Dilatational and Shear Elasticity of Gel-like Protein Layers on Air/Water Interface

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Received September 29, 1999. In Final Form: January 3, 2000

We propose a simple new method for measuring the surface shear elasticity modulus (μ) together with the dilatational modulus (K) of gel-like protein layers on an air/water boundary. The stress response to compression/expansion of the interface in a Langmuir trough is measured at two different orientations of a Wilhelmy plate, collateral and perpendicular to the movable barrier in the trough. The interfacial tension is a tensorial quantity, whence the measured values depend on the direction of the length along which the stress acts. The fact that the deformation in the trough is uniaxial, i.e., a combination of dilatation and shear, is used to determine the respective two elastic moduli (K, μ). The experiment demonstrates that adsorbed layers of β-lactoglobulin (BLG), when subjected to small deformations, exhibit a predominantly elastic rheological behavior. This proves the existence of the two-dimensional gel, as a result from partial denaturation and unfolding accompanied with entanglement of the protein molecules on the interface. Layers of this kind exhibit finite shear elasticity ($\mu \neq 0$). Data are reported for systems containing BLG at different concentrations, and for mixtures including low molecular weight nonionic surfactant Tween 20. The elastic moduli are found to increase with rising protein content (at relatively higher concentrations), which is perhaps due to reinforcement of the gel-like structure. It is proved that in all cases the presence of Tween 20 brings about a complete fluidization of the adsorbed layer, in the sense that the shear elasticity disappears and the respective modulus (μ) becomes equal to zero. The frequency dependence of the elastic moduli is discussed in view of possible exchange of protein molecules from the interface with the bulk or with the adjacent subsurface layers.

1. Introduction

The interfacial rheology has been recognized to be an important factor for stability of emulsions and thin liquid films containing proteins. The low molecular weight surfactants stabilize the fluid interfaces by the Gibbs−Marangoni mechanism (which implies a certain degree of surface mobility), whereas the stabilizing role of the proteins is based on formation of a strong viscoelastic network in which the molecules are essentially immobile.[1] This network opposes the film stretching, damps the interfacial fluctuations, and slows down the thinning.[1] For these reasons, investigations on the surface rheology of proteins, which give information for the "stiffness" of the adsorption layers, are abundant in the literature.

The material properties are characterized by dilatational and shear surface viscosities, and the respective elasticity moduli, see ref 2 for a review. In this work we shall be concerned mainly with the elastic behavior of protein layers. Common methods for studying the shear rheology rely on measuring the rotational motion of a knife-edged bob, disk, or ring, which is placed in the plane of the interface (Couette-type viscometer).[2] Typically, the shear elasticity modulus (hereafter denoted by μ) lies in the range from 0.1 to 100 dyn/cm for protein layers (ref 2). Graham and Phillips[3] reported data for BSA and lysozyme at air/water and oil/water boundary. For example, with BSA the shear elastic modulus was determined as a function of the bulk concentration; μ was found to pass through a maximum (at $\approx 5 \times 10^{-3}$ wt %), and did not exceed 5 dyn/cm (ref 3).

The techniques for studying the dilatational rheology are based on measuring the interfacial tension (σ) while the surface area is subjected to changes. We distinguishe between "trough" methods and "drop" methods.[2] The investigation of a large liquid interface in a trough is often carried out by creating periodic lateral deformations which give rise to longitudinal waves.[4] A complex viscoelastic modulus is then obtained from the analysis of the measured σ; it may be resolved into a storage modulus (connected with the elasticity), and a loss part (giving information for the dissipation processes in the layer). The latter may be due either to a "true" surface viscosity, characterizing the friction within the layer, or to relaxation effects such as diffusional exchange of molecules between the surface and the bulk[4] ("apparent" viscosity). Reported values for the dilatational elasticity modulus (denoted hereafter by K) vary from about 10 to several hundred dyn/cm with different proteins, depending on the conditions.[5,6]

In this work we propose a simple method for simultaneous determination of the two elastic moduli, K and μ,

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(1) Mackie, A. R.; Gunning, A. P.; Wilde, P. J.; Morris, V. J. *J. Colloid Interface Sci.* **1999**, *210*, 157.

(2) Murray, B. S.; Dickinson, E. *Food Sci. Technol., Int.* **1996**, *2(3)*, 131.

(3) Graham, D. E.; Phillips, M. C. *J. Colloid Interface Sci.* **1980**, *76(1)*, 240.

(4) Lucassen, J.; Van den Tempel, M. *J. Colloid Interface Sci.* **1972**, *41(3)*, 491.

(5) Graham, D. E.; Phillips, M. C. *J. Colloid Interface Sci.* **1980**, *76(1)*, 227.

(6) Murray, B. S. In *Proteins at Liquid Interfaces*; Mobius, D., Miller, R., Eds.; Elsevier: Amsterdam, 1998; Chapter 5, p 179.

10.1021/la991287k CCC: $19.00
Published on Web 03/22/2000

in an experiment in a Langmuir trough. We utilize the fact that the deformation created by the moving barrier in the trough is uniaxial, i.e., it is a superposition of well-defined dilatation and shear. In the case of solidlike layers the interfacial tension is a tensorial quantity (see the next section), so that one can measure two different values of σ if the Wilhelmy plate is oriented parallel and perpendicular to the moving barrier. The fact that σ (and K) depends on the orientation of the Wilhelmy plate has been noticed by other authors.[5] However, this was regarded as an undesired effect and was not used for extracting information about the shear modulus, μ. Recently Wijmans and Dickinson[7] performed simulations of the surface rheology of protein layers, considering a model network of spherical particles connected with flexible bonds. It was found that the two normal components (eigenvalues) of the stress tensor were substantially different in the case of uniaxial deformation.[7]

We have to emphasize that a reliable simultaneous determination of K and μ by our procedure is possible only for solidlike layers which do not exhibit significant dissipation. If the latter circumstance does not hold, the data interpretation becomes rather complicated and ambiguous.

We focus on the milk protein β-lactoglobulin, whose ability to form entangled gel-like structures on liquid surfaces is well-known.[8] The effect of the presence of a low molecular weight nonionic surfactant (Tween 20) is also discussed below. In general, the addition of Tween 20 to systems containing protein-laden interfaces has been shown to bring about a substantial fluidization of the adsorbed layer; the surface viscosity is reduced by orders of magnitude.[2,9] If the surfactant concentration is not very high, this happens without appreciable replacement and desorption of the protein.[2,9] It has been surmised that disruption of intermolecular linkages and breakage of the gel-like protein structure take place.[2,9] Replacement actually occurs at a sufficiently high ratio of surfactant to protein content.[9] We demonstrate here that BLG layers in the presence of Tween 20 become completely fluid, in the sense that the shear elasticity vanishes ($\mu = 0$). The effect of surfactant is important to practice: e.g., in ice-cream making its role is to displace a fraction of the milk proteins from the o/w interfaces and induce partial coalescence of fat droplets on the surface of air cells.[8]

2. Theoretical Background

In this section we briefly outline some considerations which are necessary for understanding the experimental results.

The constitutive relation for surface elasticity is expressed by the Hooke's law (stress is proportional to the strain). In the case when the interface exhibits linear viscoelastic rheological behavior, terms proportional to the rate of deformation should also contribute to the stress tensor, τ, which can be written in the form[10]

$$\tau = K(Tr\mathbf{u})\mathbf{U}_{II} + 2\mu\left[\mathbf{u} - \frac{1}{2}(Tr\mathbf{u})\mathbf{U}_{II}\right] + \zeta_s(Tr\mathbf{D})\mathbf{U}_{II} + 2\eta_s\left[\mathbf{D} - \frac{1}{2}(Tr\mathbf{D})\mathbf{U}_{II}\right] \quad (1)$$

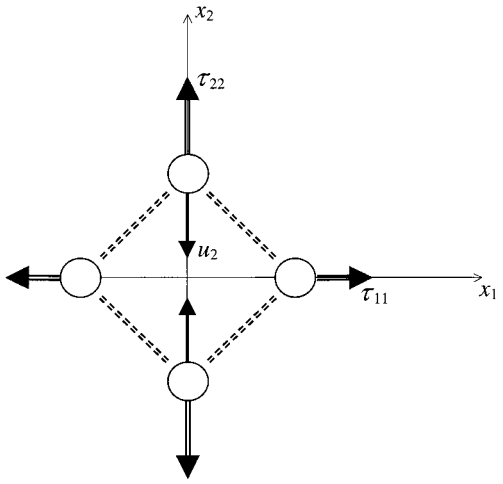

Figure 1. Schematic illustration which explains the occurrence of two main tensions (τ_{11} , τ_{22}) when a deformation along the axis 2 (u_{22}) is exerted on the protein structure (represented by the circles connected with dashed lines).

Here $\mathbf{u}$ is the strain tensor, $\mathbf{U}_{II}$ is the two-dimensional unit tensor, and K and μ represent the dilatational and shear elastic moduli, respectively.

$$\mathbf{u} = \frac{1}{2}[\nabla_{II}\underline{u} + (\nabla_{II}\underline{u})^T], \ \mathbf{D} = \frac{1}{2}[\nabla_{II}\underline{v} + (\nabla_{II}\underline{v})^T] \quad (2)$$

$\underline{u}$ is the displacement and $\underline{v}$ is the velocity of the surface material points, $\mathbf{D}$ is the rate-of-strain tensor, ∇_{II} is the two-dimensional gradient operator, and the symbol $(\,)^T$ denotes transposition. Besides, $Tr\mathbf{u} = \nabla_{II} \cdot \underline{u}$; $Tr\mathbf{D} = \nabla_{II} \cdot \underline{v}$. The constants ζ_s and η_s in eq 1 represent the dilatational and shear surface viscosities. On the basis of principal deformations eq 1 splits into the following two equations:

$$\tau_{11} = (K + \mu)u_{11} + (K - \mu)u_{22} + (\zeta_s + \eta_s)D_{11} + (\zeta_s - \eta_s)D_{22} \quad (3)$$

$$\tau_{22} = (K - \mu)u_{11} + (K + \mu)u_{22} + (\zeta_s - \eta_s)D_{11} + (\zeta_s + \eta_s)D_{22} \quad (4)$$

with τ_{kk} , u_{kk} , D_{kk} ($k = 1,2$) being the respective tensor components. Figure 1 illustrates the physical origin of the elastic stresses when a protein structure is distorted. If a deformation along the axis 2 (characterized by the displacement u_2 and the strain component $u_{22} = \partial u_2/\partial x_2$) is exerted on the entangled protein network, stresses along both axes 1 and 2 are evoked (τ_{11}, τ_{22}).

Let us now discuss the uniaxial deformation in the Langmuir trough, where the liquid interface is expanded/compressed by a laterally moving barrier. We shall be interested in the behavior typical for globular proteins (like BLG) which form a highly entangled and cross-linked network at the surface.[6] Such a network actually represents a macroscopically homogeneous elastic medium, so it is plausible to assume that the relative extension of length along the axis of deformation (x) is uniform throughout the whole area. The geometry of the system is sketched in Figure 2. Under these conditions, the expressions for the x, y components of the displacement vector (δu_x, δu_y) and the velocity (v_x, v_y) read

$$\delta u_x(x) = \frac{\delta L}{L}x; \ \delta u_y = 0; \ v_x(x) = \frac{\delta u_x(x)}{\delta t} = \frac{x}{L}\frac{\delta L}{\delta t}; \ v_y = 0 \quad (5)$$

(7) Wijmans, C. M.; Dickinson, E. *Langmuir* **1998**, *14(25)*, 7278.
(8) Dickinson, E. *J. Chem. Soc., Faraday Trans.* **1998**, *94(12)*, 1657.
(9) Courthaudon, J.-L.; Dickinson, E.; Matsumura, Y.; Clark, D. C. *Colloids Surf.* **1991**, *56*, 293.
(10) Landau, L. D.; Lifshitz, E. M. *Theory of Elasticity*; Pergamon Press: Oxford, 1984; *Fluid Mechanics*; Pergamon Press: Oxford, 1984.

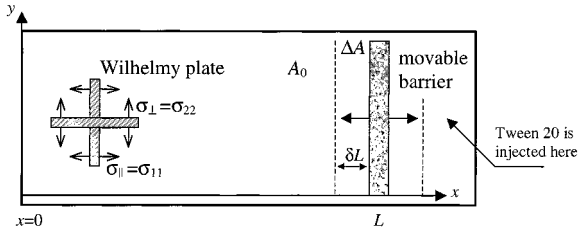

Figure 2. Geometric configuration of the system (layer in the Langmuir trough).

Here δL is an infinitesimal displacement of the barrier, and L is the length of the trough (from the barrier to the opposite wall); t denotes time. The nonzero components of the strain and rate-of-strain tensors are as follows:

$$u_{11} \equiv u_{xx} = \frac{\partial(\delta u_x)}{\partial x} = \frac{\delta L}{L} = \delta \ln A;$$

$$D_{11} \equiv D_{xx} = \frac{\partial v_x}{\partial x} = \frac{\delta \ln A}{\delta t} \quad (6)$$

(A is the area of the layer). Note that u_{11} and D_{11} are independent of the position in the surface. Next, we consider finite deformations, which are still small enough so that the linear theory (eq 1) retains its validity. For such a case we introduce the notation (cf. eq 6)

$$\alpha = u_{11} = \ln\frac{A}{A_0}; \dot{\alpha} = D_{11} = \frac{d \ln A}{dt} \quad (7)$$

where α has the meaning of relative dilatation, and A_0 is the area in a reference nondeformed state. With the help of eq 7, eqs 3 and 4 acquire the form

$$\tau_{11} = \sigma_\parallel - \sigma_{eq} = (K + \mu)\alpha + (\zeta_s + \eta_s)\dot{\alpha} \quad (8a)$$

$$\tau_{22} = \sigma_\perp - \sigma_{eq} = (K - \mu)\alpha + (\zeta_s - \eta_s)\dot{\alpha}. \quad (8b)$$

The stresses τ_{11} and τ_{22} are simply the respective deviations of the interfacial tensions σ_{11} and σ_{22} from the equilibrium value σ_{eq} (in the nondeformed state $\sigma_{11} = \sigma_{22} = \sigma_{eq}$). From Figure 2 it becomes evident that σ_{11} (or σ_{xx}) is in fact the interfacial tension which one would measure if the Wilhelmy plate is oriented parallel to the barrier, and σ_{22} (or σ_{yy}) corresponds to the perpendicular orientation. So, for the sake of clarity we use the symbols $\sigma_\parallel$ (=σ_{11}) and $\sigma_\perp$ (=σ_{22}) in eqs 8.

In our experimental setup the deformation is periodic; we apply compression−expansion cycles with low frequency (less than 0.03 Hz). It is worthwhile to point out that the speed with which the deformation propagates along the layer is rather high; from the data provided by Lucassen and van den Tempel[4] one can estimate this velocity to be about 10 cm/s. Given the dimensions of our trough (~20 cm, cf. the next section), we may conclude that the time required for the deformation induced by the barrier to reach any point is much shorter than the period of oscillations. Therefore, it seems reasonable to accept that the deformation is established almost instantaneously throughout the layer, i.e., the material follows the motion of the barrier.

Relaxation processes, e.g., the diffusional exchange of surfactant molecules between the interface and the bulk phase, are known to bring about a phase shift between stress and strain.[4] Such a shift would also arise from the dissipation connected with true surface viscosity. Here we mean the friction within the interfacial layer, characterized by constant coefficients, i.e., viscosities which do not depend on the rate of deformation (frequency). It is easy to show how ζ_s and η_s are connected with the phase shift: for a sinusoidal deformation, $\alpha = \alpha_{max} \sin(\omega t)$, eqs 8 yields $\tau_{11} = \tau_{11}^{max} \sin(\omega t + \xi_{11})$, $\tau_{22} = \tau_{22}^{max} \sin(\omega t + \xi_{22})$, where the phase angles ξ_{11} and ξ_{22} obey the following relations:

$$\tan(\xi_{11}) = \omega\frac{\zeta_s + \eta_s}{K + \mu}; \qquad \tan(\xi_{22}) = \omega\frac{\zeta_s - \eta_s}{K - \mu} \quad (9)$$

The construction of our Langmuir trough is such that during each experiment the barrier moves forward and backward with a constant speed, v_b (v_b can be varied in different runs). In this regime, if the deformation is small, the function $\alpha(t)$ is approximately linear (in other words, we have a triangular-shaped deformation). In the latter case, the stress response will also be triangular-shaped only in the absence of appreciable dissipation effects (when the viscosities are sufficiently small, and the interface behaves as a perfectly elastic body). Then, there should be no phase shift between stress and strain as well. Systems which possess such properties are discussed below.

The exchange of surfactant molecules between the adsorption layer and the adjacent bulk phase has been shown to influence the interfacial rheology considerably.[11,12] In general, it has been inferred that when the total number of adsorbed molecules, N, changes, the dilatation of the whole area, α, is not the appropriate quantity to characterize the deformation. The relative change in the *area per molecule*, ϵ, is the pertinent measure for the disturbances which determine the physical response.[11,12]

$$\epsilon = \ln\frac{a}{a_0}; \dot{\epsilon} = \frac{\mathrm{d} \ln a}{\mathrm{d}t} \quad (10)$$

where $a = A/N$, and a_0 refers to the nondeformed state. Thus, if N is not constant, eqs 8 should rather be written as

$$\tau_{11} = (K + \mu)\epsilon + (\zeta_s + \eta_s)\dot{\epsilon} \quad (11a)$$

$$\tau_{22} = (K - \mu)\epsilon + (\zeta_s - \eta_s)\dot{\epsilon} \quad (11b)$$

A connection between α and ϵ is easily derived from the material balance on the surface:

$$\frac{\mathrm{d}\Gamma}{\mathrm{d}t} + \dot{\alpha}\Gamma = \frac{1}{A}\frac{\mathrm{d}N}{\mathrm{d}t} = \Gamma(\dot{\alpha} - \dot{\epsilon}) \quad (12)$$

(the adsorption is $\Gamma = 1/a$). The relative dilatation, α, comes from the experiment, but to find ϵ may turn out to be a rather difficult task. Equation 12 suggests that the difference between $\dot{\alpha}$ and $\dot{\epsilon}$ originates from the flux of molecules to the interface. This flux depends on the particular hydrodynamic conditions for the transfer (diffusion, convection, etc.). What is important for our purpose is that the effects strongly depend on the rate of deformation (i.e., on the frequency, ν, for periodic changes). Therefore, we may anticipate that using α instead of ϵ in the constitutive relation (eq 11) (for nearly elastic layers)

(11) Horozov, T. S.; Danov, K. D.; Kralchevsky, P. A.; Ivanov, I. B.; Borwankar, R. P. *Proceedings of the First World Congress on Emulsion*; Paris, France, 1993; Vol. 2, p 137.

(12) Horozov, T. S.; Kralchevsky, P. A.; Danov, K. D.; Ivanov, I. B. *J. Dispersion Sci. Technol.* **1997**, *18*, 593.

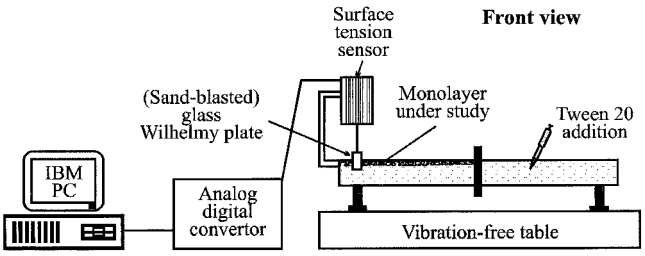

Figure 3. Scheme of the experimental setup (Langmuir trough).

would lead to apparent values of K and μ, which are affected by the frequency of deformation.

3. Experimental Section

Materials. β-Lactoglobulin (BLG, L-0130, Lot No. 114H7055, mixture of A and B variants), and Tween 20 (polyoxyethylene sorbitan monolaurate) were purchased from Sigma Co. (St. Louis, MO). Sodium azide (analysis grade) was used to prevent the appearance of bacteria during storage of the protein solutions. The water used throughout all experiments was obtained from a Milli Q purification system (Millipore, USA), and was checked for contaminants before each experiment, measuring the surface tension, σ_w , at the air−water boundary (by means of a Kruss tensiometer). In all cases σ_w turned out to be 72.3 ± 0.1 mN/m at ambient temperature (22 °C).

Method. Figure 3 shows the setup used in our experiments. It comprises a homemade Langmuir trough (manufactured from Teflon) with the following dimensions: width 211 mm, length 202 mm, depth 6 mm. The surface tension, σ, was measured by means of a sand-blasted glass plate (15 × 15 mm). The signals obtained from the surface tension sensor were converted by an analogue-digital converter (ADC) and recorded on a PC (Intel 486) for subsequent processing. The Langmuir trough only allows compression/expansion deformation at a constant rate of barrier translation in a given run. This rate may be changed in different runs.

Experimental Procedure. Before each experiment the Langmuir trough was thoroughly cleaned with chromic acid, and rinsed abundantly with Milli-Q quality water. The cleanness of the trough was additionally checked by compressing the bare air−water interface and measuring the surface pressure. If the surface pressure at maximum compression did not exceed 0.1 mN/m, the trough was assumed clean enough. After that we removed the water and poured the respective protein solution in. All solutions were freshly prepared (within no more than 24 h). In parallel, the surface tension of the same solution was measured by means of a Kruss tensiometer and the data were compared with those obtained from the pressure sensor of the Langmuir trough (see Figure 3). This precaution was taken in order to ensure that a quasi-equilibrium value of the surface tension had been reached in all cases before starting the deformations of the adsorbed protein layer. Bearing in mind the tendency of globular proteins to change very slowly their conformation on the interface, the term "quasi-equilibrium" here should be understood to mean reaching a more or less constant value of σ (absence of change for 15−20 min). This is achieved approximately 1 h after loading the aqueous solution in the trough.

In our experiments the pH was not specially regulated by additives. We worked at the "natural" pH, which results when the protein is dissolved in water. The pH of the solutions of β-lactoglobulin was measured at the three studied concentrations: at 1×10^{-3} wt % it was pH = 6.1−6.2; at 1×10^{-2} wt % it was pH = 6.2; at 1×10^{-1} wt % it was pH = 6.3−6.4. Thus, the natural pH does not essentially depend on the protein content. Regarding the influence of pH on the aggregation state of BLG, it is known that near the neutral point, pH ≈ 7, this protein in aqueous solutions exists in the form of dimers, held by noncovalent interactions.[8]

The deformation experiments were performed as follows: First, an area A_0 (Figure 2) was chosen in such a way that the deformation in both directions $\pm\Delta A$ did not exceed 5%. Second,

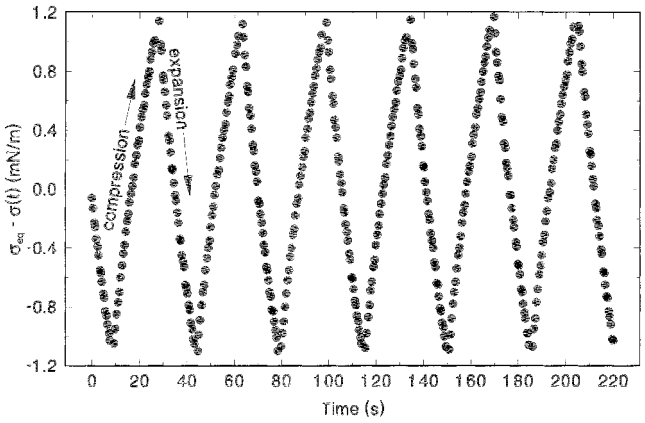

Figure 4. A typical reading of the sensor for the surface tension measurement, as a result of periodic compression/expansion of the layer. The system contains 0.1 wt % β-lactoglobulin. The barrier rate was set to 0.5 mm/s.

we chose a set of barrier rates at which the compression/expansion cycles would be carried out. The first measurement was performed at the lowest barrier rate, and the subsequent rates were changed in ascending order. Third, after completing a compression/expansion cycle (usually 2−3 compressions and 2−3 expansions) at a given rate with a collaterally positioned Wilhelmy plate, we carefully changed the plate orientation from collinear to perpendicular with respect to the moving barrier (see also Figure 2), and repeated the same compression/expansion cycle.

That scheme of performing experiments was kept during all studies. After having finished the planned compression/expansion cycles with the protein solution, we injected 1 mL of Tween 20 solution, so as to obtain an overall bulk concentration of 1×10^{-5} M (around 6 times below the cmc of this surfactant, which was reported to be cmc = 6×10^{-5} M[13]). This quantity was always put in the bulk, beyond the barrier, at the opposite side of the Langmuir trough compartment with respect to the Wilhelmy plate (see Figures 2 and 3). Thus, we prevented any direct leakage of Tween 20 solution behind the barrier, toward the Wilhelmy plate. Such a leakage may happen by surface convection, when the needle carrying the solution of Tween 20 touches the a/w interface. We wanted the adsorption of Tween 20 to take place only by bulk diffusion and subsequent replacement of the protein by the more surface active molecules of the nonionic surfactant.

In a separate experiment we have checked what would happen if even a minute drop of Tween 20 solution gets directly on the surface at the same side of the Langmuir trough compartment where the Wilhelmy plate is. Then, within a second or so the surface pressure jumps up with about 15 mN/m, which is a direct consequence of the Tween 20 molecules compressing and replacing the existing protein network because of the much higher surface pressure of the surfactant layer (Marangoni effect).

Once the Tween 20 solution had been injected in the trough (in the presence of the already formed layer of protein), the system was left overnight for a thermodynamic equilibrium to be established. On the next day we proceeded further with repeating the compression/expansion cycles in the presence of the protein−surfactant mixture. Besides, no modification of the three-phase contact angle on the Wilhelmy plate was noticed (a problem quite often encountered in studies with proteins).

4. Results and Discussion

Dilatation and Shear Elastic Moduli. Figure 4 shows typical results for the surface tension changes during a compression/expansion cycle (in a system with BLG). The rate of the barrier translation was 0.5 mm/s. What is impressive at first sight is the high degree of reproducibility and symmetry between the separate compressions and expansions. There is no appreciable deviation from the straight lines, which means that the stress response

(13) Harlow, E.; Lane, D. *Antibodies: A Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, 1988.

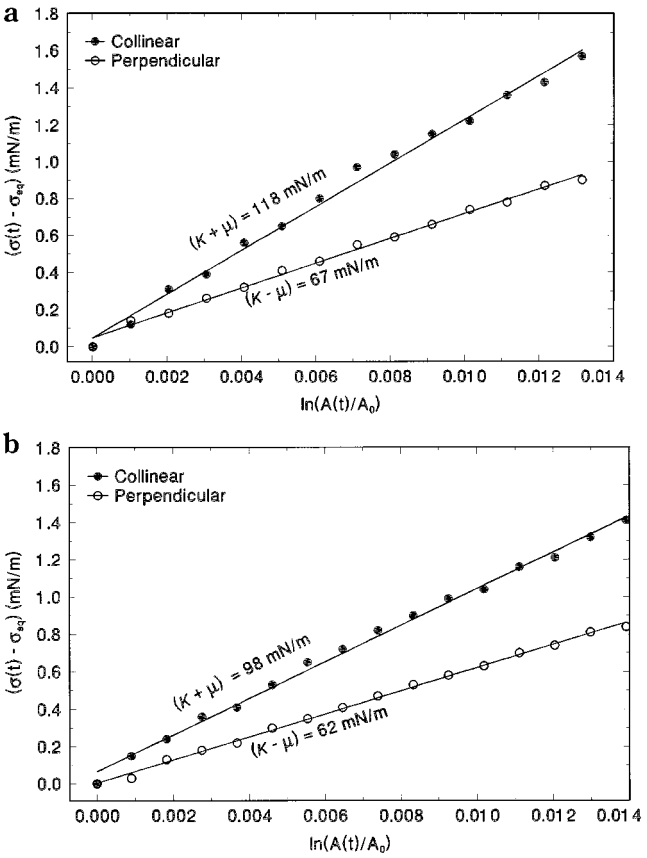

Figure 5. Results from stress vs deformation measurements. The two elastic moduli (K, μ) are obtained according to eqs 8, from the slopes of the straight lines $\tau_{11}(\alpha)$, $\tau_{22}(\alpha)$. They correspond to experiments with collinear and perpendicular orientation of the Wilhelmy plate with respect to the movable barrier. The system contains 0.1 wt % β-lactoglobulin. $T = 22$ °C. (a) expansion; (b) compression.

Table 1. Data for the Dilatational and Shear Elastic Moduli of Interfacial Layers at the Air/Water Boundary

experimental system	frequency of deformation ν (Hz)	expansion K dyn/cm	expansion μ dyn/cm	compression K dyn/cm	compression μ dyn/cm
	1.154×10^{-3}	66	10	54	12
	2.048×10^{-3}	80	15	63	11
	2.868×10^{-3}	79	21	65	13
β-LG	3.688×10^{-3}	86	16	71	16
1×10^{-1} wt %	8.196×10^{-3}	90	18	83	13
	1.229×10^{-2}	92	20	83	13
	2.048×10^{-2}	94	17	88	15
	2.868×10^{-2}	98	19	87	15
+ Tween 20	1.297×10^{-2}	65	0	45	0
1×10^{-5} M	2.121×10^{-2}	75	0	49	0
β-LG	2.048×10^{-3}	78	25		
1×10^{-1} wt %	4.835×10^{-3}	88	22	49	3
+ 0.01 M DTT	1.297×10^{-2}	91	22	70	15
	2.121×10^{-2}	79	30	74	17
	2.048×10^{-3}	51.6	3.9	73	4
β-LG	4.835×10^{-3}	60.6	12.9	56	7
1×10^{-2} wt %	1.297×10^{-2}	61	12.9	55	5
	2.121×10^{-2}	63.8	15.8	56	6
	2.048×10^{-3}	54	0	71	1
+ Tween 20	4.835×10^{-3}	63	0	67	2
1×10^{-5} M	1.297×10^{-2}	75	0	67	0
	2.121×10^{-2}	80	0	68	0
	2.048×10^{-3}	83.5	17.3	67	
β-LG	4.835×10^{-3}	112.6	33.1	84	17
1×10^{-3} wt %	1.297×10^{-2}	131.6	32.8	83	25
	2.121×10^{-2}	149.4	38.2	80	24
+ Tween 20	4.835×10^{-3}	68	0	73	0
1×10^{-5} M	1.297×10^{-2}	81	2	73	0
Tween 20	4.835×10^{-3}	56	0	56	0
1×10^{-5} M	1.297×10^{-2}	65	0	55	0
	2.121×10^{-2}	72	0	54	0
Tween 20	4.835×10^{-3}	58	0	55	0
1×10^{-5} M	1.297×10^{-2}	69	0	52	0
(second experiment)	2.121×10^{-2}	74	0	53	0

upon compression or expansion is linear (cf. also Figure 5). In addition, the response exactly follows the deformation. Let us recall that in our experiments the relative dilatation changes linearly with time; see Section 2 above. Another fact, which is worthwhile to be pointed out, is that in no experiment performed with β-lactoglobulin did we detect any delay (phase shift) of the stress with respect to the strain. These findings, along with the perfect reproducibility of the consecutive compression/expansion cycles, leads us to believe that any dissipation effects are sufficiently small in this case, and the macroscopic behavior of the BLG-laden interface resembles that of a purely elastic sheet.

To obtain quantitative information about the elastic moduli of the protein layer, we plotted the surface tension change as a function of the relative area dilatation ($\alpha = \ln(A/A_0)$; eq 7). Figure 5 shows two examples of such graphs; we work at small deformations, $\Delta A/A_0$ below ~2%. In this manner we processed all our data, obtaining the respective elastic moduli, K and μ, from the slopes of the lines ($\sigma_{eq} - \sigma(t)$) vs $\ln(A(t)/A_0)$, according to eqs 8 (without the dissipative terms). A full list of those moduli for different systems is presented in Table 1. As it was predicted by the theoretical model described in Section 2, we detected a remarkable disparity in the system response to the deformation depending on the orientation of the Wilhelmy plate. As can be seen from Figure 5a and Figure 5b, there is a considerable difference between the slopes of the two straight lines (for $\sigma_{||}$ and $\sigma_{\perp}$), which reflects the contribution of the shear elasticity.

The interfacial shear elasticity is important, e.g., for the stability of emulsions.[2,8] Higher modulus μ improves the resistance of the emulsion droplets against coalescence.

The contributions of the viscous terms in eqs 8 to the stresses in the layer are connected with the intercepts of the lines in Figure 5, since in our experiments $\dot{\alpha}$ is constant. Bearing in mind the scattering of the data (Figure 5), the intercepts could be at most ~0.1 dyn/cm. In our trough 5% relative area deformation is realized for a time interval $1/(4\nu)$, whence $\dot{\alpha} \approx 0.2\nu$, and for frequency of the order of $\nu = 0.005$ Hz this gives $\dot{\alpha} \approx 0.001$ sec^{-1}. The possible magnitude of the intercept (if it exists, Figure 5) is consistent with viscosity coefficients ζ_s and η_s smaller than about 100 dyn sec/cm (cf. eq 8). For the sake of comparison, it may be mentioned that Graham and Phillips[3] reported values of η_s between 5 and 10^4 dyn sec/cm with BSA and lysozyme. Therefore, our results indicate that the layer viscosity may be considerable, but its influence on the shape of the experimental curves (Figures 4 and 5) is rather weak, i.e., the method is not sensitive to the viscous effects. In the trough experiments the protein layer is subjected to small homogeneously distributed deformation, and under these conditions the macroscopic rheological behavior of the layer is predominantly elastic. Some effects connected with dissipation have actually been observed (and are discussed below), but the main contribution to the stress response is due to elasticity. Besides, the latter circumstance allows us to determine the elastic moduli with a satisfactory precision using a conceptually very simple method.

In our study we have assumed (and experimentally proved) that the surface pressure change does not depend on the choice of A_0 (in other words, the layer is macroscopically homogeneous, and the surface tension is considered to be uniform along the whole length of the Langmuir trough; cf. eqs 5 and 6 in Section 2). We carried out experiments applying the same procedure and only changing the initial value of A_0. The results for the surface dilatational and shear elastic moduli were not affected (within the frames of the experimental uncertainty). With most of the studied systems (Table 1) we have carried out several independent experiments (with newly prepared solutions, with the Langmuir trough having been cleaned again, etc.). Correspondingly, the numbers in Table 1 are average values. The overall uncertainty in the measured elastic moduli may be estimated to be about $\pm 3-4$ dyn/cm. On the other hand, it should be mentioned that the deviations of the results between different runs (compressions/expansions) in the same experiment with a given system are considerably smaller; these deviations are typically below 1 dyn/cm.

From Table 1 we see that at the lowest concentration of BLG (1×10^{-3} wt %) the values of K for expansion are very high. This can be attributed to incomplete adsorption: the molecules are far from each other, and possibly the protein network is formed by interconnecting already extended chains of partially unfolded molecules. Such a network would produce higher stress when stretched further. At 1×10^{-2} wt % BLG K is lower (about 60 dyn/cm for frequencies of the order of 0.005 Hz; the frequency dependence of the elastic moduli will be discussed separately below). Probably, when the neighboring molecules are closer the extension of the layer is accompanied by additional mechanical unfolding without generating too much stress. The fact that with rising protein concentration K decreases seems strange, but is in consonance with the findings of ref 3 (K passes through a maximum when the concentration increases[3]). Of course, at vanishing protein content K should ultimately go down to zero.

At 1×10^{-1} wt % BLG K increases to about 90 dyn/cm (compared to 60 dyn/cm at 1×10^{-2} wt %). We can interpret this effect of the protein content in view of a strengthening of the network structure at the highest concentration of BLG. In general, the dilatational and shear moduli of BLG layers turn out to be somewhat higher for expansion compared to the case of compression (Table 1). Although the differences are not large, they are still appreciable. It can be conjectured that K and μ are influenced to some extent by mass exchange with the bulk phase or with the subsurface layers adjacent to the a/w boundary. Murray[6] emphasized that the dominant process in the dilatational rheology of food proteins is the adsorption of new molecules to the interface, rather than the rearrangement of existing molecules in the layer. In our systems such an exchange is however not intensive enough to cause deviation of the stress response from linearity (at small deformations), or to bring about a phase shift.

We decided to check the properties of the BLG layers at larger deformations (15%). Data are presented in Figure 6, where the deviation of the signal from linearity is noticeable (one may compare with Figure 4, taken under the same experimental conditions except that $\alpha_{max} = 5\%$). We believe the increasing magnitude of the deformation induces a more intensive mass transfer of protein molecules from/to the bulk and/or the subsurface layer (or even from/to adjacent loosely packed multilayers). Other possibilities to explain the peculiar shape of the curves in Figure 6 are (i) different interactions between the protein molecules when the distance between them changes, or (ii) failure of Hooke's law at large deformations. These effects, however, are proportional to the deformation, and therefore, they seem to be inconsistent with the change of slope at the maxima of the curves in Figure 6. On the other hand, despite the deviations from linearity, the curves in Figure 6 are reproducible at consecutive expansions/compressions. The latter fact testifies that the eventual mass exchange is reversible at this time scale.

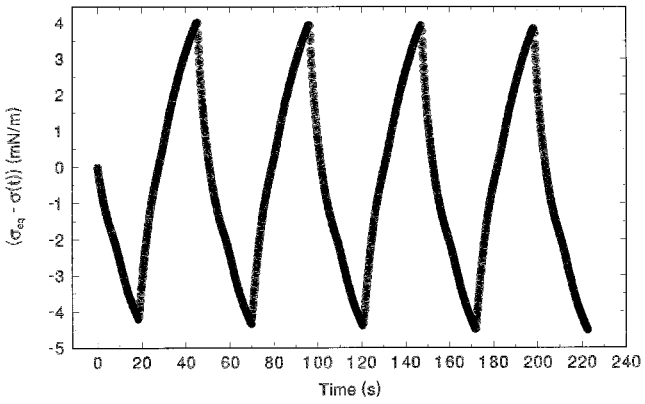

Figure 6. Stress response to periodic compression/expansion deformations of larger magnitude (the relative dilatation is 15%). The system contains 0.1 wt % β-lactoglobulin. The barrier rate was set to 0.5 mm/s.

In some experiments at the lowest barrier rates, even at small surface deformations, we do observe such deviations from linearity, probably due to comparable rates of deformation and protein adsorption/desorption. For that reason, all experimental results were processed only up to 2% relative area deformation.

Influence of Surfactant. In many food emulsions stabilized by milk proteins an essential additive is the low molecular weight nonionic surfactant Tween 20 (polyoxyethylene sorbitan monolaurate). Until now, many authors have studied its influence on the rheological properties of surface layers,[14−16] revealing that the Tween 20 decreases to a great extent the interfacial viscosity, and thus affects the overall emulsion stability. For example, Courthaudon et al.[9] have found that the surface shear viscosity (η_s) of 1×10^{-3} wt % solution of BLG is about 600 dyn sec/cm (at pH = 7, after 5 h of adsorption), and falls to zero when Tween 20 is added to the system in equimolar ratio (see Figure 1 in ref 9). Similar effects were reported by Krägel et al.;[17] Dickinson and coworkers[16] have also carried out a detailed investigation on that problem. In view of its importance, we decided to explore the impact of Tween 20 on the rheological properties of a protein layer, expecting a strong effect especially on the surface shear elasticity modulus. It has been reported in the literature[2,8] that the dilatational elasticity modulus is less sensitive to changes in the structure of the protein layer, in comparison with the surface shear elasticity modulus.

Mackie et al.[1] have demonstrated that Tween 20 replaces the proteins by the so-called "orogenic" mechanism: the surfactant is first adsorbed at defects in the protein network, the nucleated patches then grow, com-

(14) Krägel, J.; Clark, D. C.; Wilde, P. J.; Miller, R. *Prog. Colloid Polym. Sci.* **1995**, *98*, 239.
(15) Clark, D. C.; Husband, F.; Wilde, P. J.; Cornec, M.; Miller, R.; Krägel, J.; Wüstneck, R. *J. Chem. Soc., Faraday Trans.* **1995**, *91*(*13*), 1991.
(16) Dickinson, E.; Gelin, J.-L. *Colloids Surf.* **1992**, *63*, 329.
(17) Krägel, J.; Wüstneck, R.; Clark, D.; Wilde, P.; Miller, R. *Colloids Surf. A* **1995**, *98*, 127.

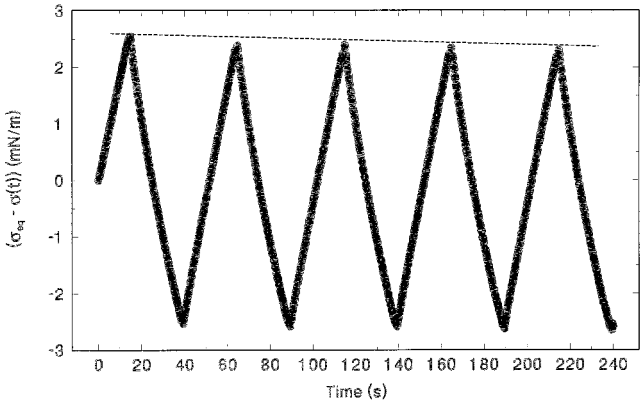

Figure 7. Stress response to periodic compression/expansion deformations. The system contains a mixture of 0.1 wt % β-lactoglobulin and 1×10^{-5} M Tween 20. The rate of barrier translation was set to 0.5 mm/s. There is a slight decrease of σ in a time scale longer than that of the oscillations. This is most probably a result of the mass transfer of Tween 20 molecules from the bulk to the interface.

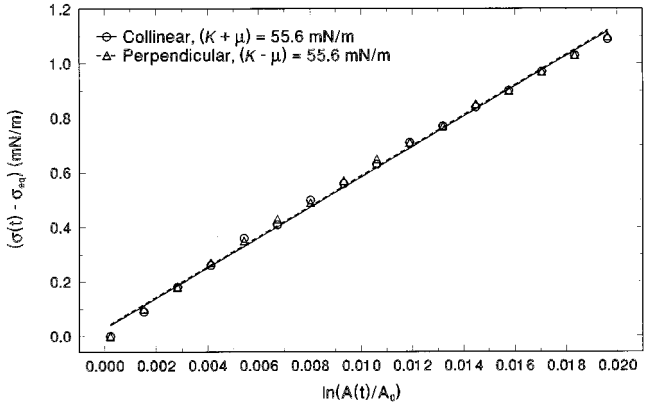

Figure 8. Stress response to expansion in a system containing 0.1 wt % β-lactoglobulin and 1×10^{-5} M Tween 20. There is no difference between the slopes of the linear $\sigma[\ln(A/A_0)]$ dependencies for collateral and perpendicular orientations of the Wilhelmy plate. This is due to the lack of any surface shear elasticity ($\mu = 0$). The layer is fluidlike.

pressing the gel-like structure until the latter is destroyed, and finally protein desorption occurs. We anticipated the protein displacement by low molecular weight surfactant to be accompanied by substantial fluidization of the layer, whence the surface shear elasticity should fall drastically.

Figure 7 presents the results from a compression/expansion cycle with a system comprising 0.1 wt % β-lactoglobulin and 1×10^{-5} M Tween 20. As can be seen, probably because of the relatively small magnitude of the deformation (below 5%) and the high barrier rate (0.5 mm/s), the linearity of the stress response is preserved. However, there is a drift of the baseline; the surface tension gradually decreases, in a time scale much longer than the period of the oscillations. We believe this drift may be due to the exchange of Tween 20 molecules with the bulk. Besides, the linearity of the curves probably stems from the relative slowness of this transfer. Kragel et al.[17] have measured the time evolution of the surface tension of Tween 20 solutions and have found a slow change during more than 1 h (at 5×10^{-6} M). We have also performed trough experiments with pure Tween 20 solutions, and obtained results quite similar to those from Figure 7.

What, however, is more important in our case is that the surface shear elasticity modulus, μ, determined from the $\sigma(\alpha(t))$ curves (e.g., Figure 7) falls dramatically in the presence of surfactant (cf. Table 1). In no system containing Tween 20 did we observe $\mu \neq 0$. Figure 8 proves that the stress response does not depend on the orientation of the Wilhelmy plate, which means that $\mu \equiv 0$. Therefore, once the entangled protein network breaks, the layer becomes entirely fluidlike, not possessing any surface shear elasticity. Another manifestation of the dominance of Tween 20 can be seen from the comparison between different mixtures (BLG + Tween 20) and a pure Tween 20 solution (Table 1). There is no marked difference (in the framework of the experimental error) between the elastic properties of the layer in the mixed systems and the layer entirely built up from Tween 20 molecules.

As we pointed out in the Experimental Procedure Section, if Tween 20 is allowed to spread over an adsorbed protein layer it literally blows the protein away, due to the differences in the surface pressure. This finding supports the orogenic mechanism of replacement, put forward in ref 1. In adsorption layers first built from BLG molecules and after that equilibrated with Tween 20 (as described in the Experimental Section) we do not exclude the possibility that some protein remains on the surface.

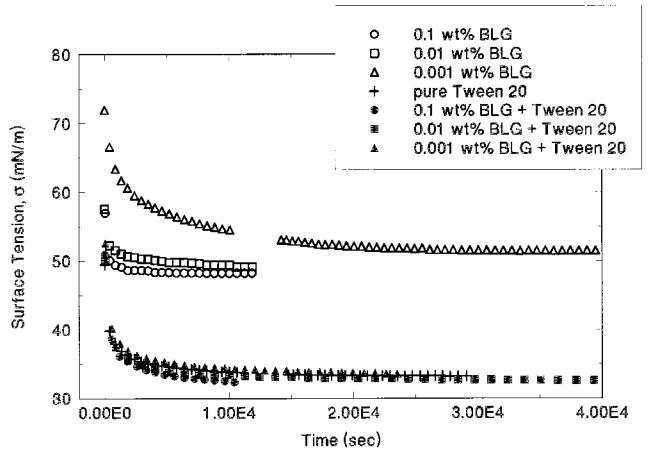

Figure 9. Kinetic measurements of the surface tension, σ, for protein solutions with three different concentrations (0.1, 0.01, and 0.001 wt % BLG), solution of 5×10^{-5} M Tween 20, and in mixed systems containing a fixed amount of the nonionic surfactant (5×10^{-5} M Tween 20) and various protein concentrations.

However, the contribution of this protein to the interfacial tension, σ, and to the elastic properties of the layer seems to be negligible. Evidently, since $\mu = 0$, the cross-linked protein network is destroyed by the surfactant; the latter is efficient in hindering the short-range interactions between the protein molecules. This inference is in line with the known ability of BLG to bind Tween 20 and form a molecular complex.[16,18]

Figure 9 presents our kinetic measurements of σ with pure Tween 20, BLG, and in mixed systems. It is obvious that for all mixtures the presence of protein practically does not influence the surface tension: the measured σ almost coincides with that of the layer of pure Tween 20. Such will be the case if the protein molecules are either collected in compact islands floating in the surfactant layer, or are dispersed sparsely as single entities among the molecules of Tween 20.

Frequency Dependence of the Elastic Moduli. The effects connected with the rate of deformation are illustrated in Figures 10 and 11, where K and μ are plotted as functions of ν. The first thing to be noticed is that in almost all cases the respective elastic parameter tends to

(18) Clark, D. C. In *Characterization of Food: Emerging Methods*; Gaonkar, A. G., Ed.; Elsevier: Amsterdam, 1995; Chapter 2, p 23.

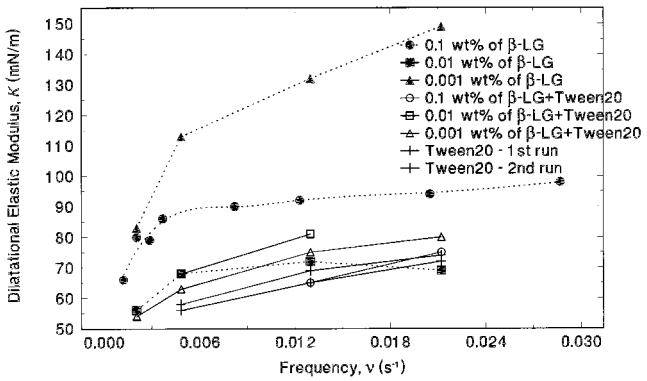

Figure 10. Surface dilatational elasticity modulus, K, as a function of the frequency of the periodic deformation ($\nu = |\nu_b|/[4(L_{max} - L_0)]$).

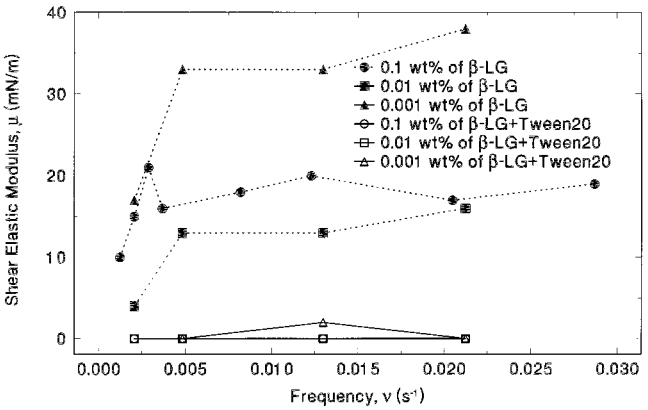

Figure 11. Surface shear elasticity modulus, μ, as a function of the frequency of the periodic deformation ($\nu = |\nu_b|/[4(L_{max} - L_0)]$).

approach a plateau value at high frequencies. This fact might be considered in the context of the competition between the adsorption/desorption of protein molecules to/from the interface and the area deformation. Obviously, at higher frequencies the exchange does not to follow the periodic disturbances: the protein layer behaves as if constituted of virtually insoluble species. Serrien et al.[19] have also discussed the frequency dependence of the elastic properties (K) of protein layers (BSA). Their curves resemble ours (Figure 10), reaching plateau values. However, the authors of ref 19 did not consider the exchange of molecules from the interface. Instead, they attributed the effect of the rate of deformation to a surface reaction−transformation of the protein molecule to another form (unfolding was envisaged). In our opinion, it is unlikely that such a transformation may manifest itself for frequencies of the order of $\nu \approx 0.005$ Hz, because the process of protein reconfiguration is known to be rather slow[20] (even taking several hours). It seems possible, however, that an exchange of protein molecules from the interface with those residing in the adjacent loosely packed multilayers (subsurface) might be regarded as an effective reaction. We have carried out ellipsometric measurements of the kinetics of BLG adsorption at the air/water boundary (unpublished results). The data demonstrate that for a time period of about a minute the surface coverage (Γ) attains half of its final (equilibrium) value. Therefore, such a relatively fast exchange may explain the effects observed at frequencies which correspond to characteristic time of a few minutes.

Second, from the data in Figures 10 and 11 we observe that at the lowest protein concentration the surface dilatational elasticity modulus does not reach a plateau value; in addition, K and μ are the highest at 1×10^{-3} wt % BLG. We think the reason for that is connected with the unsaturated protein layer in the case of 1×10^{-3} wt % BLG. The indication for incomplete coverage is provided by the fact that in the presence of 1×10^{-3} wt % β-lactoglobulin the equilibrium surface tension, σ_{eq}, is more than 2 mN/m higher compared to the respective values in the systems containing 1×10^{-1} and 1×10^{-2} wt % BLG (cf. Figure 9). Thus, the larger stress response in the trough, found at 1×10^{-3} wt % BLG, develops in a relatively more diluted layer. On the other hand, Dickinson and co-workers[21] have applied neutron reflectivity to measure the thickness of adsorbed layers of β-lactoglobulin. With increasing the protein bulk concentration a pronounced thickening of the surface layer was registered.[21] Comparing the curves which correspond to β-lactoglobulin concentrations 1×10^{-1} wt % and 1×10^{-2} wt % in Figures 10 and 11, it can be said that the thicker the layer, the higher its elasticity (and the strength of the gel-like network).

In the presence of Tween 20 we see that there is little difference (in the framework of the experimental error) between the values of the surface dilatational elastic modulus K in the studied systems with BLG (cf. Table 1 and Figure 10). Moreover, these values are close to the value of K measured for a system containing Tween 20 only (1×10^{-5} M). This finding could be interpreted as a result of the considerable extent of protein replacement from the interface, due to the high surface activity of the Tween 20 molecules. Confirmation of such a conclusion may also be seen in the results of Clark et al.,[15] who have observed that the dilatational elastic moduli of 1×10^{-5} M Tween 20 and its mixture with 4×10^{-4} wt % BLG virtually coincide (see Figure 6 in ref 15).

Importance of Disulfide Bonds. Finally, we would like to comment on the role of the covalent bonds for the strength of the protein network. β-lactoglobulin contains a hidden sulfhydryl group, which may be engaged in entanglement of the neighboring molecules via disulfide linkages.[2,8] In ref 22 it has been suggested that a two-dimensional gel of BLG is formed as a result of polymerization via sulfhydryl−disulfide interchange. On the other hand, the −S−S− bridges can easily be disrupted under the action of appropriate reducing agent, e.g., dithiothreitol (DTT). The latter is famous for its ability to break existing disulfide bonds, as well as its ability to prevent the formation of such bonds in bulk protein solutions.[2]

We performed a separate experiment adding DTT to a system with BLG (Table 1). The results imply that no significant change in the dilatational and shear elasticities of the BLG layer is induced by the presence of DTT. Hence, the disulfide bonds do not seem to be involved to a considerable extent in the formation of a strong gel-like protein structure. Probably, other intermolecular interactions, such as the hydrophobic interactions, hydration, or hydrogen bonds, are more important in this respect. The formation of −S−S− bridges is favored when the protein solution is heated for ~30 min at ~85 °C,[23] or at basic pH. Our solutions of BLG were neither preheated nor kept at

(19) Serrien, G.; Geeraerts, G.; Ghosh, L.; Joos, P. *Colloids Surf.* **1992**, *68*, 219.
(20) Graham, D. E.; Phillips, M. C. *J. Colloid Interface Sci.* **1979**, *70*(*3*), 403.
(21) Atkinson, P.; Dickinson, E.; Horne, D.; Richardson, R. *J. Chem. Soc., Faraday Trans.* **1995**, *91*(*17*), 2847.
(22) Dickinson, E. *J. Dairy Sci.* **1997**, *80*(*10*), 2607.

pH > 7. Thus, it is perhaps not surprising that no significant contribution of disulfide linkages to the surface elasticity manifests itself.

5. Conclusions

We propose a simple new method for measuring the surface shear elasticity modulus (μ) together with the dilatational modulus (K) of gel-like protein layers on the air/water boundary. The stress response to deformation of the interface in a Langmuir trough is measured at two different orientations of the Wilhelmy plate; collateral and perpendicular to the movable barrier. The fact that the deformation is a superposition of dilatation and shear is utilized to extract the values of K and μ. It is proved that layers of β-lactoglobulin (BLG) exhibit predominantly elastic rheological behavior (at small deformations). Data are provided for systems containing BLG at different concentrations, and for mixtures with the nonionic surfactant Tween 20. The elastic moduli are found to increase with rising protein content (in relatively more concentrated systems), which is perhaps a result of reinforcement of the gel-like structure.

It is proved that in all cases the presence of low molecular weight nonionic surfactant (Tween 20) brings about a complete fluidization of the adsorption layer, in the sense that the shear elasticity disappears and the respective modulus (μ) becomes equal to zero. Thus, the surfactant destroys the entangled protein network, perhaps by hampering the short-range specific interactions between the protein molecules. The frequency dependence of the elastic moduli is discussed in view of possible mass exchange with the bulk or subsurface (or with adjacent loosely packed protein multilayers).

The interfacial shear elasticity (and the existence of a strong entangled network capable of enduring considerable stresses) is probably due to a complex interplay of hydrophobic interactions, hydration, and hydrogen bonds. The disulfide linkages between BLG molecules seem to be of lesser importance.

Acknowledgment. This work was financially supported by Kraft Foods, Inc. (USA). The authors are indebted to Prof. Ivan B. Ivanov for fruitful discussions, and to Mrs. Violeta A. Oudvareva for help in carrying out some of the experimental measurements.

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(23) Dickinson, E.; Chen, J. *J. Dispersion Sci. Technol.* **1999**, *20*(*1&2*), 197.