Process Biochemistry 50 (2015) 272–278

Contents lists available at ScienceDirect

Process Biochemistry

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

Effect of surfactant structure on reverse micellar extraction of ovalbumin

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ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form
26 September 2014
Accepted 28 November 2014
Available online 12 December 2014

Keywords:
Liquid–liquid extraction
Protein recovery
Reverse micelles
Ovalbumin
Surfactant

ABSTRACT

The reports about reverse micellar extraction of protein with hydrophobicity (such as ovalbumin) have been very limited; most of the studies are focused on extraction of protein with very limited hydrophobicity, such as bovine serum albumin (BSA). In the present study, we found that similar to BSA, ovalbumin could be transferred into CTAB (cetyltrimethylammonium bromide) and DTAB (dodecyltrimethylammonium bromide) reverse micelles most efficiently when pH reached 7 and recovered into water at pH 4.3. However, the transfer of ovalbumin molecules between water and organic phases was less efficient compared with BSA transfer. Noticing the surfactants used in reverse micellar extraction have been mostly conventional surfactants, in this study, we further used gemini surfactant pentamethylene-α,ω-bis(dimethyldodecylammonium bromide) reverse micelle to extract ovalbumin. We found the flexible spacer of the gemini surfactant could enhance ovalbumin transfer between the two phases and less amount of surfactant was needed. Besides, ovalbumin could be recovered from the gemini surfactant reverse micelle under wider and milder condition than BSA. Thus far the reports about the effects of surfactant structure and/or protein properties on protein extraction have been limited yet. This study may help us know more about how to optimize surfactant structure based on protein properties.

1. Introduction

Reverse micelles are aggregates of surfactant molecules in a continuous organic solvent medium, with an inner core of water molecules. Two steps are included in the liquid–liquid reverse micellar extraction process: forward extraction and backward extraction. Firstly, protein dissolved in aqueous buffer is selectively solubilized into the inner core of reverse micelle (forward extraction) and then stripped into the aqueous phase by the addition of fresh aqueous buffer (backward extraction). It is reported that reverse micellar extraction process is affected by pH, concentrations of salt and surfactant, and content of protein in feed [1–3].

It has been considered that reverse micelles have great potential for the separation, concentration and purification of proteins [1,3–7], including enzymes [6,8–11]. However, reverse micelle extraction technology is still in the stage of laboratory. The surfactants used have been mostly conventional ionic surfactants, such as dioctylsodium sulfosuccinate (AOT) [4,9,12–14] and cetyltrimethyl ammonium bromide (CTAB) [3,8,12,15,16], and the protein with high solubility in water (such as bovine serum albumin, BSA) has been always chosen as the model protein [1,2,14,15,17]. The studies about the effect of surfactant structure on reverse micellar extraction efficiency and the relationship between reverse micelle extraction efficiency and protein properties/structures have still been very limited [1–18].

Ovalbumin (OVA), a major monomeric glycophosphoprotein representing >50% (w/w) of egg white proteins, is a typical globular protein with a diameter of 3 nm, an isoelectric point (pI) of about 4.6 [19,20], a molecular weight of approximately 45 kDa (containing 385 amino acid residues) [21], and 30.6% α-helicity [19]. BSA has a similar pI to OVA, but it is bigger than OVA; its molecular weight is ca. 66 kDa, containing 580 amino acid residues and an α-helix structure of 67% [22]. Besides, BSA is much more hydrophilic than OVA; OVA is widely used in the food industry due to its emulsifying and foaming capabilities and its ability to form gels upon heating [23].

In the present paper, OVA was chosen as model protein and extracted with reverse micelles. By comparing the

Abbreviations: OVA, ovalbumin; DTAB, dodecyl trimethyl ammonium bromide; C_{12}-5-C_{12}·2Br, pentamethylene-α,ω-bis(dimethyldodecylammonium bromide); CTAB, cetyl trimethyl ammonium bromide; E_f, forward extraction efficiency; E_b, backward extraction efficiency; CD, circular dichroism.

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http://dx.doi.org/10.1016/j.procbio.2014.11.013
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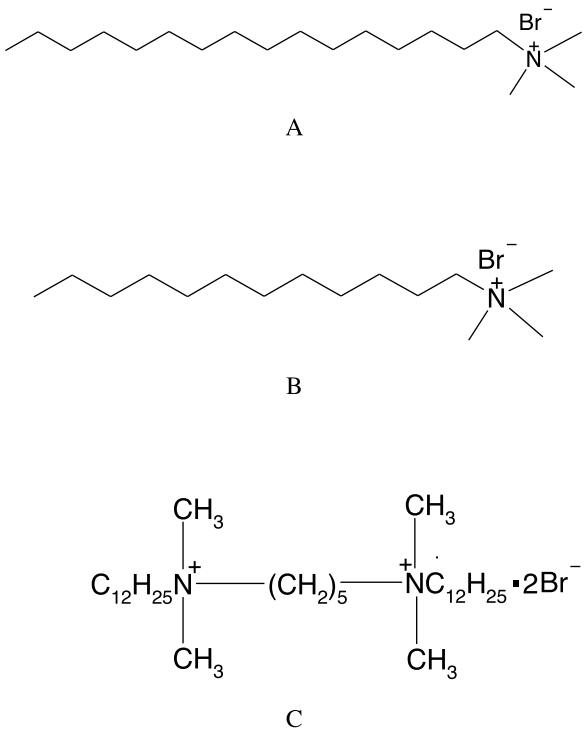

Fig. 1. Structures for CTAB (A), DTAB (B) and C_{12}-5-C_{12}·2Br (C).

extraction efficiency of OVA with that of BSA, some information about the role of protein properties in reverse micellar extraction should be obtained. Moreover, in the present study, both single-chained surfactants (CTAB and DTAB (dodecyl trimethyl ammonium bromide), shown in Fig. 1A and B) and gemini surfactant pentamethylene-α,ω-bis(dimethyldodecylammonium bromide) (designated as C_{12}-5-C_{12}·2Br, shown in Fig. 1C) were used. Gemini surfactant molecules are made up of two hydrophilic head groups, two hydrophobic chains, and 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 [24]. Hence, a comparison of extraction efficiencies with conventional surfactant reverse micelle and with C_{12}-5-C_{12}·2Br reverse micelle should be useful to optimize surfactant structure in operating reverse micelle extraction.

2. Materials and methods

2.1. Materials

DTAB and CTAB were bought from Amresco Co., USA (99%). OVA was purchased from Xibao 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}-5-C_{12}·2Br was prepared according to reference [25]. Its purity was checked by NMR and elemental analysis.

The buffer used in the forward extraction was 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 from 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. Its concentration was maintained at 1 mg/ml unless mentioned otherwise. Reverse micelle was prepared from known quantities of *n*-hexane, 1-hexanol, surfactant and water. The aqueous and organic phases were mixed with a volume ratio of 1:1 by vortex for 10 min at room temperature, and then the mixture was centrifuged at 14,000 rpm 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 and stripping phase (buffer of known pH and salt concentration) were mixed by vortex for 15 min at room temperature for backward extraction. Then, the mixture was centrifuged at 14,000 rpm for 30 min, followed by the separation of aqueous phase.

2.3. Analytical methods

2.3.1. OVA concentration and extraction efficiency

OVA concentration was determined by UV–vis spectrophotometer at 278 nm, and three scans were averaged. Efficiencies of forward (E_f) and backward (E_b) extractions were estimated using the equations given below, where $[OVA]_f$ and $[OVA]_o$ represent OVA concentrations in feed and in organic phase of forward extraction, respectively, and $[OVA]_{aq}$ and $[OVA]_s$ represent OVA concentrations in aqueous phases of forward extraction and backward extraction, respectively. V_f, V_o and V_s represent the volumes of the feed, the organic phase in forward extraction, and the stripping solution in backward extraction.

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

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

All the extraction experiments were repeated at least 3 times. The error limitation of extraction efficiencies was within ±5%.

2.3.2. Circular dichroism spectroscopy

Circular dichroism (CD) experiments were done with a JASCO J-810 spectrometer at 25.0 ± 0.1 °C (JASCO International Co., Ltd., Tokyo, Japan). CD spectra were recorded between 180 and 300 nm, using a step interval of 0.1 nm. The scanning rate was 100 nm/min and four scans were averaged.

All the experiments were repeated at least three times.

3. Results

3.1. Forward extraction with single-chained cationic surfactant reverse micelles

Cosurfactant content is an important parameter in extracting protein with reverse micelles. So, we first examined the effect of cosolvent (i.e. 1-hexanol) content on the forward extraction efficiency (E_f) (Fig. S1), from which, when the volume ratio of 1-hexanol to *n*-hexane (i.e., $V_{hexanol}/V_{hexane}$) was ca. 11%, a maximum OVA can be transferred from water to the reverse micelles. Therefore, in the following experiments, $V_{hexanol}/V_{hexane}$ was fixed to 11%.