EXTRACTION OF AMINO ACIDS BY REVERSED MICELLES

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Introduction

Separation of bioproducts from the reaction media of bioreactors is an important process in biotechnology. Extraction of bioproducts can be applied to their crude separation or purification. Recently, extraction of proteins using reversed micelles has been investigated and found to have good prospects. The extraction is promoted by an electric attractive force between the surfactant and the protein.

Amino acids are also useful bioproducts for various applications, thus their separation by means of reversed micelle extraction may present a useful step for large-scale production of amino acids. However, little research on extraction of amino acids has been carried out. The aim of this paper is to present the extraction behavior of amino acids using reversed micelles with AOT®.

1. Experimental Methods

Sodium di-2-ethylhexylsulfosuccinate, AOT®, was a product of Nakarai Chemical Co., Kyoto. Isooctane and L-arginine (Arg) were analytical-grade reagents made by Wako Chemical Co., Tokyo. L-tyrosine (Tyr) was obtained from Kyowa Hakko Kogyo, Ltd., Tokyo. Cytochrome-C from horse heart was a product of Sigma, Co., St. Louis, MO, (Type IV). The reagents were used without further purification.

The extraction equilibrium was measured using a 100-cm^3 conical flask. To mix the organic and aqueous phases in the vessel, a four-blade turbine of 2 cm diameter was used at ca. 600 rpm. To settle the droplets, the solution was centrifuged. Time necessary to reach the equilibrium distribution was measured in a preliminary experiment and it was found that one hour was sufficient. Temperature was regulated at 300 K. Amino acids in the organic phase were analyzed after extracting them into the aqueous phase. Analysis of amino acids were carried out by the ninhydrine method.[10]

Material balances of the acids were satisfied within 5 percent. The water content in the organic phase was measured by the Karl-Fischer method.

2. Results

2.1 Separation

First, the extraction of amino acids with different isoelectric points, pI, was investigated. The amino acids selected were Tyr and Arg, having isoelectric points of 5.63 and 10.8, respectively. The fraction of extracted amino acid is illustrated in **Fig. 1.** These data were obtained separately using aqueous solutions of the respective amino acids. The extracted fraction for the protein cytochrome-C is also shown in the figure for comparison. AOT has a negative charge and the amphoteric molecules are extracted at pH < pI. It is found however that the amino acid extraction was not so sensitively discriminated by pH as in the case of protein extraction[6]. The ionization equilibrium of the

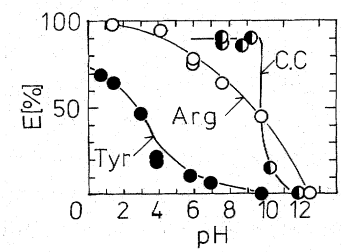

Fig. 1. Effect of pH on extraction of amino acids and protein. $c_{AOT}=157\,\mathrm{mol/m^3}$, $I=0.1\,\mathrm{kmol/m^3}$. c.c=cytochrome C

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amino acids was calculated but found to be affected by pH more sensitively than the extraction behavior. The less pH-sensitive extraction behavior could not be explained clearly and has been left for further analysis.

The extraction was carried out using 20 cm^3 of organic phase at $I=0.1$ kmol/m^3 and pH 9.0 to separate Arg and Tyr. Tyr was not extracted under this condition. The extract was back-extracted by 20 cm^3 of aqueous solution at pH 7.1 and $I=1$ kmol/m^3, and Arg was recovered at a yield of 48.5 per cent by the first back-extraction. Over 90 per cent was recovered by repeating the back-extraction three times. Thus separation of amino acids with different pI is found possible. The effect of the ionic strength on the extraction of Arg is shown in **Fig. 2.** When the ionic strength is higher, the electric interaction is decreased due to a decrease in the range of the Debye ionic atmosphere. This qualitatively can explain the decrease in E with the increase of I shown in the figure.

2.2 Distribution equilibrium

The distribution of Arg between the organic and aqueous phases was investigated. As shown above, distribution into the organic phase increases at higher AOT concentration and lower ionic strength (**Fig. 3**). The pH dependence of the distribution is shown in **Fig. 4.** The distribution was mostly expressed by a Freudlich-type equation. The equilibrium at higher pH is less favorable for extraction.

3. Discussion

In extraction by the reversed micelles, AOT molecules are considered to exist mostly in the organic phase. Using this assumption and an analytical measurement of H_2O concentration in the oil phase, the ratio of water and AOT concentrations in the oil phase, R, was obtained. This R value is claimed to affect the size of reversed micelles. Eicke[2] expressed this relation in terms of average molecular weight, M_o, of the reversed micelles by Eq. (1).

$$M_o=(19+2.1R)^3 \tag{1}$$

From the M_o value the diameter of micelles can be calculated using the molecular volume of water with the assumption that AOT is negligible in the inner water phase, and the diameter was compared with measurements in the literature. The diameter of the inner water phase of micelles at $R=19.0$ was 6.4 nm by Eq. (1), about the same as the experimental values by Mori *et al.*[9] (5.8 nm) and Zulauf and Eicke[12] (6.2 nm). Harada *et al.*[5] used the relation proposed by Wong *et al.*[3,11] and from their result the diameter became ca. 7.5 nm considering the AOT molecular length to be 1.1 nm. Thus, it can be assumed that Eq. (1) is applicable to estimate the number of AOT

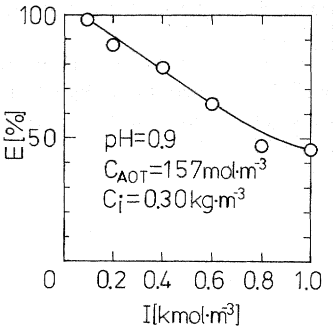

Fig. 2. Effect of ionic strength on extraction of Arg

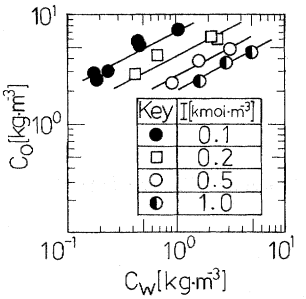

Fig. 3. Distribution of Arg. Effect of ionic strength $c_{AOT}=157$ mol/m^3, pH = 1.4

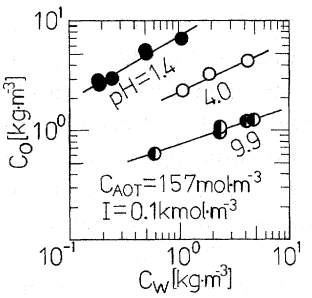

Fig. 4. Distribution of Arg. Effect of pH

molecules constituting the reversed micelle. The value $M_o/(M_{AOT}+RM_W)$ is the association number of AOT molecules in a micelle, n_{AOT}. Using this value, the number of micelles, n_m, in the organic phase can be calculated by Eq. (2).

$$n_m=\frac{(c_{AOT}-cmc)(M_{AOT}+RM_W)N_{Avo}}{M_{AOT}M_o} \tag{2}$$

Then the number of Arg molecules trapped in a reversed micelle, N_{AA}, is given by Eq. (3).

$$N_{AA}=\frac{c_oM_{AOT}M_o}{(c_{AOT}-cmc)(M_{AOT}+RM_W)M_{Arg}} \tag{3}$$

The ratio of the number of AOT molecules to the number of the Arg molecules in the reversed micelles, $N_{AOT/Arg}$, was calculated from the concentrations of AOT and Arg in the organic phase.

The calculated values of N_{AA} and $N_{AOT/Arg}$ in the case of $C_{AOT}=157$ mM and pH = 1.4 are shown in **Figs. 5** and **6**. The number of Arg molecules in a micelle is much higher than that in the case of protein

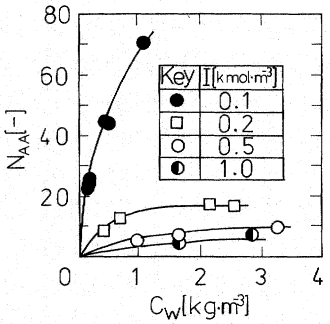

Fig. 5. Number of Arg molecules entrapped in a micelle. $c_{AOT}=157\,\mathrm{mol/m^3}$, pH = 1.4

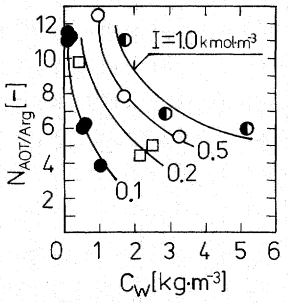

Fig. 6. Ratio of number of AOT molecules to number of Arg molecules in the micelles. $c_{AOT}=157\,\mathrm{mol/m^3}$, pH = 1.4

extraction,[1,4,8] where in general one molecule is entrapped. Also, it may be noted that five or ten molecules of AOT correspond to one Arg molecule. The Arg concentration in the aqueous phase in the micelle, c_m, was calculated by Eq. (4) where c_{H_2O} is the water concentration in the organic phase, assuming that water in the organic phase existed inside the micelle. It is given in **Fig. 7**.

$$c_m=\frac{c_o\rho_w}{c_{H_2O}} \tag{4}$$

The concentration of Arg in the aqueous phase inside the micelle was higher than that in the bulk aqueous phase. Thus Arg concentration may be possible by reversed micelle extraction if appropriate procedures are adopted to recover the aqueous phase in reversed micelles. This may be possible by back-extraction using high ionic concentrations.[5]

Conclusions

Extraction of amino acid by means of reversed micelles was investigated. Extraction was not so sensitively dependent upon pH value compared to the case of protein extraction. The distribution equilibrium was obtained. Amino acid enrichment is prossible if the aqueous phase inside micelles is adequately recovered.

Nomenclature

c_{AOT}	=	concentration of AOT in organic phase	[kg/m³]
c_i	=	initial concentration of extractant	[kg/m³]
c_{H_2O}	=	concentration of water in organic phase	[kg/m³]

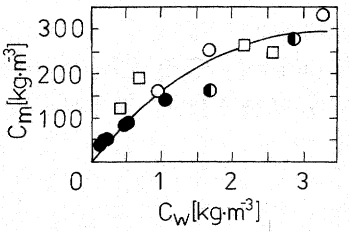

Fig. 7. Arg concentration in the inner aqueous phase of reversed micelles. Keys are the same as those in Fig. 6. $c_{AOT}=157\,\mathrm{mol/m^3}$, pH = 1.4

c_m	=	concentration of amino acid in the aqueous phase of reversed micelle	[kg/m³]
c_o	=	concentration of amino acid in organic phase	[kg/m³]
c_w	=	concentration of amino acid in aqueous phase	[kg/m³]
cmc	=	critical micelle concentration of AOT in organic phase	[kg/m³]
E	=	fraction of amino acid extracted into organic phase	[%]
I	=	ionic strength	[kmol/m³]
M	=	molecular weight	[kg/kmol]
M_w	=	molecular weight of water	[kg/kmol]
M_o	=	apparent molecular weight of a reversed micelle considering the micelle as a molecule	[kg/kmol]
$N_{AOT/Arg}$	=	ratio of the number of AOT molecules to the number of Arg molecules in reversed micelle	[—]
N_{AA}	=	number of amino acid molecules entrapped in a micelle	[—]
N_{Avo}	=	Avogadro's number	[—]
n_{AOT}	=	association number, i.e. number of AOT molecules composing a reversed micelle	[—]
n_m	=	number of reversed micelles in organic phase	[—]
R	=	ratio of water to AOT moles in organic phase	[—]
ρ_w	=	density of water	[kg/m³]

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