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Selective Transport of Histamine from a Mixture of Histidine and Histamine by the Use of Organic Liquid Membrane Systems

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Selective Transport of Histamine from a Mixture of Histidine and Histamine by the Use of Organic Liquid Membrane Systems

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Abstract

The transport behaviors of histidine (His) and its related compounds through organic liquid membranes were examined. The organic liquid membranes system was composed of two aqueous phases (Phases I and II) which were put on both sides of an organic layer containing a carrier. Chloroform and sodium di-2-ethylhexyl sulfosuccinate (AOT) were used as the organic layer and the carrier, respectively. No transport reaction occurred without the carrier. The amounts of removal into the organic layer increased with an increase in the concentration of AOT up to 5 m*M* and maintained at higher concentrations. His and carnocine, which possess the carboxyl group, could be removed into the organic layer from Phase I at pH 4–5 but could not be removed at pH values higher than 5. On the other hand, histamine (Hm) and histidinol, which lack the carboxyl group, could be removed into the organic layer from Phase I at pH 7. Also, the compounds in the organic layer could be removed into Phase II at pH 10. On the basis of these results, the separation of Hm from the Hm–His mixture occurred with pH 7 in Phase I and pH 10 in Phase II through the organic layer containing 5 m*M* of AOT. Hm was effectively transported from Phase I to Phase II through an organic layer using some molar ratios (1, 50, and 100) of mixtures (molar ratio = concentration of His/concentration of Hm).

INTRODUCTION

Removal studies of biological-related compounds by organic liquid membrane systems are useful not only for the simulation of transport across biological membranes (*1*) but also for the development of biosensors (*2–4*).

There are many reports about the transport of amino acids and their derivatives (*5–9*) and of nucleotides (*10*) through organic liquid membrane systems. These systems are composed of two aqueous phases (Phases I and

II) which are put on each side of an organic layer containing a carrier. The carriers, which have ionic (anionic, cationic, and zwitterionic) and nonionic properties, are selected according to the properties of samples to be transported.

In the case of amino acids, their zwitterionic nature conspires against such transport under neutral conditions because desolvation of the double ion is a costly energetic proposition. As in binding studies, the transport is largely limited to either acidic or basic conditions.

A single exception has been provided by Sumamoto (*11*) who showed that a merocyanine dye permits the transport of phenylalanine across a liposomal bilayer. Since phenylalanine methyl ester is also transported, the selectivity of this intriguing system is unclear.

Moreover, with the relatively high concentration of the bilayers, the photochemical event may be expected to alter the actual structure of the bilayer and its permeability.

Both Lehn (*5*) and Cram (*6*) have transported the ammonium salts of amino acids and esters under acidic conditions, and asymmetric crown ethers have been successful in chiral recognition under these conditions. Luisi (*12*) indicated that tryptophan and its derivatives can be removed into the organic solvent of cyclohexane containing liphophilic tertiary ammonium salts in the neutral pH region (pH 6–8).

On the basis of these results, various amino acids could be transported through organic liquid membrane systems containing a suitable carrier.

We have investigated the transport behaviors of histidine (His) and its derivatives, mainly histamine (Hm), through organic liquid membrane systems containing sodium di-2-ethylhexyl sulfosuccinate (AOT) (Fig. 1) as the carrier and chloroform as the organic layer. The difference between

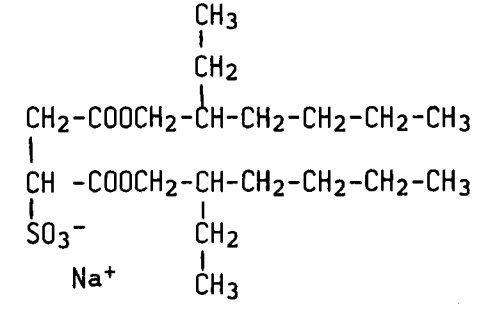

FIG. 1. Structure of sodium di-2-ethylhexyl sulfosuccinate (AOT).

Hm and His removal across a biological system is interesting because Hm is released from mast cells after decarboxylation of His.

EXPERIMENTAL

Materials

Histamine dihydrochloride, L-histidine (free base), sodium di-2-ethylhexyl sulfosuccinate, and chloroform were purchased from Nakarai Tesque. Histidinol, histidinol phosphate, and carnocine (*N* β-alanyl-L-histidine) were purchased from Sigma Chemical Company. The other reagents used were guaranteed grade reagents.

Procedure

The transport experiments were carried out at room temperature in U-tube glass cells as shown in Fig. 2. A chloroform solution of AOT (25 mL) was placed at the bottom of the tube. A solution of histidine or its derivative (20 mL) and a suitable solution (20 mL) were put into one side (Phase I) and the other side (Phase II), respectively.

The organic layer was stirred with magnetic stirrers. The amounts of

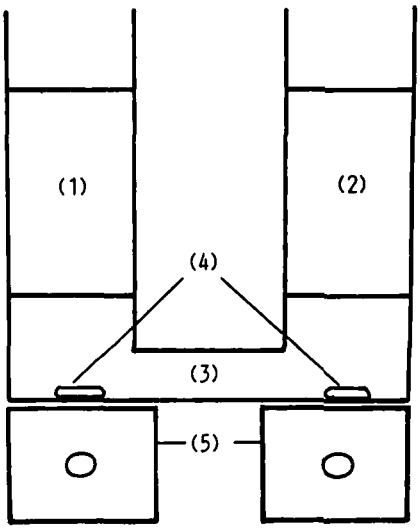

FIG. 2. Outline of the organic liquid membranes system. (1) Phase I, (2) Phase II, (3) the organic layer containing a carrier, (4) spinners, (5) magnetic stirrers.

samples in Phases I and II were measured by fluorometry by the use of *o*-phthalaldehyde-mercaptoethanol (*13*). 0.1 *M* Acetate buffer (pH 4–6) and 0.1 *M* borate buffer (pH 7.2–10) were used for pH control in Phases I and II.

The amounts of sample removed from Phase I to the organic layer and from the organic layer to Phase II were indicated as the residual ratio (R.R.) and the transported ratio (T.R.), respectively:

$$\text{R.R.} = C_i1/C_i \times 100; \qquad \text{T.R.} = C_i2/C_i \times 100$$

where C_i is the initial concentrations of samples, and the amounts of sample in Phase I (C_i1) and in Phase II (C_i2) after various times have passed are indicated.

A Shimadzu fluorophotometer FR-540 was used for the fluorescent determination. The HPLC apparatus was pump unit PN-101 of Yanagimoto

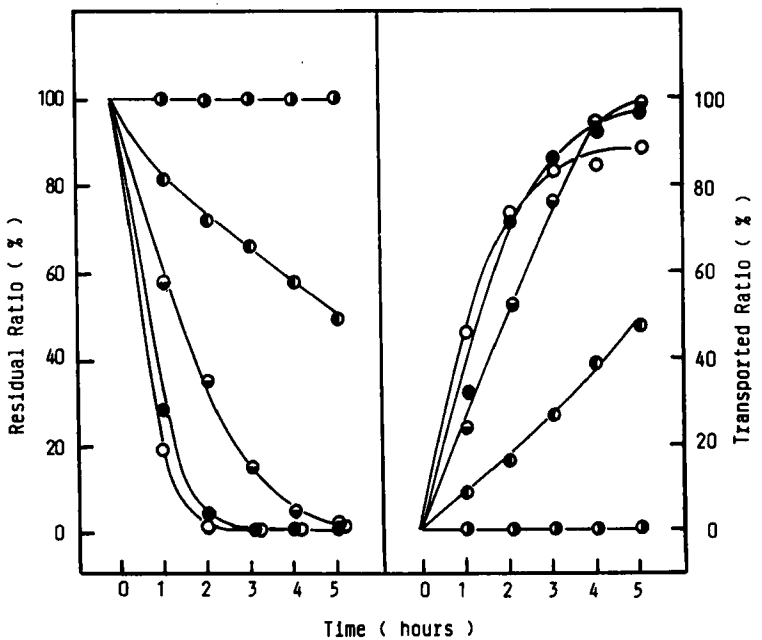

FIG. 3. Relationship between the concentration of AOT in the organic layer and the transport of histamine. Phase I pH: 5.0. Phase II pH: 10.0. Initial concentration of histamine: 1 m*M*. (◑) None, (◐) 0.4 m*M*, (◒) 1 m*M*, (●) 5 m*M*, (○) 10 m*M*.

liquid chromatograph Model L-2000 equipped with a Nihonbunko UV-spectrophotometer DE-100 IV. The detection wavelength was 220 nm.

The analytical column (15 × 0.4 cm) was prepared with Nucleosil 5 C_{18} (5 μm) by using a high-pressure slurry-packing technique. Separations were performed by eluting with 0.05 *M* phosphoric acid and 5 m*M* sodium hexanesulfonate containing 15% (v/v) methanol.

RESULTS AND DISCUSSION

The Concentration Effect of a Carrier

The removal behavior from Phase I to the organic layer and from the organic layer to Phase II are indicated as the residual ratios and transported ratios in the figures. No removal reaction from Phase I to the organic layer was observed without the carrier, and the amount of Hm increased as the concentration of AOT increased to 5 m*M*, but the amount of Hm from the organic layer to Phase II decreased at 10 m*M* of AOT compared with

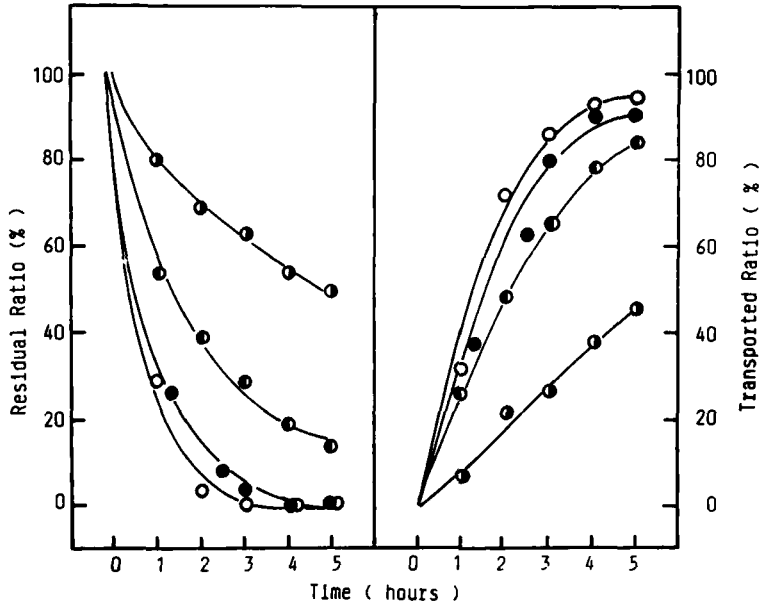

FIG. 4. Relationship between the pH of Phase I and the transport of histamine. Phase II pH: 10.0. Concentration of AOT: 5 m*M*. Initial concentration of histamine: 1 m*M*. (○) pH 5.0, (●) pH 7.2, (◐) pH 8.0, (◑) pH 9.0.

5 m*M* (Fig. 3). This phenomenon suggests that the reextraction reaction occurred at higher concentrations of AOT.

Effect of pHs on Removal Behavior

Hm and His which possess amino and imidazole groups, and their net charges, are expected to be changed by varying the pH of the medium. Figures 4 and 5 show the transport behaviors of Hm and His at various pH conditions in Phase I. In this case the pH's of Phase II were kept at 10. Hm could be transported at pH 5–9 in Phase I, and the amounts of Hm transported in Phase II increased depending on the amount of removal from Phase I to the organic layer. On the other hand, His could not be transported at pH 6–7.2 in Phase I. The reason for the different transport behaviors between His and Hm are suggested to be as follows: In the case of His, a positive charge sufficient to cause electrostatic interactions with the negative charge of AOT which contains sulfonyl groups is not expected at pH 6–7.2 because the protonation reaction of imidazole (pK_a of the

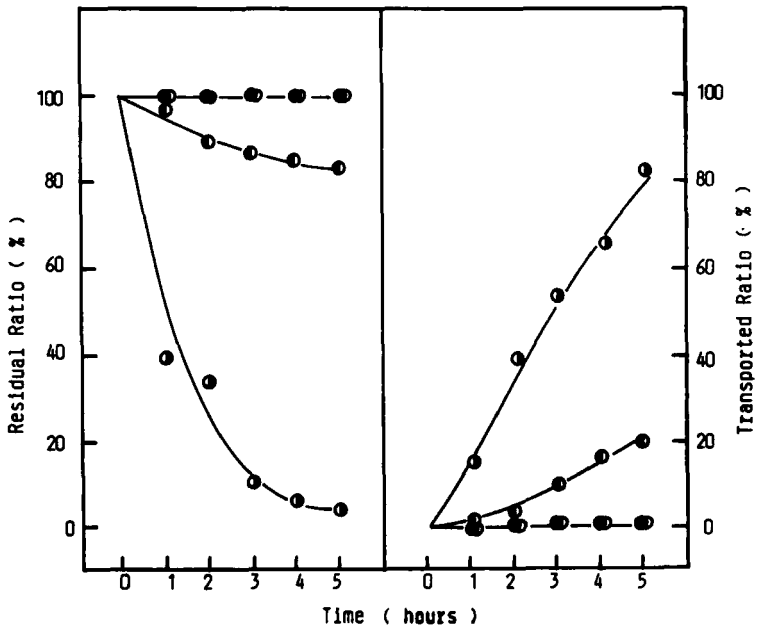

FIG. 5. Relationship between the pH of Phase I and the transport of histidine. Phase II pH: 10.0. Concentration of AOT: 5 m*M*. Initial concentration of histidine: 1 m*M*. (◑) pH 4.0, (◐) pH 5.0, (○) pH 6.0, (●) pH 7.2.

imidazole group of His is 6.12) (*14*) and the compensating reaction between the negative charge of the carboxyl group and the positive charge of the amino group of His occurs. As a result, the net charge of His is zero. However, a sufficient positive charge was obtained below pH 5, and His could be transported (Fig. 5). A similar mechanism is suggested for the case of Hm which is difficult to transport at pH 9 in Phase I because the net charge of Hm is almost zero due to the protonation of the imidazole and amino groups of Hm (pK_a of the amino and imidazole groups of Hm are 9.88 and 6.13, respectively) (*15*).

The opposite reaction is suggested for removal from the organic layer to Phase II. The effects of pH in Phase II were examined at pH 5 in Phase 1 (Fig. 6). Hm could be transported at pH 9–10 in Phase II because electrostatic interaction disappears in this pH region due to protonation of imidazole and partial protonation of the amino group.

These explanations of the transport mechanism are illustrated in Fig. 7 and are also supported by the experimental data of the related compounds of His in Table 1. Hm and histidinol, which lack carboxyl groups, were

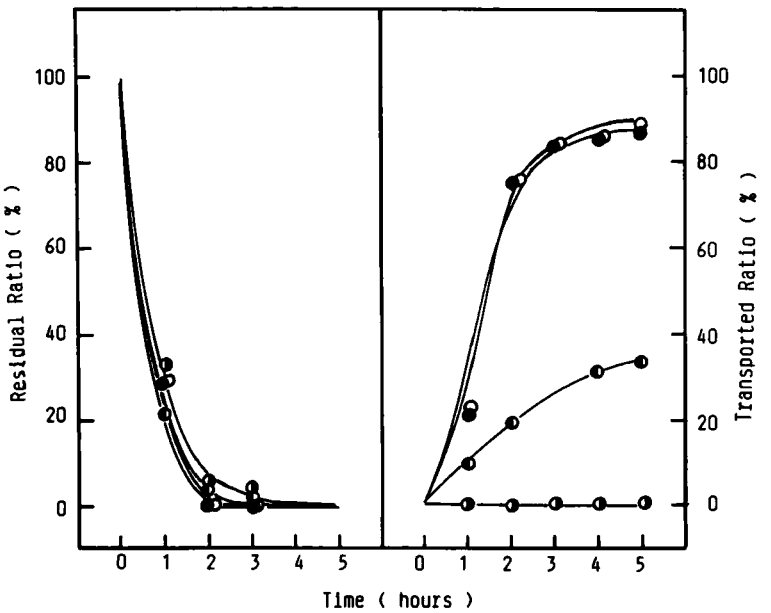

FIG. 6. Relationship between the pH of Phase II and the transport of histamine. Phase I pH: 5.0. Concentration of AOT: 5 m*M*. Initial concentration of histamine: 1 m*M*. (◑) pH 7.2, (◐) pH 8.0, (●) pH 9.0, (○) pH 10.0.

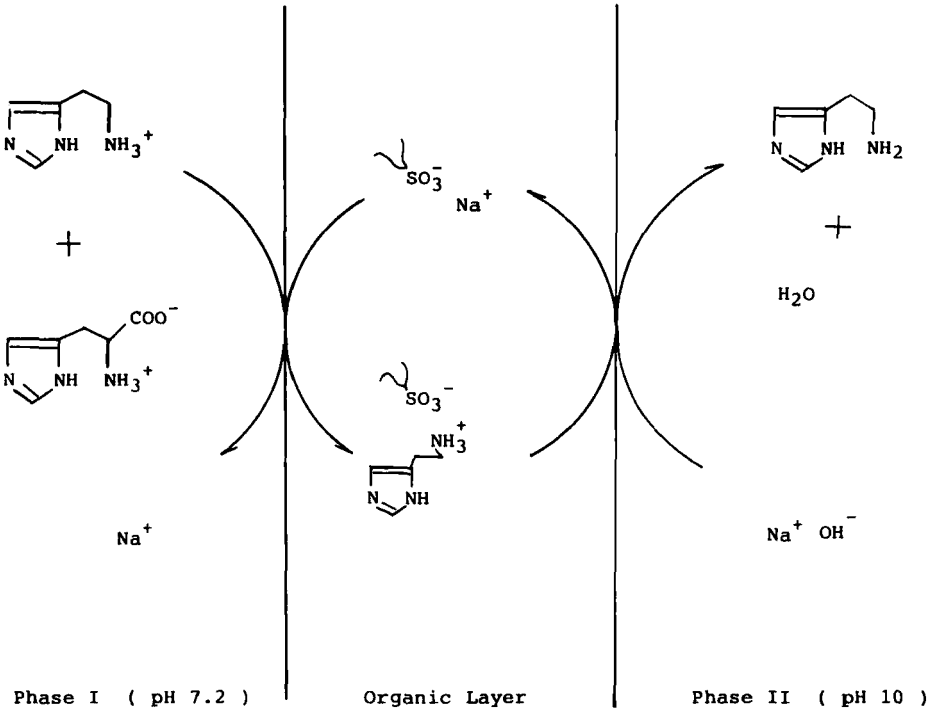

FIG. 7. Possible mechanism of selective transport of histamine from a mixture of histidine and histamine through the organic liquid membranes system.

transported at pH 5 and pH 7.2 in Phase I, but His and carnosine (*N*-β-alanyl-L-histidine), which possess carboxyl groups, were not transported at pH 7.2 in Phase I. In the case of pH 5 in Phase I, small amounts of them were observed (about 20%) in Phase II. On the other hand, histidinolphosphate was not transported at either pH in Phase I. It is suggested that the two negative charges of the phosphate group, compared with the single negative charge of the carboxyl group, prevent removal into the organic layer.

Arginine, lysine, and histidine, which are basic amino acids, can be transported through organic liquid membranes containing 5 m*M* AOT under the conditions of pH 5 in Phase I and pH 10 in Phase II. The amounts transported are in the order: arginine > histidine >> lysine (*16*). Only a small amount of lysine was removed into the organic layer compared with arginine or histidine.

TABLE 1

Transported Ratios of Histamine and Its Related Compounds after 2 and 5 hours[a]

	Transported ratios			
	pH 5 in Phase I		pH 7 in Phase I	
Compounds	2 h	5 h	2 h	5 h
Histamine	72%	95%	63%	90%
Histidine	4%	20%	—	—
Histidinol	63%	95%	64%	98%
Histidinol phosphate	—	—	—	—
Carnocine	10%	19%	—	—

[a]Initial concentrations of samples, 1 m*M*; concentration of AOT, 5 m*M*; pH of Phase II, 10.0; —, not detected.

In this way, the transport of amino acids can be controlled by varying the pH, and selective transport is possible if suitable conditions are selected.

Luisi (*12*) indicated that tryptophan and its related compounds can be removed from an aqueous phase of pH 6–9 to an organic phase of cyclohexane containing methyltrioctylammonium chloride as the carrier. Compounds of large molecular weight, such as proteins and enzymes, can also be removed into an organic layer containing this carrier.

Application for the Separation of Hm

Selective transport might be possible to use for the different removals of His and Hm by changing the pH of the solution. We examined the selective transport of Hm from a His–Hm mixture by having pH 7.2 in Phase I and pH 10 in Phase II (Table 2). The amounts of Hm and His transported from Phase I to Phase II were measured by a HPLC apparatus.

When a solution of a His-Hm mixture containing a molar ratio of 1 was put into Phase I, only Hm was detected (T.R. = 90%) in Phase II after 5 h. Hm (T.R. = 80–90%) and small amounts of His (T.R. < 0.5%) in Phase II were observed after 5 when solutions containing molar ratios of 50 or 100 were put into Phase I. In this way it is suggested that this is a usable system for the separation of Hm after the decarboxylation reaction of histidine. This selectivity is an interesting point to consider in the different behaviors of Hm and His in biological systems.

AOT used in this experiment is the anionic surfactant, and other surfactants which have cationic, nonionic, and zwitterionic characters have been synthesized and used as the carriers for biomimetic membranes (*17–19*).

TABLE 2

Selective Transport of Histamine from a Mixture of Histidine and Histamine at Some Molar Ratios in Phase I[a]

Molar ratios (His/Hm)[b]		Transported ratios[c] 2 h	5 h
1	Hm	60%	91%
	His	—	—
50	Hm	55%	83%
	His	—	0.2%
100	Hm	65%	90%
	His	0.2%	0.4%

[a]pH of Phase I, 7.2; pH of Phase II, 10.0; concentration of Hm, 1 m*M*; concentration of AOT, 5 m*M*.

[b]Molar ratio = concentration of His/concentration of Hm.

[c]Measured by HPLC; —, not detected.

Some of them were used as bioseparation tools (*20*). The transport of such high molecular weight compounds as proteins will be possible if surfactants which can form reversed micelles are selected as carriers because the water pools in reversed micelles are large enough to enclose proteins (*21*). The transport of some proteins through organic liquid membranes containing AOT as the carrier was recently reported, and selective transport could be performed by changing the pH or the ionic strength (*22*, *23*).

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