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Reverse micellar extraction of bovine serum albumin – A comparison between the effects of gemini surfactant and its corresponding monomeric surfactant

Jing Xiao, Juan Cai, Xia Guo *

School of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou, Jiangsu 225002, PR China

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ABSTRACT

Gemini surfactant displayed distinct advantages over monomeric surfactant in the liquid–liquid reverse micellar extraction process. First, less amount of gemini surfactant than monomeric surfactant was needed for transferring almost complete bovine serum albumin (BSA) into organic phase from aqueous phase. Second, the loading capacity of gemini surfactant reverse micelle phase was much higher than that of the corresponding monomeric surfactant reverse micelle. Third, efficient backward extraction (75–92%) of BSA could be effected in a wide pH range from 4 to 9 with gemini surfactant reverse micelle while a pH of *ca.* 4.3 is prerequisite to the recovery of BSA from monomeric surfactant reverse micelle. So far, the reports about the effect of surfactant structure on protein extraction have been limited. This study indicates the important role of the spacer of gemini surfactant in protein extraction process and may provide more knowledge on how to optimise surfactant structure.

1. Introduction

Reverse micelles are aggregates of surfactant molecules containing an inner core of water molecules, dispersed in a continuous organic solvent medium. They are optically transparent and thermodynamically stable. Reverse micelles have great potential for the separation, concentration and purification of bioactive proteins, including enzymes (Hatton, 1989; Kadam, 1986). Some of the advantages of reverse micellar extraction are no loss of native function/activity, high capacity, ease of scale-up, and potential for continuous operation (Harikrishna, Srinivas, Raghavarao, & Karanth, 2002; Hatton, 1989; Kadam, 1986; Lu, Chen, Li, & Shi, 1998).

In the liquid–liquid reverse micellar extraction process, a target protein is selectively solubilised into the organic phase (forward extraction) and subsequently stripped into the aqueous phase (backward extraction) by the addition of fresh aqueous buffer (Dungan, Bausch, Hatton, Plucinski, & Nitsch, 1991; Harikrishna et al., 2002; Lu et al., 1998). The electrostatic interaction between protein and surfactant plays a very important role in protein transfer. The kinetic study suggests that the behaviour of the two-phase surfactant-laden interface should also contribute to protein extraction (Dungan et al., 1991; Goklen, 1986).

Factors affecting the performance of the reverse micelle system are rather complicated, including the nature and concentration of target protein, pH, the concentration and species of ions in aqueous phase, the structures of organic solvent and cosolvent (i.e. alcohol) in reverse micellar solution, type and concentration of surfactant, content of cosolvent, and so on (Chen, Su, & Chiang, 2006; Harikrishna et al., 2002; Hebbar & Raghavarao, 2007; Imm & Kim, 2009; Lu et al., 1998; Noritomi, Kowata, Kojima, Kato, & Nagahama, 2006).

Albumins have been used as a model protein for diverse biophysical, biochemical and physicochemical studies (Carter & Ho, 1994; Jones, 1975). The molecular size of bovine serum albumin (BSA) is 3.5–3.6 nm and its isoelectric point (pI) is 4.9 (Carter & Ho, 1994; Jones, 1975). It should be noted that the surfactants used in protein extraction so far have been almost exclusively AOT (di-2-ethylhexyl sodium sulfosuccinate) and CTAB (cetyl trimethyl ammonium bromide) (Hebbar & Raghavarao, 2007; Lu et al., 1998; Shiomori, Ebuchi, & Kawano, 1998). As far as we know, there have been just a couple of reports where other surfactants were used (Imm & Kim, 2009; Noritomi et al., 2006), and research about the effect of surfactant structure (for example, the hydrophobic chain length, the size and structure of head group) on protein extraction efficiency has been limited. Therefore, to study whether and/or how the surfactant structure affects protein extraction efficiency

Abbreviations: BSA, bovine serum albumin; DTAB, dodecyl trimethyl ammonium bromide; C_{12}-8-C_{12}·2Br, octamethylene-α,ω-bis(dimethyldodecylammonium bromide); CTAB, cetyl trimethyl ammonium bromide; E_f, forward extraction efficiency; E_b, backward extraction efficiency; CD, circular dichroism; cmc, critical micelle concentration.

* Corresponding author. Tel.: +86 0514 87975590x9513; fax: +86 0514 87975244.
E-mail address: guoxia@yzu.edu.cn (X. Guo).

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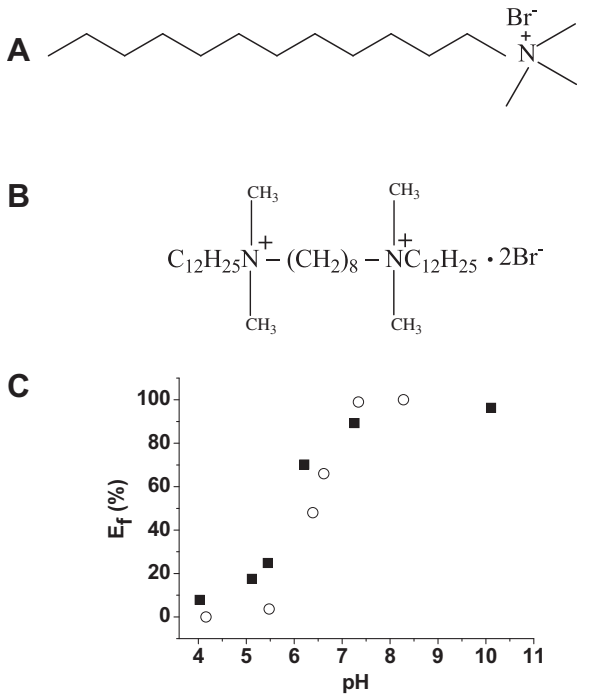

Fig. 1. Panels A and B show the structures of DTAB and C_{12}-8-C_{12}·2Br, respectively. Panel C shows the relationship between the forward extraction efficiency E_f of BSA with reverse micelle and pH of the aqueous phase (with 0.1 M NaCl). Reverse micelle: DTAB/hexane/hexanol (symbol ■), C_{12}-8-C_{12}·2Br (symbol ○). Surfactant concentration: 20 mg/ml. V_{hexane}:$V_{hexanol}$: 9:1.

should be meaningful for liquid–liquid reverse micellar extraction process.

Since the extraction of BSA with CTAB reverse micelles has been reported, in the present paper, we will observe the extractions of BSA with DTAB (dodecyl trimethyl ammonium bromide, shown in Fig. 1A) reverse micelle and gemini surfactant octamethylene-α,ω-bis(dimethyl-dodecylammonium bromide) (designated as C_{12}-8-C_{12}·2Br, shown in Fig. 1B) reverse micelle. DTAB has a shorter hydrophobic alkyl chain than CTAB. By comparing protein extraction using DTAB reverse micelle with that using CTAB reverse micelle, we could get some information about the effect of alkyl chain length. C_{12}-8-C_{12}·2Br molecules are made up of two hydrophilic head groups, two hydrophobic chains, and a spacer (octamethylene) linking to the two head groups via covalent bonds; DTAB can be considered as the corresponding monomer. The spacer of gemini surfactant can considerably change the physical and chemical properties, including both the densities of the head group charge and of the alkyl chain as well as the molecular geometry. As a result, gemini surfactants exhibit superior surface activity compared to that of the corresponding conventional (monomeric) surfactants (Menger & Keiper, 2000; Zana, 2002; Zana & Xia, 2004). Therefore, a comparison between the extraction efficiency with DTAB reverse micelle and that with C_{12}-8-C_{12}·2Br reverse micelle should help us know more about how to optimise the surfactant structure in operating reverse micellar extraction.

2. Materials and methods

2.1. Materials

Dodecyl trimethyl ammonium bromide (DTAB) and cetyl trimethyl ammonium bromide (CTAB) were bought from Amresco Co., Solon, OH (99% purity). BSA was purchased from Shenggong Co. (biological grade, Shanghai, China). *n*-Hexane and 1-hexanol were bought from Chinese Chemicals (analytical grade, Sinopharm chemical reagent Co., Ltd, Shanghai, China). The water used was ultrapure prepared by Milli-Q system (Millipore Co., Billerica, MA).

C_{12}-8-C_{12}·2Br was prepared according to Zana, Benrraou, and Rueff (1991). Its purity was checked by NMR and elemental analysis.

The buffer used in the forward extraction was prepared from disodium hydrogen phosphate (10 mM)/citric acid (pH ⩽8.0) and glycine (10 mM)/sodium hydroxide (pH >8.0). The stripping solution in the backward extraction was prepared from disodium hydrogen phosphate (10 mM)/citric acid (pH ⩽8.0), or by acetic acid/sodium acetate (10 mM, pH ⩽5.7) and disodium hydrogen phosphate/sodium dihydrogen phosphate (10 mM, pH 5.8–8.0). The stripping solution with pH higher than 8.0 was prepared from glycine (10 mM)/sodium hydroxide.

2.2. Methods

2.2.1. Forward extraction

Forward extraction experiments were carried out by mixing aqueous and organic phases, followed by vortex for 10 min at room temperature. The aqueous phase was prepared by dissolving BSA in buffer of known pH and salt concentration. BSA concentration was maintained at 1 mg/ml unless mentioned otherwise. The organic phase was prepared from known quantities of *n*-hexane, 1-hexanol, surfactant (the content is higher than its critical micelle concentration) and water. For all the experimental runs, the volume ratio of aqueous phase to organic phase was 1:1. Phase separation was carried out in a laboratory centrifuge at 3000*g* for 20 min. The organic phase separated from the mixture of the forward extraction was used for the backward extraction.

2.2.2. Backward extraction

Backward extraction was carried out by mixing the organic phase of the forward extraction with an equal volume of stripping phase (buffer of known pH and salt concentration), followed by vortexing for 15 min at room temperature, then centrifuging at 3000*g* for 20 min and separation of the two phases.

2.3. Analytical methods

2.3.1. BSA concentration and extraction efficiency

BSA concentrations were determined by UV–Vis spectrophotometer at 278 nm. The readings were taken in triplicate and average values were reported. Efficiencies of forward (E_f) and backward (E_b) extractions were estimated using the equations given below, where $[BSA]_f$ and $[BSA]_o$ represent BSA concentrations in feed and in organic phase of forward extraction, respectively, and $[BSA]_{aq1}$ and $[BSA]_{aq2}$ represent BSA concentrations in aqueous phases of forward extraction and backward extraction, respectively.

$$E_f(\%) = \frac{[BSA]_o(mg/ml)}{[BSA]_f(mg/ml)} \times 100 = \frac{[BSA]_f - [BSA]_{aq1}(mg/ml)}{[BSA]_f(mg/ml)} \times 100$$

$$E_b(\%) = \frac{[BSA]_{aq2}(mg/ml)}{[BSA]_f(mg/ml)} \times 100$$

2.3.2. Circular dichroism spectroscopy

Circular dichroism (CD) experiments were carried out on a JASCO J-810 spectrometer at 25.0 ± 0.1 °C (JASCO International Co., Ltd., Tokyo, Japan). CD spectra were recorded between 200 and 250 nm, using a step interval of 0.5 nm, an integration time of 0.5 s, and a bandwidth of 1.0 nm. The scanning rate was 100 nm/min and four scans were averaged. The path length of the quartz cuvette used was 1 cm.

All the experiments were repeated at least three times.