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Competitive adsorption of the protein hydrophobin and an ionic surfactant: Parallel vs sequential adsorption and dilatational rheology

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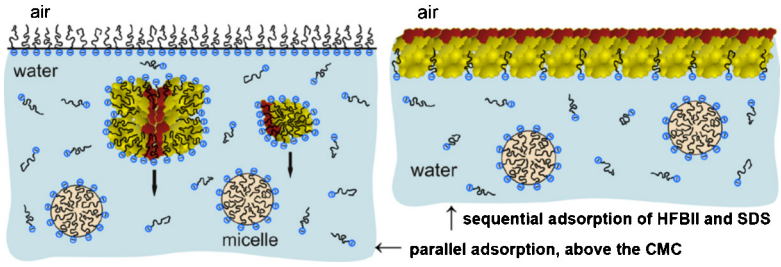
HIGHLIGHTS

- At parallel adsorption, HFBII is hydrophilized and SDS occupies the interface.
- At sequential adsorption, the pre-adsorbed HFBII cannot be washed out by SDS.
- The co-adsorption kinetics of protein and surfactant below the CMC is quantified.
- Dilatational elasticity of adsorption layers is determined by oscillating bubbles.
- Two types of behavior of elasticity, linear and non-monotonic, are identified.

GRAPHICAL ABSTRACT



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ABSTRACT

The competitive adsorption of the protein HFBII hydrophobin and the anionic surfactant sodium dodecyl sulfate (SDS) is investigated in experiments on parallel and sequential adsorption of the two components. The dynamic surface tension and the surface storage and loss dilatational moduli are determined by the oscillating bubble method. A new procedure for data processing is proposed, which allows one to collect data from many different runs on a single master curve and to determine more accurately the dependence of the dilatational elasticity on the surface pressure. Experiments on sequential adsorption are performed by exchanging the HFBII solution around the bubble with an SDS solution. Experiments with separate thin foam films bring additional information on the effect of added SDS. The results indicate that if HFBII has first adsorbed at the air/water interface, it cannot be displaced by SDS at any concentration, both below and above the critical micellization concentration (CMC). In the case of parallel adsorption, there is a considerable difference between the cases below and above the CMC. In the former case, SDS cannot prevent the adsorption of HFBII at the interface, whereas in the latter case adsorption of HFBII is absent, which can be explained with hydrophilization of the hydrophobin aggregates by the SDS in the bulk. The surface dilatational elasticity of the HFBII adsorption layers markedly decreases in the presence of SDS, but it recovers after washing out the SDS. With respect to their dilatational rheology, the investigated HFBII layers exhibit purely elastic behavior, the effect of dilatational viscosity being negligible. As a function of surface tension, the elasticity of the investigated interfacial layers exhibits a high maximum, which could be explained with the occurrence of a phase transition in the protein adsorption layer.

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1. Introduction

Hydrophobin HFBII is an amphiphilic protein produced by filamentous fungi [1]. Its adsorption layers at the air/water interface solidify fast. They possess elasticity, which is higher than that measured for all other investigated proteins [2–7]. The hydrophobin molecules are "sticky" – they adhere to each other, as well as to other macromolecules and solid walls. The hydrophobin is an excellent stabilizer of foams [8] and emulsions [9]; its adhesive properties lead to jamming in fluid dispersions [10]. The hydrophobins are used also for immobilizing functional molecules at surfaces [11], and as coating agents for surface modification [12].

Hydrophobins are relatively small and stable protein molecules. HFBII (70 amino acids) is rich in cysteine and is interconnected with four disulfide bonds. The hydrophobins are very hard to denature – their aqueous solutions have been heated to 90 °C without any sign of protein denaturing [1,13]. There may be some minor changes in shape for hydrophobins when they self-assemble, but this should not be confused with denaturing [1]. There are no evidences for denaturing of hydrophobins by surfactants.

The investigations of foam films stabilized with HFBII showed the formation of self-assembled hydrophobin bilayers due to the strong cohesion of the HFBII monolayers on the two film surfaces [14,15]. The adsorption kinetics and the dilatational rheology of HFBII interfacial layers have been studied by compression–expansion cycles in a Langmuir trough [16], and with oscillating drops [3], and an exceptionally high dilatational elasticity was measured. Very high shear elasticity has been determined for HFBII adsorption layers and interpreted in the framework of an appropriate rheological model [4,5]. The self-assembly of HFBII/surfactant complexes in the bulk of aqueous solutions and the adsorption behavior of HFBII/surfactant mixtures at the air/solution interfaces were investigated by neutron reflectivity and small-angle neutron scattering [17,18]. At surfactant concentrations below the CMC, the formation of mixed protein/surfactant adsorption layers was detected. However, the effect of surfactants on the dilatational surface rheology of hydrophobin adsorption layers has not been investigated so far. This is important for the stability and rheology of the foams and emulsions produced from the respective mixed solutions [2,7–10].

Our goal in the present study is to investigate the effect of sodium dodecyl sulfate (SDS) (i) on the kinetics of adsorption from mixed solutions with HFBII, and (ii) on the dilatational rheology, as well as (iii) to compare the properties of interfacial layers formed by parallel vs. sequential protein/surfactant adsorption.

In many studies [19–24], it has been established that at sufficiently high concentrations the surfactants are able to displace proteins, such as β-casein, β-lactoglobulin (BLG) and bovine serum albumin (BSA), from liquid interfaces. Experiments on dilatational surface rheology, including measurements with oscillating bubbles and drops [25,26], represent a sensitive method for the investigation of protein–surfactant interactions [27,28]. Two approaches to the realization of sequential adsorption with drops and bubbles have been realized: (i) exchange of the solution *inside* the drop by using coaxial capillaries [29,30] and (ii) exchange of the phase *outside* the bubble/drop by pumping out the old solution with a simultaneous supply of a new one [31,32]. Theoretical models of the kinetics of adsorption from mixed protein/surfactant solutions have been also proposed [33].

In Section 3, the kinetics of co-adsorption of HFBII and SDS is studied by experiments with buoyant bubbles. The measured surface-tension relaxation is quantitatively interpreted. In Sections 4 and 5, parallel and sequential adsorption of HFBII and SDS is investigated using phase exchanges. At different stages of the experiments, the surface dilatational moduli are measured by oscillatory experiments. Experiments with thin foam films are also carried out to confirm the conclusions from the rheological measurements. The HFBII/SDS system is compared with the BLG/SDS and BSA/SDS systems under similar experimental conditions. In Section 6, the dependencies of the dilatational elasticity of HFBII layers on the surface tension in the presence or absence of SDS are compared and discussed. A physical picture of the interfacial processes, which govern the surface pressure and elasticity, emerges from the analysis of the experimental data.

2. Materials and methods

2.1. Materials

In our experiments, we used class II hydrophobin (HFBII) of molecular weight 7.2 kDa isolated from the fungus *Trichoderma reesei* following a procedure described elsewhere [14]. Aqueous solutions with concentration 0.001 wt% (0.01 g/L) were used with a natural pH = 5.6. This relatively low protein concentration was selected on the basis of our previous experiments [3], which showed that at 0.001 wt% the HFBII adsorption layer at the air/water interface is relatively diluted and does not solidify. This is necessary, because we are using the oscillatory bubble method (OBM), which is applicable only to fluid interfaces. Indeed, in OBM the surface tension is determined by fitting the bubble profile with the Laplace equation. However, the profile of bubbles with solidified surfaces (with nonzero surface shear elasticity) could significantly deviate from the Laplacian shape, which would make the OBM inapplicable.

In comparative experiments, we used bovine serum albumin (BSA) – a globular protein of molecular weight 66.4 kDa, composed of 580 amino acid residues with 17 disulfide bonds (product of Sigma, A7511). The other used protein was β-lactoglobulin (BLG, Sigma, L0130) – a milk protein of molecular weight 18.4 kDa, composed of 162 amino acid residues with 2 disulfide bonds. Aqueous solutions of concentration 0.01 wt% were prepared and used overnight. Their natural pH was 6.1 ± 0.1. Sodium dodecyl sulfate (SDS, product of Acros Organics, Pittsburgh, PA) was used at concentrations varying from 1 to 50 mM.

All solutions were prepared with a deionized water of specific resistivity 18.2 MΩ cm (Elix purification system, Millipore). 3 mM NaCl (Merk) were added to the used water to have a defined ionic strength. All experiments were carried out at a room temperature of 25 ± 1 °C.

2.2. Surface tension and surface dilatational rheology

We formed and observed buoyant bubbles on the tip of a J-shaped hollow needle dipped in the aqueous solution by means of the instrument DSA100R (Krüss GmbH, Germany). The surface tension σ was determined by axisymmetric drop shape analysis with the software DSA1 (Krüss GmbH). The surface dilatational storage and loss moduli, E' and E'', were determined using the OBM [34]. For this goal, the variation of σ was recorded for sinusoidal oscillations of the bubble area A with a period of 2 s.

A sketch of the experimental setup [32] for exchange of the aqueous phase is shown in Fig. 1a. A cartridge pump simultaneously supplies the new solution and sucks out the old one with the same flow rate thus keeping the volume of liquid in the cuvette constant. The estimates [31] show that when the volume of the inserted new solution reaches 10 times the volume of the cuvette, the resulting solution becomes practically identical with the newly supplied one. In our experiments, the pump was stopped when the volume of the supplied new solution became 12 times the cuvette volume. The bubble was observed in transmitted light during the whole experiment and its profile was processed to calculate the surface tension, bubble area, volume, and the error of the Laplace fit.