: (*) native protein, (∇) heat-treated protein at 80 °C for 60 min, with sinusoidal oscillation movement, and (—) heat-treated protein at 80 °C for 60 min, without sinusoidal oscillation movement. (B) Protein concentration in the drop bulk phase: 10^{-1}%, w/w: (○) native protein and (Δ) heat-treated protein at 80 °C for 60 min, without sinusoidal oscillation movement.

of convection is the same as that in the absence of convection. As reported by Damodaran (1989), the interfacial adsorption of proteins is not only dependent on diffusion to the interface, but the interfacial energy must be enough to overcome the activation energy for protein penetration and rearrangement into fluid−fluid interfaces. Thus, the surface forces, both of shear and dilational characteristics, may provide a means of altering protein interactions at the air−water interface (Phillips et al., 1995; Prins, 1988; Rodríguez Niño et al., 1997b) and, probably, at the oil−water interface as well. However, this effect was not observed from data in Figure 6, at least for the dilational deformation used in this work (at a frequency of 100 mHz and an amplitude of compression/expansion cycle of 15%).

7. Effect of Heat-Treated WPI. Figure 7 gives an example of the time dependence of the interfacial pressure for heat-treated and native WPI at two representative concentrations in the bulk phase. The characteristic parameters derived from application of eqs 9 and 10 to results of WPI adsorption at the oil−water interface are included in Table 2. The greater time dependence of the interfacial pressure may be associated with the fact that for heat-treated β-lactoglobulin, the amount of protein unfolding is maximum, although some protein aggregation could take place (de Wit and Swinkels, 1980; Cayot and Lorient, 1997).

The protein unfolding upon adsorption, especially for heat-treated protein, increases the accessibility of the

sulfhydryl group and the formation of intermolecular disulfide cross-links which are responsible for the high E value of heat-treated adsorbed WPI films (data not shown). As observed by Das and Kinsella (1990), the surface hydrophobicity—a measure of alteration of the native structure of a protein—of β-lactoglobulin increases with heating at 80 °C just as does the amount of protein adsorbed on emulsion droplets with the formation of multilayers. Similar behavior was observed by Dickinson and Hong (1994) for heat-treated β-lactoglobulin at 70 °C in relation to time-dependent surface shear viscosity and interfacial surface coverage in emulsion droplets. The effect of gelation on viscoelastic characteristics of adsorbed protein films at the oil–water interface is of theoretical and practical importance. This study is under way at present and will be published in a forthcoming paper.

CONCLUSIONS

In this paper we present surface dynamic properties (interfacial tension and surface dilational properties, i.e., surface dilational modulus, elastic and viscous component, and phase angle) of a whey protein isolate with a high content of β-lactoglobulin adsorbed at the oil–water interface as a function of adsorption time. The surface dynamic properties were measured as a function of WPI concentration in solution (ranging from 1×10^{-1} to 1×10^{-5}% w/w) and under different processing conditions (effect of convection and heat treatment). We found that the interfacial pressure and surface dilational modulus increase and the phase angle decreases with time, which should be associated with WPI adsorption. A normalization in a single master curve of E vs π data reflects the interfacial behavior of WPI adsorbed films for different protein concentrations, at different adsorption times, and under different processing conditions, which indicates that the interfacial behavior of WPI films is mainly due to the amount of adsorbed protein. The film displayed viscoelastic behavior which was practically elastic, especially at high adsorption time. The rate of WPI adsorption at the oil–water interface increases with protein concentration in solution, in the presence of convection, and for heat-treated protein. The adsorption kinetics of WPI at short adsorption times is controlled by the diffusion of the protein toward the interface, in agreement with the Ward and Torday model. However, at long-term adsorption, a first-order kinetic model is a satisfactory mathematical description of the rheokinetic data (π vs θ and E vs θ) for WPI adsorption and unfolding at the interface. These phenomena have been related to the protein unfolding and/or protein–protein interactions as a function of protein concentration in solution and processing conditions.

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