Effect of Diethanollauramide on Extraction of Water and Proteins by AOT Reverse Micelles

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Effect of the addition of diethanollauramide (LDEA), which is expected to interact moderately with AOT molecule, into the organic phase on the extraction of water and bovine serum albumin (BSA) into the reverse micellar phase was investigated. The water concentration extracted into the organic phase increased with an increase in the LDEA concentration. The increase in the water concentration become larger by the decrease in the NaCl concentration in the aqueous phase. The extraction of BSA was carried out using the LDEA-AOT reverse micellar solution. In the absence of LDEA, the maximum of BSA extraction is obtained at around pH6 and the extraction decreases sharply with further increase in pH. In the presence of LDEA, the extraction of BSA increases with the concentration of LDEA added and the pH range extracting BSA is extended to higher pH range.

INTRODUCTION

Reverse micelles, which are self-aggregates of surfactant molecules in apolar organic phase, have the ability to extract proteins from aqueous phase [1]. One intensively studied surfactant is sodium bis(2-ethylhexyl) sulfosuccinate (AOT). Extraction of proteins into AOT reverse micelles is mainly controlled by the pH and salt concentration in the aqueous phase. The mechanism of protein extraction has been explained by the interaction between protein and reverse micelles, such as electrostatic, steric and hydrophobic interactions [4]. The development of new effective reverse micellar systems for protein extraction have been expected. Previously, we reported that the addition of either long chain alkyl amines [2] or water-soluble amines [3] to the extraction system using AOT reverse micelles is effective for the extraction of proteins by the control of pH.

The micellar-micellar interaction and the interaction of reverse micelles with the liquid-liquid interface between organic and aqueous bulk phases would affect to extraction of proteins [5, 6]. The percolation process clearly reflects the micellar-micellar interaction and it can be quantified by the measurement of electrical conductivity of the reverse micellar solution as a function of water content or temperature.

It is well known diethanollauramide (LDEA), whose structure is shown in **Fig. 1**, interacts moderately with anionic surfactants due to its weakly cationic hydrophilic head group. The interaction of LDEA with anionic surfactants is known to enhance the formation and the stability of foam. Hence, LDEA is also expected to interact moderately with AOT molecule and to affect the structure and the extraction behavior of the AOT reverse micelles. In this study, the effect of the addition of diethanollauramide in the organic phase on the extraction of water and bovine serum albumin (BSA) into the reverse micellar phase and the percolation phenomena was investigated under various condition.

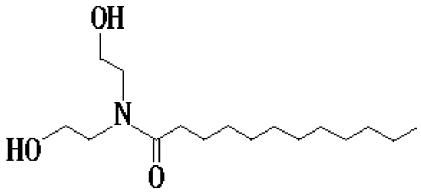

Fig. 1 Structure of diethanollauramide (LDEA)

EXPERIMENTAL

Reagents

The surfactant constructing reverse micelles was sodium bis(2-ethylhexyl) sulfosuccinate (AOT) from Nacalai Tesque Co. Diethanollauramide (LDEA) was used as additives for the reverse micellar system, was obtained from Wako Pure Chemical Co. AOT was dissolved in isooctane. A small amount of water was added to the AOT/isooctane solution to form reverse micelles, and then LDEA was dissolved in the solution which was used as an organic phase. A buffer solution containing NaCl (0.1 - 1.0 kmol/m^3) was used as an aqueous phase. Acetic acid-sodium acetate (pH 3-6), dimethyl glutamate-NaOH (pH 5-8), glycine-NaOH (pH 9-11),

and Na_2HPO_4-NaOH (pH 11-12) were used as buffers at 50 mol/m^3 concentrations in all experiments. Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. and dissolved in the buffer solution containing salts.

Methods

Extraction and back-extraction of BSA were carried out by the phase transfer method. Same volumes of the organic and aqueous phases were placed in a screw capped sample tube. The two phases were dispersed completely by a magnetic stirrer for 30 min. at 298 K. After mixing, the solution was separated into two phases by centrifugation at 3,500 rpm for 15 min and the organic and aqueous phases were collected, respectively. Back-extraction of BSA from the micellar phase was carried out by contacting the organic phase extracted BSA with a new aqueous phase in the same manner as for the forward extraction.

The measurement of percolation phenomena of the reverse micellar phase was measured by the same method in the previous paper [5]. The water concentration in the micellar organic phase was determined by Karl-Fisher titration using a Kyoto Electronics MKS-1S. The concentration of BSA in the aqueous and organic phases, was measured by adsorption at 280 nm with a Hitachi UV 3200.

RESULTS AND DISCUSSION

Extraction behavior of water

Diethanollauramide(LDEA) was dissolved in the AOT/isooctane solution containing small amount of water, which formes reverse micelles, however, not dissolved in isooctane or the AOT/isooctane solution without water. The formation of the reverse micelles would be essentioal to dissolve diethanolamide. This means that LDEA is dissolved into the membrane layer of AOT forming reverse micelles.

The effect of LDEA concentration on the water concentration extracted in the reverse micellar phase at various NaCl concentration in the aqueous phase and 0.1 M AOT in the organic phase is shown in **Fig. 2**. The water concentration extracted into the organic phase increased with an increase in the LDEA concentration. The increase in the water concentrations become larger by the decrease in the NaCl concentration in the aqueous phase. LDEA can interact with AOT by weak electrostatic interaction because the hydrophilic group of LDEA is weak cationic one by its polarization. Hence, it is considered that the interaction cause the swelling of the reverse micelles. It is, however, considered that the interaction between AOT

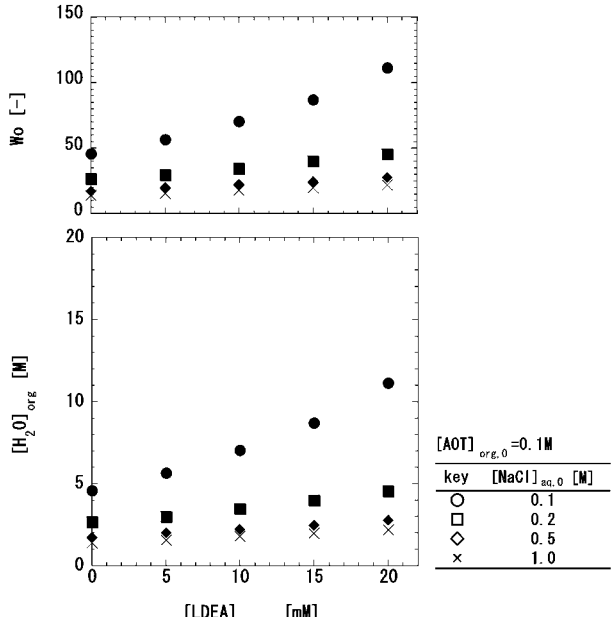

Fig. 2. Effect of LDEA concentration on the water extraction into the reverse micellar phase at various NaCl concentration in the aqueous phase and 0.1 M AOT in the organic phase

and LDEA is difficult to occur by the electrostatic shielding effect at high salt concentration.

The effect of the molar ratio of LDEA to total surfactant concentration in the organic phase on the water extraction is shown in **Fig. 3**. The concentration of water extracted into the organic phase increased with the molar ratio of LDEA. The water content, *Wo*, was, however, almost same value independent on the AOT concentration. This means the LDEA molecules added are form the mixed reverse micelles together with AOT molecules.

It is clear that the addition of LDEA cause a large increase in the water extraction. This would be caused by the insertion of LDEA molecule to the membrane layer of AOT reverse micelles to induce a expansion of the membrane layer.

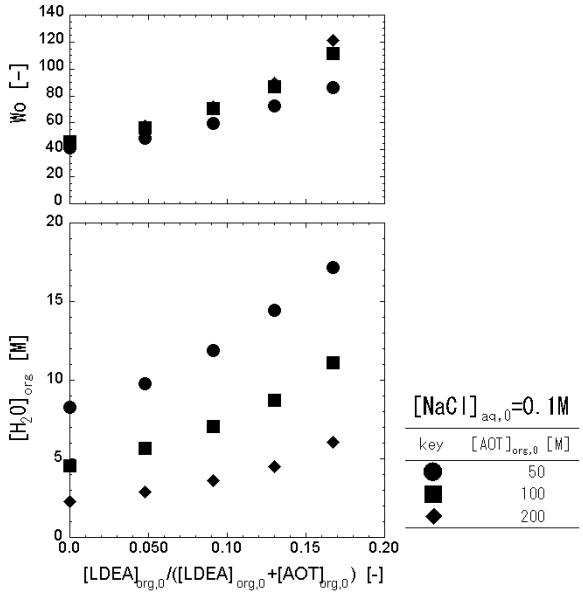

Fig. 3. Effect of the molar ratio of LDEA to total surfactant concentration in the organic phase on the water extraction

Percolation behavior of the reverse micellar solution of AOT and LDEA

The electrical conductivity of the reverse micellar solution at various LDEA concentration plotted against the volume fraction of water in the organic phase, ϕ_{aq}, is shown in **Fig. 4**. At low range of ϕ_{aq}, the conductivities were insensitive to the value of ϕ_{aq}. However, the conductivities increased sharply when the ϕ_{aq} exceed respective threshold values, ϕ_P, indicating percolation phenomena. An increase in LDEA caused a decrease in the percolation threshold. This result

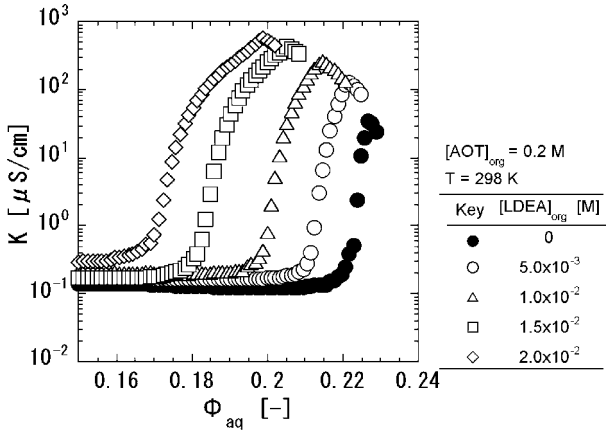

Fig. 4. Effect of the LDEA concentration on the percolation process of the mixed reverse micellar solution of AOT and LDEA.

indicates the destabilization of reverse micelles by the addition of LDEA.

The difference of percolation threshold, $\Delta\phi_P$, in the presence and the absence of LDEA reflects the effect of the LDEA concentration on the percolation processes. $\Delta\phi_P$ plotted against the LDEA concentration in **Fig. 5**. There is a linear correlation between $\Delta\phi_P$ and the LDEA concentration. The negative slope means the destabilization of the reverse micelles by the addition of LDEA. This results quite similar to the addition of propanol and buthanol [6]. Long chain alcohols, such as hexanol and octanol, show positive large slopes which means the stabilization of the reverse micelles by the addition of these alcohols. LDEA also has long alkyl chain. The effect of LDEA on the percolation phenomena is quite opposite direction with that of long chain alcohols. It is considered the interaction of hydrophilic group of LDEA with sulfonic group of AOT would be larger than that of the alcohols. Hence, LDEA may affect strongly to the membrane structure of the reverse micelles to cause destabilization. Further investigation is needed for the interaction of LDEA with AOT reverse micelles.

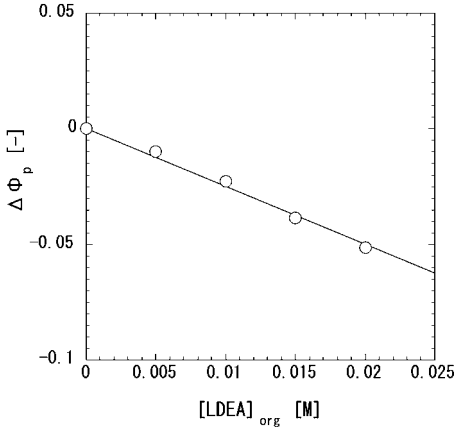

Fig. 5. Effect of the LDEA concentration on the percolation threshold, $\Delta\phi_P$.

Extraction behavior of BSA

The extraction of BSA was carried out using the LDEA-AOT reverse micellar solution. The effect of pH on the BSA extraction using the reverse micellar solution at various LDEA concentrations is shown in **Fig. 6**. In the absence of LDEA, the maximum of BSA extraction is obtained at around pH 6 and the extraction decreases sharply with further increase in pH. In the presence of LDEA, the extraction of BSA increases with the concentration of LDEA added and the pH range extracting BSA is extended to higher pH range. The electrostatic repulsion, which induces the decrease of the extraction, between the reverse micelles and BSA may be reduced by the addition of LDEA.

The effect of initial BSA concentration on the BSA concentration using the LDEA-AOT and the AOT reverse micellar solution is shown in **Fig. 7**. In the case of the AOT reverse micellar system, the BSA concentration extracted into the reverse micellar phase increased with the AOT concentration and decreased moderately with an increase in initial BSA concentration at its high concentration rage. On the other hand, The BSA concentration extracted using the LDEA-AOT reverse micellar solution was considerable larger than that using the AOT system. The extraction behavior of BSA was observed large difference between the LDEA-AOT and the AOT systems. It is clear that the addition of LDEA in the organic phase is effective for the extraction of BSA with the reverse micelles.

Back-extraction of BSA

The back-extraction of BSA extracted into the LDEA-AOT reverse micellar solution was carried out by the change of NaCl concentration in the aqueous solution used for the back-extraction. The effect of the NaCl concentration on the back-extraction at pH 9.0-9.1 is shown in **Fig. 8**. BSA was successfully back-extracted to the aqueous phase. Though the reduced fraction from the organic phase, R_b, increased with an increase in the NaCl concentration, the back-extracted fraction to the aqueous phase, E_b, decreased. This suggests that BSA was denatured at high NaCl concentration. In reality, a small amount of aggregate of

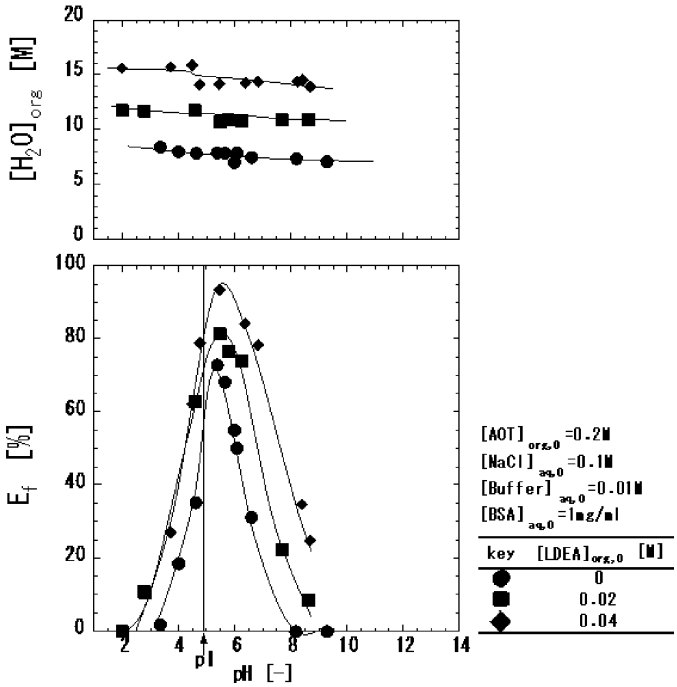

Fig. 6. Effect of pH on the extraction of BSA at various concentrations of LDEA.

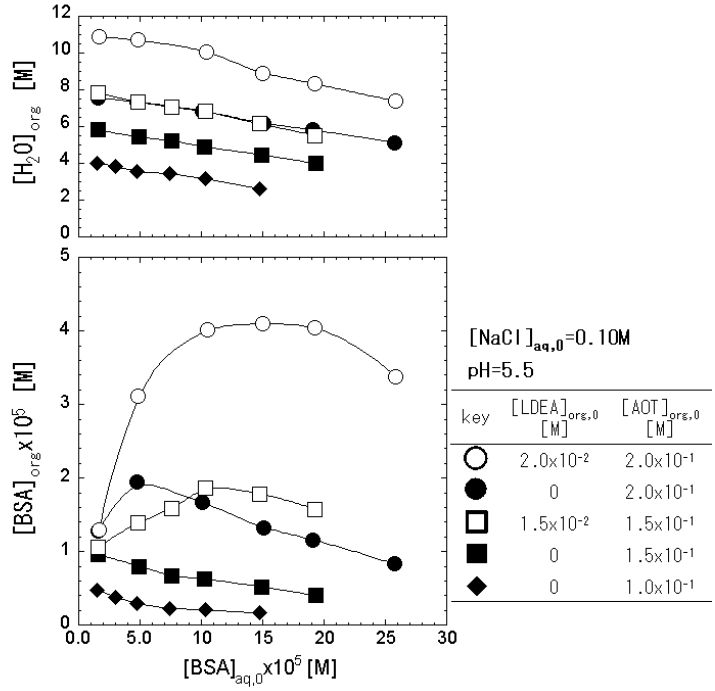

Fig. 7. Effect of initial BSA concentration on the extraction of BSA at various concentrations of LDEA and AOT.

protein was observed at the interface after the back-extraction process at high NaCl concentration.

COMCLUSIONS

Diethanollauramide was added to the extraction system of AOT reverse micelles. The

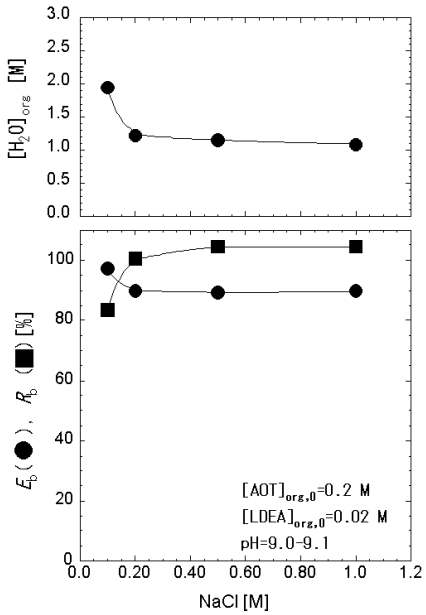

Fig. 8. Effect of NaCl concentration on the Back-extraction of BSA

extraction of water increased by the addition of LDEA. The increase in the water extraction would be caused as a result of the interaction of LDEA with the reverse micelles by electrostatic interaction between anionic head group of AOT and weakly cationic head group of LDEA.

The percolation phenomena of the AOT reverse micellar solution were also affected by the addition of LDEA. The difference of percolation threshold, $\Delta\phi_P$, had a linear correlation with the LDEA concentration with a negative slope value, which means the destabilization of the reverse micellar system.

The extraction of BSA was enhanced by the addition of LDEA. The extraction behavior of BSA with LDEA-AOT mixed system was different from that with AOT alone system. The back-extraction of BSA was successfully achieved by contacting with a new aqueous phase containing NaCl at pH 9.

NOMENCLATURE

E_f = extracted fraction of protein into organic phase [%]
E_b = back extracted fraction of protein into aqueous phase [%]
R_b = removed fraction of protein from organic phase [%]
Wo = molar ration of water to AOT [-]
ϕ_P = percolation threshold in the presence of LDEA
$\Delta\phi_P$ = difference of percolation threshold in the presence and the absence of LDEA
[ ] = molar concentration in bracket [kmol/m3]

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