PROTEIN EXTRACTION BY REVERSED MICELLES USING DIOLEYL PHOSPHORIC ACID

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Introduction

A separation technique using reversed micelles is an attractive approach to the separation and purification of bioproducts from fermentation media or very dilute solutions. Recently, extraction of proteins (including enzymes)[3,4,7,8,10] or amino acid[5] using reversed micelles were undertaken by many investigators and a few applications of liquid membrane systems have also been studied[1,9]. However, little attention has been paid to searching for a suitable surfactant for protein separation. The surfactant plays a key role in the separation process because the surfactant's aggregate in a nonpolar solvent forming a water-pool provides a unique environment for the proteins. In this study, we synthesized a series of dialkyl phosphoric acid surfactants. The extraction behavior of some proteins from an aqueous phase into their reversed micelles was also investigated in comparison with that with the conventional surfactant, AOT.

1. Experimental

1.1 Reagent

A series of dialkyl phosphoric acids, $2C_nPA$, (carbon number, $n=8$, 12, 14, 16, 18, $18\Delta^9$ (oleyl)) were synthesized as described in a previous paper[6]. Sodium di-2-ethylhexyl sulfosuccinate (AOT) of reagent grade supplied from American Cyanamid Co. was used without further purification. Organic solvent was isooctane of analytical grade. The proteins used (lysozyme, myoglobin, hemoglobin and γ-globulin) were products of Sigma Chemical Co. and were used as received.

1.2 Protein extraction

The pH and ionic strength of the aqueous solution were adjusted using phosphate buffer solution and potassium chloride. Equal volumes ($10\,cm^3$) of aqueous and organic solutions of known concentrations were shaken in flask immersed in a thermostatted water bath (303K), and allowed to reach equilibrium. After about 24 hours the two phases were separated and the concentration of proteins was determined by UV spectrophotometer at 280 nm (lysozyme and γ-globulin) or 406 nm (myoglobin an hemoglobin). The water content in the organic phase was measured by the Karl-Fischer method. The mean diameter of reversed micelles was measured with a Photal (Otuka Elec.) dynamic light-scattering spectrometer (DLS-700). The distribution ratio of surfactants between aqueous and organic phases was determined by measuring the concentration of **P** or **S** atom in the aqueous phase with an ICP-atomic emission spectroscope (SEIKO SPS-1200VR).

2. Results and Discussion

Table 1 shows the result of protein extraction (lysozyme and hemoglobin) using reversed micelles made with different surfactants. A phosphoric acid surfactant having saturated long alkyl chains more than $n=14$ was not sufficiently dissolved in an isooctane solution. However, dioleyl phosphoric acid (DOLPA) was easily dissolved and the water content

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Table 1. Protein extraction using reversed micelles formed by various surfactants

Surfactant	AOT	D2EHPA	$2C_8PA$	$2C_{12}PA$	$2C_{18}\Delta^9PA$ (DOLPA)
C_w[mol/m^3]	128	3.1	4.5	63.7	406
D [—]	0.031	0.025	—	—	$\ll 10^{-4}$
Lysozyme E [%]	98.9	0	0	24.1	99.8
Hemoglobin E [%]	reddish precipitate	reddish precipitate	emulsified	74.1	99.7

$C_{prot.}=1$ kg/m^3, $C_{KCl}=100$ mol/m^3 ($pH=6.1$), $C_S=20$ mol/m^3, $D=C_{S,aq}/C_{S,org}$, $E=(C_{prot.,0}-C_{prot.})/C_{prot.,0}$

of the reversed micelles was larger than that of the other reversed micelles. Bhattachryya *et al.*[2] reported that D2EHPA formed an aggregate like a reversed micelle in *n*-heptane at high concentration. However, surfactants of phosphoric acid type having short alkyl chains did not form reversed micelles under this experimental condition. Among them, only DOLPA reversed micelle could dissolve both proteins and it was confirmed that no denaturation of the proteins in the reversed micelle observed by CD or ESR spectrum. Denaturation of proteins is caused by complex formation with aqueous solved surfactants[8]. This indicates that DOLPA having a low distribution ratio is a suitable surfactant for protein extraction.

Figure 1 shows the relation between water content dissolved by reversed micelles in isooctane and the pH of the aqueous solution. The water content using AOT was held constant throughout the range of experimental pH. However, in the case of DOLPA, the water content rapidly increases about pH = 4 and was kept constant above pH = 6. Under the condition below pH = 4, it was confirmed that DOLPA mainly exists as a dimer in an isooctane solution by vapor-phase osmometry. The reversed micelle can be formed only by anionic species of DOLPA. The formation of reversed micelles using DOLPA can be controlled by adjusting the aqueous pH. The relation between the mean diameter (Stokes diameter) of reversed micelles and the surfactant concentration is shown in **Fig. 2.** In this concentration range, the size of reversed micelles formed by AOT was almost constant, but that of the reversed micelles formed by DOLPA increased with increasing surfactant concentration. Further, the diameters of both reversed micelles decreased with increasing salt concentration. From the results of diameter measurement, a separation of proteins of different size can be carried out by adjusting the optimal concentration of DOLPA. **Figure 3** shows the extraction results for proteins which have almost the same isoelectric point but different molecular weight. Myoglobin, with the smallest molecular weight (17,000) was extracted in a lower concentration range than the other proteins. Further, the DOLPA reversed micelles could easily extract hemoglobin at a concentration of more than

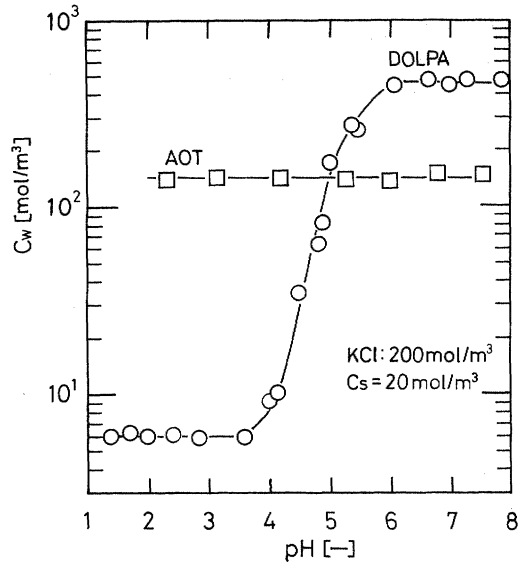

Fig. 1. Relation between water content in organic solution and pH of aqueous solution

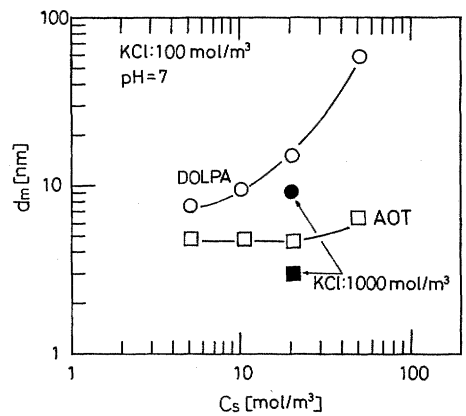

Fig. 2. Relation between mean diameter of reversed micelles and surfactant concentration

20 mol/m^3. However, γ-globulin, having the largest molecular weight (156,000) could not be extracted, even in the DOLPA reversed micelle.

Conclusion

Protein extraction by reversed micelles using dioleyl phosphoric acid was studied in comparison with that of AOT. When DOLPA was used the formation of

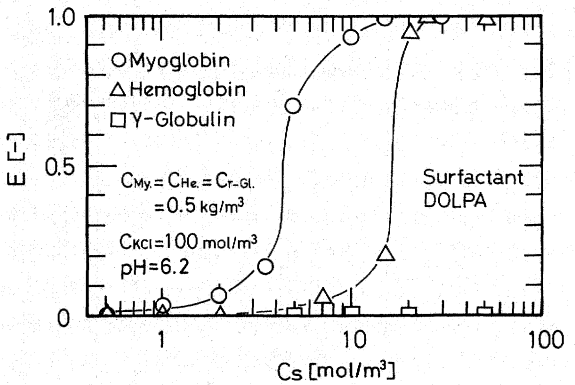

Fig. 3. Protein extraction by reversed micelles using DOLPA

reversed micelles could be controlled by adjusting the pH of aqueous solution. The DOLPA reversed micelles could extract hemoglobin of more than 50,000 of molecular weight, which is difficult to extract by the conventional AOT reversed micelles. Further, this reversed micelle system can provide new reaction sites of enzyme reactions in organic media.

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Nomenclature

C_{KCl}	=	concentration of KCl	[mol/m^3]
$C_{Prot.}$	=	concentration of protein	[kg/m^3]
C_s	=	concentration of surfactant	[mol/m^3]
C_w	=	water content in reversed micelles	[mol/m^3]
D	=	distribution ratio of surfactant between aqueous and organic solution	[—]
E	=	extraction ratio of protein	[—]

〈subscripts〉

0	=	initial value
aq	=	aqueous phase
org	=	organic phase

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