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Comparison of positional surfactant isomers for displacement of rubisco protein from the air–water interface

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ABSTRACT

Protein–surfactant interaction, which is a function of the protein and surfactant characteristics, is a common phenomenon in a wide range of industrial applications. In this work, we used rubisco, the most abundant protein in nature, as a model protein and sodium dodecylbenzenesulfonate (SDOBS), one of the most widely used commercial surfactants, with two positional isomers (SDOBS-2 and SDOBS-6), as a model surfactant. We first examined the surface tension and the mechanical properties of interfacial mixed rubisco–SDOBS films adsorbed at the air–water interface. The concentration of rubisco in solution was fixed at 0.1 mg mL^{-1} while the SDOBS concentration varied from 0 to 150 μM. Both the surface tension and the mechanical strength of the interfacial film decreased with increasing SDOBS concentration. Overall, the surface tension of a rubisco–SDOBS-6 mixture is lower than that of rubisco–SDOBS-2, while the mechanical strength of both systems is similar. Neutron reflection data suggest that rubisco protein is likely denatured at the interface. The populations of rubisco and SDOBS of the mixed systems at the interface were determined by combining non-deuterated and deuterated SDOBS to provide contrast variation. At a low surfactant concentration, SDOBS-6 has a stronger ability to displace rubisco from the air–water interface than SDOBS-2. However, when surfactant concentration reaches 50 μM, SDOBS-2 has a higher population than SDOBS-6, with more rubisco displaced from the interface. The results presented in this work suggest that the extent of protein displacement from the air–water interface, and hence the nature of the protein–surfactant interactions at the interface, are strongly affected by the position of surfactant isomerisation, which might allow the design of formulations for efficient removal of protein stains.

1. Introduction

The properties of mixed layers of proteins and surfactants are of direct relevance to a wide range of applications. For example, protein–surfactant mixed systems could provide better foam and emulsion stability than individual components. Protein displacement from interfaces is also correlated with the extent of protein–surfactant interactions (i.e. properties of the mixed protein–surfactant films) [1,2]. Different techniques have been employed to improve understanding of the complex behaviour of these mixed systems. These include surface tension and rheology measurements, which give the macroscopic behaviour of a mixed system, and fluorescence microscopy and Brewster angle microscopy, which provide microscopic properties at interfaces [2,3]. Knowledge at a molecular level can be achieved by neutron reflection, which reveals structural details of a mixed layer at an interface [4].

Previous interfacial studies on protein–surfactant systems have focussed on models using proteins including lysozyme, β-lactoglobulin and BSA with non-ionic and ionic surfactants [5–9]. The reason for the selection of such systems is perhaps, in part, because detailed knowledge of each component is well documented and these proteins are commercially available. In this work, we focussed our study on a system closely related to laundry cleaning by using a mixture of ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) and sodium dodecylbenzenesulfonate (SDOBS). Rubisco exists in bacteria and all green plants and non-green algae, and is the most abundant protein in nature [10]. This makes it a good representative of the class of grassy stains. Recently, Onaizi et al. have studied the disassembly of pre-adsorbed rubisco

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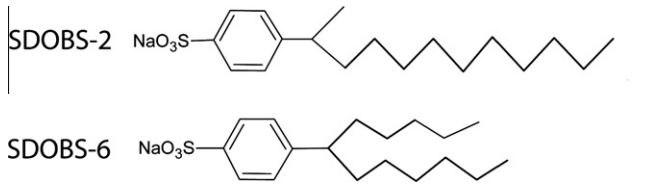

Fig. 1. Chemical structure of two positional isomers of sodium dodecylbenzenesulfonate (SDOBS), SDOBS-2 and SDOBS-6.

network by commercial SDOBS at the air–water interface by directly measuring the mechanical properties of the rubisco network at the interface [11].

SDOBS, on the other hand, represents the group of surfactants linear alkylbenzene sulfonate, which are one of the most important groups of synthetic surfactants in laundry detergents. Commercially-produced SDOBS often exists as a mixture of head group positional isomers (see representative isomers in Fig. 1). There is increasing interest in comparing the physicochemical properties of these individual isomers using different techniques in order to guide rational design of surfactant products. Ma et al. have studied phase behaviours of SDOBS isomers and their interfacial activity at the air–water interface [12]. Neutron reflection and small angle neutron scattering have been used to investigate the interfacial and solution behaviours of mixtures of SDOBS isomers with non-ionic surfactants [13,14]. The cooperative interaction between SDOBS isomers and a designed peptide has also been recently studied [15]. Molecular dynamic simulation has recently been used to analyse phase behaviour of SDOBS isomers [16].

In this work, we selected two positional isomers SDOBS-2 and SDOBS-6, and compared their interaction with rubisco protein at the air–water interface. We first measured the interfacial tension and the mechanical properties of the mixed systems at the interface, followed by neutron reflection study on the mixed film structure. In order to measure the surface excess of SDOBS and rubisco independently, we performed contrast variation by a combination of deuterated SDOBS/rubisco and protonated SDOBS/rubisco.

To our best of knowledge, this work is the first study directly comparing positional isomers of an important industrial surfactant for their interaction with a protein. Furthermore, studies of surfactant–protein interaction in literature are mainly focused on model proteins (e.g. lysozyme or BSA) or food proteins, for instance milk proteins (β-casein and β-lactoglobulin) [17], and these studies are of direct interest for food processing and food formulations [3]. In contrast, representing protein stains from vegetables and grasses, the rubisco protein studied in this work is closely related to a laundry process. Thus, the different physicochemical properties of SDOBS isomers revealed in this study are useful not only in fundamentally understanding protein–surfactant interactions, but also in designing detergent formulations to improve detergency performance.

2. Materials and methods

2.1. Rubisco purification and SDOBS synthesis

Rubisco was extracted from spinach leaves and purified as described previously [11]. The purification process includes several precipitation steps followed by ion-exchange chromatography. The purified rubisco was aliquoted into small samples, lyophilized and stored at −80 °C until use. After redissolving into buffer, lyophilized samples showed similar interfacial behaviour to fresh samples.

SDOBS-2 and SDOBS-6, two representative isomers of SDOBS, were prepared by chemical synthesis [15]. In addition to non-deuterated samples, alkyl chain deuterated SDOBS-2 and SDOBS-6 were also prepared. A high purity of SDOBS isomers and their precursors was confirmed by gas chromatography - mass spectrometry (GC - MS).

2.2. Surface tension

Axisymmetric bubble shape analysis was carried out with a DSA10 tensiometer (Krüss GmbH, Hamburg, Germany). The solution of interest (8 mL) was placed into a quartz cuvette and a *ca.* 8 μL air bubble was formed inside the solution. Monitoring of the surface tension continued for 3600 s after bubble formation or until the rate of change in the surface tension was less than 0.05 mN m^{-1} per minute.

2.3. Interfacial rheology

Mechanical properties of films assembled at the air–water interface were determined using the Cambridge Interfacial Tensiometer (CIT) [18–22]. Stress transmitted through an interfacial layer in response to an applied strain was measured, and the interfacial elasticity (*Et*) was extracted from the stress–strain curves by taking the gradient in the elastic region (up to 1% strain). The sample was subjected to non-destructive (5%) strain disturbances once every minute for the period of an hour. After final aging (typically 3 h), the 1% gradient of eight tensile tests to 5% strain were averaged to obtain a final value of *Et*.

2.4. Neutron reflection

Neutron reflection experiments were carried out using SURF time-of-flight reflectometer at the Rutherford Appleton Laboratory (ISIS, Oxfordshire, UK). An incident angle of 1.5° was used to give a momentum transfer between 0.048 and 0.6 $Å^{-1}$ as the incident neutron wavelength ranges from 0.55 to 6.8 Å. The scale factor was obtained from the measurements of pure D_2O, and was used to calibrate reflectivity data to an absolute scale. A constant background was subtracted using the average reflectivity measured between 0.27 and 0.6 $Å^{-1}$. Samples were prepared in 20 mM Na^+ phosphate, pH 8.0, ionic strength 56 mM. For mixed solutions of rubisco and SDOBS, contrast variation was obtained by combining deuterated SDOBS (*d*-SDOBS) and unlabeled SDOBS (*h*-SDOBS) in null reflecting water (NRW, 8.1% (v/v) D_2O). Deuterated SDOBS was used for single component measurements. Freshly prepared solution was poured into a Teflon trough (SURF reflectometer), and aged for 3.5–6 h before measurements. Repeat experiments showed that there was no measurable signal difference (value within error bars) between samples aged for 1.5 h and 8 h. A kinematic approximation was used to calculate theoretical reflectivity based on a partial-structure-factor approach [23], which simultaneously fits reflectivity data at different contrast variations. Theoretical reflectivity was further corrected by a Crowley formulation to improve the calculation near the critical angle [24]. Key structural parameters such as area per molecule and film thickness were obtained from the fitting of reflectivity data. Combination of *d*-SDOBS/rubisco and *h*-SDOBS/rubisco data measured at null reflecting water ensures that high precision value of area per molecule for two components were obtained as fitting is most sensitive to this parameter under this condition. However, there are uncertainties for other parameters such as distance between two components because of the limited data sets (two sets of data of *d*-SDOBS/rubisco and *h*-SDOBS/rubisco in NRW). We are thus cautious not to over-interpret these parameters obtained from model fitting.