Separation and Purification Technology 158 (2016) 367–373

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Extraction of ovalbumin with gemini surfactant reverse micelles – Effect of gemini surfactant structure

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ARTICLE INFO

Article history:
Received 10 December 2015
Accepted 22 December 2015
Available online 24 December 2015

Keywords:
Liquid–liquid extraction
Reverse micelles
Separation
Ovalbumin
Surfactant

ABSTRACT

So far, the protein with high solubility in water (such as bovine serum albumin) has been always chosen as the model protein in the studies on reverse micellar extraction; to recover the protein with considerable hydrophobicity from organic phase may not be easy. Noticing that the surfactants used have been mostly conventional ionic surfactants, in the present study, we extract ovalbumin (OVA), a typical globular protein exhibiting emulsifying and foaming capabilities and forming gels upon heating, with a series of gemini surfactant (C_m-*s*-C_m·2Br with m being 12, *s* being 2, 8, 12 or m being 16, *s* being 5, 8) reverse micelles. Results show that C_{12}-*s*-C_{12}·2Br reverse micelle can load more OVA than C_{16}-*s*-C_{16}·2Br reverse micelle. Under optimum condition, ca. 90% of OVA can be transferred from water into all these gemini surfactant reverse micelles, while the backward extraction efficiency (i.e. the recovery of OVA from reverse micellar phase) is dependent on gemini surfactant structure; about 59–73% of OVA can be transferred back into water from C_{12}-2-C_{12}·2Br, C_{16}-5-C_{16}·2Br and C_{16}-8-C_{16}·2Br reverse micelles, but very little OVA can be recovered from C_{12}-8-C_{12}·2Br and C_{12}-12-C_{12}·2Br reverse micelles.

1. Introduction

Reverse micelles consist of surfactant, oil, cosurfactant (i.e. alcohol) and water. They are aggregates of surfactant molecules with an inner core of water molecules dispersed in a continuous organic solvent medium. Proteins can be solubilized into the inner water droplets and hence, shielded from the organic medium, which helps maintain the native function/activity of proteins [1–4]. Nowadays, more and more people pay attention to extract proteins with reverse micelles, owing to their impressive potential for continuous operation and scaling up [5–7]. There are two steps in the liquid–liquid reverse micellar extraction process: forward extraction and backward extraction. Forward extraction is the first step, in which a target protein is selectively solubilized into the organic phase (also called reverse micellar phase), and backward extraction is the second step, in which the target protein is stripped into the aqueous phase from organic phase by addition of fresh aqueous buffer [1–8].

So far, a number of studies have indicated that the electrostatic interaction between protein and surfactant generally plays a very important role in protein transfer and many factors affect the performance of a reverse micelle system, including the nature and concentration of target protein, pH, the concentration and species of ions, the composition of reverse micelles, and so on [9–12]. Currently, reverse micelle extraction technology is still in the stage of laboratory; the protein with high solubility in water (such as bovine serum albumin, BSA) has been always chosen as the model protein [4,7,9,13–15]. It seems reasonable to imagine that to recover the protein with considerable hydrophobicity from organic phase may not be easy.

Ovalbumin (OVA) is a typical globular protein with an isoelectric point (pI) of about 4.6 [16,17]. OVA exhibits emulsifying and foaming capabilities and can form gels upon heating, which makes it used widely in the food industry [18]. When using conventional surfactants dodecyl trimethyl ammonium bromide (DTAB) and cetyltrimethyl ammonium bromide (CTAB), although OVA can be transferred from water to organic phase, almost no OVA can be recovered from the organic phase (i.e. transferred from organic phase back to aqueous phase) [19].

In addition to conventional ionic surfactants, such as CTAB, DTAB and dioctyl sodium sulfosuccinate (AOT) [6–15,20–24], which have been mostly used in protein extraction so far, gemini surfactant should be worth trying as a potential candidate for extracting protein [4,19]. Gemini surfactant molecules are made up of two hydrophilic head groups, two hydrophobic chains, and

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http://dx.doi.org/10.1016/j.seppur.2015.12.042
1383-5866/

a spacer linking to the two head groups via covalent bonds. It has been concluded that gemini surfactants exhibit superior surface activity compared to single-chained surfactants [25], and the spacer chain length can show an obvious effect on the interaction between protein and gemini surfactant [26–29]. In the present paper, we will extract OVA with a series of gemini surfactant (C_m-s-C_m·2Br with m being 12 and s being 2, 8, 12 or m being 16 and s being 5, 8, shown in Fig. 1A) reverse micelles, based on which, the role of gemini surfactants in OVA extraction should be elucidated.

2. Materials and methods

2.1. Materials

OVA was bought from Xibao Co. (biological grade, Shanghai, China). n-Hexane and 1-hexanol were purchased from Chinese Chemicals (analytical grade, Sinopharm chemical reagent Co., Ltd., Shanghai, China). Gemini surfactants C_m-s-C_m·2Br (Fig. 1A, for simplicity, C_m-s-C_m·2Br is represented by C_m-s-C_m) were prepared according to Ref. [25]. Its purity (>97%) was checked by ^{1}H NMR and elemental analysis. Figs. S1 and S2 of Supplementary material exemplified the ^{1}H NMR spectra.

The buffer used in the forward extraction was prepared using disodium hydrogen phosphate (10 mM)/citric acid (pH: 3.0–8.0) and glycine (10 mM)/sodium hydroxide (pH: 9.0–10.0). The stripping solution in the backward extraction was prepared using acetic acid/sodium acetate (10 mM, pH = 4.3) and disodium hydrogen phosphate/sodium dihydrogen phosphate (10 mM, pH = 7.0).

2.2. Methods

2.2.1. Forward extraction

OVA was dissolved in buffer of known pH and salt concentration and its content was 1 mg/ml unless mentioned otherwise. Reverse micelle was prepared from known quantities of n-hexane, 1-hexanol, gemini surfactant and water, with the volume ratio of n-hexane to 1-hexanol being 9:1. Aqueous and organic phases were mixed with a volume ratio of 1:1 and the mixture was vortexed for 10 min at room temperature. Phase separation was done by centrifuging at 17,968g for 30 min. The organic phase separated from the mixture was used for the backward extraction.

2.2.2. Backward extraction

The organic phase of the forward extraction was mixed with stripping phase (i.e. buffer of known pH and salt concentration) and the volume ratio of organic phase and stripping phase was changed from 1:1 to 1:10. The mixture was vortexed for 15 min at room temperature. Then the mixture was centrifuged at 17,968g for 30 min, followed by the separation of the two phases.

2.3. Analytical methods

2.3.1. OVA concentration and extraction efficiency

OVA concentration was determined by UV–Vis spectrophotometer at 278 nm. The experiments were taken in triplicate and average values were reported. Efficiencies of forward (E_f) and backward (E_b) extractions were calculated from Eqs. (1) and (2), where $[OVA]_f$ and $[OVA]_o$ represented OVA concentrations in feed and in organic phase of forward extraction, respectively, and $[OVA]_{aq}$ and $[OVA]_s$ represented OVA concentrations in aqueous phases of forward extraction and backward extraction, respectively. V_f, V_o and V_s represented the volumes of the feed, the organic phase in forward extraction, and the stripping solution in backward extraction.

$$E_f\ (\%) = \frac{[OVA]_o\ (mg/ml)}{[OVA]_f\ (mg/ml)} \times \frac{V_o\ (ml)}{V_f\ (ml)} \times 100 = \frac{[OVA]_f \times V_f - [OVA]_{aq} \times V_f}{[OVA]_f \times V_f} \times 100 \quad (1)$$

$$E_b\ (\%) = \frac{[OVA]_s\ (mg/ml)}{[OVA]_o\ (mg/ml)} \times \frac{V_s\ (ml)}{V_o\ (ml)} \times 100 = \frac{[OVA]_s \times V_s}{[OVA]_f \times V_f - [OVA]_{aq} \times V_f} \times 100 \quad (2)$$

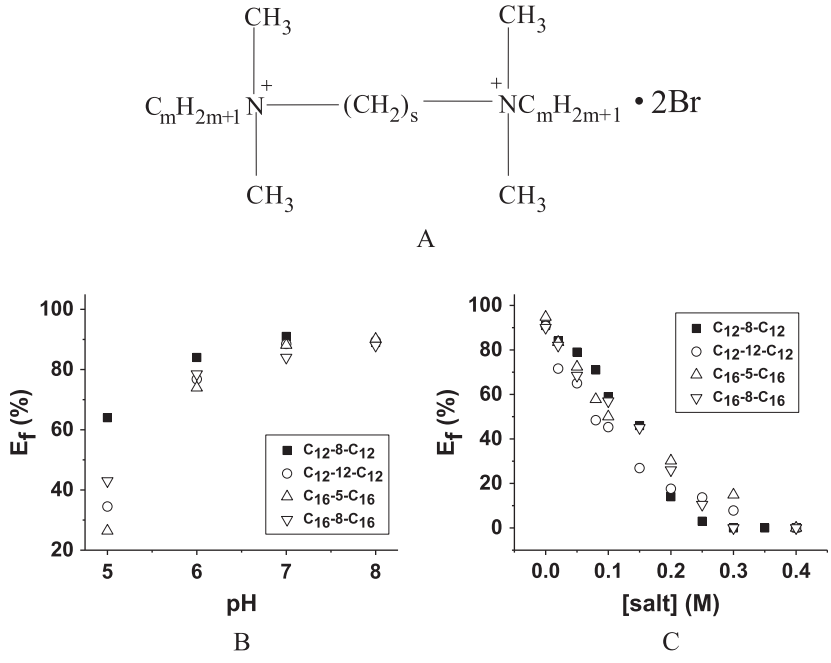

Fig. 1. Panel A shows the structure for C_m-s-C_m·2Br (m = 12, s = 2, 8, 12 or m = 16, s = 5, 8). Panel B shows the relationship between pH and the forward extraction efficiency (E_f) of OVA in the absence of salt. Panel C illustrates the effect of salt content on E_f at pH 7.3. Surfactant content (B and C): 10 mg/ml.