Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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Shigeo Takeshima[a]; Shinichiro Wada[a]; Hiromu Sakurai[a]
[a] KYOTO PHARMACEUTICAL UNIVERSITY, KYOTO, JAPAN

To cite this Article Takeshima, Shigeo , Wada, Shinichiro and Sakurai, Hiromu(1994) 'Transport Behavior of Basic Amino Acids through an Organic Liquid Membrane System', Separation Science and Technology, 29: 16, 2117 — 2129
To link to this Article: DOI: 10.1080/01496399408002193
URL: http://dx.doi.org/10.1080/01496399408002193

SEPARATION SCIENCE AND TECHNOLOGY, **29**(16), pp. 2117–2129, 1994

Transport Behavior of Basic Amino Acids through an Organic Liquid Membrane System

SHIGEO TAKESHIMA,* SHINICHIRO WADA,
and HIROMU SAKURAI
KYOTO PHARMACEUTICAL UNIVERSITY
NAKAUCHI-CHO MISASAGI YAMASHINA-KU, KYOTO 607, JAPAN

ABSTRACT

The transport behavior of basic amino acids (BAA), such as arginine (Arg), histidine (His), and ornithine (Orn), through an organic liquid membrane system (LMS) was investigated. The LMS was composed of two aqueous phases (Phases I and II) separated by an organic phase of chloroform containing sodium di-(2-ethylhexyl) sulfosuccinate (Aerosol OT, AOT). The amount of BAA that moved from Phase I at pH 3 into the organic phase increased with increasing AOT concentration (2–10 mM). The relative amount of extracted BAA was in the following order: Arg > His > Orn. On the other hand, the release of BAA from the organic phase into Phase II at pH 10 did not depend upon their amount in the organic phase. Arg was difficult to release. The relative amount of released BAA was in the following order: Arg = His > Orn. BAA were extracted from Phase I at pH 5 into the organic phase containing 4 mM AOT because they exist as cationic species. Other amino acids possessing nonionic residues were untransportable under these conditions except leucine, tryptophan, and phenylalanine, which have highly hydrophobic residues. However, they were transportable in their cationic forms at pH 1. These transport phenomena are essentially controlled by the interaction of the anionic group of AOT and a cationic form. These results suggested that BAA can be separated from most amino acids under an appropriate pH by using AOT.

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* To whom correspondence should be addressed.

INTRODUCTION*

The organic liquid membrane system (LMS) has been widely used as a biomimetic model for studying membrane transport (1–4) and as a separation tool (5–8). Various carriers in LMS have been selected depending upon the property of the compound to be transported. Anionic and cationic surfactants would be necessary for the transport of cationic and anionic species, respectively. Amino acids usually possess amino and carboxyl groups which are ionized at a physiological pH to form zwitterions. Thus, amino acids having nonionic residues are electrically neutral. The transport of these amino acids is generally difficult owing to these zwitterionic characteristics, and the conditions for their transport are either highly acidic (0.1 M HCl) or alkaline (0.1 M NaOH) to generate their positive or negative forms. For example, a quaternary ammonium salt (Aliquat 336) as a cationic surfactant has been used to transport amino acids in their anionic form. The relative transported amounts were in the following order: Phe > Trp > Leu > Tyr > Val >> Ala > Ser (9). Various lipophilic amines have been used to examine the transport of *N*-benzoylamino acids and primary amines possessing long alkyl groups. Furthermore, among amine carriers with the same number of carbon atoms, the order of transport was tertiary > secondary >> primary (10).

The relative rates of amino acid transport are generally dominated by the nature of their residues. Phe, which possesses a highly hydrophobic residue, is transported much more readily through LMS than Ser, which has a less hydrophobic residue. Tsukube (11, 12) reported the transport of *N*-benzoylamino acids and their derivatives through LMS containing lipophilic metal complexes as the anionic carriers. The transport of amino acids in their anionic forms has been investigated in ternary complexes with azacrownether (13) or valinomycin (14) in which alkaline metals or alkaline earth metals were entrapped. On the other hand, the cationic form of amino acids can also be transported through LMS containing a lipophilic anionic surfactant such as dinonylnaphthalene sulfonate (9) and tributyl or tripentyl phosphate (15). In these studies, hydrophobic amino acids or their derivatives were mainly used. In contrast, little attention had been directed toward the transport of less hydrophobic amino acids such as arginine (Arg), histidine (His), and ornithine (Orn), which are interesting compounds from a biochemical perspective. These are basic amino acids (BAA).

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* Abbreviations: Ala, alanine; AOT, sodium di-(2-ethylhexyl) sulfosuccinate; Arg, arginine; Gly, glycine; His, histidine; Hm, histamine; Leu, leucine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine. BAA, basic amino acid; LMS, an organic liquid membrane system.

Recently, in studies of protein and amino acid extraction into the organic phase of isooctane containing sodium di-(2-ethylhexyl) sulfosuccinate (Aerosol OT, AOT), Arg was extractable at neutral pH, and the degree of extraction increased with increasing net positive charge (16).

It is thus evident that amino acids can be extracted when they possess a net positive charge, which governs the degree of extraction. We investigated the selective transport of histamine from a mixture of histidine and histamine through LMS containing AOT, and histamine was separated efficiently at pH 7.2 (17). These results suggested that the transport was controlled by the degree of the net positive charge between histidine and histamine. Therefore we investigated the fundamental transport behavior of BAA through LMS compared with neutral amino acids which have nonionic residues. Chloroform and AOT were used as the organic phase and the carrier, respectively.

EXPERIMENTAL

Chemicals

L-Arginine, L-histidine, L-ornithine monohydrochloride, and other L-amino acids and sodium di-(2-ethylhexyl) sulfosuccinate (Aerosol OT, AOT, Fig. 1) were obtained from Nacalai Tesque Co. Other chemicals were of special reagent grade. Chloroform was distilled before use.

Transport Procedure

The transport studies proceeded at room temperature in a U-tube glass cell as described previously (17). A chloroform solution of AOT (25 mL) was placed in the bottom of the U-tube, and a buffer containing 1 mM of amino acid (Phase I, 20 mL) and another buffer (Phase II, 20 mL) were placed in both arms of the U-tube, floating on the chloroform phase. The organic phase was agitated slowly at a constant speed with two magnetic

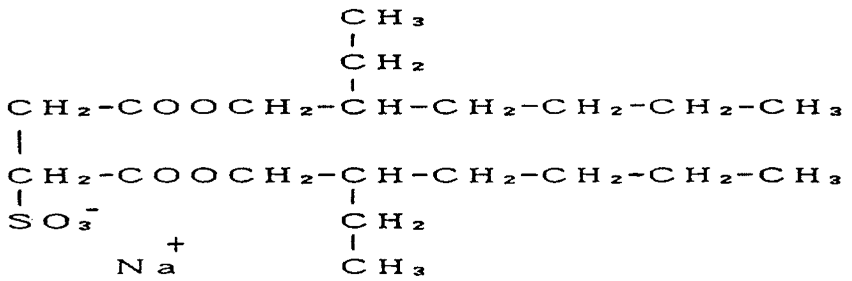

FIG. 1 The structure of sodium di-(2-ethylhexyl) sulfosuccinate (Aerosol OT, AOT).

stirrers. The concentrations of the amino acids in Phases I and II were monitored periodically by fluorometry using *o*-phthalaldehyde and mercaptoethanol (18). A Shimadzu spectrofluorophotometer RF-540 was used for the fluorescent determination.

Various buffers were prepared by mixing 0.1 M HCl with 0.1 M sodium acetate (pH 2–3), 0.1 M acetic acid with 0.1 M sodium acetate (pH 4–6), 0.1 M sodium tetraborate with 0.1 M HCl (pH 7.2–9), and 0.1 M sodium tetraborate with 0.1 M NaOH (pH 10–12). The 0.1 M HCl was used as pH 1.

The transport behavior was evaluated by means of the residual (R.R.) and transported ratios (T.R.) as shown in Eqs. (1) and (2):

$$\text{R.R.} = (C_{1t}/C_i) \times 100\ (\%) \tag{1}$$

$$\text{T.R.} = (C_{2t}/C_i) \times 100\ (\%) \tag{2}$$

where C_i is the initial concentration of an amino acid and C_{1t} and C_{2t} are those of the amino acid in Phases I and II after a period of t hours, respectively.

RESULTS AND DISCUSSION

The Organic Liquid Membrane System (LMS)

The organic liquid membrane system (LMS) was composed of two aqueous phases separated by an organic phase. At the interface of aqueous Phase I and the organic phase, an anionic and lipophilic surfactant, AOT, binds with the cationic species of an amino acid via electrostatic interaction to extract it into the organic phase. The amino acid in the cationic form in the organic phase is released to aqueous Phase II by an exchange reaction with an antiport cation (4, 12). AOT is a typical surfactant which aggregates in the apolar medium to form reversed micelles.

These have an external shell consisting of hydrocarbon chains of amphiphatic molecules and a polar charged head group, with the counterion being localized in the interior of the aggregate (19). Under appropriate conditions, these reversed micelles containing aqueous solutions (water pool) can exist in equilibrium with the bulk aqueous phase, and they can be used to extract polar materials (water-soluble compound) from the bulk aqueous phase (20–22).

The Concentration Dependence of the Carrier upon the Transport of BAA

Charged solutes can permeate the organic membrane phase via the carrier/solute complex in a facilitated transport system. The carrier is thus a vital component in the membrane formulation for this system. The transport behavior of BAA through LMS containing AOT as a carrier was examined. Phases I and II were set at pH 3 and 10, respectively, and the concentration of AOT was 4 mM (Fig. 2). At pH 3, BAA are positively charged. Their extraction into the organic phase from Phase I increased over time. Arg was totally extracted into the organic phase after 4 hours. However, His and Orn were not completely extracted after 6 hours. Thus, the relative extracted ratio of BAA from Phase I into the organic phase was in the order: Arg > His > Orn. The release of BAA from the organic phase to Phase II almost depends upon the amount of BAA in the organic phase, and the relative released ratio was in the following order: Arg = His > Orn. Furthermore, the effect of the carrier concentration on the transport behavior of BAA was investigated. As shown in Fig. 3, the pH values of Phases I and II were fixed at 3 and 10, respectively, and the data after 6 hours of reaction were plotted. The extraction of BAA increased with increasing carrier concentration. Arg and His were completely extracted from Phase I at AOT concentrations of 4 and 10 mM,

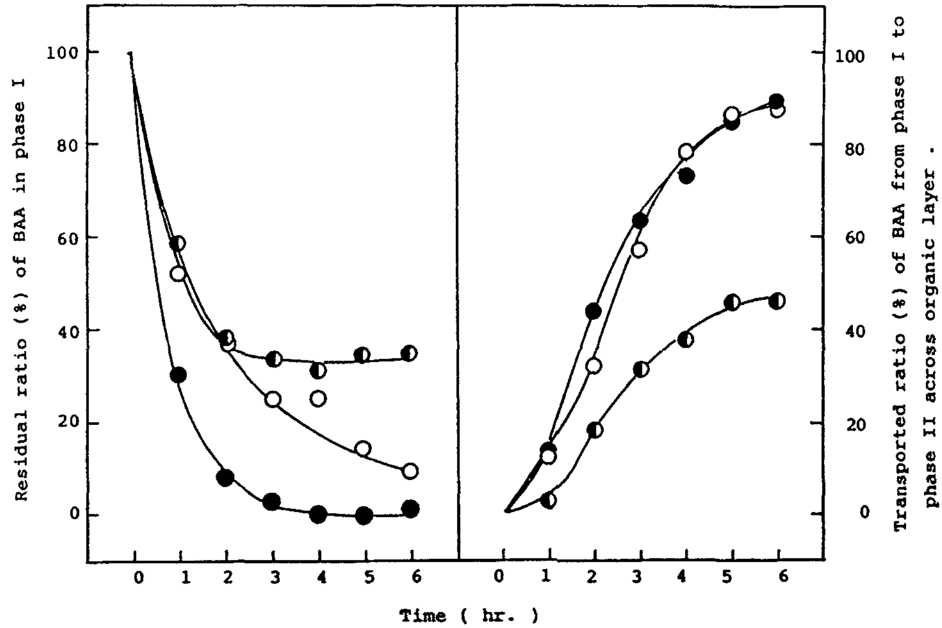

FIG. 2 The time-dependent transport of basic amino acid. The BAA and AOT concentrations were 1 and 4 mM, respectively. The pH values of Phases I and II were maintained at 3 and 10, respectively. Both residual and transported ratios of BAA were plotted against the reaction period. (●) Arg, (○) His, (◐) Orn.

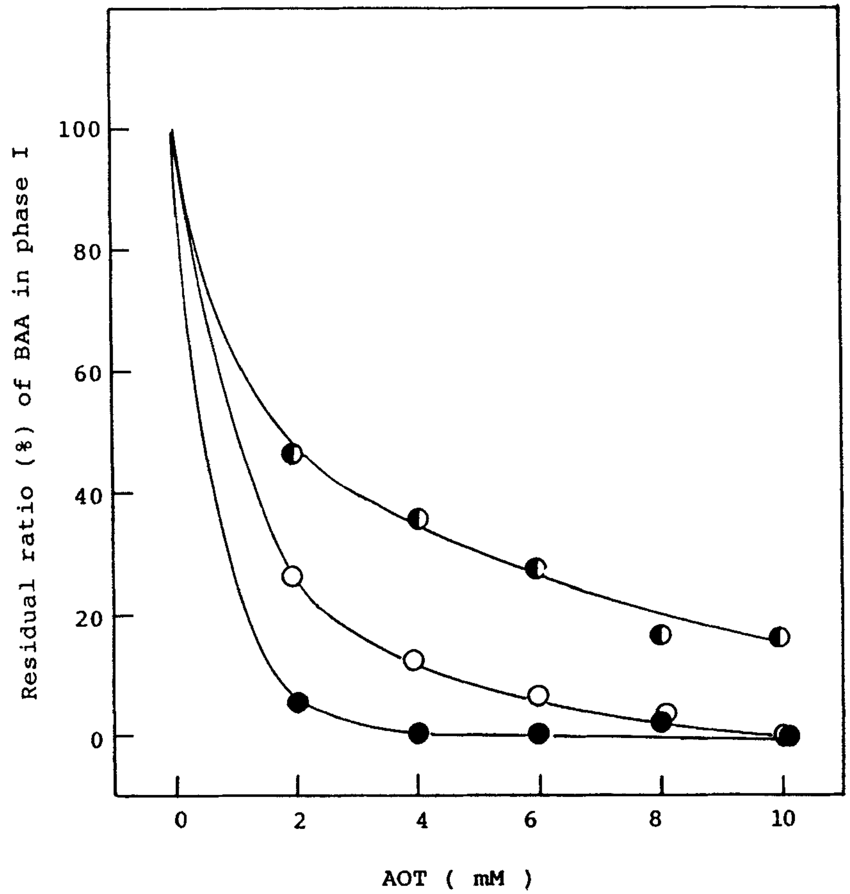

FIG. 3 The concentration dependence upon AOT of the extraction of basic amino acids from Phase I to the organic phase. The BAA concentration was 1 mM. The pH values of Phases I and II were maintained at 3 and 10, respectively. The residual ratio of BAA was plotted against the concentration of AOT after 6 hours. (●) Arg, (○) His, (◐) Orn.

respectively. On the other hand, Orn could not be completely extracted at these AOT concentrations. These results suggest that the participation of the hydrophobicity of BAA in their removal is less important because the partition coefficient reflecting their hydrophobicities are similar in their charged forms (Arg: −4.20; His: −4.15; Orn: −4.22) (23). At pH 3, they have the same net positive charge. It appears that different types of electrostatic interactions occur between each residue of BAA and the sulfonate group of AOT, although they remain unknown.

However, the extraction of amino acids such as BAA into the organic phase is assumed to be controlled as follows: BAA are soluble in the reversed micellar solution after electrostatic interactions between the anionic surfactant head group and the positively charged amino acid.

Judging from the cmc (critical micellar concentration) of AOT (0.4 mM at 20°C in chloroform) (24), the concentrations of AOT (2–10 mM) used in our experiments are sufficient to form reversed micelles in chloroform.

The pH-Dependent Transport of BAA

Amino acids possessing both an amino group and a carboxyl group can exist as cations, anions, and zwitterions depending upon the pH of the medium. At the physiological pH, amino acids possessing nonionic residues are ionized and electrically neutral.

On the other hand, BAA possessing basic residues have a net positive charge at their physiological pH. Orn possesses a positively charged amino group at the ϵ-position in its side chain. Arg has a positively charged guanidium group, and His has a weakly basic imidazolium residue. In Table 1 the proton dissociation constants (pK_a) of BAA are summarized. BAA are positively charged below pH 5, which means that they will probably be transported. The transport behavior of BAA was investigated by changing the pH of Phase I (pH 1–7, Fig. 4). In these experiments the pH of Phase II was maintained at 10, the concentration of AOT was 10 mM, and data were plotted after 6 hours of reaction. Three BAA were completely extracted into the organic phase at pH 1–2 from Phase I, and over 80% of BAA were released into Phase II from the organic phase.

At pH 3, Arg and His were totally extracted into the organic phase from Phase I whereas a small amount of Orn remained in Phase I. Orn was difficult to extract at a pH above 3, although it was extractable when sufficiently positively charged. At pH 5, about 90% of Arg, 40% of His,

TABLE 1
The Proton Dissociation Constants (pK_a) of Basic Amino Acids (25°C)

Amino acid (reference)	pK_{a_1}	pK_{a_2}	pK_{a_3}
Arginine (31)	1.96[a]	9.02[b]	12.07 (guanidino)
Histidine (32)	1.96[a]	6.12 (imidazole)	9.07[b]
Ornithine (31)	1.75[a]	9.14[b]	10.67 (ϵ-amino)

[a] Carboxyl group.
[b] α-Amino group.

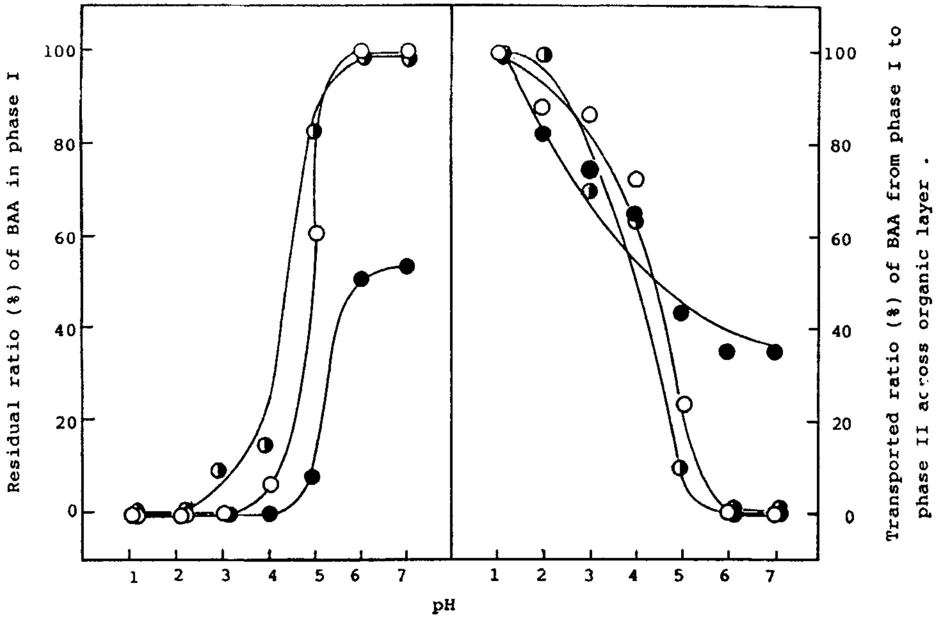

FIG. 4 The pH-dependent transport behavior of basic amino acid. The BAA and AOT concentrations were 1 and 10 mM, respectively. The pH value of Phase II was maintained at 10 and that of Phase I was changed. Both the residual and transported ratios of BAA were plotted against the pH value after 6 hours. (●) Arg, (○) His, (◑) Orn.

and 20% of Orn were extractable. However, at pH 6 and 7, His and Orn could not be extracted whereas Arg was extractable. At pH 6 and 7, about 50% and less than 10% of the imidazole groups are positively charged, respectively (the pK_a value of the imidazole group is 6.12) (32). According to these results, the degree of the positive charge of His was reduced at these pH values. The guanidium group of Arg has a high affinity for the sulfonate group of AOT. The guanidium group reportedly has a very high pK_a value (12.07) (31). Thus, its proton dissociation is little affected by a pH change (25).

Furthermore, the effect of pH on Phase II upon the release of BAA from the organic phase was investigated. Figure 5 shows the release of BAA into Phase II at alkaline pH (pH 8–12) when the concentration of AOT was 10 mM, the pH of Phase I was maintained at 3, and the reaction proceeded for 6 hours.

Significant changes in the release reaction from the organic phase into Phase II were not insured by changing the pH, indicating that the effect of the pH of Phase II was negligible and that these amino acids were

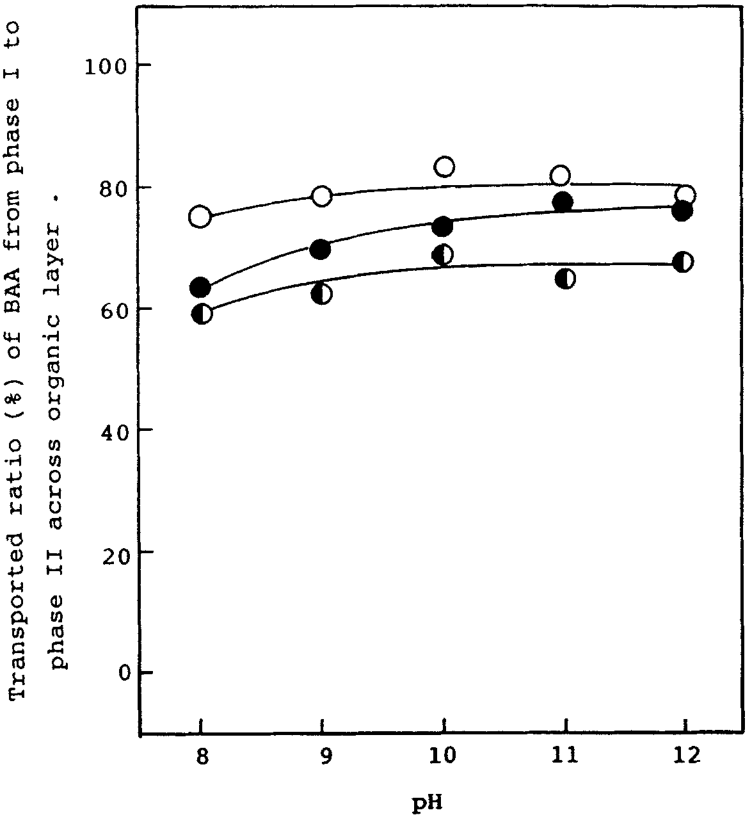

FIG. 5 The release of basic amino acids from the organic phase into Phase II. The BAA and AOT concentrations were 1 and 10 mM, respectively. The pH value of Phase I was maintained at 3 and that of Phase II was changed. The transported ratios of BAA were plotted against the pH value of Phase II after 6 hours. (●) Arg, (○) His, (◐) Orn.

probably exchanged by the antiport cations in Phase II between the organic phase and Phase II (4, 11).

The Transport Behavior of Various Amino Acids

The degree of extraction of amino acids into the organic phase depended upon their hydrophobicity. Trp, Phe, and Leu, which have highly hydrophobic residues, are easily extractable into the organic phase (9). The transport of organic compounds through the organic phase depends primarily upon their hydrophobicity as determined by their partition coefficients between octanol and water. These values are affected by the pH of the medium for compounds which possess ionizable groups. The partition coefficients are usually described by log P (P = the concentration in an apolar solvent/the concentration in water) (26–28). Considering these facts, the transport behaviors of neutral amino acids and BAA were investigated at various pH values in Phase I. The results are summarized in Table 2, where the partition coefficients of amino acids at their zwitterions are also summarized. The concentration of AOT was 4 mM, the pH of Phase II was maintained at 10, and reaction proceeded for 6 hours.

All the amino acids studied were extracted into the organic phase at pH 1 because they have net positive charges at this pH due to the suppression of ionization of the carboxyl groups (pK_a of the carboxyl group is about 2) (29). At pH 3, the amount of amino acids removed from Phase I into the organic phase decreased because their net positive charge was reduced. A small net positive charge remained in neutral amino acids, since their carboxyl groups were not completely ionized. Among neutral amino acids, the amounts transported depended upon their hydrophobicity, and were in the following order: Trp > Phe > Leu > Met > Val > Ala > Thr > Gly. These results were also indicated in Fig. 6 where the extraction ratio (E.R.) (E.R. = 100 − R.R.) is plotted against the partition coefficient. Good linearity between the E.R. value of neutral

TABLE 2
The Transport Behavior of Various Amino Acids through the Organic Liquid Membrane System after 6 Hours[a]

Amino acids		pH 1 (Phase I) R.R. (%)	pH 1 (Phase I) T.R. (%)	pH 3 (Phase I) R.R. (%)	pH 3 (Phase I) T.R. (%)	pH 5 (Phase I) R.R. (%)	pH 5 (Phase I) T.R. (%)	log P
Glycine	(Gly)	87	13	97	0	100	0	−3.03
Threonine	(Thr)	87	12	92	0	97	0	−2.91[b]
Alanine	(Ala)	78	19	86	13	97	0	−2.74
Ornithine	(Orn)	0	88	37	46	84	11	−2.89
Histidine	(His)	0	95	13	86	72	24	−2.86
Arginine	(Arg)	0	94	0	87	49	43	−2.59
Valine	(Val)	21	77	53	44	100	0	−2.10
Methionine	(Met)	19	84	46	59	100	0	−1.84[b]
Leucine	(Leu)	12	88	15	82	91	5	−1.71
Phenylalanine	(Phe)	0	97	8	88	89	9	−1.43
Tryptophan	(Trp)	0	88	3	94	84	7	−1.04

[a] The amino acid and AOT concentrations were 1 and 4 mM, respectively. The pH value of Phase II was 10 and that of Phase I was varied. Reproducibility less than ±15%.

[b] From Reference 23; all other log P values are from Reference 26.

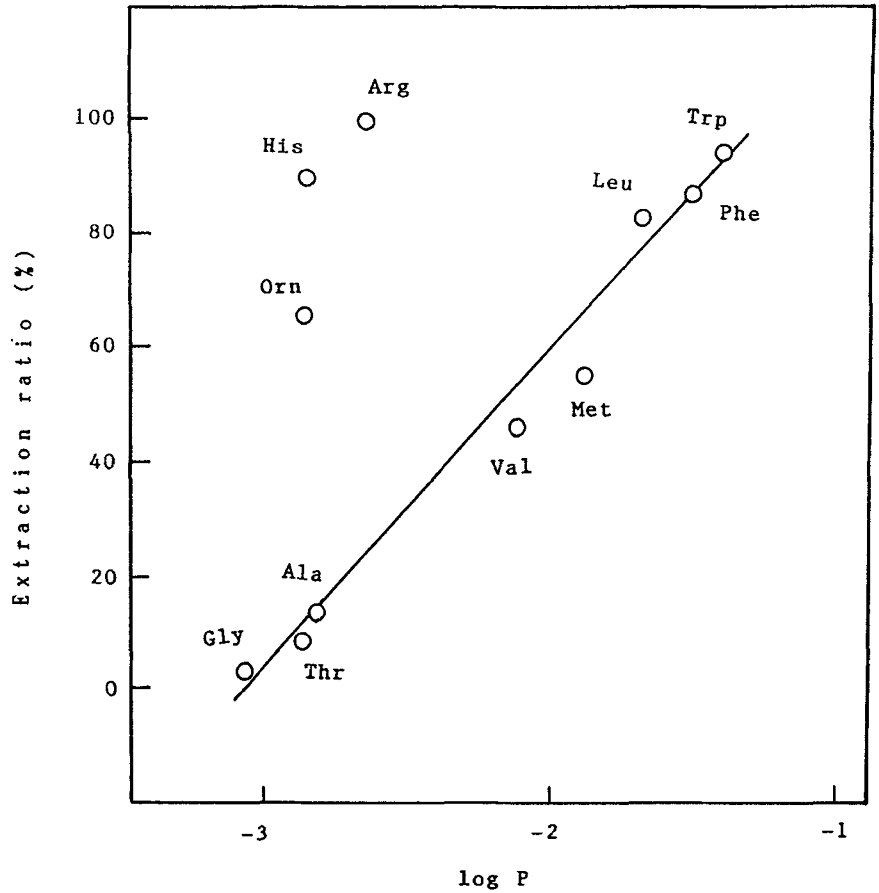

FIG. 6 The relationship between the extraction ratio of amino acid and the partition coefficient. The amino acid and AOT concrntrations were 1 and 4 mM, respectively. The pH values of Phases I and II were 3 and 10, respectively. The extraction ratio of amino acids was plotted against the partition coefficient after 6 hours. Extraction ratio (%) = 100 − residual ratio (%).

amino acids and the partition coefficient is indicated. On the other hand, BAA, which has higher positive charges than neutral amino acids, lay outside of this linearity. At pH 5, neutral amino acids are difficult to extract except for Leu, Phe, and Trp, which have highly hydrophobic residues. On the other hand, BAA are extractable because they still have net positive charges on their residues at pH 5. These results suggest that BAA can be separated from most amino acids under appropriate pH conditions. Leu, Phe, and Trp were extracted into an organic phase containing methyltrioctylammonium cation (MTO) as the carrier at pH 10.

On the other hand, BAA were difficult to extract under these conditions (30). Thus, amino acids or other ionic compounds can be separated by changing the pH of the medium and by using anionic or cationic carriers.

CONCLUSION

The transport behavior of neutral amino acids and BAA in an organic liquid membrane system (LMS) containing sodium di-(2-ethylhexyl) sulfosuccinate (Aerosol OT, AOT) was examined. BAA was transported from Phase I at pH 5 to Phase II at pH 10 through an organic phase. BAA were probably extracted from Phase I into the organic phase by electrostatic interaction between cationic amino acids and the anionic sulfonate group of AOT. They were released from the organic phase into Phase II by the exchange reaction of an antiport cation in Phase II to the organic phase. The degree of BAA extracted from Phase I into the organic phase was in the following order: Arg > His > Orn. Most neutral amino acids were not extracted from Phase I at pH 5 into the organic phase because they exist as zwitterions. However, these amino acids were extracted from Phase I at pH 1 because their net charges were positive. These results suggest that BAA can be separated from various amino acids at the appropriate pH by using AOT or another carrier.

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Received by editor December 28, 1993
Revised March 14, 1994