Molecularly Imprinted Polymeric Membranes Containing DIDE Derivatives for Optical Resolution of Amino Acids†

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ABSTRACT: Molecularly imprinted polymeric membranes, bearing the tetrapeptide derivative H−Asp(OcHex)−Ile−Asp(OcHex)−Glu(OBzl)−CH_2−, were prepared during the membrane preparation (casting) process in the presence of print molecule Boc−L-Trp. The molecularly imprinted membranes thus obtained showed adsorption selectivity toward a print molecule family, such as L-Trp, L-Phe, L-Ala, L-Arg, and L-Glu. The tetrapeptide derivative in the molecularly imprinted membranes preferentially recognized the L-amino acid from the D-isomer. Enantioselective permeation was attained with the present membrane, and the D-isomer was permeated in preference to the L-isomer by using the concentration difference as a driving force for membrane transport. Electrodialysis of racemic amino acid showed the possibility that permselectivity directly reflects its adsorption selectivity. It was made clear that the optical resolution was attained by the molecularly imprinted polymeric membranes.

Introduction

L-Glutamic acid is tasty, while D-glutamic acid savors of sour. This is given as a familiar example, a lot of optically active substances show different bioactivities depending on their mirror image isomers. Much attention has been given to enantioselective separation in the field of pharmaceuticals, foods, agricultural chemicals, and so on. Enantioselective separation originated from the resolution of sodium ammonium tartarate in 1848 by Pasteur.[1] Conventional optical resolution methods, such as fractional crystallization, microbiological methods, kinetic enzymatic resolution technology, and high-performance liquid chromatography, are batch processes, and only a small amount of optically active compound can be treated in one batch operation. On the contrary, optical resolution with a permselective membrane might be the ultimate technique to obtain optically active compounds since the membrane separation technique can be operated continuously and easily and the energy efficiency is high compared with the other optical resolution methods mentioned above.

Optical resolution with synthetic membranes was first investigated by liquid membranes containing chiral crown ethers.[2] The stability and strength of liquid membranes are, however, lower than those of polymeric membranes. There have been pioneering reports on polymeric membranes for optical resolution, such as plasma-polymerized membranes of *d*-camphor and *l*-menthol,[3] polymeric membranes having cyclodextrin moieties,[4] polymeric chiral crown ethers,[5] poly(amino acid) membranes having amphiphilic side chains,[6] an enantioselective ultrafiltration membrane bearing an amino acid condensate,[7] a (+)-poly{1-[dimethyl(10-pinanyl)silyl]-1-propyne} membrane,[8] polysulfone membranes with immobilized bovine serum albumin,[9] a sericine membrane,[10] a cellulose tris(3,5-dimethylphenyl)carbamate membrane,[11] a poly{γ-[3-(pentamethyldisiloxyl)propyl]-L-glutamate} membrane,[12] and PMMA membranes containing (−)-oligo{methyl(10-pinanyl)siloxane}.[13]

As is well-known, excepting optical activity, optically active substances give the same physicochemical properties. From this, physical stereoselectivity might be an important factor to recognize and separate optically active substances. All synthetic membranes for optical resolution already reported[2−13] possess such chiral microenvironments in them. Oligopeptide is one of the candidates to render a chiral microenvironment to synthetic membrane. On the basis of this idea, the authors prepared polymeric membranes bearing a tetrapeptide derivative, H−Asp(OcHex)−Ile−Asp(OcHex)−Glu(OBzl)−CH_2−, as a molecular recognition site and investigated its permselectivity toward racemic α-amino acids.

Strategy of Membrane Design

Crown ethers,[14] cyclophanes,[15] cyclodextrins,[16] calixarenes,[17] molecular clefts,[18] and amphiphilic complexes[19] have been actively studied in connection with molecular recognition. From reported results,[2−13] it is necessary to introduce a chiral microenvironment into the molecular recognition site in the membrane so that the membrane can show optical resolution. It might, however, require a great effort and take much time to complete such an approach. Molecular imprinting[20] is the other promising way to introduce a chiral recognition site into synthetic membranes for optical resolution without difficulty. Such an approach has been practiced for the transport of nucleic acid components.[21] In this case, the polymeric membrane was prepared by radical polymerization in the presence of template molecules.

In the present paper, oligopeptide, which is expected to render a chiral microenvironment to the polymeric membrane, was adopted as a recognition site for optical resolution. The same molecular imprinting technique applied in ref 21 cannot be applied in the present case. This led us to the alternative molecular imprinting technique.[22] Our concept of alternative molecular imprinting is schematically depicted in Figure 1. To be brief, "molecular memory" of the substrate to be separated is induced in the membrane at the same time that the membrane is cast from the polymer solution as

† This article is dedicated to Professor Takeo Shimidzu on the occasion of his retirement from the Division of Molecular Engineering, Kyoto University.

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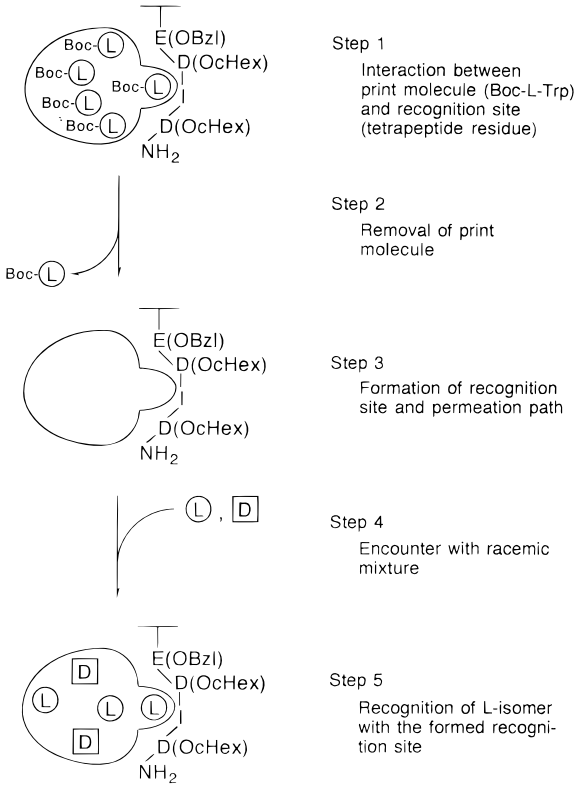


Figure 1. Concept of alternative molecular imprinting polymeric membrane.

follows: the tetrapeptide residue, in the figure, H−Asp(OcHex)−Ile−Asp(OcHex)−Glu(OBzl)−CH_2− (DIDE derivative), is in contact with the print molecule, Boc−L-tryptophan to interact specifically before (and during) the membrane preparation process (step 1). The print molecule is removed from the membrane to complete the preparation of molecularly imprinted polymeric membranes (step 2). The presence of the print molecule during the membrane preparation process might be effective not only for the introduction of a recognition site but also for the formation of the permeation path. That is, Boc−L-tryptophan is expected to work as a print molecule and as a porogen. If the polymeric membrane thus obtained is in contact again with racemic mixtures of tryptophan, the membrane could recognize L-tryptophan.

In the present study, the authors aim at preparing polymeric membranes for optical resolution of α-amino acids. Separation will be done in aqueous solution or a solution similar to aqueous one. If the hydrophobic protecting groups, such as benzyl and cyclohexyl moieties are removed from DIDE derivative, the tetrapeptide residue will be swollen by the solution applied and the molecular memory will be lost. To prevent from structural deformation of recognition site and to retain the "molecular memory" in the membrane, protecting groups of side chain carboxyl groups were not removed in the present study. From this, the sequence of H−Asp(OcHex)−Ile−Asp(OcHex)−Glu(OBzl)−CH_2− was first chosen as a tetrapeptide rather than other sequences.

Experimental Section

Materials. Protected amino acids, Boc−L-Glu(OBzl), Boc−L-Asp(OcHex), and Boc−L-Ile, were kindly provided by Kyowa Hakko Kogyo Co., Ltd. Chloromethylated polystyrene resin (Cl-resin) (1% divinylbenzene), of which the Cl content was 0.78 mequiv/g, and dicyclohexylcarbodiimide (DCC) were purchased from Peptide Institute, Inc., Osaka, Japan, and used without further purification. Dichloromethane,[23] trifluoroacetic acid (TFA),[23] diisopropylethylamine (DIEA),[24] and 2-propanol[23] were purified by the usual methods. The copolymer from acrylonitrile and styrene (AS), of which the weight fraction of acrylonitrile was 0.33, was kindly supplied by Ube Cycon, Ltd. D-Trp, L-Trp, D-Phe, L-Phe, D-Ala, L-Ala, D-Arg, L-Arg, D-Glu, L-Glu, Boc−D-Trp, Boc−L-Trp, sodium azide, and ethanol were used without purification. Distilled water was employed.

Table 1. Schedule for Performed Anhydride Coupling

step	operation and reagent[a]	mixing time/min
1	CH_2Cl_2, 50 cm^3 (five times)	1
2	20% TFA/CH_2Cl_2, 40 cm^3 (once)	2
3	20% TFA/CH_2Cl_2, 50 cm^3 (once)	20
4	CH_2Cl_2, 50 cm^3 (five times)	1
5	5% DIEA/CH_2Cl_2, 40 cm^3 (three times)	2
6	CH_2Cl_2, 50 cm^3 (five times)	1
7	"pre-mix" reaction mixture (once)	90
8	0.5 mol dm^{-3} DIEA/CH_2Cl_2, 1 cm^3 (once)	15
9	CH_2Cl_2, 50 cm^3 (three times)	2
10	2-PrOH, 40 cm^3 (three times)	2
11	repeat steps 4−10	

[a] Percentages express vol/vol ratios.

Preparation of Membrane Materials. The membrane materials were prepared by Merrifield's technique of solid phase peptide synthesis.[25,26] Boc−L-Glu(OBzl)−$OCH_2C_6H_4$-resin was prepared from 1.000 g (2.96×10^{-3} mol) of Boc−L-Glu(OBzl) and 0.726 g (5.66×10^{-4} unit mol of chloromethyl moiety) of chloromethylated polystyrene resin (1% divinylbenzene).[27]

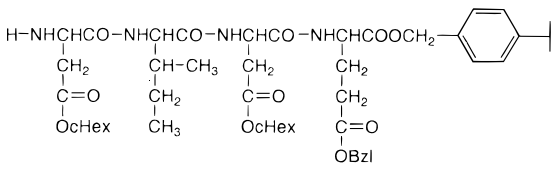
Boc−L-Glu(OBzl)−$OCH_2C_6H_4$-resin (0.878g, 5.05×10^{-4} unit mol of Boc−L-Glu(OBzl) moiety) was placed in a manual reactor similar to one reported[28] and carried through the schedules[29] shown in Table 1. The "pre-mix" reaction mixture used in all other couplings (Table 1) was prepared as follows: 3.15 mmol of Boc−amino acid in 9 cm^3 of CH_2Cl_2 was cooled at 0 °C and mixed with 326 mg (1.58 mmol) of DCC in CH_2Cl_2 (6 cm^3). After the mixture was stirred for 30 min at 0 °C, the precipitate was filtered at ambient temperature and washed with 2 cm^3 of CH_2Cl_2. The combined filtrate and washings were added immediately to the resin manually. The completeness of coupling was monitored by qualitative ninhydrin tests.[30] Acylation was judged complete in the coupling of Boc−L-Asp(OcHex) after two cycles. In the case of Boc−L-Ile, three cycles of the coupling reaction were carried out to be judged complete. Following the schedule mentioned above, we obtained the polystyrene resin bearing the tetrapeptide derivative, Boc−Asp(OcHex)−Ile−Asp(OcHex)−Glu(OBzl)−CH_2−(Boc−DIDE-resin). The polystyrene resin bearing H−Asp(OcHex)−Ile−Asp(OcHex)−Glu(OBzl)−CH_2− (DIDE-resin) was derived from Boc−DIDE-resin by treatment with trifluoroacetic acid in dichloromethane.[29]

From the hydrolysis of the polystyrene resin thus obtained and the derivatization with phenyl isothiocyanate[31] or (dimethylamino)azobenzenesulfonyl chloride,[32] the concentration of the tetrapeptide derivative thus introduced into the chloromethylated polystyrene resin was estimated to be 0.28 mmol/g of DIDE-resin or 0.27 mmol/g of Boc−DIDE-resin.

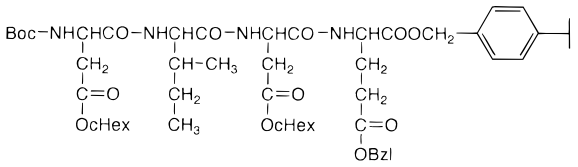
Preparation of Molecularly Imprinted Membranes. Each polymeric membrane studied in the present paper was prepared from tetrahydrofuran solution, containing corresponding components. The copolymer of acrylonitrile and styrene (AS), of which the weight fraction of acrylonitrile was 0.33, was adopted as a membrane matrix because DIDE- and Boc−DIDE-resins, and chloromethylated polystyrene resin (Cl-resin) do not form a membrane by themselves. Boc−L-Trp or Boc−D-Trp was adopted as a print molecule.

The typical membrane preparation process will be described using that for the Boc−L-Trp imprinted membrane, of which the mole ratio of the print molecule to DIDE derivative was 5, from DIDE-resin and AS: 6 mg of print molecule Boc−L-Trp, the amount being 5 times that of DIDE derivatives in

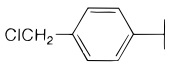
DIDE-Resin



Boc-DIDE-Resin



Cl-Resin



cHex- : C_6H_{11}-

Bzl- : $C_6H_5CH_2$-

Boc- : $(CH_3)_3COCO$-

the resin, was dissolved in 2 cm^3 of THF with 10 mg of DIDE-resin. And then a 190 mg of AS was dissolved in the previous THF solution. The THF solution thus prepared was poured into a flat laboratory dish (8.9 cm diameter) and the solvent allowed to evaporate at ambient temperature for 24 h. The obtained membrane was dried at 50 °C for an additional 2 h. After drying, the print molecule was removed from the resultant membranes by a known large volume of methanol till the print molecule could be hardly detected in methanol by UV analysis. In this case, it was found that approximately 96% of added print molecules were recovered from the membrane. Other membranes were prepared in a manner similar to that mentioned above. The thickness of the membranes thus obtained was 140−150 μm.

Enantioselective Permeation of Racemic Mixtures. The membrane, which effective membrane area was 3.0 cm^2, was fixed tightly with Parafilm between two chambers of a permeation cell. The volume of each chamber was 40cm^3. A 50 vol.% aqueous ethanol solution of racemic amino acids was placed in the left-hand side chamber (L side) and an 50 vol.% aqueous ethanol solution in the right-hand side chamber (R side). Each concentration of racemic amino acid was fixed to be 1.0mmol dm^{-3}. In both chambers, 0.02 wt.% of sodium azide was added as a fungicide. All experiments were done at 40 °C with stirring.

The amounts of D- and L-isomers that permeated through the membrane were estimated on an HPLC instrument (JASCO PU 980) equipped with a UV detector (JASCO UV 970) by using a TSKgel Enantio L1 column (150 × 4.6 (i.d.) mm) (Tosoh Corp.) or CROWNPAK CR(+) column (250 × 4.0 (i.d.) mm) (Daicel Chemical Industries, Ltd.), with aqueous copper sulfate solution as the eluent for Trp analysis with TSKgel Enantio L1 or aqueous perchloric acid solution as the eluent for HPLC analyses with CROWNPAK CR(+).

The permeability coefficient, P (cm^2 h^{-1}), is defined as

$$P = Q\delta/\Delta CAt$$

where Q (mol) is the amount of permeated amino acid, δ (cm) is the thickness of the membrane, ΔC (mol cm^{-3}) is the difference in concentration of the amino acid between the compartment L and R, A (cm^2) is the effective membrane area, and t (h) is time.

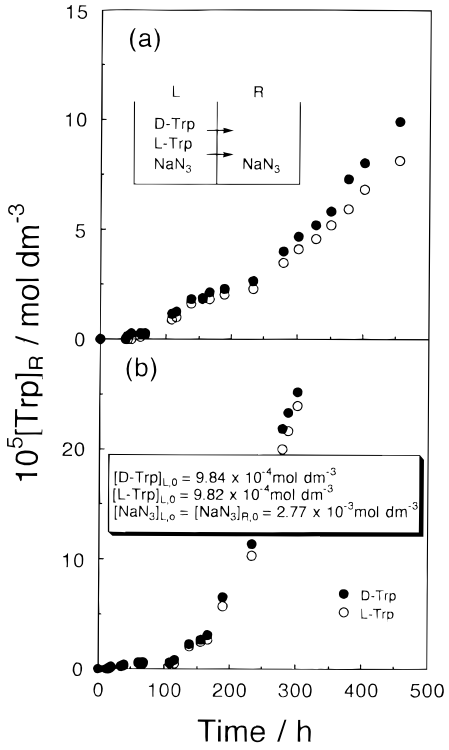


Figure 2. Time-transport curves of D- and L-Trp through Boc−L-Trp (a) and Boc−D-Trp (b) imprinted membrane, prepared from DIDE-resin and AS, at 40 °C. (The mole ratio of print molecule to DIDE derivative was fixed to be 10.)

The flux value, J (mol cm^{-2} h^{-1}), is defined as

$$J = Q/At = P\Delta C/\delta$$

The separation factor $\alpha_{i/j}$ is defined as the flux ratio J_i/J_j divided by the concentration ratio C_i/C_j in the feed side.

$$\alpha_{i/j} = (J_i/J_j)/(C_i/C_j) = P_i/P_j$$

Adsorption of Racemic Mixtures to the Membranes. The molecularly imprinted polymeric membranes from DIDE-resin were immersed in the racemic compounds solution, which was the same mixtures studied in the enantioselective permeation, and the mixture was allowed to be equilibrated at 40 °C during 216 h. Aliquots of the solution at the initial stage and after 216 h were used for quantitative estimation by the HPLC instrument described above. The amount of amino acid in the supernatant subtracted from the amount initially in the solution gave the amount of amino acid adsorbed by the membrane.

Adsorption selectivity $S_{A(i/j)}$ is defined as

$$S_{A(i/j)} = ((AA)_i/(AA)_j)/(C_i/C_j)$$

where $(AA)_i$ and C_i are the amount of amino acid adsorbed in the membrane and the concentration in the solution after equilibrium was reached, respectively.

Electrodialysis. A 50 vol % aqueous ethanol solution of racemic amino acids was placed in both chambers of the permeation cell. Each concentration of racemic amino acid was fixed to be 1.0 mmol dm^{-3}. The electrodialysis was carried out with an applied voltage of 2.5 V for the study of Glu and Arg between platinum black electrodes (10 mm square; distance between the electrodes, 65 mm) at 40 °C with stirring.

Results and Discussion

Effect of Print Molecules on Optical Resolution. Figure 2 shows the time-transport curves of D- and L-Trp

Table 2. Results of Enantioselective Permeation[a]

membrane component/template	permeant	$10^9 J$/mol cm^{-2} h^{-1}	$10^4 P$/cm^2 h^{-1}	$\alpha_{D/L}$ (=P_D/P_L)
DIDE-resin/Boc−D-Trp	L-Trp	29.7	4.29	1.0
	D-Trp	30.4	4.39	
DIDE-resin/Boc−L-Trp	L-Trp	3.8	0.55	1.4
	D-Trp	5.1	0.74	
Boc−DIDE-resin/Boc−D-Trp	L-Trp	18.0	2.72	1.0
	D-Trp	17.2	2.60	
Boc−DIDE-resin/Boc−L-Trp	L-Trp	15.3	2.22	1.0
	D-Trp	15.3	2.22	
Cl-resin/Boc−D-Trp	L-Trp	21.0	3.06	1.0
	D-Trp	20.8	3.03	
Cl-resin/Boc−L-Trp	L-Trp	26.0	3.97	1.0
	D-Trp	25.9	3.95	

[a] The mole ratio of print molecule, Boc−L-Trp or Boc−D-Trp, to DIDE derivative in the membrane preparation process was fixed to be 10.

through Boc−L-Trp (Figure 2 a) and Boc−D-Trp (Figure 2 b) imprinted membranes, prepared from DIDE-resin and AS, of which the mole ratio of print molecule to DIDE derivative was 10. In the permeation through the Boc−D-Trp imprinted membrane, permselectivity toward racemic Trp mixtures was scarcely observed. On the other hand, D-Trp was preferentially permeated compared with L-Trp through Boc−L-Trp imprinted polymeric membranes and the separation factor at steady state was calculated to be 1.4, as summarized in Table 2.

In order to study the effect of membrane materials on permeation, the following permeation experiments were conducted in addition to the permeation experiment mentioned above: the optical resolution experiments of Trp through the membrane from DIDE-resin and AS without a print molecule, Boc−L-Trp and Boc−D-Trp imprinted membranes from Boc−DIDE-resin and AS, Boc−L-Trp and Boc−D-Trp imprinted membranes from Cl-resin and AS were investigated. As for the membrane from DIDE-resin and AS, which was prepared without a print molecule, permeation of Trp was scarcely observed over a period of 500 h. On the contrary, D- and L-Trp were permeated through other membranes, which were prepared in the presence of Boc−L-Trp or Boc−D-Trp as a print molecule. From these, it can be concluded that the addition of Boc−L-Trp or Boc−D-Trp in the membrane preparation process, at least, plays an important role as a porogen to form permeation path for the permeant. Flux values, permeability coefficients, and permeability coefficient ratios (α) for these membranes were summarized in Table 1. Optical resolution was observed only in the case of the Boc−L-Trp imprinted membrane from DIDE-resin and AS shown in Figure 2a. In this case, the presence of Boc−L-Trp in the membrane preparation process might be effective not only for the formation of the permeation path but also for that of the molecular recognition site. In other words, it is expected that Boc−L-Trp, in the present case, can work as a porogen to form the permeation path and as a print molecule to shape the molecular recognition site. If the presence of Boc−L-Trp in the membrane preparation process led to the formation of a molecular recognition site, the Boc−L-Trp imprinted membrane from DIDE-resin and AS should show preferential sorption toward L-Trp rather than D-Trp.

On the basis of this idea, the affinity between D- or L-Trp and Boc−L- or Boc−D-Trp imprinted membranes from DIDE-resin and AS were investigated by adsorption experiments. The results were given in Table 3 together with those for Phe and Ala, which will be described in the next section. In Table 3, amounts of

Table 3. Adsorption of Amino Acids in Molecularly Imprinted Membranes[a]

template	substrate	$10^6(AA)_M$/mol	$(AA)_M$/(DIDE)
Boc−D-Trp	D-Trp	0.57 ± 0.01	1.6
	L-Trp	0.54 ± 0.02	1.5
Boc−L-Trp	D-Trp	3.06 ± 0.02	8.7
	L-Trp	3.46 ± 0.02	9.9
Boc−D-Trp	D-Phe	0.46 ± 0.02	1.3
	L-Phe	0.45 ± 0.02	1.3
Boc−L-Trp	D-Phe	2.14 ± 0.04	6.1
	L-Phe	2.42 ± 0.03	6.9
Boc−D-Trp	D-Ala	0.40 ± 0.03	1.1
	L-Ala	0.38 ± 0.02	1.1
Boc−L-Trp	D-Ala	1.54 ± 0.03	4.4
	L-Ala	1.76 ± 0.03	5.0

[a] The mole ratio of print molecule, Boc−L-Trp or Boc−D-Trp, to DIDE derivative in the membrane preparation process was fixed to be 10.

Trp adsorbed in the membrane are given not only in absolute ones but also in relative ones, which were converted to those of the DIDE derivative basis for the convenience of the following discussion. In the case of the Boc−D-Trp imprinted membrane from DIDE-resin and AS, the membrane did not discriminate between D-Trp and L-Trp. In the case of the Boc−L-Trp imprinted membrane, the result was, however, different from that of the Boc−D-Trp imprinted one. The amount of L-Trp adsorbed in the membrane was more than that of D-Trp, an excess of 1.2 DIDE derivative over D-Trp adsorbed in the membrane. This suggests that the recognition site toward L-Trp was produced in the Boc−L-Trp imprinted membrane by the print molecule of Boc−L-Trp. In other words, gross structural deformation of the recognition site in the membrane was not introduced during the experiment and the imprinted membrane can exhibit "memory" for the original print molecule family, as expected. As was described in the section Strategy of Membrane Design, this memory might be due to the fact that carboxyl residues in the side chains in the tetrapeptide were protected by hydrophobic cyclohexyl or benzyl moieties and the swelling by solution was suppressed. Among D- and L-Trp adsorbed in the Boc−L-Trp imprinted membrane, it can be said that L-Trp, for which the amount was around 1.2 times that of the DIDE derivative, was preferentially incorporated in the recognition site, which was formed by the print molecule Boc−L-Trp in the membrane preparation process. D-Trp and the remaining L-Trp adsorbed in the membrane, for which the amount was around 8.7 times that of the DIDE derivative, might be found in the permeation path, which was also produced by the presence of Boc−L-Trp. When Boc−D-Trp was adopted as a print molecule, there might not be a specific interaction between Boc−D-Trp and the

Table 4. Results of Enantioselective Permeation[a]

template	permeant	$10^9 J$/mol cm^{-2} h^{-1}	$10^4 P$/ cm^2 h^{-1}	$\alpha_{D/L}$ (=P_D/P_L)
Boc−D-Trp	L-Trp	29.7	4.29	1.0
	D-Trp	30.4	4.39	
Boc−L-Trp	L-Trp	3.8	0.55	1.4
	D-Trp	5.1	0.74	
Boc−D-Trp	L-Phe	19.6	2.91	1.0
	D-Phe	19.5	2.81	
Boc−L-Trp	L-Phe	13.3	2.12	1.2
	D-Phe	15.3	2.44	
Boc−D-Trp	L-Ala	10.7	1.70	1.0
	D-Ala	10.4	1.66	
Boc−L-Trp	L-Ala	6.3	1.02	1.1
	D-Ala	7.1	1.16	

[a] The mole ratio of print molecule, Boc−L-Trp or Boc−D-Trp, to DIDE derivative in the membrane preparation process was fixed to be 10.

DIDE derivative, which consists of L-amino acids. As a result, Boc−D-Trp worked only as a porogen in the membrane preparation process. The adsorption selectivity was opposite to permselectivity. This might be due to the suppression of the permeability of L-Trp by the relatively high affinity to the membrane. Similar phenomena were observed in enantioselective permeation.[3,4,5,7,12]

In addition, the results summarized in Table 2 suggest that not only the tetrapeptide derivative but also the primary amine moiety of N-terminal amino acid in the DIDE derivative are essential to optical resolution.

Optical Resolution of Other Amino Acids. Enantioselective permeation of Trp was attained with the molecularly imprinted polymeric membranes, as described in the previous section. It is expected that the membrane performance is dependent on both the dimension[33] and hydrophobicity[34] (or hydrophilicity) of the side group of the amino acid. On the basis of this idea, the enantioselective permeation of Phe and Ala was conducted in addition to that of Trp. The results of enantioselctive permeation are given in Table 4. D-Amino acids were preferentially permeated through the molecularly imprinted membranes like the permeation of Trp mixtures.

These results suggest that L-amino acids, such as L-Phe and L-Ala, were adsorbed in the membrane in preference to corresponding D-isomers like the selective adsorption of Trp previously described. The results of selective adsorption of Phe and Ala are summarized in Table 3. As is expected, L-isomers were adsorbed in preference to D-isomers. In the case of the adsorption of Phe, the amount of L-Phe was also more than that of D-Phe, an excess of 0.8 DIDE derivative over D-Phe in the membrane. As for Ala, the situation was the same and L-Ala was preferentially incorporated into the membrane, an excess of 0.6 times DIDE derivative over D-Ala adsorbed in the membrane.

As for the distribution of each amino acid between the outside and the inside of the membrane, two different kinds of distributions, K_M and K_{DIDE}, can be anticipated, as schematically shown in Figure 3: the distribution between the outside of the membrane and the permeation path, K_M, and that between the permeation path and the recognition site shaped by the print molecule of Boc−L-Trp, K_{DIDE}. Distribution ratios are summarized in Table 5. It can be seen from the table that the extent for the distribution of each amino acid between the outside and the permeation path was dependent on hydrophobicity of the side groups of these

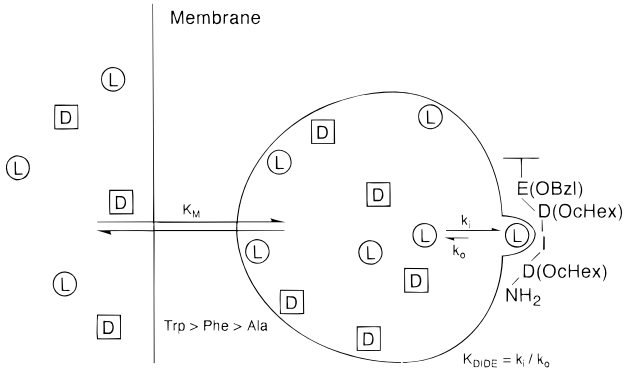


Figure 3. Schematic presentation of the distribution of amino acids between the outside and the inside of the membrane.

Table 5. Distribution Ratios among Trp, Phe, and Ala[a]

AA	$(D\text{-AA})_M/(D\text{-Ala})_M$	$(L\text{-AA})_M/(L\text{-Ala})_M$	$\Delta(L\text{-AA})_M/\Delta(L\text{-Ala})_M$[b]
Trp	2.0	2.0	2.0
Phe	1.4	1.4	1.3

[a] The mole ratio of print molecule, Boc−L-Trp, to DIDE derivative in the membrane preparation process was fixed to be 10. [b] $\Delta(L\text{-AA})_M = (L\text{-AA})_M - (D\text{-AA})_M$; $\Delta(L\text{-Ala})_M = (L\text{-Ala})_M - (D\text{-Ala})_M$.

three kinds of amino acids[34] and the order was as follows: Trp > Phe > Ala. The distribution ratios of D-Trp to D-Ala and that of L-Trp to L-Ala gave 2.0 and that of D-Phe to D-Ala and that of L-Phe to L-Ala were calculated to be 1.4. The more hydrophobic the amino acid, the more it was incorporated into the permeation path in the membrane. And this distribution, K_M, did not depend on the kind of isomer; it was only dependent on the property of side group of the amino acid. The distribution ratios for L-amino acids ($\Delta(L\text{-AA})_M/\Delta(L\text{-Ala})_M$; AA = Trp or Phe), which were considered to be adsorbed on the recognition site, gave quite similar values of the distribution ratios between the outside and the permeation path. From this, it can be said that the distribution of L-amino acid between the permeation path and the recognition site was same for each L-amino acid, and the distribution ratios reflected their distribution ratios between the outside of the membrane and the permeation path, K_M.

Through the adsorption experiments, it was made clear that the recognition site, which was prepared by the print molecule, was effective in discriminating L-amino acid from the D-isomer, and this ability of discrimination was effective for any amino acid.

Effect of Imprinting Conditions on Membrane Performance. So far, in the present paper, the mole ratio of print molecule to DIDE derivative was fixed to be 10 in the molecular imprinting process. It can be expected that the reduction of the dimension of the permeation path wil lead to the increase in the affinity toward the L-amino acid, because the area ratio of the nonspecific wall to the recognition site toward the L-isomer is decreased. In other words, the decrease in the ratio of the print molecule to DIDE derivative in the membrane preparation process is expected to lead to the increase in the contribution of recognition site toward the adsorption selectivity. On the basis of this idea, the effect of imprinting conditions on membrane performance was investigated. That is, the mole ratios of print molecule to tetrapeptide derivative in the membrane preparation process were changed from 10 to 0.5 as follows: 10, 5, 3, 1, and 0.5, and each membrane performance toward Trp was examined.

Table 6. Effect of Imprinting Condition on Adsorption of Amino Acids in Molecularly Imprinted Polymeric Membranes

(Boc−L-Trp)/(DIDE)	substrate	$10^6(AA)_M$/mol	$(AA)_M$/(DIDE)
10	D-Trp	3.06 ± 0.02	8.7
	L-Trp	3.46 ± 0.02	9.9
5	D-Trp	1.12 ± 0.03	3.2
	L-Trp	1.54 ± 0.03	4.4
3	D-Trp	0.53 ± 0.03	1.5
	L-Trp	0.91 ± 0.04	2.6
1	D-Trp	0.07 ± 0.02	0.2
	L-Trp	0.32 ± 0.03	0.9
0.5	D-Trp	0.03 ± 0.02	0.1
	L-Trp	0.18 ± 0.03	0.6

The dependence of adsorption of D-/L-Trp on imprinting conditions is given in Table 6. In Figure 4, the results in Table 6 are shown visually. Each amount of isomer adsorbed in the membrane was plotted as a function of imprinting condition. Both plots gave straight lines; that is, adsorbed amounts increased linearly with the increase in the molecular imprinting ratio. This suggests that the increase in the amount of print molecule in the membrane preparation process led to the enlargement of the permeation path, as expected. Even though molecular imprinting conditions were changed, L-Trp was always incorporated in the membrane in preference to D-Trp, and an excess of DIDE derivative over D-Trp was adsorbed in the membrane. Below the mole ratio of the print molecule to the tetrapeptide derivative of 1, most of the amino acid adsorbed was L-isomer, L-Trp. The adsorption selectivity toward L-Trp is plotted against the membrane preparation conditions and shown in Figure 5. The selectivity increased with the decrease in (Boc−L-Trp)/(DIDE) ratio and reached 6 at the mole ratio of 0.5. The adsorption selectivity toward L-Trp was increased with the reduction of nonspecific wall by the decrease in mole ratio of print molecule to tetrapeptide derivative in the membrane preparation process.

Figure 6 shows the separation factor, $\alpha_{D/L}$, of the enantioselective permeation of racemic Trp solutions through these five kinds of membranes. All membranes gave permselectivity toward D-Trp, showing a maximum at around the print molecule−DIDE derivative ratio of 3. This might be explained as follows: obeying the solution-diffusion theory, permselectivity depends on both adsorption selectivity and diffusivity selectivity. In the present case, adsorption selectivity toward D-Trp decreased with the decrease in (Boc-L-Trp)/(DIDE) ratio, while the diffusivity selectivity toward the D-isomer increased with the decrease in molecular imprinting conditions. These two phenomena are due to the relatively high affinity between L-Trp and the membranes, as mentioned above. As a result, the permeability coefficient ratio gave a profile showing a maximum, as shown in Figure 6.

Electrodialysis. When their concentration difference was used as a driving force for the enantioselective permeation of racemic amino acids, the permeability of one isomer, which is incorporated into the membrane in preference to the other isomer, had a tendency to be suppressed by the relatively high affinity toward the membrane. It is an interesting and crucial subject to selectively permeate the isomer, which is preferentially adsorbed in the membrane, in other words, to construct the membrane transport system, reflecting adsorption selectivity. As one way to attain such a membrane transport system, electrodialysis of racemic amino acids through the present membrane, Boc−L-Trp imprinted membranes from DIDE-resin and AS, was investigated. The pH values of racemic Trp mixtures in the present study were around 6.4. Namely, during the enantioselective permeation study in the present paper, racemic Trp was assumed to be a zwitterion to give, on the whole, a neutral form.[33] In order to use the potential difference as a driving force for membrane transport, racemic arginine, having a basic side chain, and racemic glutamic acid, bearing an acidic side chain, were adopted as permeants instead of racemic Trp, Phe, or Ala, which were used in the previous sections. The pH values for

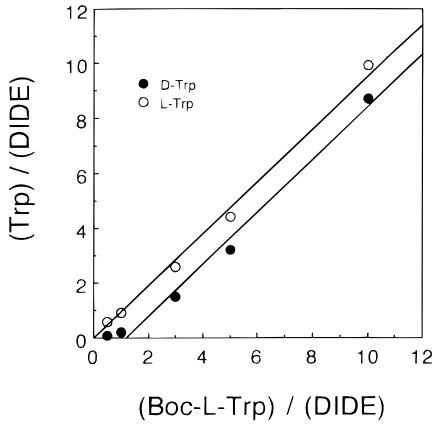


Figure 4. Effect of the membrane preparation condition on adsorption of D-/L-Trp. (Each concentration of racemic Trp was fixed to be 1.0×10^{-3} mol dm^{-3}.)

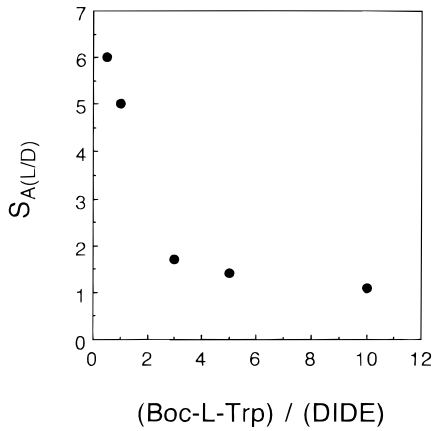


Figure 5. Effect of the membrane preparation condition on adsorption selectivity toward L-Trp. (Each concentration of racemic Trp was fixed to be 1.0×10^{-3} mol dm^{-3}.)

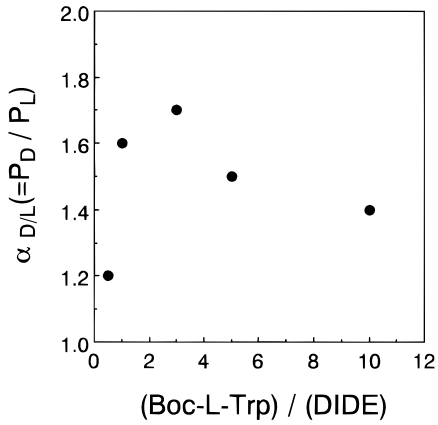


Figure 6. Effect of the membrane preparation condition on permselectivity toward D-Trp. (Each concentration of racemic Trp was fixed to be 1.0×10^{-3} mol dm^{-3}.)

Table 7. Results of Enantioselective Permeation[a]

ΔE/V	permeant	$10^8 J$/mol cm^{-2} h^{-1}	$\alpha_{L/D}$	$S_{A(L/D)}$
2.5	L-Glu	0.43	1.2	1.2
	D-Glu	0.37		
2.5	L-Arg	0.29	1.3	1.3
	D-Arg	0.23		

[a] The mole ratio of print molecule, Boc−L-Trp, to DIDE derivative in the membrane preparation process was fixed to be 3.

racemic Arg and Glu in the present study were around 7.9 and 5.4, respectively. Arg was positively charged in the present conditions and the net charge per Arg is calculated to be +0.93.[33] On the other hand, the net charge of Glu was determined to be −0.93, conversely, negatively charged.[33]

Arg was transported to the cathode and Glu to the anode. The results of enantioselective electrodialysis are summarized in Table 7. As is expected, L-isomers, which were selectively adsorbed in the membrane, were permeated in preference to the corresponding D-isomers by electrodialysis. From these results, it was made clear that electrodialysis of racemic amino acids shows the possibility that permselectivity directly reflects its adsorption selectivity.

Conclusion. Molecularly imprinted polymeric membranes, bearing a tetrapeptide derivative, were prepared during the membrane preparation (casting) process in the presence of a print molecule. The Boc−L-Trp imprinted polymeric membranes thus obtained showed adsorption selectivity toward a print molecule family, such as L-Trp, L-Phe, L-Ala, L-Arg, and L-Glu. The tetrapeptide derivative in the molecularly imprinted membranes discriminated the L-amino acid from the D-isomer, that is, showed L-specificity. Enantioselective permeation was attained with the present membrane, and the D-isomer was permeated in preference to the L-isomer by using the concentration difference as a driving force for membrane transport. Electrodialysis of racemic amino acid showed the possibility that permselectivity directly reflects its adsorption selectivity. The present study suggests that the molecularly imprinted polymeric membranes have potential to attain the optical resolution of amino acids.

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