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Extraction of bovine serum albumin with reverse micelles from glucosylammonium and lactosylammonium surfactants

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ABSTRACT

Reverse micelle extraction is still in the stage of laboratory. Major limitation associated with use of synthetic surfactants in reverse micelle extraction process is the unfolding or denaturation of proteins. Sugar surfactants are thought non-toxic and environmentally benign, and can exhibit interesting interfacial properties, but the application of sugar-based surfactants in protein extraction is still limited. In the present study, we extracted bovine serum albumin (BSA) by using reverse micelles from glucosylammonium (**GA**) and lactosylammonium (**LA**) surfactants (with dicarboxylate as counter ion). It was found that under optimum condition, (1) the maximum forward extraction efficiency was ca. 86% with **GA**, while only around 50% with **LA**, and (2) almost all BSA solubilized in reverse micelles prepared from **GA** could be recovered into aqueous phase, while the recovery of BSA from the reverse micelles of **LA** was lower. In addition, the optimum extraction parameters were closely related to surfactant structure. Therefore, the electrostatic interaction, H-bonding and sugar head size should be important for BSA transfer.

1. Introduction

Reverse micelle is a kind of surfactant aggregate. It is prepared in nonpolar media by surfactant, oil and cosurfactant. The polar head of surfactant is toward the reverse micelle core and the hydrophobic tail in the continuous oil phase; water can be solubilized into reverse micelle core. Reverse micellar extraction of protein has potential for continuous operation and is easy to scale up with no loss of native function/activity and high capacity of protein; it has now been considered as an alternative to conventional separation and purification procedures for bioactive proteins [1–7].

There are two steps in liquid–liquid reverse micellar extraction process, which are called forward extraction and backward extraction. In forward extraction, a target protein is selectively solubilized into the organic phase. In backward extraction, the protein solubilized into the organic phase is stripped into water phase by addition of fresh aqueous buffer, also called stripping solution [3,7–9]. Although there have been lots of reports about this technology, it is still in the stage of laboratory. So far, it has been concluded that the electrostatic interaction between reverse micelle and protein plays a key role in protein extraction and many extraction parameters, including aqueous phase pH, ionic strength, nature and concentration of target protein, and reverse micelle composition, affect the extraction result; to increase the attraction between protein and reverse micelle is important for protein transfer into reverse micelle and to destroy the attraction is necessary for protein back into water phase from reverse micelle [1,4,6,9–15]. In addition, the binding of protein with surfactant can change protein conformation, which generally results in the variations of polarity and stability of protein [16–18]. Major limitation associated with use of synthetic surfactants is the unfolding or denaturation of protein. Therefore, how to recover protein not only with high efficiency but also with high activity (or unchanged conformation) still attracts researchers' interests, and thus, surfactant which is nontoxic should be worthy of attention.

Sugar surfactants are a relatively new class of surfactants, comprising sugar headgroup and alkyl chain. They may occur in nature (as certain sugar esters, for example) or be synthesized chemically or enzymatically [19–27]. Sugar surfactants are thought non-toxic and environmentally benign, and can exhibit interesting interfacial properties, so they have engrossed increased attention recently in areas of food, medicine, self-assembly and molecular recognition in biological systems [28–32]. However, the application of sugar-based surfactants in protein extraction is still limited.

We [33,34] recently studied the micellizations of glucosylammoniums and lactosylammoniums (with different carboxylates as the counter ions) and found that the structures of sugar and counter ion both controlled the aggregation behaviors. Further, their interaction with DNA was affected by the structures of sugar and counter ion.

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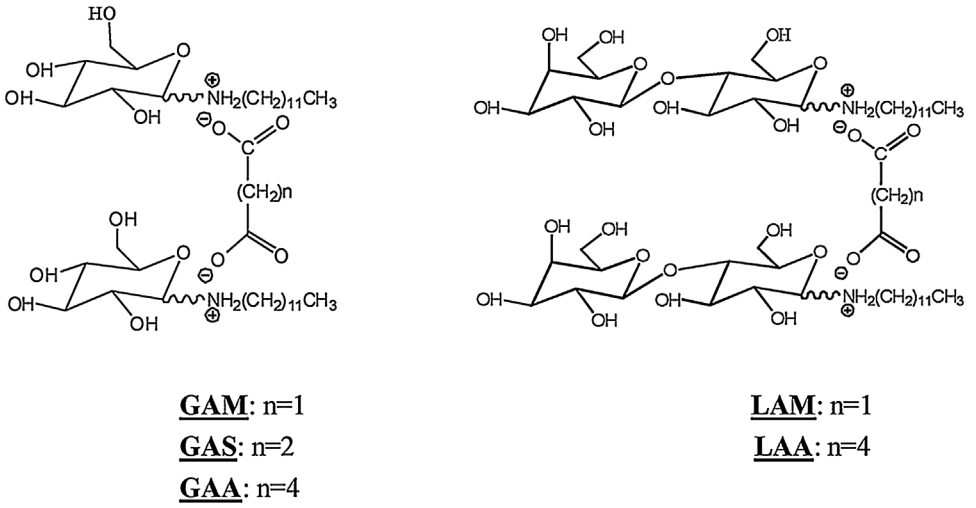

Scheme 1. Structures for di(N-dodecylglucosylammonium) dicarboxylate and di(N-dodecyllactosylammonium) dicarboxylate.

In the present study, we prepared reverse micelles using these cationic sugar surfactants and observed their performance on the extraction of model protein bovine serum albumin (BSA), from which the effects of sugar head and counter ion on extraction efficiency were illustrated.

2. Experimental section

2.1. Materials

BSA was purchased from Shenggong Co. (biological grade, Shanghai, China). *n*-Octane and 1-hexanol were bought from Chinese Chemicals (analytical grade, Sinopharm, Shanghai, China). The water used was deionized. Cationic sugar surfactants di(N-dodecylglucosylammonium) malonate (**GAM**, molecular weight: 799.05), di(N-dodecylglucosylammonium) succinate (**GAS**, molecular weight: 813.08), di (N-dodecylglucosylammonium) adipate (**GAA**, molecular weight: 841.13), di(N-dodecyllactosylammonium) malonate (**LAM**, molecular weight: 1123.33) and di(N-dodecyllactosylammonium) adipate (**LAA**, molecular weight: 1165.42) were prepared according to our previous reports [33,34], and the structures were shown in Scheme 1. Their critical micelle concentrations in water were 1.25 (for **GAM**), 0.73 (**GAS**), 1.58 (for **GAA**), 4.69 (for **LAM**), and 4.12 mg/ml (for **LAA**) [33,34].

2.2. Methods

2.2.1. Forward extraction

The aqueous phase in the forward extraction was prepared by dissolving BSA (1 mg/ml) in buffer of known pH and salt concentration; the buffer was prepared by disodium hydrogen phosphate (10 mmol L^{-1})/citric acid (pH: 3.0–8.0) or glycine (10 mmol L^{-1})/sodium hydroxide (pH: 8.0–10.0), and the salt concentration was lower than 0.60 M.

The organic phase (i.e. reverse micellar phase) was prepared by known quantities of *n*-octane, 1-hexanol, surfactant and water. The volume ratio of *n*-octane to 1-hexanol was between 1 and 9. The surfactant content was between 1 and 30 mg/ml. The molar ratio of water to surfactant was 100; the addition of water could make surfactant dissolved easily and quickly. The organic phase obtained was clear and optically transparent.

The aqueous and organic phases were mixed in equal volumes and vortexed for 15 min at 25 °C. After centrifugation at 17,968*g* (Allegra 64R centrifuge, Beckman Coulter, USA) for 20 min, the interface between the aqueous and organic phases were clear. Then, the organic phase was separated, which was used for the subsequent backward extraction.

2.2.2. Backward extraction

The stripping solution was prepared by acetic acid/sodium acetate (10 mmol L^{-1}, pH 4.3–5.7) or disodium hydrogen phosphate/sodium dihydrogen phosphate (10 mmol L^{-1}, pH: 5.8–8.0). The organic phase of the forward extraction was mixed with an equal volume of stripping solution. The salt concentration in the stripping solution was changed from 0 to 1.0 M. The mixture was vortexed for 15 min at room temperature and then, centrifuged at 17968*g* (Allegra 64R centrifuge, Beckman Coulter, USA) for 20 min. Then, the two phases could be separated.

2.2.3. Determination of extraction efficiency of BSA

BSA concentrations were determined by Uv–vis spectrophotometer at 278 nm. Efficiencies of forward (E_f) and overall (E_o) 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.

$$E_f(\%)=\frac{[BSA]_o(mg/ml)}{[BSA]_f(mg/ml)}\times 100=\frac{[BSA]_f-[BSA]_{aq1}}{[BSA]_f}\times 100 \quad (1)$$

$$E_o=\frac{[BSA]_{aq2}(mg/ml)}{[BSA]_f(mg/ml)}\times 100 \quad (2)$$

The extraction experiments were repeated at least 3 times and the error limitations for E_f and E_o were within ± 5%.

2.2.4. Circular dichroism spectroscopy

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

2.2.5. Determination of reverse micelle size

The reverse micelle size was measured by using dynamic light scattering on a Malvern Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK). The temperature of the sample cell was 25.0 °C, and the scattering angle was 90°. All the experiments were repeated at least three times and the error for the size was not higher than ± 10%.