EXTRACTION EQUILIBRIA OF AMINO ACIDS WITH QUATERNARY AMMONIUM SALT

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The extraction equilibria of various amino acids with tri-*n*-octylmethylammonium chloride (TOMAC) were studied in the high pH range. TOMAC reacted with the anion of amino acid and OH⁻ according to the stoichiometric ratio determined by ionic valency. The extraction equilibrium constants K_A of various amino acids differed considerably and could be correlated well with each hydrophobicity scale. The highest K_A value obtained for tryptophan was larger than the lowest value for glycine by a factor of about 260. These findings suggested that separation of amino acids is possible by utilizing the differences in K_A values. The extraction equilibrium constants of inorganic anions which were present in the fermented broth was comparable to those of amino acids exhibiting a low degree of extraction.

Introduction

Liquid-membrane processes, which were first proposed by Li⁹⁾ as an innovative separation technique, have been applied to a variety of fields such

as hydrometallurgy and wastewater treatment. The recovery of metals has been investigated⁷⁾ extensively due to the high possibility of developing practical processes, and a zinc recovery test plant has been put into operation at Austria.²⁾ Recently, the application of this approach to the downstream processing of various bioproducts has become of interest owing to the potential for simultaneous

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separation and enrichment together with the avoidance of pretreatment.¹⁶⁻¹⁸⁾ The biochemicals treated by liquid-membrane processes so far are amino acids,^{1,4,8,13-18,20)} organic acids,^{5,11,12)} antibiotics⁶⁾ and cholesterol.¹⁹⁾ This paper discusses the recovery of amino acids by extraction.

Amino acids, which are typical bioproducts produced on a large scale, are mainly separated and concentrated by ion exchange and crystallization. However, the drop in resin adsorption power results in the reduction of purification efficiency. The separation of amino acids by liquid-membrane processes has been performed by use of extractants since amino acids are hardly soluble in organic solvents. Two types of extractants have been employed so far. One is used in the low pH range to extract amino acid cations and the other in the high pH range to extract anions. Phosphoric acid esters like di-2-ethylhexyl phosphoric acid^{15,20)} and quaternary ammonium salts like tri-*n*-octylmethylammonium chloride (abbreviated as TOMAC)^{4,13,17,18)} are typical extractants for cation and anion amino acids respectively. The quaternary ammonium salt, a well-known liquid ion-exchanger first used by Behr and Lehn¹⁾ in the carrier-mediated transportation of amino acids, was employed in this study. Since liquid-membrane processes involve extraction, it is necessary to investigate the extraction equilibria and kinetics to establish the optimum recovery system.^{1,3,15)} However, the extraction behavior of amino acids has not been investigated in detail. The objects of the present study were to analyze the extraction equilibria of amino acids with TOMAC and to make clear the factors that control the degree of extraction. The coextractions of various inorganic anions present in the fermented broth is also discussed.

1. Experimental

The extraction equilibrium at 298 K was measured by mixing 25 cm³ of aqueous amino acid solution and 25 cm³ of kerosene solution in which Capriquat® TOMAC, from Dojinodo Lab., Kumamoto, was dissolved as an extractant. The aqueous amino acid solutions were adjusted to pH 11 to 12 by KOH in order to shift amino acids to the anion type.

The concentration of amino acids in the aqueous solution was determined by HPLC (Japan Spectroscopic Co.) equipped with a fluorescence detector using the OPA-post-labeling method. The concentration of Cl⁻ was measured by an ionmeter and H⁺ by a pH meter. Aqueous solutions of KOH, MgSO₄, K₂HPO₄ and Na₂CO₃ were also used to examine the extraction equilibrium of coexisting inorganic anions. The concentration of PO₄³⁻ was determined by the molybdate blue method and SO₄²⁻ and CO₃²⁻ by the nephelometry method.

The hydrophobicity scales of isoleucine (Ile) and arginine (Arg) were determined by measuring the solubilities of these amino acids in ethanol at 298 K according to the method of Nozaki and Tanford.¹⁰⁾

2. Evaluation of equilibrium constants

The results of the extraction equilibrium were analyzed as follows, considering the simultaneous extraction of amino acids and OH⁻.

First, amino acids dissociate into anions in the high pH range.

The dissociation equilibrium constant K_2 is expressed by Eq. (2).

$$K_2 = [\text{H}^{+}][\text{A}^{-}]/[\text{HA}] \quad (2)$$

The extractant TOMAC was assumed to form salts with monovalent anions of amino acid and OH⁻ by equimolar reactions.

The extraction equilibrium constants, K_A and K_{OH} , are expressed as follows.

$$K_A = [\text{QA}^{*}][\text{Cl}^{-}]/[\text{QCl}^{*}][\text{A}^{-}] \quad (5)$$

$$K_{OH} = [\text{QOH}^{*}][\text{Cl}^{-}]/[\text{QCl}^{*}][\text{OH}^{-}] \quad (6)$$

The asterisk * in the above equations indicates the species in the oil phase. The values of K_A and K_{OH} were calculated by solving Eqs. (2), (5) and (6) simultaneously, together with the following material balance equations of TOMAC, amino acids and Cl⁻.

$$(\text{Amino acid}) \quad [\text{HA}]_0 = [\text{HA}] + [\text{A}^{-}] + [\text{QA}^{*}] \quad (7)$$

$$(\text{TOMAC}) \quad [\text{QCl}^{*}]_0 = [\text{QCl}^{*}] + [\text{QA}^{*}] + [\text{QOH}^{*}] \quad (8)$$

$$(\text{Cl}^{-}) \quad [\text{QCl}^{*}]_0 = [\text{QCl}^{*}] + [\text{Cl}^{-}] \quad (9)$$

The above material balance equations are based on equal volumes of water and oil phases during extraction.

3. Results and Discussion

3.1 Amino acids extraction equilibria

In Figs. 1 and 2, the experimental results are plotted according to Eqs. (5) and (6) for some typical amino acids: phenylalanine (Phe), tryptophan (Trp), leucine (Leu), glycine (Gly), methionine (Met) and histidine (His). These amino acids clearly formed the salt with TOMAC in a ratio of 1:1 since the slopes of all the plotted data were nearly equal to one. The amount of OH⁻ extracted simultaneously was smaller than those of the amino acids. Table 1 lists the extraction equilibrium constants K_A of the amino acids

Fig. 1. Extraction equilibria of Trp, Phe and Leu

Fig. 2. Extraction equilibria of Met, His and Gly

investigated in this study together with their isoelectric points pI . All the amino acids except tyrosine (Tyr) exhibited equimolar reaction with TOMAC under the condition of pH 11 to 12. Tyr dissociates into divalent anion in the pH range higher than 13. Therefore, experimental data gave a straight line having a slope of two in the equilibrium plot of $[Q_2A^*][Cl^-]^2/[A^{2-}]$ vs. $[QCl^*]$.

Although the values of pI in Table 1 are not appreciably different, a considerable difference was observed in K_A values. Among the amino acids studied, the highest K_A value observed for Trp was about 260 times that of the lowest one for Gly. Consequently, the selective separation of amino acids is possible by extraction with TOMAC when there is a large difference in the K_A values of the amino acids coexisting in the fermented broth.

Figure 3 shows the effect of the organic solvent on the extraction equilibrium of Trp. Since the extraction

Table 1. Extraction equilibrium constants and isoelectric points of various amino acids

Amino acid	Structure	pI	K_A
Glycine (Gly)	NH_2-CH_2-COOH	6.0	0.036
Alanine (Ala)	$CH_3-CH-COOH$ $ $ NH_2	6.0	0.038
Valine (Val)	$(CH_3)_2CH-CH-COOH$ $ $ NH_2	6.0	0.089
Leucine (Leu)	$(CH_3)_2CH-CH_2-CH-COOH$ $ $ NH_2	6.0	0.29
Isoleucine (Ile)	$CH_3(CH_2)CH-CH-COOH$ $ $ NH_2	6.0	0.24
Methionine (Met)	$CH_3-S-(CH_2)_2-CH-COOH$ $ $ NH_2	5.7	0.21
Phenylalanine (Phe)	$\text{C}_6\text{H}_5-CH_2-CH-COOH$ $ $ NH_2	5.5	0.97
Tryptophan (Trp)	$\text{Indol-3-yl}-CH_2-CH-COOH$ $ $ NH_2	5.9	8.89
Tyrosine (Tyr)	$HO-C_6H_4-CH_2-CH-COOH$ $ $ NH_2	5.7	0.40
Histidine (His)	$\text{Imidazol-5-yl}-CH_2-CH-COOH$ $ $ NH_2	7.6	0.083
Arginine (Arg)	$HN(H_2N)-C(=NH)-NH-(CH_2)_3-CH-COOH$ $ $ NH_2	10.8	0.062
Serine (Ser)	$CH_2-CH-COOH$ $ $ OH	5.7	0.049
Threonine (Thr)	$CH_3-CH-CH-COOH$ $ $ OH	6.2	0.071

Fig. 3. Effects of organic solvent on extraction behavior

results obtained in various solvents follow the same straight line, the solvents in the oil phase may have no influence on the amino acid extraction.

3.2 Relation between equilibrium constants and hydrophobicity scales

It is apparent from Table 1 that the values of K_A of aliphatic amino acids tend to increase as the number of carbon atoms increases. Further, the values of aromatic amino acids are fairly high compared to those of aliphatic amino acids. These findings suggest that the hydrophobicity of amino acids affects the degree of extraction. Thien *et al.*,¹⁸⁾ investigated the extraction of various amino acids by liquid surfactant membrane using TOMAC as a mobile carrier, and reported a linear correlation between the hydrophobicity scale and the initial extraction flux. They concluded that the hydrophobicity is a dominant

Fig. 4. Relation between equilibrium constants and hydrophobicity scales

factor in the extraction of amino acids with TOMAC. In this paper, the relation between K_A and the hydrophobicity scale was examined to confirm the role of hydrophobicity since the extraction flux observed in the liquid surfactant membrane process is also affected by mass transfer rates. **Figure 4** is a plot of $\log K_A$ against the hydrophobicity scale proposed by Nozaki and Tanford.¹⁰⁾ These hydrophobicity scales are defined as the free energy change when each amino acid transfers from water to ethanol phase. As a reference substance for this scale, glycine was arbitrarily assigned. Thus the hydrophobicity scale is expressed as the relative difference against glycine. The hydrophobicity scales of Ile and Arg in Fig. 4 were measured by the authors according to the methods of Nozaki and Tanford. As clearly shown in these plots, a good correlation was obtained between $\log K_A$ and each hydrophobicity scale. Therefore, the linear correlation of extraction flux reported by Thien *et al.* may be due to the strong influence of the hydrophobicity scale on the extraction equilibrium constants.

The logarithm of the equilibrium constant is equal to the negative free energy change divided by the gas constant and absolute temperature. Consequently, the linear correlation observed in Fig. 4 demonstrates that the free energy change in the amino acids extraction with TOMAC is proportional to that in the transfer from water to ethanol phase.

3.3 Extraction equilibria of coexisting inorganic anions

Since various inorganic salts are added as nutrients for microbial growth in the fermented broths, the coextraction of inorganic anions together with amino acids takes place to some extent. In the present study, extraction equilibria were investigated for OH^- ,

Fig. 5. Extraction equilibrium of OH^-

SO_4^{2-} , CO_3^{2-} and PO_4^{3-} which were commonly present in fermented broths. **Figure 5** shows the extraction equilibrium of OH^- using KOH. It is clear to form the salt with TOMAC in the ratio of 1:1, since the slope of the plotted data is one. The value of K_{OH} from Fig. 5 is almost the same as those obtained by analyzing the extraction data of amino acids. **Figure 6** shows the extraction equilibria of SO_4^{2-} , CO_3^{2-} and PO_4^{3-} measured by using MgSO_4 , Na_2CO_3 and K_2HPO_4 respectively. The slopes of the correlating lines for SO_4^{2-} , CO_3^{2-} and PO_4^{3-} are 2, 2 and 3, respectively, showing that inorganic anions react with TOMAC according to their ionic valencies. The extent of coextraction of these inorganic salts is comparable to those of hydrophilic amino acids. Therefore, in the recovery of such amino acids as Gly, Ala, Val, Arg and His, it is necessary to remove the inorganic salts by some means after extracting amino acids from fermented broths.

Conclusion

The extraction equilibria of various amino acids with tri-*n*-octylmethylammonium chloride (TOMAC)

Fig. 6. Extraction equilibria of SO_4^{2-} , CO_3^{2-} and PO_4^{3-}

were studied and the following results were obtained.

1) TOMAC reacted with amino acid anions in the ratio of 1 : 1 or 1 : 2 depending on ionic valency. There was a considerable difference in the extraction equilibrium constants of amino acids investigated. These findings suggested that the selective separation of amino acids was possible by extraction. The solvents in the oil phase had no influence on extraction behavior.

2) The extraction equilibrium constants could be correlated well with the hydrophobicity scale of each amino acid. The relative free energy change in the extraction of amino acids with TOMAC was approximately equal to that in their transfer from water to ethanol phases.

3) Inorganic anions OH^- , CO_3^{2-} , SO_4^{2-} and PO_4^{3-} which are present in fermented broths were also extracted with TOMAC, and the extent of extraction was comparable to those of hydrophilic amino acids.

Nomenclature

K_A = extraction equilibrium constant of amino acid anion [—]

K_{OH} = extraction equilibrium constant of OH^- anion [—]
 K_2 = dissociation equilibrium constant of amino acid [$\text{mol} \cdot \text{dm}^{-3}$]
 pI = isoelectric point [—]

<Subscript>

0 = initial

<Superscript>

* = species in oil phase

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