ENANTIOSELECTIVE PERMEATION OF AMINO ACID ISOMERS THROUGH L-PHENYLGLYCINE-FIXED MEMBRANES AT PRESSURE GRADIENT

TERUYUKI MASAWAKI, SATOSHI MATSUMOTO, AND SETSUJI TONE*
Department of Chemical Engineering, Faculty of Engineering Science, Osaka University, Toyonaka 560

Key Word: Membrane Separation, Optical Resolution, Amino Acid, Chiral Recognition, Optical Isomer

Three types of L-phenylglycine-fixed membranes were prepared by varying casting conditions. The optical resolution of racemic phenylglycine (DL-Pgly) was performed by pressure gradient. L-Pgly-fixed membranes were permeable in preference to D-Pgly to L-Pgly, and the maximum separation factors of respective membrane under the operating conditions were 9.1, 8.6 and 2.7. Apparent self-association constants were evaluated from the dialysis data. By applying these values to the basic solute flux equations at a given pressure gradient, the values of the viscous parameters were evaluated. The ratio of viscous parameter of D-Pgly to that of L-Pgly depended on the apparent self-association constant. The separation factor was successfully represented on the basis of the basic solute flux equations including the apparent self-association constants, and the separation factor became larger in denser membranes with low volume flux.

Introduction

The increase in demand of physiologically active materials such as amino acids useful for the pharmaceuticals and food additives, has increased the importance of optical resolution of such active isomers. The optical resolution by membrane separation processes has attracted much attention as an energy-saving technique with no phase change, and its operation is favorable for industrial separation of such materials because continuous separation can be performed at high speed[6]. However, because the driving force is the concentration gradient and the solute flux is considerably small, most of the membrane separation techniques for the optical resolution reported[1-3, 5, 7] are not suitable for the treatment of large quantities of racemate solutions.

In the previous study[4], we prepared an enantioselective polymer membrane by introducing L-phenylalanine (L-Phe) into the membrane matrix, and the optical resolution of DL-phenylalanine from aqueous solutions was performed at concentration and pressure gradients across the membrane, respectively. The solute permeability of each enantiomer was evaluated from the data obtained by dialysis experiment. Introducing the concept of a self-association interaction between L-Phe in the solution and L-Phe fixed membrane at concentration gradient, the apparent self-association constant was evaluated, and we showed that the optical resolution was favorably achieved by the difference of affinities between D- and L-amino acids for the fixed L-Phe in the membrane.

In the present work, three types of L-phenylglycine-fixed polymer membranes were prepared with different casting conditions in order to clarify the effect of membrane structure on the optical resolution. The optical resolution of an amino acid from aqueous solutions was performed with a pressure gradient and the permeation flux and the separation factor of each membrane were observed. Apparent self-association constants were evaluated from dialysis data. Applying apparent self-association constants to the solute flux equation at a given pressure gradient, viscous parameters were evaluated, and the relationship between apparent self-association constants and viscous parameters was examined. Furthermore, the effects of the volume flux, apparent self-association constant and solute permeability on the separation factor of amino acid isomers were examined from experimental data on the basis of the basic equations of solute flux.

* Received December 8, 1993. Correspondence concerning this article should be addressed to S. Tone.

1. Experimental

1.1 Materials

Polysulfone (P-1700, Union Carbide) was supplied by Daicel Chemical Industries, LTD. DL-Phenylglycine (DL-Pgly) of special grade was purchased from Wako Pure Chemical Industries, LTD. Glutaraldehyde (25 wt% aqueous solution), N-methyl-2-pyrrolidone, and lithium nitrate were special grade and used without further purification.

1.2 Preparation of the L-phenylglycine-fixed membrane

The L-Pgly condensate was obtained from the reaction of L-Pgly (0.5 wt% aqueous solution, 0.4 dm^3) and glutaraldehyde (25 wt% aqueous solution, 0.1 dm^3) by a

Table 1. Casting conditions and the physical properties of membranes

Membrane	M1	M2	M3
Solvent	NMP	NMP	NMP
Swelling agent	$LiNO_3$	$LiNO_3$	$LiNO_3$
Weight ratio of PSF/NMP/$LiNO_3$/A	1/5/0.2/0.1	1/5/0.2/0.1	1/5/0.2/0.1
Solvent evaporation temperature [K]	333	333	333
Solvent evaporation period [min]	65	50	45
Membrane thickness [μm]	193	227	15
Water content [-]	0.60	0.68	0.75
Pure water permeability [m·Pa^{-1}·s^{-1}]	1.06×10^{-13}	1.61×10^{-13}	4.41×10^{-13}

NMP: N-methyl-2-pyrrolidone, PSF: polysulfone, A: amino acid condensate

Table 2. Parameters evaluated from the experimental data

	M1		M2		M3		L-Phe-fixed membrane[4]	
	D-Pgly	L-Pgly	D-Pgly	L-Pgly	D-Pgly	L-Pgly	D-Phe	L-Phe
$Ds_{i,app} \times 10^{13}$ [m^2·s^{-1}]	5.7	3.6	8.8	6.4	1.6	1.2	6.1	2.1
$Ks_{i,app}$ [-]	2.0	2.1	1.8	1.8	1.3	1.5	3.5	4.1
$Ps_{i,app} \times 10^{12}$ [m^2·s^{-1}]	1.1	0.74	1.6	1.2	2.1	1.9	2.1	0.85
H [-]	0.54		0.32		0.18		1.49	
$k_{2i} \times 10^7$ [m·s^{-1}]	1.3	1.6	0.29	0.42	0.18	0.36	4.0	9.3
$k_{3i} \times 10^7$ [m·s^{-1}]	1.2	1.2	0.13	0.048	0.12	0.045	2.9	7.9
k_{2i}/k_{3i} [-]	1.0	1.4	2.1	8.7	1.5	8.0	1.4	1.2

method similar to that described in previous paper[4].

The membranes were cast from a solution of polysulfone(PSF)/N-methyl-2-pyrrolidone(NMP)/$LiNO_3$/amino acid condensate, whose weight ratio was 1/5/0.2/0.1. Evaporation of the solvent was done in an oven at 333 K with evaporation periods of 65, 50, and 45 min. The gelation was done in an ice-cold water bath for more than 12 h. The casting conditions and the physical properties of the membranes are listed in **Table 1**.

1.3 Dialysis of DL-Pgly through the L-Pgly-fixed membranes

The dialysis experiment was carried out using the same apparatus as described previously[4] at 310 K. Cell compartment 1 was filled with an aqueous solution of racemic phenylglycine(DL-Pgly) with a concentration, C_{1i} (i = D, L), of 1 mol·m^{-3}. Cell compartment 2 was filled with distilled water. The solutions in both cell compartments were stirred well. During the measurement, the concentration of aqueous amino acid solution in compartment 1 remained constant at its initial value owing to the transport of a very small amount of solute from compartment 1 to compartment 2.

1.4 Optical resolution of enantiomers at elevated pressure

The permeation experiments were carried out using a batch-type cell[4]. The solution in the cell was stirred with a rotating impeller. The effective area of the membrane was 28.3 cm^2. The temperature was kept at 310 K and the operating pressure was varied in the range of 0.05-2 MPa. The solute used in aqueous solutions was DL-Pgly and its

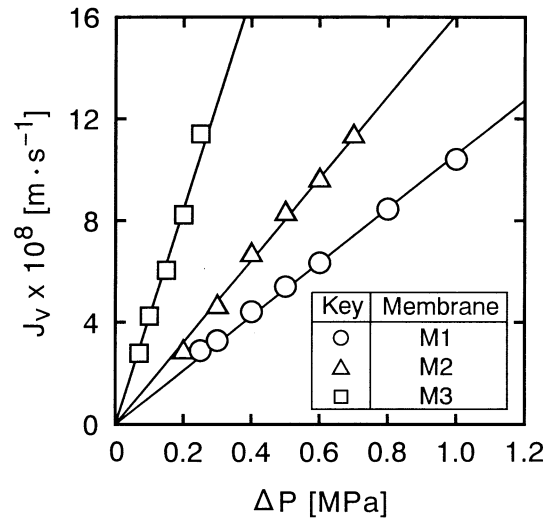

Fig. 1 Effect of the operating pressure on the volume flux

concentration, C_{bi} (i = D, L), was 1 mol·m^{-3}.

The separation factor, α, of DL-Pgly was defined as follows.

$$\alpha = \frac{C_{pD} / C_{bD}}{C_{pL} / C_{bL}} \tag{1}$$

The analysis of the concentration of D- or L-Pgly in permeate solution was performed by HPLC using an optical resolution column (Crownpak CR (+), Daicel Chemicals Industries, LTD.).

2. Results

2.1 Evaluation of solute permeability

In the dialysis experiments, the total amount of diffusing solute of component i, Q_{ti}, which has passed through the membrane over time t becomes linearly dependent on process time t. Q_{ti} can be expressed as follows[4]:

$$Q_{ti} = \frac{Ps_{i,app} C_{1i}}{\delta}\left(t - \frac{\delta^2}{6Ds_{i,app}}\right) \qquad (i = D, L) \tag{2}$$

where the apparent solute permeability, $Ps_{i,app}$, is the product of the apparent diffusion coefficient, $Ds_{i,app}$, and the apparent partition coefficient, $Ks_{i,app}$.

By fitting Eq. (2) to the experimental data, $Ds_{i,app}$ and $Ks_{i,app}$ were evaluated with a procedure similar to that used previously paper[4]. These values are shown in **Table 2**.

2.2 Effects of operating pressure on volume flux and solute flux

Figure 1 shows the effect of operating pressure, ΔP, on the volume flux, J_V. The volume flux increased linearly with an increase in operating pressure in each membrane

Since the amino acid solution was very dilute, the volume flux was related to operating pressure as follows.

$$J_V = Lp \cdot \Delta P \tag{3}$$

Figure 2 shows the effect of operating pressure on solute flux J_i (i = D, L). D-Pgly had a greater permeabil-

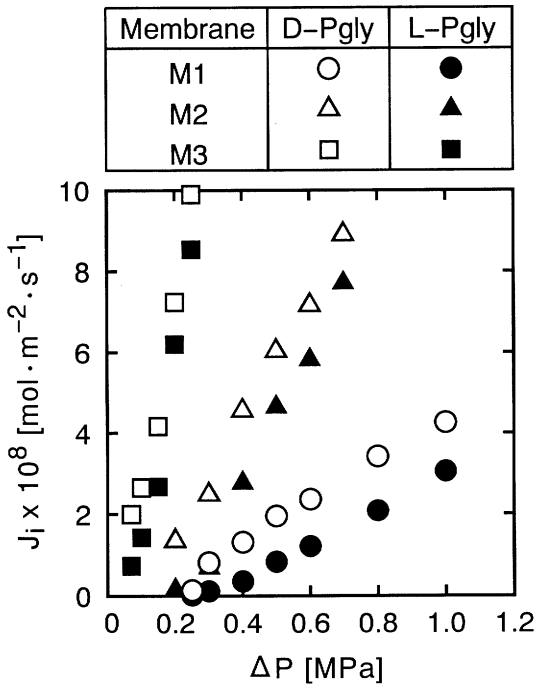

Fig. 2 Effect of the operating pressure on the solute flux

ity than L-Pgly through the membranes. Both the volume flux and the solute flux through the membrane decreased with an increase in the solvent evaporation period for membrane preparation. This may be attributed to the reduction of the size of membrane pore for a longer solvent evaporation periods.

2.3 Effect of operating pressure on the separation factor

Figure 3 shows the effect of ΔP on the separation factor, α. The value of α was larger than unity, and decreased with an increase in operating pressure. A comparison of the separation factor of each membrane at the same operating pressure showed that the membrane with smaller *Lp*, *i.e.*, having a smaller size of membrane pore, had a considerably higher value of α.

3. Discussion

3.1 Evaluation of apparent self-association constant

The polymer membrane used in the present study was constructed from polysulfone and L-Pgly condensate. Although L-Pgly is merely adsorbed on the surface of the membrane pores in the polysulfone matrix, L-Pgly is bound strongly on to the surface of the membrane pore in the amino acid condensate matrix, subject to the self-association interaction between L-Pgly fixed in the membrane and that in the solution. A certain type of chemical interaction may be established by considering the exchange reaction between "adsorbed" (or free) solute molecules, S_{LF}, and "associated" (or bound) solute molecules, S_{LB}.

$$S_{LF} \rightleftarrows S_{LB}$$

The apparent self-association constant, H, of the exchange reaction between S_{LF} and S_{LB} is defined by Eq.

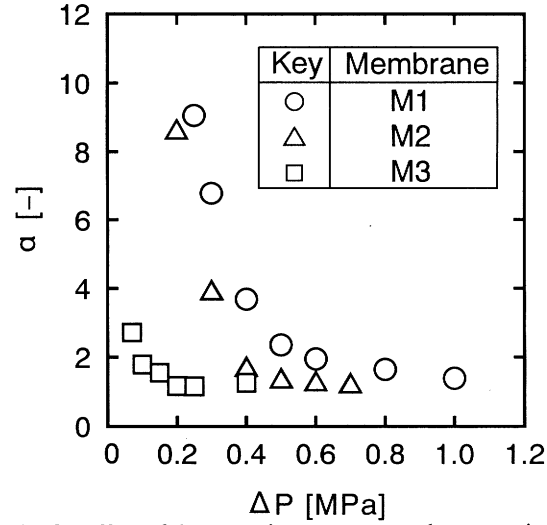

Fig. 3 Effect of the operating pressure on the separation factor

(4), with their respective concentrations, Cm_{LF} and Cm_{LB}.

$$H = Cm_{LB} / Cm_{LF} \tag{4}$$

By assuming that the "associated" solute molecule does not diffuse, the solute flux of L-Pgly can be expressed as shown previously[4]:

$$J_L = -\frac{Ps_L}{1+H}\frac{\partial C_L}{\partial x} \tag{5}$$

Thus, the apparent solute permeability of L-Pgly, $Ps_{L,\,app}$, can be expressed by the following equation:

$$Ps_{L,\,app} = Ps_L / (1+H) \tag{6}$$

There is no interaction between D-Pgly in the solution and the L-Pgly sites fixed within the membrane. D-Pgly is merely adsorbed on the surface of the membrane pore, and the solute flux of D-Pgly can be represented by:

$$J_D = -Ps_D \frac{\partial C_D}{\partial x} \tag{7}$$

As there is no self-association interaction between D-Pgly in the solution and L-Pgly fixed in the membrane, the apparent solute permeability of D-Pgly, $Ps_{D,\,app}$, will be equal to Ps_D:

$$Ps_{D,\,app} = Ps_D \tag{8}$$

This relation corresponds to the case of L-Pgly when $H = 0$. For the case of D-Pgly and L-Pgly existing as free the solute molecules, the values of solute permeability for both D-Pgly and L-Pgly must be the same because they have similar physical properties. If the solute permeability is denoted as Ps^*, we have

$$Ps_D = Ps_L = Ps^* \tag{9}$$

From Eqs. (6), (8) and (9), the following expression for apparent self-association constant is obtained:

$$H = \frac{Ps_{D,\,app}}{Ps_{L,\,app}} - 1 \tag{10}$$

Using Eq. (10), the values of H are evaluated from

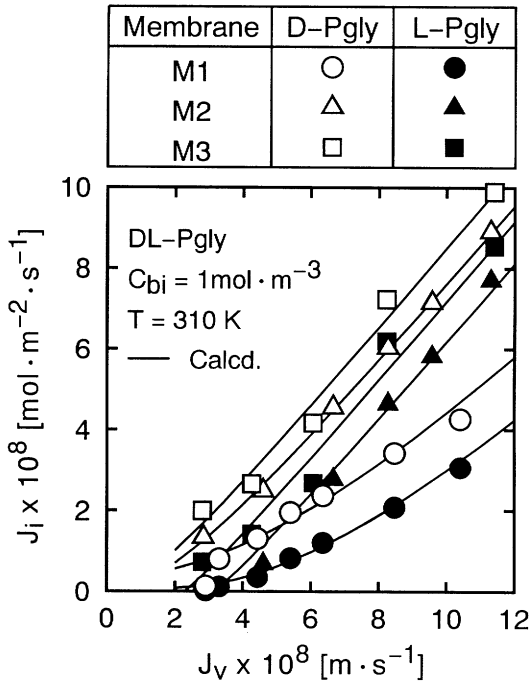

Fig. 4 Effect of the volume flux on the solute flux

the solute permeabilities of D-Pgly and L-Pgly, which were obtained from dialysis experiments. There are summarized in Table 2. Different values of the apparent self-association constants were obtained for each membrane. It was found that the apparent self-association constant was highly dependent on the membrane structure, *i.e.* the geometrical structure of L-Pgly at the fixed site on the surface of the membrane pore. From Table 2, the following results were obtained: the values of the partition coefficients of D-Pgly and L-Pgly are similar in magnitude. The ratio of the diffusion coefficient of D-Pgly to L-Pgly nearly equals the ratio of the solute permeability of D-Pgly to that of L-Pgly.

3.2 The effect of the viscous flow on solute flux

As proposed in the previous paper[4], the solute flux was described as the sum of the diffusing flow induced by the concentration gradient and the viscous flow induced by the pressure gradient. In the present experiment, the volume flux was low and intermediate between diffusing flow and the viscous flow. Combining Eqs. (5), (7) and (9) with the solute flux equations proposed previously, the following equations are obtained:

$$J_D = -Ps^* \frac{dC_D}{dx} + \left(1 - \frac{k_{2D}}{J_V + k_{3D}}\right) J_V C_{bD} \tag{11}$$

$$J_L = -\frac{Ps^*}{1+H} \frac{dC_L}{dx} + \left(1 - \frac{k_{2L}}{J_V + k_{3L}}\right) J_V C_{bL} \tag{12}$$

For the second term of the right hand side of Eqs. (11) and (12) to be a positive, the volume flux must be larger than J_V^{**} ($= k_{2i} - k_{3i}$). Therefore, the solute flux in the present study was analyzed in the range above J_V^{**}.

Since C_{bi}, C_{pi} and J_V, are constant at steady state, the following boundary conditions apply:

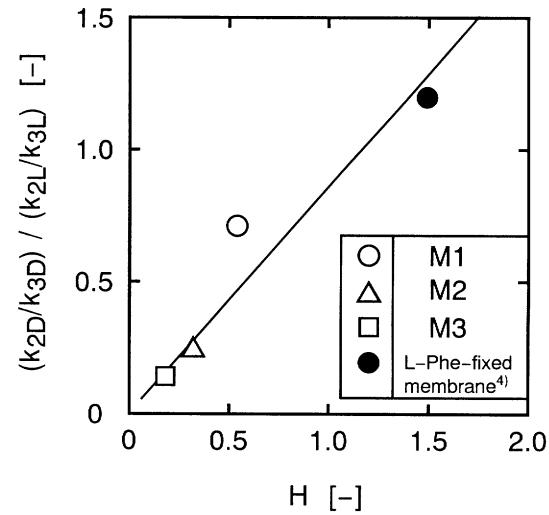

Fig. 5 Relation between the apparent self-association constant and the ratio of viscous parameters

$$\text{B.C.: } C_i = C_{bi} \quad at \quad x = 0 \tag{13-a}$$

$$C_i = C_{pi} \quad at \quad x = \delta \tag{13-b}$$

Under the experimental conditions, the amino acid solution is dilute. Thus, the following relation holds:

$$J_i = C_{pi} \cdot J_V \tag{14}$$

Integration of Eqs. (11) and (12) using Eq. (14) with the boundary conditions of Eq. (13) across the membrane thickness yields the following:

$$J_D = \frac{\frac{Ps^*}{\delta} + \left(1 - \frac{k_{2D}}{J_V + k_{3D}}\right) J_V}{Ps^*/\delta + J_V} J_V C_{bD} \tag{15}$$

$$J_L = \frac{\frac{Ps^*}{\delta(1+H)} + \left(1 - \frac{k_{2L}}{J_V + k_{3L}}\right) J_V}{Ps^*/\delta(1+H) + J_V} J_V C_{bL} \tag{16}$$

Figure 4 shows the effect of J_V on J_i. As shown in Fig. 4, J_i increased with an increase in J_V.

The viscous parameters, k_{2i} and k_{3i}, in Eqs. (15) and (16) were evaluated from experimental data of solute fluxes by curve fitting their values are shown in Table 2 together with the values of L-Phe-fixed membrane as previously reported[4].

The value of the viscous parameter M3, which has the largest membrane pore size, was the smallest among three membranes. From Table 2, it was found that the evaluated value of viscous parameter of L-Pgly for each membrane was larger than that of D-Pgly. It can be considered that a kind of flow restriction arises owing to the stronger interaction between L-Pgly in the solution and L-Pgly fixed in the membrane than that between D-Pgly in the solution and L-Pgly fixed in the membrane.

In the membrane pore, there will be two kinds of viscous flows: (1) flow near the membrane pore wall and (2) flow along the center of the membrane pore. Near the membrane pore wall, the L-Pgly solute interacts with L-Pgly fixed in the membrane pore wall and the diffusion of L-Pgly solute may be restricted. At the center of the

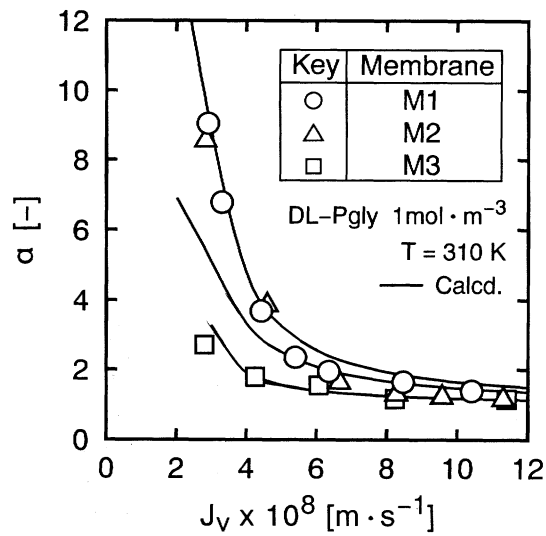

Fig. 6 Effect of the volume flux on the separation factor

membrane pore, however, the feed solution freely permeates through the membrane pore.

In the case when J_V is extremely low, $k_{2i}/(J_V + k_{3i})$ $(i = D, L)$ reduces to k_{2i}/k_{3i} $(i = D, L)$. The ratio of viscous parameters of D-Pgly, k_{2D}/k_{3D}, is affected by the physical interaction between D-Pgly in the solution and the membrane pore wall. On the other hand, k_{2L}/k_{3L} is dependent on both the physical interaction and the self-association interaction between the L-Pgly in the solution and that fixed in the membrane. As shown in Table 2, the values of k_{2D}/k_{3D} for all membranes ranged within 1.0 to 2.1 despite the variation in apparent self-association constants. On the contrary, the values of k_{2L}/k_{3L} were 1.4, 8.7, 8.0 and 1.2 for respective membranes, *i.e.*, M1, M2, M3, together with L-Phe-fixed membrane. This indicates that the values of k_{2L}/k_{3L} changed considerably with variation in the apparent self-association constants. The ratio of these parameters, *i.e.* $(k_{2D}/k_{3D})/(k_{2L}/k_{3L})$, is considered to be a function of H. These values are shown in Table 2 for the membranes of M1, M2, M3 and L-Phe-fixed membrane, respectively. The plots of $(k_{2D}/k_{3D})/(k_{2L}/k_{3L})$ against H gave a straight line as shown in **Fig. 5**. From Fig. 5, it was confirmed that the apparent self-association constants, which depended on the membrane pore structure, contributed to the relative magnitude of viscous parameters for L-Pgly-fixed membranes as well as L-Phe-fixed membranes.

3.3 Effect of apparent self-association constant and viscous parameter on separation factor

From Eqs. (1) and (14), the separation factor, α, can be expressed as follows:

$$\alpha = \frac{C_{bL}}{C_{bD}} \cdot \frac{J_D}{J_L} \tag{17}$$

Combining Eqs. (15)-(17) yields Eq. (18).

$$\alpha = 1 + \frac{J_V \delta \left\{ \frac{HPs^*}{1+H} + J_V \delta (\beta_L - \beta_D) + Ps^* \left(\beta_L - \frac{H\beta_D}{1+H} \right) \right\}}{(Ps^* + J_V \delta) \left\{ \frac{Ps^*}{1+H} + (1 - \beta_L) J_V \delta \right\}} \tag{18}$$

where $\beta_i = k_{2i}/(J_V + k_{3i})$.

Figure 6 shows the effect of volume flux on the separation factor. The value of α increased with decreasing J_V. The solid curves show the calculated values obtained from Eq. (18) using the parameter values in Table 2. These values could be fitted to the experimental values well.

As shown in Fig. 6, the maximum values of α obtained at $J_V = 2.9, 2.8, 2.8 \times 10^{-8}$ m·s^{-1} were 9.1, 8.6 and 2.7 for M1, M2 and M3, respectively. On the other hand, the values of α from the dialysis data were 1.54, 1.32 and 1.18, respectively, which were evaluated from the ratio of $Ps_{D,app}$ to $Ps_{L,app}$. In the region of lower volume flux in ultrafiltration, the membrane is preferably permeable to D-Pgly in the solution compared with dialysis. In the region of low volume flux, there exists a transition region between the diffusing flow and the viscous flow. However, this flow behavior is very complex owing to the mutual interaction between D- and L-Pgly fluxes and the membrane pore structure. Further investigation will be necessary for satisfactory description of the permeation characteristics.

In ultrafiltration, the higher values of α, which were more than five times those obtained by dialysis, were at flux values that were about two times larger than those in dialysis. By applying the L-Phe-fixed membrane to the construction of hollow-fiber type membrane module, the available membrane area per unit volume becomes larger. Consequently, it will be possible to perform the highly selective and high speed separation of optical isomers under multistage operation.

Conclusion

The optical resolution of DL-Pgly from aqueous solution was examined with a pressure gradient using three types of L-phenylglycine-fixed membranes prepared by varying the solvent evaporation period. The membranes had a preferred permeability of D-phenylglycine to L-phenylglycine. Comparison of the separation factor of each membrane at the same operating pressure showed that the membrane with smaller Lp had a considerably higher value of α.

The apparent self-association constant and the viscous parameters of D- and L-Pgly were evaluated on the basis of the solute flux equations. It was found that the ratio of viscous parameters, *i.e.* $(k_{2D}/k_{3D})/(k_{2L}/k_{3L})$, was proportionally related to the apparent self-association constant.

In the range of low volume flux, α showed a larger value than that at a given concentration gradient. The membranes will be effective for optical resolution of amino acids when operation is conducted under low operating pressures.

Acknowledgement

The authors wish to thank Mr. Kazuhisa Toubou for his assistance in the experimental work. This work was supported in part by a Grant-in-Aid for Developmental Scientific Research (No. 04555224) from the Ministry of Education, Science and Culture, Japan.

Nomenclature

C	=	concentration of Pgly in the solution	[mol·m^{-3}]
Cm	=	concentration of Pgly in the membrane	[mol·m^{-3}]
C_1	=	concentration of Pgly in compartment 1 of the dialysis cell	[mol·m^{-3}]
Ds	=	diffusion coefficient	[m^2·s^{-1}]
H	=	apparent self-association constant	[-]
J	=	solute flux	[mol·m^{-2} s^{-1}]
J_V	=	volume flux	[m·s^{-1}]
J_V**	=	critical volume flux	[m·s^{-1}]
k_2	=	viscous flow parameter of Eqs. (15) and (16)	[m·s^{-1}]
k_3	=	viscous flow parameter of Eqs. (15) and (16)	[m·s^{-1}]
Ks	=	partition coefficient	[-]
Lp	=	pure water permeability	[m·Pa^{-1} s^{-1}]
ΔP	=	pressure difference	[Pa]
Ps	=	Ds·Ks, solute permeability	[m^2·s^{-1}]
Ps*	=	solute permeability without self-association interaction	[m^2·s^{-1}]
Q_t	=	total amount of diffusing solute	[mol·m^{-2}]
T	=	temperature	[K]
t	=	time	[s]
x	=	coordinate in the membrane	[m]
α	=	separation factor	[-]
β	=	$k_{2i}/(J_V + k_{3i})$	[-]
δ	=	membrane thickness	[m]

<subscripts>

app	=	apparent value evaluated from dialysis data by using Eq. (2)
B	=	associated solute
b	=	feed solution
D	=	D-amino acid
F	=	adsorbed solute
i	=	*D* or *L*
p	=	permeate solution
L	=	L-amino acid

Literature Cited

1) Ishihara, I., N. Suzuki, and K. Matsui: *J. Chem. Soc. Japan*, 446-451, (1987)
2) Kikuchi, H., J. Hattori, Y. Mori, and T. Kajiyama: *Kagaku Kogaku Ronbunshu*, **15**, 617-622 (1989)
3) Maruyama, A., N. Adachi, T. Takatsuki, M, Torii, K. Sanui, and N. Ogata: *Macromolecules*, **23**, 2748-2752 (1990)
4) Masawaki, T., M. Sasai, and S. Tone: *J. Chem. Eng. Japan.*, **25**, 33-39 (1992)
5) Matson, S. L. and J. A. Quinn: Paper presented at the 72th. AIChE Annual Meeting, San Francisco (1979)
6) Tone, S. and H. Nakamura: *Kagaku Kogaku Ronbunshu*, **14**, 347-352 (1988)
7) Yamaguchi, T., K. Nishimura, T. Shinbo, and M. Sugiura: *Maku (Membrane)*, **10**, 178-182 (1985)