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# Identifying changes in chemical, interfacial and foam properties of β-lactoglobulin–sodium dodecyl sulphate mixtures

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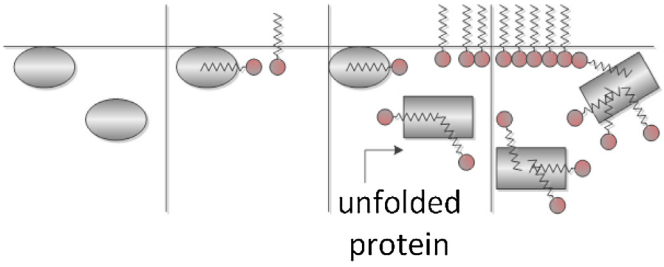
## HIGHLIGHTS

- Binding of surfactants to proteins determines the foam stability of the mixture.
- Interfacial, foam and bulk properties of BLG-SDS mixtures depend on the mixing ratio.
- At low molar ratio (<10), SDS significantly decreases protein foam stability.
- Electrospray ionization mass spectrometry can be used to identify BLG-SDS complexes.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Techno-functional properties of proteins, such as foam stability, can be affected by the presence of low-molecular-weight surfactants. In order to understand and control the foam properties of such protein–surfactant mixtures, a thorough characterization of foam and interfacial properties needs to be supplemented by a detailed analysis of the structural changes of the protein and possible complexation with the surfactant. In this study, β-lactoglobulin (BLG) was mixed with sodium dodecyl sulphate (SDS) in different molar ratios (MRs). The foam half-life time of BLG-SDS mixtures decreased from that of pure BLG (315 min at MR 0) to 44 min at MR 20, which is close to the half-life of SDS at the respective concentration. With a further increase in the MR, the foam stability of the mixture increased, similar to the stability of SDS, to 250 min at the highest MR (MR 100). The minimum in the foam stability curve was not reflected in the interfacial properties ($\Pi$ and $E_d$). $\Pi$ decreased and $E_d$ increased continuously with increasing MR from values close to those of protein towards values typically found in pure surfactant solutions.

The results show no clear correlation between the interfacial and foaming properties. In addition, it was shown by isothermal titration calorimetry and mass spectrometry that SDS molecules bind to the BLG. This leads to the formation of BLG-SDS complexes. These complexes have large influence on the foam properties in the mixture. The combination of analytical methods that were used give insights about protein complexation and the resulting change of foam properties of the mixture.



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## 1. Introduction

When investigating foam properties, a general rule of thumb is that the foam properties will improve with increasing amount of surface active substances. However, when mixtures of the latter of different types of surfactants are used, this observation may change. For mixtures of surfactants and proteins, it has, under certain conditions, been observed that the foam stability of the mixture is lower than of the protein alone [1]. This could be explained by assuming an interaction between the proteins and the surfactant as mentioned in literature [2]. This shows that the foam properties of protein surfactant mixtures are not simply explained by a weighted average of the contributions of the individual compounds. One reason for this effect could be the different mechanisms of foam stabilization of proteins and surfactants or the interaction of the material in the bulk solution. The aim of this study is to describe the effect of protein surfactant interaction on functional properties of the protein by using chemical (bulk interaction, etc.) as well as the physical (interfacial properties, foam, etc.) techniques. Combining these techniques leads to a more detailed understanding of the functional properties of the protein surfactant mixture.

The current literature on mixed proteins and surfactants shows a broad separation into two major categories (Fig. 1). One research category focuses on bulk interactions between proteins and surfactants, while the other category focuses on the interfacial and foam properties of mixed protein–surfactant solutions. The following section focusses mainly on studies, which use BLG and SDS as examples.

The approach in the first category (Fig. 1, category 1) is to determine specific aspects such as the binding of surfactants to protein. Further studies in this field determine the effects of binding on changes of the protein structure [3]. For instance, isothermal titration calorimetry (ITC) was used to show that 1 mol of sodium dodecyl sulphate (SDS) binds to one molecule of β-lactoglobulin (BLG), which is a molar ratio (MR) of 1 [4]. The binding of SDS to BLG was also shown by X-ray diffraction analysis of BLG-SDS crystals [5]. The binding site is a cavity formed by nine β-sheets (calyx) and is located on the inside of the BLG molecule. Another study found no changes in the secondary structure of BLG up to a concentration of 5 mM SDS (which corresponds to a molar ratio (MR) of 91 in this study) using circular dichroism (CD). At higher MR (between MR 100 and 200; concentrations are above the critical micelle concentration (CMC) of SDS in this case), however, the predominantly β-sheet-rich secondary structure of BLG changed into a more helical structure. Simultaneously, unfolding of the tertiary structure occurred in the range of MR 18–91 [6]. Surprisingly, the binding to surfactants increases the heat stability of BLG against denaturation from 80 °C at MR 0 to 88 °C at MR 1.

The research on interfacial behaviour of mixed systems (Fig. 1, category 2) has been reviewed extensively, e.g. by [7,8]. The reviews emphasize the important improvement of foam properties that mixing proteins and surfactants can have. They also indicate the lack of quantitative understanding on the relation between foam stability and interfacial rheology of the protein–surfactant mixtures. The adsorption of molecules at the air–water interface can be studied directly (i.e. using ellipsometry, infrared reflection–adsorption spectroscopy (IRRAS) or Brewster angle microscopy (BAM)) or indirectly, from the measured interfacial properties (surface pressure ($\Pi$) and dilatational elasticity ($E_d$)). The direct studies are used to investigate the interfacial composition and structure of molecules adsorbed at the interface. The methods to study adsorbed monolayers are described in reviews [9–12]. The composition of interfacial layers is also studied using atomic force microscopy on Langmuir Blodgett (LB) films [13]. In such studies, first a protein adsorption layer is made, and sequentially surfactants are added to the bulk solution. Then, an LB film is made by transferring the adsorbed layer onto a solid surface. Results of sequential adsorption studies indicate the expulsion of proteins from the interface at high surfactant concentration, which is explained by the orogenic displacement model [13]. However, the observations of protein displacement is not generally true but depend on the type of protein and surfactant, the respective concentrations and on the experimental procedure, since, for instance, HFBII hydrophobin cannot be displaced from the interface [14].

Other studies characterize air/water interfaces indirectly, e.g. by determining the interfacial tension or surface elastic modulus of protein–surfactant mixtures. Proteins and surfactants stabilize interfaces in two counteracting mechanisms. Protein form elastic networks [15], while surfactant interfaces are stabilized by the Marangoni mechanism [16]. Those different ways of stabilizing are, for instance, reflected in different interfacial properties, such as the surface pressure ($\Pi$) and the complex dilatational modulus ($E_d$). $\Pi$ is related to adsorption of material onto the interface by the equation of state [17], while the dilatational modulus is related to interfacial interactions between molecules in the interface as well as to the adsorption and desorption of material from the interface [18]. Studies investigating these parameters are usually related to adsorption of the mixtures and to the interfacial interactions between the protein and surfactant molecules [19,20]. These indirect methods are usually applied in competitive adsorption studies. In these studies, proteins and surfactants are combined and both can adsorb to the interface at the same time. However, the only common conclusions among these studies are related to high MR, when the concentration of surfactants is higher than the CMC and proteins seem to be completely removed from the interface [21]. Another possibility to use indirect techniques is to determine the change in interfacial properties during or after sub-phase exchange with buffer. The buffer displaces the surfactant solution from the bulk of the droplet, resulting in desorption from the interface. A comparison of the interfacial properties before and after exchange gives insights about the initial interfacial composition [19].

The occurrence of changes in chemical properties of proteins after mixing with surfactants is generally accepted by researchers from the first category. Only recently, the researches belonging to the second category are beginning to consider this. The common picture on protein–surfactant mixtures at the interface can be summarized by picturing two models (Fig. 2A and B). In model A, the simplest model of a mixture of any protein and surfactant, both substances are considered as separate, non-interacting entities. With increasing concentration, the surfactants displace the proteins from the interface and determine the interfacial properties of the mixture (Fig. 2A). Since for certain systems (e.g. BLG-SDS) it is known that there is an interaction between both substances, an alternative model (B) has been described. In this model, the proteins and surfactants are considered to form complexes. With increasing surfactant concentration, more surfactants bind to the protein. At concentrations higher than a critical concentration, the interface is mostly covered by surfactants, while the influence of the protein–surfactant complexes on the interfacial properties decreases (Fig. 2B).

Both research categories investigate similar types of surfactants and proteins. However, their focus as well the used mixing ratios in their studies are different. Studies from the interface category are performed with SDS concentrations ranging from $10^{-9}$ mol/L up to concentrations of $10^{-1}$ mol/L, which is above the critical micelle concentration ($8 \times 10^{-3}$ mol/L). Usually, the largest changes in interfacial properties, such as the surface tension, are observed between MR 10 and 100, after which the interfacial properties of the mixed system become similar to those of the pure surfactant [22].

A few of the above-mentioned studies from both categories use experimentally similar techniques. These studies, however, mainly