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Enhancing protein self-association at the gas–liquid interface for foam fractionation of bovine serum albumin from its highly diluted solution

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A B S T R A C T

Improving foam stability is an important issue in foam fractionation of proteins from their aqueous solutions with low foam properties. In this work, enhancing protein self-association at the gas–liquid interface by sodium citrate (Na-citrate), instead of adding surfactants, was used to improve the stability of protein foams. Using bovine serum albumin (BSA) as a model protein, the role of Na-citrate in enhancing self-association of BSA at the gas–liquid interface was studied at the molecular level. Then, the role of the enhanced protein self-association in intensifying foam fractionation of BSA was studied. The results show that by weakening electrostatic repulsion between the BSA molecules and unfolding their structures, Na-citrate induced the formation of 12 and 18 BSA aggregates at the gas–liquid interface. The enhanced protein self-association effectively increased the interfacial adsorption of BSA and improved the foam stability. At BSA concentration 50 mg/L, the recovery percentage of BSA with 30 mmol/L Na-citrate increased by 4 folds while its enrichment ratio just decreased by 11.5%, compared to those without Na-citrate. The results give a clear understanding of the role of protein self-association in foam fractionation of proteins.
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1. Introduction

As the security issues of environment, food and medicine become increasingly prominent, green techniques of low energy consumption and free pollution are heavily needed in the chemical industry to yield products closely related to human health. Foam fractionation is just such a promising one that uses rising foam to separate surface-active compounds from their diluted aqueous solutions (Chen and Parlar, 2013). Due to its unique characteristics, foam fractionation has wide applications in the separation of proteins in the fields of biochemical engineering (Khalesi et al., 2013). Therefore, lots of

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efforts for process intensification of foam fractionation have been made to achieve its large-scale applications (Burghoff, 2012).

For a successful foam fractionation of a protein, the most essential requirement is a relatively stable foam flow which can continuously separate the protein molecules from the bulk solution. However, the requirement is often unobtainable when the protein concentration is too low or the protein has very poor surface activity (Gerken et al., 2006; Burapatana et al., 2005). In this context, improving the stability of the protein foams becomes an important issue. In previous works, various surfactants have served as foam stabilizers to achieve satisfactory separation performances in the separation of proteins with very low concentrations or surface activity (Mukhopadhyay et al., 2010; Schinke and Germani, 2013; Rosa et al., 2007). Unfortunately, the subsequent isolation of proteins from surfactants needs great efforts, because the two compounds often interact with each other to form complexes (Gelamo et al., 2002; Otzen, 2011; Tucker et al., 2014). Furthermore, many surfactants can cause serious pollution to the environment and even do harms to human bodies (Hwang et al., 2014; Tehrani-Bagha et al., 2015). Finally the recovered proteins will lose usability. So it is necessary to develop new strategies for stabilizing the unstable protein foams in foam fractionation.

In fact, the optimal strategy to improve the stability of unstable protein foams is to use themselves as foam stability enhancers. It is well known that proteins suffer structural unfolding to make their hydrophobic groups exposed to the gas phase in their adsorption towards the gas–liquid interface (Barackov et al., 2012). The unfolded molecules readily interact with each other to form a network to stabilize the interface (Poole et al., 1984). If the protein self-association is effectively enhanced, the stability of protein foams will largely increase (Martin et al., 2002; Nicorescu et al., 2009). In this case, proteins themselves will serve as the stability enhancers for their own foams and the subsequent separation due to the addition of surfactants will not be necessary. Then, how to enhance protein self-association at the gas–liquid interface becomes the key to successful foam fractionation of proteins from their solutions with poor foam properties.

Sodium citrate (Na-citrate) will be developed to enhance protein self-association at the gas–liquid interface. Liu et al. (2013) have reported that a trace of citric acid effectively changed the structure of polyphenoloxidase and inhibited its bioactivity. Furthermore, Le Floch-Fouéré et al. (2015) have observed that citrate allowed ovotransferrin to self-assemble at the gas–liquid interface, thus enhancing the interfacial adsorption of the protein. The above results indicate that Na-citrate has the possibility to enhance protein self-interaction at the gas–liquid interface. In addition, Na-citrate is a widely used acidity regulator and flavouring agent in the food industry (Gibbs et al., 1982), so it will be a green foam stability enhancer.

In this work, the role of Na-citrate-enhanced protein–protein association in foam fractionation of bovine serum albumin (BSA) from its highly diluted solution will be investigated. The selection of BSA as a model protein is because of its well-understood structure, high purity and wide applications in the pharmaceutical industry (Shi et al., 2012; Tang and Shen, 2013). Firstly, the effect of Na-citrate on the BSA structure will be studied. Subsequently, the effect of Na-citrate on the aggregation of BSA and its adsorption at the gas–liquid interface will be investigated and explained at a molecular level. Finally, the role of Na-citrate-enhanced protein–protein association in foam fractionation of BSA will be discussed.

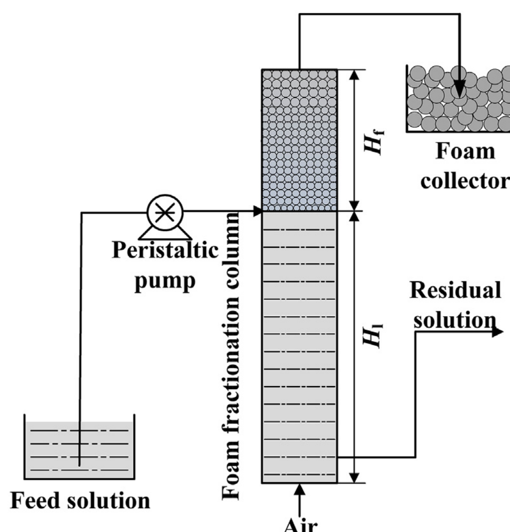

Fig. 1 – Schematic diagram of continuous foam fractionation of BSA from its solution of 50 mg/L.

2. Materials and methods

2.1. Materials

BSA with purity >99% was purchased from Tianjin Unite Stars Biotechnology Co. Ltd., China. It was dissolved in a 50 mmol/L NaH_2PO_4–Na_2HPO_4 buffer solution (pH 7.0). 1,8-Anilinonaphthalenesulfonate, a fluorescence probe, (ANS, HPLC purity ≥97%) was purchased from Sigma–Aldrich, USA. Na-citrate and other reagents with the analytical grade were purchased from Tianjin Yingdaxigui Co. Ltd., China. Ultrapure water (electrical resistance = 18.25 MΩ) prepared by UPR-II-10T water purification system (Chengdu Ultrapure Technology Co. Ltd., China) was used in all the experiments.

2.2. Equipment

A continuous foam fractionation was used for separating BSA from its solution of 50 mg/L. Its schematic diagram is shown in Fig. 1. The foam fractionation column was constructed by a transparent plexiglass tube of 40 mm in internal diameter with H_l in liquid height and H_f in foam height. Through the feed inlet near the foam-liquid interface, the feed solution was loaded into the column by a peristaltic pump (BT100-2J, Baoding Longer Precision Pump Co. Ltd., China). At the column bottom, a gas distributor made of polyethylene was installed with pore diameters of $100 \pm 20\,\mu m$ (not shown in Fig. 1). Through the distributor, the air was injected into the column to generate numerous bubbles. All the foam fractionation experiments were carried out at feed flow rate 3.0 mL/min, air flow rate 200 mL/min and temperature $25 \pm 1\,^{\circ}C$. The foam out of the foam fractionation column flowed into a collector, of which the bottom was evenly coated with Tween-20. Then the foam was quickly collapsed by Tween-20 assisted with slow agitation. Tween-20 was widely used to prevent protein aggregation (Chou et al., 2005), so the further aggregation of BSA in the foamate could be prevented. It is noted that the final content of Tween-20 in the foamate was about 0.05% (v/v) and at this content, Tween-20 did not affect the measurement of BSA concentration.

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