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Stabilizing and destabilizing protein surfactant-based foams in the presence of a chemical surfactant: Effect of adsorption kinetics

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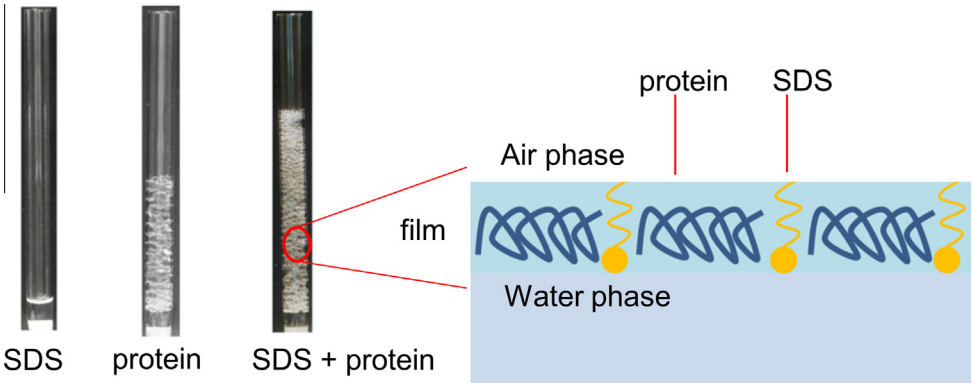
HIGHLIGHTS

- Effects of chemical surfactants and metal ions on protein-based foams are studied.
- Adsorption kinetics is critical for stabilization–destabilization of protein foams.
- Sodium dodecylsulfate enhances protein adsorption kinetics, improving foam stability.
- Addition of Zn^{2+} causes protein aggregation, lowering adsorption kinetics.
- Slowed adsorption kinetics destabilizes foams.

GRAPHICAL ABSTRACT



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ABSTRACT

Stimuli-responsive protein surfactants promise alternative foaming materials that can be made from renewable sources. However, the cost of protein surfactants is still higher than their chemical counterparts. In order to reduce the required amount of protein surfactant for foaming, we investigated the foaming and adsorption properties of the protein surfactant, DAMP4, with addition of low concentrations of the chemical surfactant sodium dodecylsulfate (SDS). The results show that the small addition of SDS can enhance foaming functions of DAMP4 at a lowered protein concentration. Dynamic surface tension measurements suggest that there is a synergy between DAMP4 and SDS which enhances adsorption kinetics of DAMP4 at the initial stage of adsorption (first 60 s), which in turn stabilizes protein foams. Further interfacial properties were revealed by X-ray reflectometry measurements, showing that there is a re-arrangement of adsorbed protein–surfactant layer over a long period of 1 h. Importantly, the foaming switchability of DAMP4 by metal ions is not affected by the presence of SDS, and foams can be switched off by the addition of zinc ions at permissive pH. This work provides fundamental knowledge to guide formulation using a mixture of protein and chemical surfactants towards a high performance of foaming at a low cost.

1. Introduction

Foams play a significant role in both domestic and industrial sectors where the demand of controlling surfactant foams

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underpins a market of tens of billion dollars each year and is growing sharply [1]. However, this huge market is still based mostly on chemical surfactants, which are mainly produced from unsustainable chemical processes using petroleum and animal or vegetable fats [2]. Alternative technologies, such as stimuli-responsive peptide/protein-based foaming materials which can be made from renewable sources are promising to provide a sustainable solution to reduce the need for chemical surfactants [3–5]. Another significant advantage of stimuli-responsive peptide/protein is that stabilization and destabilization of foams can be achieved by change of pH or metal ions [5–8]. Control of foam by this approach is reversible, and does not change the composition of the foaming systems significantly. In contrast, using conventional defoaming agents often causes changes in composition of the foaming system and thus permanent loss of foamability as well as contamination leading to waste treatment necessity [9].

Despite their technical advantages in control of foam functions, there are significant challenges that prevent widespread applications of stimuli-responsive peptide/protein surfactants. One of these challenges is the high manufacturing cost when compared to conventional surfactants [10–12], although new bioprocessing approaches are simplifying process flowsheets promising significant cost reduction [13]. In order to decrease the required amount of peptide/proteins, peptide/protein–surfactant mixing systems have attracted significant research efforts [14–16]. Formulation of protein surfactants with a small peptide or chemical surfactant offers advantages including: (1) reducing the overall formulation cost, (2) lowering the environmental impact of chemical surfactants; and (3) introducing the functional benefits of the biosurfactant, such as foam switchability by pH or metal ions addition, to the formulation. Neutron reflectometry studies on protein–surfactant mixing systems indicated that the presence of surfactant may: (1) increase the final adsorbed amount of the peptides at the liquid–air interface [6], (2) also increase the final adsorbed amount of the chemical surfactant itself [17,18], or (3) cause conformation changes of polypeptides [15].

Another significant challenge in application of protein/peptide-based biosurfactant is the slow adsorption kinetics of large protein surfactants [19]. Small chemical surfactant molecules adsorb to the interface, within seconds, to achieve desired surface coverage and thus provide stable foams [20–22]. In contrast, due to their relatively large hydrodynamic molecular size and required structural changes at the interface, the adsorption of protein/peptide-based biosurfactants to interfaces normally takes tens of minutes, even hours to reach the equilibrium coverage [23,24]. In a study of a designed protein DAMP4 and its analogue DAMP1, Dwyer et al. showed the adsorption of the larger molecule protein DAMP4 is accompanied with structural changes at the surface, and the adsorption kinetics is much slower than that of its smaller analogues DAMP1 [24].

To address these two challenges, this work studied how adsorption kinetics of designed protein surfactant DAMP4 is affected by addition of a small chemical surfactant sodium dodecylsulfate (SDS) in the absence and presence of zinc ions, and its correlation with stabilization and destabilization of foams. Such knowledge will be highly valuable to guide the design of next generation protein surfactant materials and their formulation for better control of foams for widespread applications.

2. Experimental

2.1. Materials

Sodium dodecylsulfate (⩾98.5%, GC), ethylenediaminetetraacetic acid disodium salt (analytical), Kanamycin sulfate, and N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic sodium salt (HEPES, analytical) were purchased from Sigma–Aldrich (Sydney, Australia); Isopropyl-β-D-1-thiogalactopyranoside (IPTG) was purchased from Astral Scientific Pty Ltd (Sydney, Australia); Sodium chloride (analytical), sodium hydroxide (analytical), trifluoroacetic acid (for HPLC, ⩾99.0%), and imidazole (analytical) were purchased from VWR International Pty Ltd (Melbourne, Australia); Immobilized metal ion affinity chromatograph (IMAC) media Ni Sepharose™ High Performance, and RP-HPLC media Source™ 15 RPC were purchased from GE Life Science, and anion exchange media UNOsphereTM Q was purchased from Bio-rad (Sydney, Australia).

2.2. Protein expression and purification

DAMP4, the protein surfactant with a molecular weight of 11116.5 Da, was expressed in *Escherichia coli* BL21 (DE3) transformed with the pET-48b plasmid containing the DAMP4 gene [3]. The *E. coli* was cultivated in 25 g/L LB broth under 37 °C and protein expression was induced with a final concentration of 1 mM IPTG at OD_{600} ~0.6 followed by another 4 h cultivation. Then cell mass was collected by centrifugation (10,000*g*, 5 min) and resuspended in 0.5 M Na_2SO_4 solution to a final OD_{600} = 20. Cell lysis and DAMP4 extraction was performed with a heat treatment method [13] using 90 °C water bath for 20 min. The heat treated sample was centrifuged at 10,000*g* for 5 min, and pellet was discarded.

In order to obtain high purity of DAMP4, the supernatant solution after heat treatment were further purified by three steps of adapted chromatographic methods, similarly described in [3], including immobilized metal ion affinity chromatograph (IMAC), anion exchange chromatography, and reverse HPLC (RP-HPLC). Typically, 10 mL Ni Sepharose™ High Performance media or anion exchange media UNOsphere™ Q was packed in an Omnifit Benchmark Column (inner diameter I.D. = 10 mm, length *L* = 150 mm, two adaptors) from Diba Industries Ltd (Cambridge, UK). For packing 10 mL RP-HPLC media Source™ 15 RPC, an Omnifit SolventPlus Column (inner diameter I.D. = 25 mm, length *L* = 100 mm, one fixed and one adjustable ends) was used. DAMP4 eluted from RP-HPLC was lyophilized and stored at −80 °C before use. For typical experiments, lyophilized DAMP4 was dissolved in 25 mM sodium 4-(2-hydroxyethyl)-1-piperazineethanesulphonate (HEPES) buffer which included 200 μM ethylenediamine tetraacetic acid (EDTA) and 10 mM sodium chloride (NaCl), pH 8.5.

2.3. Foaming assay

Foaming assay was performed with a custom-made foam preparation apparatus described elsewhere [5]. Air is pumped into 1 mL of sample solution through a porous glass frit at the base of the column using syringe pump (NE-1600 6-channel syringe pump SDR Scientific, Sydney, Australia) operating at 10 mL/min. Foaming stability of DAMP4 samples was observed over a time course of 10 min.

2.4. Dynamic surface tension

Pendent drop method (Dataphysics OCA20, Germany) was used to measure dynamic surface tension at 23 °C. 10 μL of air was pushed through a U-shaped needle to form a bottom up bubble in 8 mL sample placed in a cubic quartz cuvette. The accuracy of method was confirmed by measuring the surface tension of MilliQ water (72.0 ± 0.2 mN/m at 23 °C). The dynamic surface tension of samples of interest was collected at an average rate of two measurements per second over a period of 3600 s.