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Shigeo Takeshima[a]; Shingo Hirose[a]
[a] Kyoto Pharmaceutical University Misasagi, Kyoto, Japan

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An Organic Liquid Membrane System for the Separation and Production of Histamine

SHIGEO TAKESHIMA* and SHINGO HIROSE
KYOTO PHARMACEUTICAL UNIVERSITY
MISASAGI, YAMASHINA, KYOTO 607, JAPAN

Abstract

The recovery of histamine (Hm) produced by histidine decarboxylase (HD) from histidine (His) by using an organic liquid membrane system was investigated. The system was composed of two aqueous phases (phase I of pH 4.5 and phase II of pH 7.2) separated by a third organic chloroform phase containing di-(2-ethylhexyl) phosphoric acid (EHP) as a carrier. His could not be moved from phase I of pH 4.5, the optimum pH of HD into the organic layer. On the other hand, Hm was moved from phase I into the organic layer, and Hm was released from the organic layer into phase II. The removal of Hm from phase I into the organic layer increased at dose dependency with EHP concentration up to 50 m*M* and decreased at concentrations above 50 m*M*. According to these results, the recovery of Hm from His by HD was investigated. In this experiment phase I was pH 4.5 containing 1 m*M* His, 40 μg/mL pyridoxal-5-phosphate, and a suitable concentration of HD; and phase II was set at pH 7.2. 170 μ*M* Hm (3 h later) and 760 μ*M* (8 h later) were transported into phase II by using 120 μg/mL HD and 50 m*M* EHP, as confirmed by HPLC.

INTRODUCTION

The unique method using an organic layer containing carriers (organic liquid membrane) has been studied as a separation tool and a model of biological membranes.

The former function as a separation system for useful compounds (metal ions, organic acids, carbohydrates, etc.) from source mixtures (*1–4*). The latter is of note in studies in the transport of biologically active compounds through biological membranes with properties of active transport or in recognition of these compounds (*4–8*).

There have been many investigations regarding the transport of amino acids and their derivatives which possess both amino and carboxyl groups.

*To whom correspondence should be addressed.

It is of interest to note that under physiological conditions, both amino and carboxyl groups are ionized, so that amino acids will be electrically neutral for nonionic residues. This zwitterionic character makes these amino acids difficult to move into the organic phase. They are only transported by strong acids (0.1 *N* HCl) or strong alkalies (0.1 *N* KOH), in which they are either negatively or positively charged, by cationic or anionic surfactants (*9*, *10*).

On the other hand, histidine has a cationic character below pH 6 because this residual group (pK_a of imidazole group = 6.12) (*11*) has a positive charge in this pH region.

We previously reported the removal behavior of histidine (His) and its related compounds, mainly histamine (Hm), and both His and Hm were transported from one aqueous phase (phase I) of pH 5 to another aqueous phase (phase II) of pH 10 through the organic chloroform layer containing di-2-ethylhexyl sodium sulfosuccinate (Aerosol OT, AOT). However, His could not be moved into the organic layer from phase I of pH 7.2. On the other hand, Hm could be moved into the organic layer under this condition. On the basis of these different removal behaviors, Hm was selectively transported from a mixture of His and Hm (*12*). According to this study, the separation of Hm after decarboxylation of His by histidine decarboxylase (HD) should be possible. However, both His and Hm were moved into the organic layer at pH 4.5, which is the optimum pH condition of HD (*13*). To separate Hm under this condition, a different system will be needed. We have sought an effective method for separating Hm after decarboxylation of His by HD by means of an organic liquid membrane system.

The separation of a product from source compounds is an important step, and studies of a system with both product synthesis and separation is necessary in chemical technology.

EXPERIMENTAL

Reagents

Histamine dihydrochloride, histidine (free base), di-(2-ethylhexyl) phosphoric acid (Fig. 1, EHP) and chloroform were purchased from Nacalai Tesque Co. Histidine decarboxylase (HD) from Lactobacillus 30α (0.33 unit/mg protein) was purchased from Sigma Chemical Co. Other reagents used were guaranteed grade reagents.

Procedures

Chloroform containing EHP (25 mL) was placed on the bottom of a U-tube glass cell (Fig. 2). The buffer solutions for HD containing 1 m*M* His or Hm (20 mL) and a suitable pH solution (20 mL) were added into phases

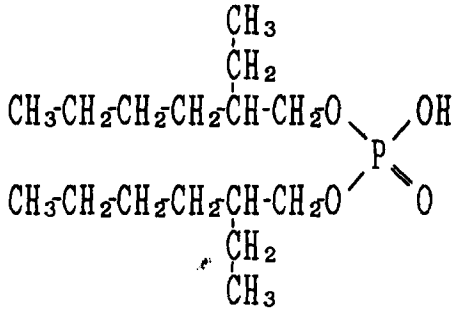

FIG. 1. Structure of di-(2-ethylhexyl) phosphoric acid.

I and II, respectively. His and Hm in both aqueous phases were measured at constant times by fluorometry by using *o*-phthalaldehyde-mercaptoethanol (*14*). Experiments were carried out at room temperature (20°C).

The movement of a sample from phase I into the organic layer and from the organic layer to phase II were indicated by the residual ratio (RR) and the transported ratio (TR), respectively:

$$RR = (C_{t1}/C_i) \times 100$$
$$TR = (C_{t2}/C_i) \times 100$$

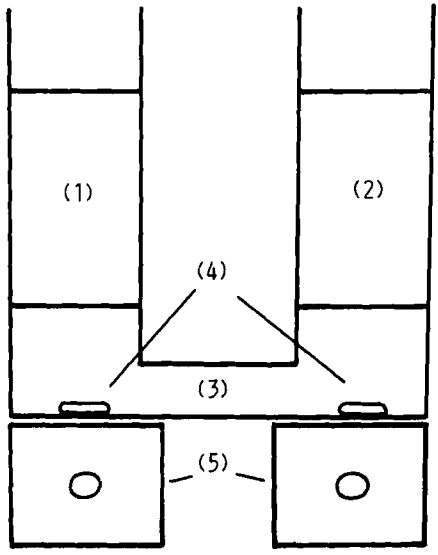

FIG. 2. Schematic diagram of transport apparatus: (1) phase I, (2) phase II, (3) the organic layer containing a carrier, (4) spinners, (5) magnetic stirrers.

where C_i is the initial concentration of a sample and C_{r1} and C_{r2} are the levels in phases I and II, respectively, at various times.

Enzyme reactions were carried out according to Bermeyer (*13*), and Hm and His were measured by high performance liquid chromatography (HPLC) by using a UV detector at 220 nm. The buffer solution for HD (pH 4.5) was prepared as follows: 11.5 mL acetic acid and 0.9 g EDTA were dissolved in distilled water, 56 mL of 1 *N* KOH was added, and the mixture was diluted to 1000 mL with distilled water.

Other buffer solutions were prepared by mixing 0.1 *M* sodium tetraborate and 0.1 *M* HCl (pH 7.2, 8, and 9) or 0.1 *M* NaOH (pH 10).

Apparatus

A Shimadzu fluorophotometer RF-540 was used for fluorescent determinations. The HPLC apparatus was pump unit PRS-2000 from Kurahashigiken equipped with a Nihonbunko UV-spectrophotometer UVIDEC-100 IV. Separations were carried out by eluting through an analytical column (15 × 0.4 cm) containing Nucleosil $5C_{18}$ (5 μm) with 0.05 *M* phosphoric acid and 5 m*M* sodium haxanesulfonate containing 15% (v/v) methanol.

RESULTS AND DISCUSSION

AOT, which possesses a sulfonyl group, is not a suitable carrier with which to perform the separation of Hm after decarboxylation of His by HD at optimum pH 4.5, since both Hm and His were moved into the organic layer containing AOT at this pH according to our previous paper (*12*).

To separate Hm after the reaction with HD, a different surfactant instead of AOT was necessary. We selected EHP which is a dialkylphosphate ester. EHP is a cation exchanger for various metals in solvent extraction. Uranium ions have been separated with EHP and condensed from crude phosphoric acid (*15*).

Physicochemical investigations concerning transport metal ions such as Eu^{3+} (*16*), Am^{3+} (*17*), and Cd^{2+} (*18*) through an organic liquid membrane containing EHP have been reported. Not only metal ions, but also such an important amino acid as lysine can be transported through an organic liquid membrane containing cationic EHP at low pH regions (pH 2–3) (*19*).

Influence of pH upon the Transport

Basic studies concerning the transport of Hm from phase I to phase II through an organic layer containing 50 m*M* EHP were carried out by changing the pH in phase II (Fig. 3). In this case the pH of phase I was

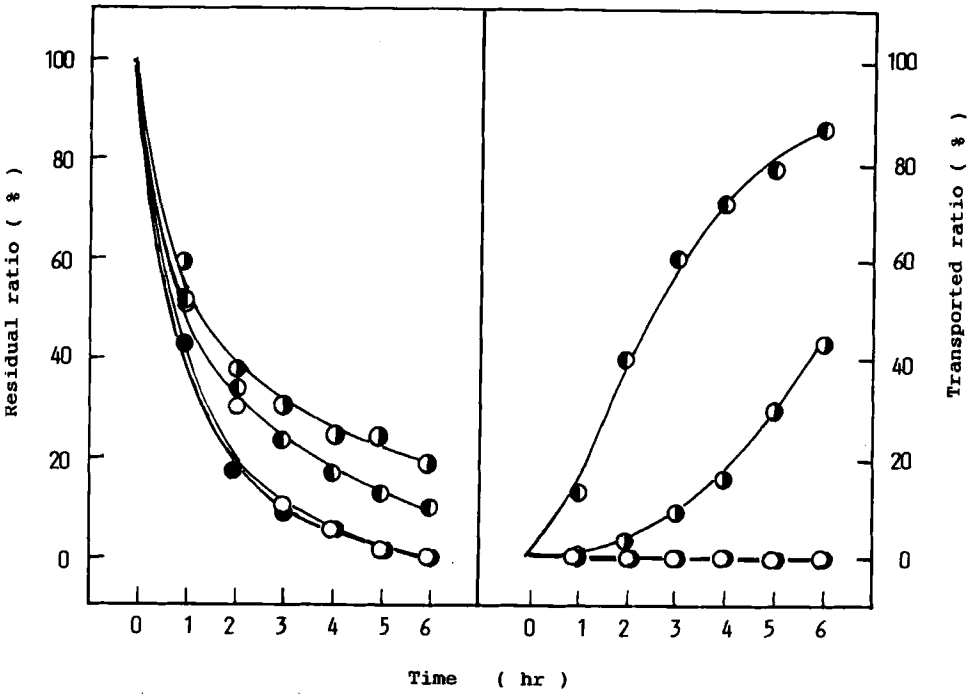

FIG. 3. Relationship between pHs in phase II and transport behaviors of Hm. Initial concentration of Hm: 1m*M*. Concentration of EHP: 50 m*M*. pH of phase I: 4.5. pH values: (◐) 7.2, (◑) 8, (○) 9, (●) 10.

maintained at 4.5 by an HD buffer solution. Hm was moved into the organic layer from phase I, and the rate increased with increasing pH in phase II. All the Hm had moved into the organic layer in phase II at pH 9–10 after 6 h. Removal of Hm from the organic layer to phase II at pH 9–10 was not observed; furthermore, phase II became turbid under these pH conditions. However, Hm was removed from the organic layer at pH 7–8 in phase II. A higher TR at pH 7.2 than at pH 8 was observed. These results demonstrate the different manners of transport with AOT as the carrier, enabling Hm to be moved from the organic layer to phase II at pH 9–10. One of the reasons for these different transport behaviors may be the different affinities between Hm and AOT or EHP, but more detailed information will be needed for clarification of this phenomena. In the case of His, there was no transport at pH 4.5 in phase I and pH 7.2 in phase aII as described in Fig. 6. According to these results, Hm after His decarboxylation was effectively transported through the organic layer under these pH conditions.

Effect of EHP Concentration

The amount of transported molecules into the organic layer should increase with increasing carrier concentration. The influence of EHP concentration on Hm transport behavior was investigated at pH 4.5 in phase I and at pH 7.2 in phase II. Hm could not be transported without EHP, RR decreased with increasing concentrations of EHP, and all Hm was moved into the organic layer after 6 h (Fig. 4). On the other hand, TR with 100 m*M* EHP was not higher than that with 50 m*M* EHP, and the best result was observed at this concentration. These results are plotted in Fig. 5 at 3 and 6 h versus some concentrations of EHP. This reduction of TR at 100 m*M* EHP probably means that a reextraction of Hm occurred between the organic layer and phase II at this high concentration of EHP. A similar tendency was indicated with the use of AOT. In this case, such a high concentration of AOT was not required for satisfactory transport of Hm (about 5 m*M*) (*12*).

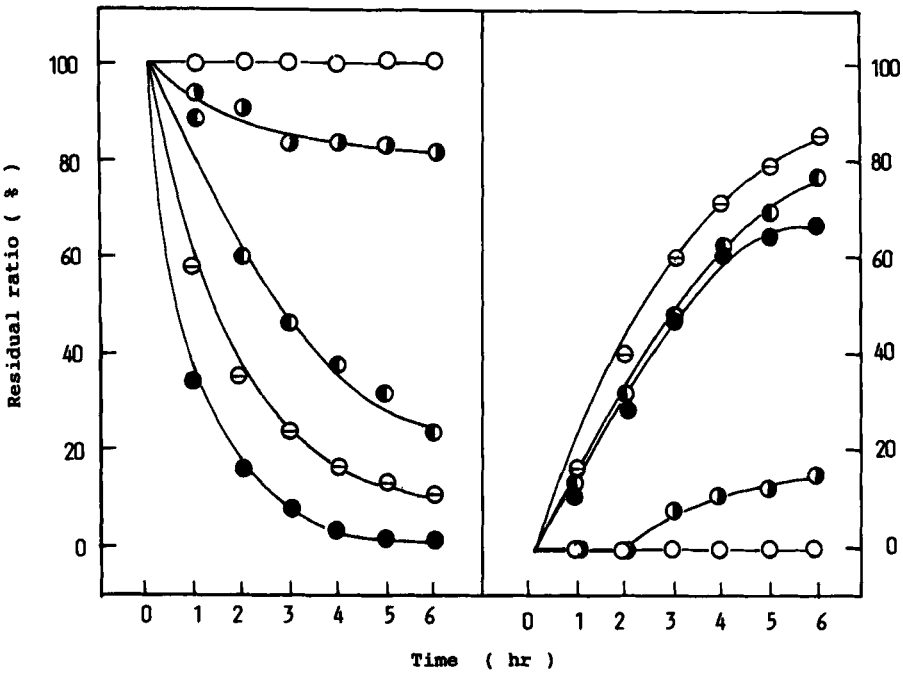

FIG. 4. Relationship between concentrations of EHP and transport behaviors of Hm. Initial concentration of Hm: 1 m*M*. pH of phase I: 4.5. pH of phase II: 7.2. Concentrations (in m*M*): (○) 0, (◑) 10, (◐) 30, (⊖) 50, (●) 100.

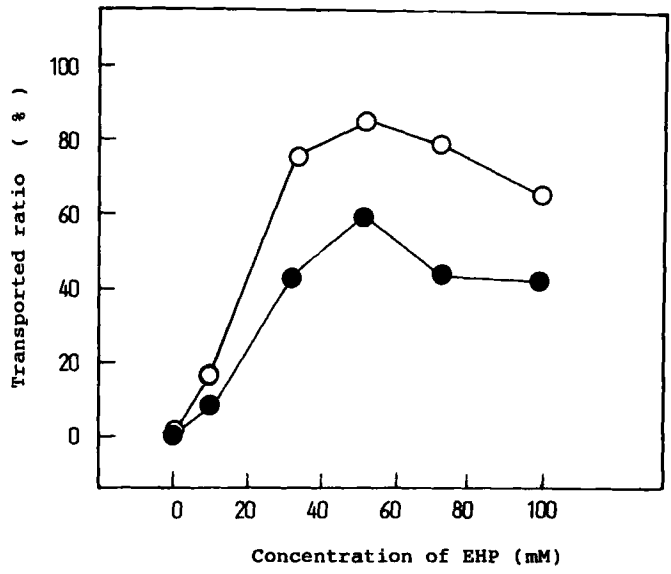

FIG. 5. Plots of transported ratio of Hm at 3 and 6 h later versus some EHP concentrations. Initial concentration of Hm: 1m*M*. pH of phase I: 4.5. pH of phase II: 7.2. Time (in hours): (●) 3, (○) 6.

Recovery of Hm after His Decarboxylation by HD

On the basis of these results, we applied this organic liquid membrane system to the recovery of Hm after His decarboxylation by HD.

In this system, HD buffer solution (pH 4.5) containing 1 m*M* His, 40 μg/mL pyridoxal-5-phosphate, and a suitable concentration of HD was put into phase I. A buffer solution of pH 7.2 was used as phase II, and the EHP concentration was 50 m*M*. The temperature was maintained at 35°C to facilitate enzyme reaction. As shown in Fig. 6, a significant change in transport was not observed at 35°C as opposed to room temperature, and His was not transported.

Progress curves for the formation of Hm by HD and the removal behavior of Hm from the organic layer into phase II are shown in Fig. 7. The complete transformation from His to Hm by HD was indicated after 6 h of treatment with 40 μg/mL HD. TR of Hm increased over time, and approximately 60% TR was observed 6 h later. This TR was not expected from the basic studies of the transport behavior of Hm as shown in Fig. 6 (TR = about 95%, 6 h, 35°C). It is suggested that some species in the reaction mixture prevent satisfactory Hm transport.

Transport behavior at various concentrations of HD are indicated in Fig.

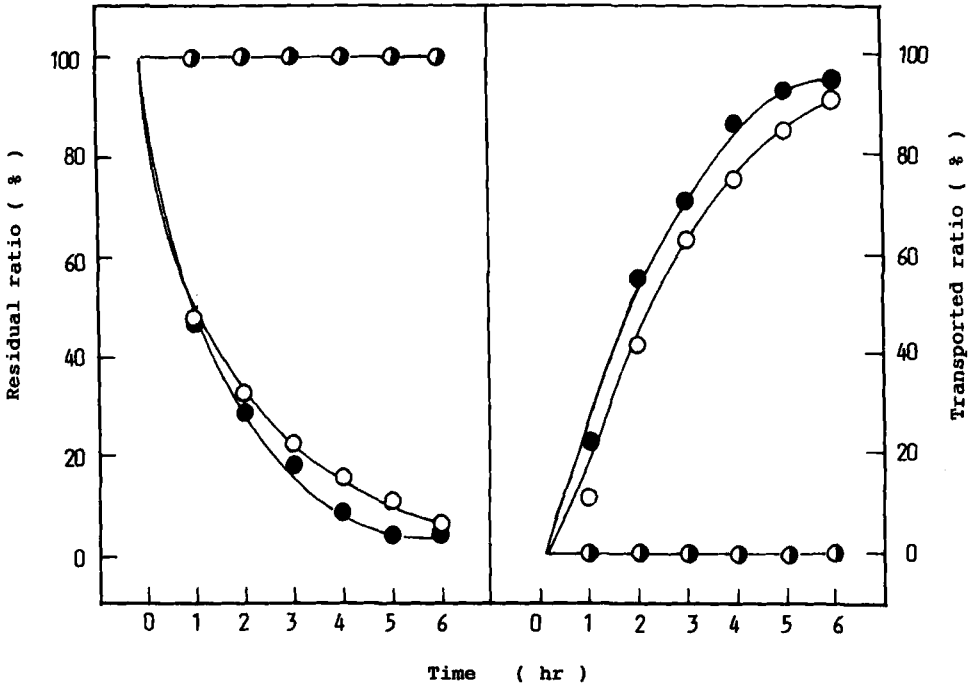

FIG. 6. Transport behaviors of Hm and His. Initial concentration of His or Hm: 1 m*M*. Concentration of EHP: 50 m*M*. pH of phase I: 4.5. pH of phase II: 7.2. (○) Hm at room temperature, (●) Hm at 35°C, (◑) His at 35°C.

8. Further, the experimental data using our liquid membrane system with varying EHP or HD concentration are shown in Table 1. Similar Hm levels (about 700 μ*M*) in phase II were observed 8 h later at higher concentrations of 40 μg/mL HD.

On the other hand, lower concentration (170 μ*M*) with 40 μg/mL HD rather than at 370 μ*M* using 120 μg/mL HD were detected 3 h later because the formation of Hm from His was insufficient, as shown in Fig. 7.

The influence of EHP concentration on Hm transport behavior was similar to that in the basic studies: a lower concentration of Hm (510 μ*M*) by using 0.1 *M* EHP rather than 670 μ*M* by using 0.05 *M* EHP was observed. Under these conditions, Hm decarboxylated by HD from His was effectively transported in phase II, and His was not detected in phase II throughout the experiments. It is pointed out that in our system both procedures consisting of the production of Hm by HD in phase I and the

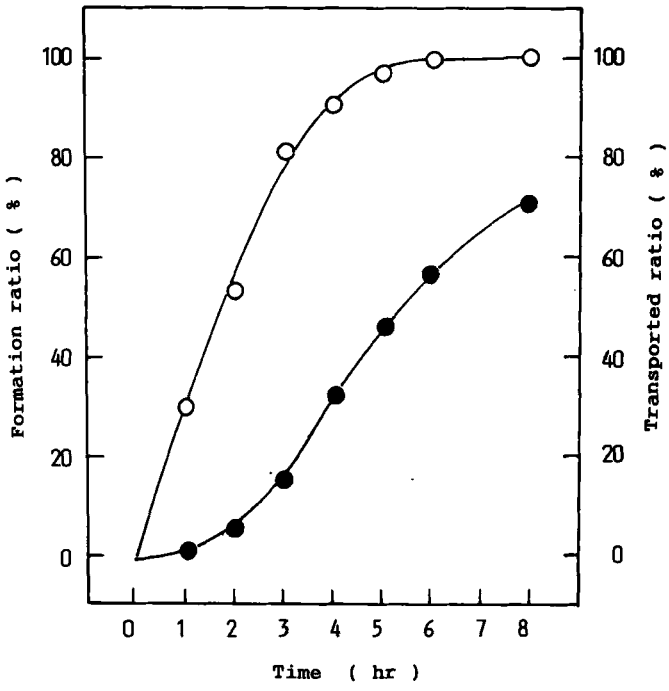

FIG. 7. Relationship between production of Hm by HD and amounts of transported Hm in phase II. Initially, phase I contained 1 m*M* His, 40 μg/mL pyridoxal-5-phosphate, and 40 μg/mL HD at pH 4.5. pH of phase II: 7.2. Concentration of EHP: 50 m*M*. (○) Production of Hm by HD, (●) transported Hm in phase II. Formation ratio = (concentration of Hm/hypothetical concentration of Hm which is perfectly transformed from His) × 100. In this figure the transported ratio was calculated by using the amount of Hm at the 100% formation ratio as the initial concentration.

transport of Hm from phase I to II were selectively performed continuously in the simple system indicated in Fig. 2. The organic layer acts not only as a separating membrane but also as a barrier between the reaction phase and the recovery phase. In the case of organic syntheses, enzyme are useful reactors compared with others because they are highly specific and the reaction can be performed under mild conditions. In these cases they are useful for preparing various organic compounds (*20*, *21*). With decarboxylases such as HD, it will be possible to prepare other important amines from their corresponding amino acids. These amines may be transported through the organic liquid membrane system containing a suitable carrier.

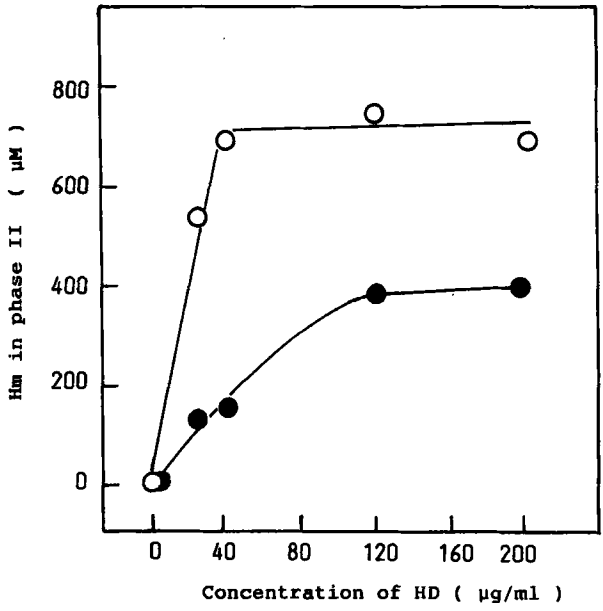

FIG. 8. Effect of HD concentration on the transport behavior of Hm in phase II. pH at phase I: 4.5. pH of phase II: 7.2. Concentration of EHP: 50 m*M*. 40 μg/mL pyridoxal-5-phosphate was added to phase I. Time (in hours): (●) 3, (○) 8.

TABLE 1
Recoveries of Hm in Phase II at Various Concentrations of HD and EHP[a]

HD (μg/mL)	EHP (m*M*)	Concentration of Hm (μM)	
		3 h	8 h
0	50	—[b]	—[b]
40	50	170 ± 40	700 ± 20
120	50	370 ± 40	760 ± 90
200	50	390 ± 50	670 ± 50
200	10	30 ± 10	170 ± 40
200	100	220 ± 50	510 ± 90

[a]Initial concentration of His, 1 m*M*; concentration of pyridoxal-5-phosphate, 40 μg/mL; pH of phase I, 4.5; pH of phase II, 7.2; 35°C. Measured by HPLC apparatus.

[b]Not detected.

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