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Comparing foam and interfacial properties of similarly charged protein–surfactant mixtures

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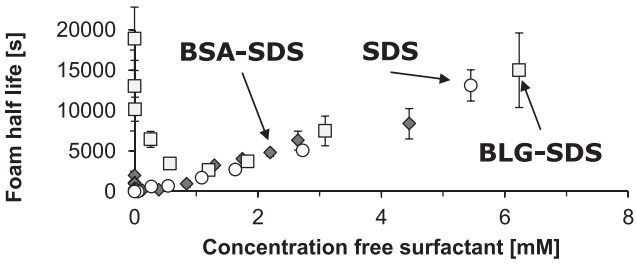
HIGHLIGHTS

- Identifying similarities in different protein–surfactant mixtures.
- Free surfactant related to foam and interface properties of mixtures.
- Free SDS concentration in mixtures depends on high and low affinity binding sites.

GRAPHICAL ABSTRACT



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ABSTRACT

The foam stability of protein–surfactant mixtures strongly depends on the charge of the protein and the surfactant, as well as on their mixing ratio. Depending on the conditions, the mixtures will contain free proteins, free surfactants and/or protein–surfactant complexes. To be able to compare different protein–surfactant mixtures, generic knowledge about the occurrence of each of these states and their relative contribution to foam stability is essential. In this work, the foam stability and interfacial properties of bovine serum albumin (BSA) mixed with sodium dodecyl sulphate (SDS) as well the binding of SDS to BSA as are studied at different molar ratios (MR). A comparison is made with β-lactoglobulin (BLG) mixed with SDS. Both proteins and SDS are negatively charged at pH 7. The foam stability in the presence of small amounts (up to MR 1) of SDS is half the value of the pure protein solutions. The foam stability for both protein surfactant mixtures reaches a minimum at MR 20. A further increase of the MR leads to an increase of foam stability. The foam stability of BLG–SDS at MR >20 follows the foam stability of pure SDS solutions at equivalent concentrations, while BSA–SDS mixtures have an offset and begin to increase from MR >50. This behaviour was also reflected in the surface pressure and complex dilatational elastic moduli, and could be linked to the binding of the surfactant to the proteins. Both proteins bind SDS at high and low affinity binding sites. BSA's high affinity binding sites have a binding stoichiometry of 5.5 $mol_{SDS}/mol_{protein}$, and BLG's high affinity binding site has a stoichiometry of 0.8 $mol_{SDS}/mol_{protein}$ (determined by isothermal titration calorimetry). Binding to the low affinity binding sites, occurs with a binding ratio, leading to an accumulation of free surfactants. While the basic mechanisms underlying the foam properties of mixed systems are not explained in detail by this approach, the foam stability plots of both protein surfactant mixtures could be superimposed using the concentration of free SDS.

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1. Introduction

For similarly charged mixtures of proteins and surfactants it is well-known that the surfactants binds to the proteins, which in return changes the foam properties of the mixtures depending on molar ratio (MR = $mol_{surfactant}/mol_{protein}$). Recently, it was shown that the foam stability of different BLG–SDS mixtures can be understood in terms of the MR and consequently the amount of bound and unbound surfactant in the mixtures [1]. Additional quantative data on foam stability for similarly charged protein surfactant mixtures is lacking, however earlier work indicated the importance of free surfactant in relation to the foam properties. If indeed the binding of the surfactant to the protein determines the foam properties, a comparison of different systems, e.g. different proteins and the same surfactant, can be obtained by accounting for the number of bound surfactant molecules per protein. Hence, the hypothesis is that the foam stability of similarly charged protein–surfactant mixtures can be described in terms of the amount of bound and free surfactant. To investigate this, two proteins with different binding capacities are used in the present study. Both proteins used, bovine serum albumin (BSA) and β-lactoglobulin (BLG) are known to bind surfactants, such as sodium dodecyl sulphate (SDS). They have both been used in studies on the effect of mixing on bulk interaction (BSA–SDS [2,3], BLG–SDS [4–6]), showing the binding of SDS to the proteins. Typically, at low MR, a small number of SDS molecules are bound to high affinity binding sites, while at higher MR more SDS will be bound in non-specific sites. A detailed discussion on the effect of surfactant binding on protein structure and conformation of the resulting complex was previously published [7]. Next to the effect of mixing on the bulk properties, interfacial properties and foam properties of those protein surfactant mixtures have been studied. It was shown that BSA and SDS cooperatively adsorb and cover the interface [8] and that the formation of protein surfactant complexes is reflected by changes in the interfacial tension of the mixtures [9]. Similar observations of coexistence of protein–surfactant complexes as well as free surfactant have been reported for BLG–SDS mixtures [10,11]. The foam ability of BSA–SDS mixtures increases with concentration of SDS [12]. However, the foam stability of this mixture has not been reported. In case of BLG–SDS, the foam stability first decreases upon increased SDS concentration until (MR 20) and starts to increase from that MR onwards [1].

Although both proteins are commonly used model proteins, only one study compared BSA- and BLG surfactant mixtures directly, finding co-adsorption of proteins and surfactant as well as destruction of protein structure at SDS concentration higher than 1 mM in case of BLG–SDS and 3 mM in case of BSA SDS [13]. In the present study, results on BSA–SDS mixtures are complemented with prior results of BLG–SDS mixtures [1]. The quantative link between the two protein–surfactant mixtures and the free surfactant results in a broader description of foam stability of similarly charged mixtures.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA) (Lot number 041M1801V, containing 91% protein based on DUMAS ($N \times 6.07$ [14] and molecular mass of 66.46 kDa determined by electrospray ionization mass spectrometry) and β-Lactoglobulin (BLG) (Lot number 030M7025V, containing 90% protein based on DUMAS ($N \times 6.33$ [14], molecular mass 18.35 kDa determined by electrospray ionization mass spectrometry) were purchased from Sigma Aldrich (Zwijndrecht, The Netherlands). Sodium dodecyl sulphate (SDS, 288.99 Da) was obtained from Merck (Schiphol-Rijk, The Netherlands). MilliQ grade water (Millipore, EMD Millipore Corp., Billerica, MA, USA), free of surface active contaminants was used in all experiments (resistivity 18.6 MΩ cm, total organic carbon 3 ppb and surface tension 72.6 ± 0.3 mN/m at 20 °C).

2.2. Methods

2.2.1. Sample preparation

Stock solutions of BSA (6.56 g/L) and BLG (1.8 g/L) with a protein concentration of 0.89 mM and SDS concentration of 10.9 mM for solution MR 0–100 and 54.5 mM for solutions MR 100–500 were made by separately dissolving protein powder and SDS in sodium phosphate buffer (pH 7.0, 10 mM). Solutions were prepared 30–120 min before use by mixing the stock solutions and buffer, while stirring slowly (without foaming) for 15 min at room temperature. The protein concentrations for solutions of different MR, after mixing with different amounts of stock solution of SDS and buffer, were always 44.5 μM for BSA and BLG, respectively, unless stated otherwise. The pH of the solutions after mixing was adjusted to pH 7.0 by adding 1 M NaOH or 1 M H_3PO_4.

Sample solutions were named according to the molar ratio of surfactant to protein. This is denoted as MR *X*, where *X* is the ratio of the SDS concentration (μM) over the protein concentration (μM). For each mixed solution of a MR, a similar pure SDS solution at an equivalent concentration of SDS (EC = [SDS]/44.5 × 10^{-6}) was used as a reference. The MRs below MR 125 were below the assumed critical micelle concentration of SDS at 20 °C (8×10^{-3} M) [15].

2.2.2. Isothermal titration calorimetry (ITC)

A microcalorimeter (ITC_{200}, GE Healthcare, Piscataway, NJ, USA) was used to titrate SDS solution (in 10 mM sodium phosphate buffer, pH 7.0) stepwise into the above described protein solutions at 20 °C. The surfactant concentrations were 10.9 mM SDS for titration into BSA and 5.45 mM SDS for titration into BLG. The solutions in the titration vessels were stirred at 600 rpm to ensure optimal mixing of the SDS in the measuring cell. In each titration step, 1.4 μL, or 1.2 μL SDS solution were injected into the BSA or BLG solution, respectively, followed by an equilibration period of 120 s until the final concentration of SDS was reached corresponding to an MR of 20 (BSA) and 2 (BLG). The area under the heat flow curve was integrated to obtain the total enthalpy change (ΔH) per injection. Each experiment was corrected with respective titration of SDS solution into buffer. A model assuming one set of identical (non-interacting) binding sites was fit to the integrated curve with a non-linear regression procedure with ITC data analysis software add in (GE Healthcare) for the Origin software suite (Origin 7.0, OriginLab Corp., Northampton, MA, USA). A more detailed description of the model and its theory can be found elsewhere [16]. The model used the theoretical stoichiometry (n [–]), which corresponds to the number of binding sites, the binding constant (K [M^{-1}]), and the binding enthalpy (ΔH [kJ/mol]) as fitting parameters.

2.2.3. Foam experiments

Foam (from solutions described above) was prepared by sparging nitrogen through a metal frit (60 mm diameter, pore size 27 ± 2 μm, 100 μm distance between centres of pores, square lattice) in an automated foaming device (Foamscan, Teclis IT-Concept, Longessaigne, France). The flow rate of gas was 400 mL/min and the maximum foam volume was 600 cm^3. The decay of the foam was monitored by a camera and the foam volume was determined by image analysis performed by software provided with the Foamscan. The time it took the foam to decay to half of the initial volume (foam half-life or $t_{1/2}$) was used as an indicator for the foam stability.