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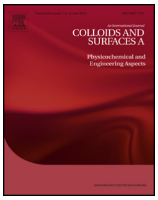

β-Lactoglobulin adsorption layers at the water/air surface: 2. Dilational rheology: Effect of pH and ionic strength

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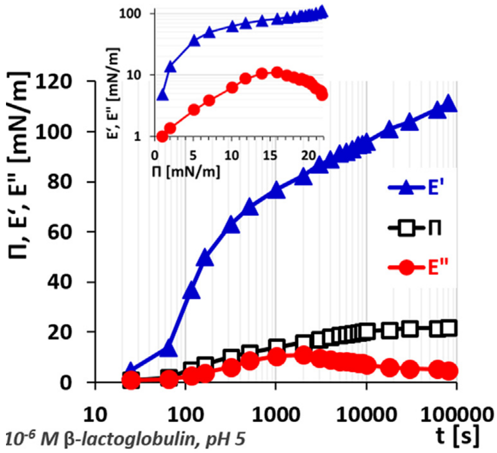
HIGHLIGHTS

- Real part E' of dilational modulus monotonically increases with surface pressure Π.
- E' vs. Π data follow master curves for pH 3 and 7.
- E' vs. Π data shift from a master curve for pH 5.
- Imaginary part E" of dilational modulus vs. Π data exhibits a maximum.
- Height of the E"(Π) maximum depends on the protein concentration for any pH.

GRAPHICAL ABSTRACT



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ABSTRACT

The dynamic surface pressure Π and the two components E' (real part) and E" (imaginary part) of the dynamic surface dilational modulus of adsorbed β-lactoglobulin (BLG) layers at the water/air interface were measured by oscillating drop/bubble profile analysis tensiometry with the aim to enlarge information on the surface rheology of solutions of this practically important protein. The effects of the solution pH and ionic strength (represented by the buffer concentration C_{buff}) on the kinetic dependences of Π, E' and E" were monitored for various protein concentrations in the range 5×10^{-9}–2×10^{-4} M. Combining the E'(t) and E"(t) data with the Π(t) data allowed for obtaining the dependences E'(Π) and E"(Π). The real part E' was found to increase monotonically with increasing Π, while the E"(Π) data exhibit a maximum in the surface pressure range 14–18 mN/m. The height of the maximum in the E"(Π) dependence was found to increase with increasing protein concentration. Apparently, in these interfacial layers, relaxation processes take place and their relaxation strengths depend on the history of the layer formation, i.e. the rate of adsorption. After the maximum in the E"(Π) dependence the E"-values progressively diminish, which can be assumed to occur due to solidification of the protein gel-like network accompanied by less energy dissipation

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and high elasticity of the interfacial layer. BLG layers at pH 5 (close to the isoelectric point pI ≈ 5.1) exhibit the highest E'-values and the lowest E''-values measured in this study, which suggest the formation of a strong protein network at these (isoelectric) conditions. Variations of the buffer concentration strongly influence the kinetic dependences of E' and E'' for BLG layers at pH $\neq$ pI, while at pH → pI no effect was detected. The viscoelastic characteristics of BLG layers adsorbed from buffer-free solutions containing NaCl or $CaCl_2$ were also studied. For the first time, hysteresis in the frequency dependence of the surface dilational modulus of protein layers measured during repeating cycles of oscillations is reported. Such hysteresis is typical for BLG layers adsorbed at any solution conditions used in this study.

1. Introduction

Along with traditional tensiometry measurements of the surface pressure, experimental methods of surface rheology are often used to gain deeper information about the interfacial behavior of surface active agents and proteins, in particular [1–5]. In a recent paper [6] we studied the adsorption kinetics at the water/air interface for one of the main milk proteins, namely the globular protein β-lactoglobulin (BLG), as affected by the solvent conditions. The results revealed pronounced effects of the solution pH and ionic strength on the adsorption kinetics and on the final steady-state values of the surface pressure Π (the surface pressure isotherm). It was concluded that the observed effects are caused either by variation of the molecular net charge |*Z*| of the protein, as dictated by the solution pH, or by screening of |*Z*| by increasing electrolyte concentrations C_{el} [7,8]. Reduction of |*Z*| and/or increase of the electrolyte concentration lead to faster adsorption kinetics and higher Π-values in the surface pressure isotherm. Hence, BLG in aqueous solution [9,10], like other proteins [11–14], exhibits the highest surface activity at pH-values close to its isoelectric point. However, deviations from this tendency were observed at low BLG concentrations [6].

Apparently, the surface dilational modulus of BLG adsorption layers at the water/air interface is dominated by its elastic component while the viscous part is usually relatively small [15–17]. Measurements of the kinetic dependences of the dilational elasticity of BLG layers show monotonic increases with time in accordance with the increase of the surface pressure due to adsorption [15–21]; however, most of these measurements have been performed with solutions only at or slightly below pH 7. Few other studies, where experiments at different pH-values have been conducted, revealed an increase of the dilational [9,10,14] and shear [11,12] elasticity when approaching the protein isoelectric point. In the present study, we report on surface dilational rheology results for buffered β-lactoglobulin (BLG) solutions with different pH and buffer concentrations (C_{buff}). Results on buffer-free solutions containing $CaCl_2$ and NaCl are also presented.

2. Experimental

The results presented here are based on previously obtained experimental data for the dynamic surface pressure Π of 10-μl buoyant air bubble in BLG solutions [6]. The preparation of the buffered solutions (Na_2HPO_4/citric acid) with different pH is described also in [6]. In the case of buffer-free solutions the natural pH of the samples was ~6.3; addition of $CaCl_2$ up to 100 mM resulted in a decrease in pH to ~ 6.0. The surface dilational viscoelasticity modulus E was evaluated by Fourier transform of the recorded surface pressure response to harmonic area A oscillations of the bubble (see Fig. 1) [22,23]. The oscillations were performed at fixed area deformation ΔA/A = 7 ±0.3% and different frequencies *f* in the range 0.01–0.2 Hz. The frequency dependent dilational modulus E can be expressed as a complex quantity [3,24]: |E| = E'+ iE'', where E' is the real part accounting for the conservation of energy in the system and E'' is the imaginary part accounting for the dissipation of energy due to relaxation processes; hereafter denoted simply as real E' and imaginary E'' parts of the dilational modulus. Unless stated otherwise, the reproducibility of the measurements was estimated to be within ±1 mN/m for Π, ±4 mN/m for E' and ±2 mN/m for E''.

Fig. 1 illustrates the experimental protocol. It contains sets of three area oscillations (triplets) at a fixed frequency of 0.1 Hz applied in the course of adsorption (80 000 s). For each triplet, a single Π-value was calculated as the average of all Π-values that correspond to the undisturbed constant bubble area (circles in Fig. 1). These Π-values are plotted in Fig. 1 (squares) as a function of the time moment in the middle of a triplet. By this presentation of the data, we are able to plot accurately the evaluated values of the dilational modulus E as a function of time t and surface pressure (see Fig. 2). After 80 000 s, a set of oscillations with different frequencies (frequency sweep) was applied. It consisted of the last oscillation triplet at 0.1 Hz, followed by a period of 10 s (during which the bubble rested undisturbed) and five subsequent quintuplets with different oscillation frequencies (see Fig. 1-b). Before each new quintuplet a resting period of 10 s was applied.

3. Results and discussion

3.1. Effect of pH

Fig. 2 presents data obtained for different BLG concentrations at pH 3 and C_{buff} = 10 mM. Fig. 2-a1 shows the measured dynamic surface pressure as a function of time and the surface pressure response to a number of area oscillations applied in the course of adsorption. Increasing the BLG concentration leads to faster adsorption kinetics and to reduction of the induction time, which are general features for BLG (and proteins in general) adsorption layers [6,16]. Fig. 2-b1 shows plots of the dynamic dilational elasticity E' as a function of time. For a certain BLG concentration E' increases monotonically with time (after having passed the induction period) in accordance with the increase of surface pressure similarly to previously reported results obtained either for buffered solutions at pH 7 [15,16,18,21] or for salt-free solutions [19,20]. Combining the $E'(t)_f$ data in Fig. 2-b1 with the corresponding Π(t) data [6] results in $E'(\Pi)_f$ plots as shown in Fig. 2-c1. The E'(Π) data for the presented BLG concentrations virtually collapse onto a master curve. Such behavior was previously found for BLG layers adsorbed from buffered solutions of pH 6.7 or 7 [21,24].

Regarding the imaginary part E'', the experimental results in Fig. 2 show different behavior. The E''-values change non-monotonically with time (Fig. 2-b2) and with the concomitant Π-increase, respectively (Fig. 2-c2). For the lowest BLG concentration of 5×10^{-8} M, the E''-values are rather low (< 2 mN/m) and scatter. For the higher BLG concentrations of 10^{-6} and 10^{-5} M, the $E''(\Pi)_f$ data either level off or show a shallow maximum, respectively. A similar trend was observed for BLG layers adsorbed from

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