the chain–chain correlation hole. Nor has the model yet been extended to treat other solution transport properties, such as probe diffusion, sedimentation, or the plateau modulus. A rigorous justification of the self-similarity approximation—one capable of predicting correction terms in a systematic way—is presently lacking.

References and Notes

(1) Wheeler, L. M.; Lodge, T. P. *Macromolecules* **1989**, *22*, 3399.
(2) Lodge, T. P.; Markland, P.; Wheeler, L. M. *Macromolecules* **1989**, *22*, 3409.
(3) de Gennes, P.-G. *Scaling Concepts in Polymer Physics*; Cornell University Press: Ithaca, NY, 1979.
(4) Hess, W. *Macromolecules* **1986**, *19*, 1395.
(5) Hess, W. *Macromolecules* **1987**, *20*, 2587.
(6) Skolnick, J.; Yaris, Y.; Kolinski, A. *J. Chem. Phys.* **1987**, *88*, 1407.
(7) Skolnick, J.; Yaris, Y. *J. Chem. Phys.* **1987**, *88*, 1418.
(8) Phillies, G. D. J. *Macromolecules* **1986**, *19*, 2367.
(9) Phillies, G. D. J. *Macromolecules* **1987**, *20*, 558.
(10) Phillies, G. D. J. *Macromolecules* **1988**, *21*, 3101.
(11) Phillies, G. D. J. *J. Phys. Chem.* **1989**, *93*, 5029.
(12) Fujita, H.; Einaga, Y. *Polym. J. (Tokyo)* **1985**, *17*, 1131.
(13) Onogi, S.; Kimura, S.; Kato, T.; Masuda, T.; Miyanaga, N. cited in ref 14 as *J. Polym. Sci., Part C* **1966**, 381.
(14) Doi, M.; Edwards, S. F. *The Theory of Polymer Dynamics*; Clarendon Press: Oxford, 1986.
(15) Grest, G. S.; Kremer, K. Springer Proceedings in Physics. *Computer Simulation Studies in Compressed Matter Physics*; Springer-Verlag: Berlin, 1988; Vol. 33, p 76.
(16) Daoud, M.; Cotton, J. P.; Farnoux, B.; Jannink, G.; Sarma, G.; Benoit, H.; Duplessix, R.; Picot, C.; de Gennes, P.-G. *Macromolecules* **1975**, *8*, 804.
(17) Wheeler, L. M.; Lodge, T. P.; Hanley, B.; Tirrell, M. *macromolecules* **1987**, *20*, 1120.
(18) Phillies, G. D. J.; Gong, J.; Li, L.; Rau, A.; Zhang, K.; Rollings, J.; Yu, L-P., *J. Phys. Chem.* **1989**, *93*, 6219.

Enantioselective Permeation of α-Amino Acid Isomers through Poly(amino acid)-Derived Membranes

Atsushi Maruyama,* Noriyuki Adachi, Takehisa Takatsuki, Masanori Torii, Kohei Sanui, and Naoya Ogata

Department of Chemistry, Faculty of Science and Technology, Sophia University, 7-1 Kioi-cho, Chiyoda-ku, Tokyo 102, Japan

ABSTRACT: Poly(L-glutamates) having amphiphilic side chains of (n-nonylphenoxy)oligo(oxyethylene) (NONx-PLG) were observed to possess potential properties as membrane materials for resolving optical isomers of α-amino acids. Permeation rate ratios of >8.0 for the D isomer to L isomer of tyrosine and tryptophan were achieved. Permeation of amino acids for times >500 h did not cause any drop in selectivity. Operating temperature considerably affected the permselectivity of the isomers. Since NONx-PLG adopted mainly an α-helical structure in its cast film and formed a liquid crystalline (nematic) type mesophase, this ordered structure of poly(amino acid) derivatives was considered to be the reason for the enantioselectivity of the membrane.

Introduction

With the rapid production of novel specialty (highly functional) materials such as pharmaceutics, bioactive substances, and liquid crystals, improvement of preparative separation methods for geometrical isomers including optical isomers is currently demanded. Although the membrane separation process has marked advantages in production such as ease of handling, instrumental simplicity, and efficiency in energy as compared to the chromatographic separation process, a practical membrane having the capability of separating optical isomers has not yet appeared except in the form of liquid membranes. These are triphasic systems composed of aqueous–organic–aqueous solutions utilizing optically active crown ethers, originally demonstrated by Cram et al.[1]

The permeation of substances through a membrane is determined by the partitioning behavior of the substances with respect to the membrane and their diffusibility (or mobility) in the membrane. Thus, to improve enantioselectivity of a membrane, considerable effort should be made to optimize these two factors. The partitioning behavior of optical isomers is largely influenced by the structure and number of recognition sites in the membrane. However, diffusibility is difficult to control because optical isomers have identical molecular sizes.

To refine the selectivity of polymeric membranes, we paid particular attention to the microenvironmental nature in the vicinity of the recognition sites as well as the distribution (or assembling) mode of the recognition sites.

Aqueous liquid membranes with amphiphilic substances such as surfactants were reported to discriminate geometric isomers of amphiphilic substances or of hydrocarbons in terms of their permeation or partitioning behavior based on the differential solubilizing effect.[2] In this case, the hydrophilic–hydrophobic balance of amphiphilic substances was considered to play a significant role. Colonna et al.[3] reported the successful asymmetric oxidation of electron-poor olefins such as chalcones to optically active oxiranes in the triphase system (water/organic solvent–poly(amino acid)). In this case, the poly(amino acid), which provides the stereoselectivity of the reaction, was placed at the interface of an emulsion of the organic phase in the aqueous phase. The hydro-

$+COCHNH+_n$
|
$(CH_2)_2$
|
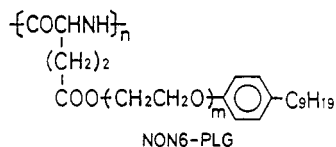$COO+CH_2CH_2O+_m$⟨O⟩$-C_9H_{19}$

NON6-PLG

Figure 1. Structural formula of NON6-PLG. The number average of repeating units of oligo(oxyethylene) was 6.0.

philic–hydrophobic environmental nature around the poly-(amino acid) was considered to play a complementary role in the stereoselectivity of the reaction. Additionally, highly enantioselective reactions in micelles[4] or bilayer membranes[5] have been reported. Thus, we expected that the resolving power of recognition moieties (or sites) fixed in a membrane would be improved by regulating the hydrophilic–hydrophobic nature around the moieties (sites). In other words, membranes having optically active recognition moieties placed in a controlled hydrophilic–hydrophobic microenvironment would discriminate optically active substances with high selectivity in terms of their partitioning or permeation behavior.

In addition to the microenvironment, the distribution and assembling modes of recognition sites in a membrane may be another influential factor on both the resolving power of recognition sites and the diffusibility of substances.

According to this concept, we designed poly(amino acid) derivatives having amphiphilic side chains, consisting of oligo(oxyethylene) and long alkyl chains, as membrane materials. The hydrophilic–hydrophobic balance in the membrane could be feasibly regulated by changing the number of constituting repeating units of oligo(oxyethylene) or by changing the length of the alkyl chain. Moreover, since rodlike rigid polymers such as α-helical poly-(amino acids) are known to orient along their long axes in their cast films or in concentrated solution,[5] chiral fields formed around the helical structure must be orderly situated in the membrane.

In this paper, we report the preparation and structural characterization of poly(amino acids) with amphiphilic side chains as well as their permselectivity to α-amino acid isomers.

Experimental Section

Materials. Poly(γ-methyl L-glutamate) (PMLG: MW = 10⁵) was kindly supplied by Ajinomoto Co. Inc. (*n*-Nonylphenoxy)-oligo(oxyethylene) (NON6: number average of constituting repeating units of oligo(oxyethylene) = 6.0 with molecular weight distribution (M_w/M_n) = 1.2) was obtained from Sanyo Chemical Industries, Ltd. DL-Tryptophan (Trp) and DL-tyrosine (Tyr) were purchased from Wako Pure Chemical Industries, Ltd., and used without further purification.

Synthesis of Poly(L-glutamate) with Amphiphilic Side Chains (NON6-PLG). The structural formula of NON6-PLG is shown in Figure 1. NON6-PLG was prepared as follows: 3 g of *p*-toluenesulfonic acid monohydrate was dissolved in an ethylene dichloride (EDC) solution (5.54 wt %, 244 g) of PMLG. To the PMLG solution, an EDC solution (50 wt %, 400 g) of NON6 was added dropwise over more than 2 h. The mixture was allowed to stand at 70 °C. To promote ester-exchange reaction, MeOH formed during the reaction was removed from the reaction mixture with EDC by evaporation. Another 220 g of NON6 was gradually added to the reaction mixture during the course of the reaction. After a 20-day reaction, a large excess of AcCN was poured into the reaction mixture, and the polymer that precipitated was collected. The resulting polymer was repeatedly washed with AcCN, followed by drying in vacuo. ¹H NMR, FT-IR, CD, DSC, and a polarized microscope were used to characterize the structure of the polymer.

Membrane Preparation. As NON6-PLG did not offer self-standing membranes, an anisotropic ultrafilter (cutoff molecu-
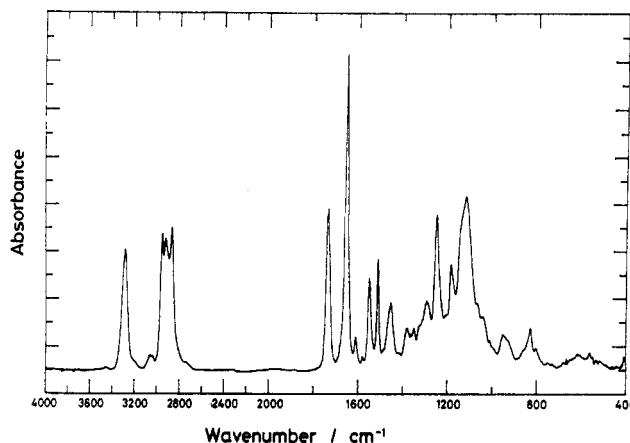

Figure 2. FT-IR spectrum of NON6-PLG film. NON6-PLG film cast on a KBr plate from EDC solution was measured.

lar weight = 2 × 10⁴, Advantec Toyo Co.) made of poly(*m*-phenyleneisophthalamide) was used as a membrane support. An EDC solution (ca. 1.0 wt %) of NON6-PLG was placed on the dense side of the ultrafilter and dried under a nitrogen atmosphere. This coating process was repeated several times (normally 5 times) to avoid pinhole formation. The polymer-coated membrane (polymer thickness: ca. 1 μm) was further dried at 40 °C for 24 h, followed by thorough drying at 40 °C in vacuo for 48 h.

Permeation Experiment. A disproportionate two-chamber cell whose chamber volumes on the donor side and acceptor side, respectively, were 100 and 4 cm³ was used for permeation experiments. The polymer-coated membrane (8.0 cm²) after equilibration in distilled water for more than 7 days was attached between the chambers with silicone-rubber packings. An aqueous solution (Trp: 4.9 mmol/L; Tyr: 2.0 mmol/L) of racemic amino acids and water, respectively, were placed in the donor- and acceptor-side chambers. The permeation experiment was carried out at various temperatures (1–40 °C) with stirring. To observe the permeation behavior of the amino acid isomers in a steady state, the solution in the acceptor-side chamber was exchanged with pure water at each sampling time. The amount of amino acid that permeated through the membrane was determined at 277 nm by a UV spectrometer, and the composition of D and L isomers was measured by means of a liquid chromatograph equipped with a Chiralpak-WH column (Daicel Chemical Industries, Ltd.), an optical resolution column, and a UV spectrometer.

Results and Discussion

Synthesis and Characterization of NON6-PLG. The FT-IR spectrum of the resulting polymer (NON6-PLG) is shown in Figure 2. The characteristic peaks of NON6 and polyglutamate moieties were observed in the IR spectrum of NON6-PLG. Furthermore, the absence of absorption at 3500 cm⁻¹ in the spectrum of the resulting copolymer corresponded to the disappearance of hydroxyl groups of NON6 due to the exchange reaction with PMLG to form ester linkages.

Peaks due to both polyglutamate and NON6 moieties were observed in the ¹H NMR spectrum of the resulting polymer in CF₃COOD. Ninety-five percent of the pendant methyl groups of PMLG were found to be exchanged with NON6 after a 20-day reaction as determined from the ratio of the peak areas between the ether methylenes in the pendant chains and the α- and β-methylenes of the polyglutamate main chains.

The elemental analysis for NON6-PLG was also in agreement with the values calculated for the structure shown in Figure 1 (%C, 64.0 (calcd 64.9); %H, 9.1 (calcd 8.9); %N, 2.4 (calcd 2.8)).

The second-order structure of NON6-PLG film cast on a KBr cell from EDC solution was evaluated by FT-IR

Figure 3. Microphotograph of NON6-PLG film between crossed polarizers at room temperature.

Figure 4. DSC curve of NON6-PLG under a heating rate of 20 K/min.

analysis. As seen from Figure 2, characteristic absorptions of the α-helix appeared at 1654 cm^{-1} (amide I) and 1551 cm^{-1} (amide II). The peaks deconvoluted from the amide I band were assigned as those of the α-helical, β-sheet, and random structures, respectively, according to Byler's report.[7] The calculated α-helix contents of NON6-PLG and PMLG films, respectively, were ca. 60% and ca. 70%, showing a slight drop in α-helix content upon introduction of NON6 pendant chains. The deconvoluted spectrum also revealed negligible amount of β-structure of NON6-PLG film.

A polarized microscope was used to evaluate the ordered structure in NON6-PLG films. As shown in Figure 3, a schliering nematic texture was observed in NON6-PLG film under crossed polarizers. This result indicates that NON6-PLG aligned along its helical long axis and adopted a well-ordered structure in its cast film. However, no remarkable structural change in texture was observed when temperature was varied from room temperature to 200 °C. A similar schliering texture was also observed in EDC solution with high concentration (>20 wt %); thus, the ordered structure of NON6-PLG film is constructed from the ordered structure formed in its solution stage. The ordered structure of NON6-PLG films changed slightly when the films were immersed in water. Thus, NON6-PLG was expected to hold its ordered structure during the course of subsequent permeation experiments carried out under aqueous conditions.

The DSC curve of NON6-PLG is shown in Figure 4. The shoulder peak at −29.5 °C was due to the relaxation of polymer pendant chains. An endothermic peak was observed at 49.8 °C, at which NON6-PLG gained fluidity. Since the ordered structure of the NON6-PLG membrane was slightly changed at this temperature as shown by polarized microscopic observation, the endothermic peak was presumably related to the change in structure or molecular motion of the pendant NON6 moieties in the polymer film. This consideration was further proved by the temperature dependence of the CD spectrum.

It is significant to note that CD signals due to the phenoxy groups in the pendant chains were observed for NON6-PLG films cast on a quartz plate, as shown in Figure 5, curve a. Induced CD signals of achiral chromophore dopes in chiral matrices, such as cholesteric liquid crystals, were reported by several researchers.[8] However, it has been pointed out that samples with a macroscopic anisotropy which caused linear dichroism
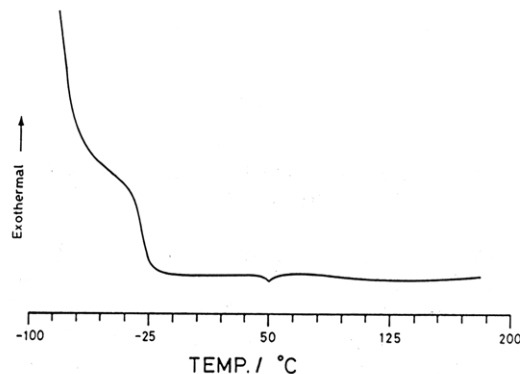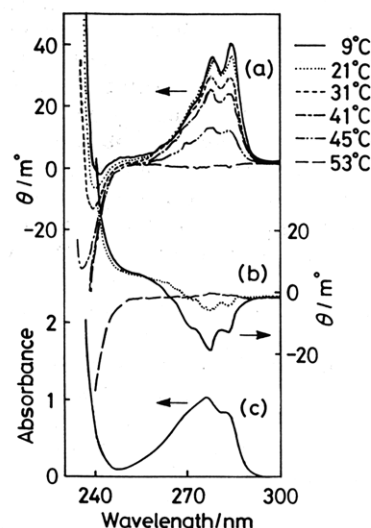

Figure 5. CD signals of NON6-PLG films cast on quartz plates at various temperatures. Curve a: the polymer film surface was directed to the light source. Curve b: the quartz surface was directed to the light source. Curve c: UV spectrum.

(LD) and linear birefringence (LB) showed apparent (artifact) CD signals.[9] To gain further information on the observed CD signals, we measured the angular dependency of the signals. As shown in Figure 5, curve b, the CD signal was considerably changed when the film-cast quartz plate was turned over. The result clearly showed that the CD signal was influenced by LB and LD, which could have resulted from the anisotropy of the phenoxy groups in the film, although we cannot completely dismiss the possibility of an induced CD.

The temperature dependence of the CD signal is depicted in Figure 6, where the differential peak intensity between the 277- and 295-nm peaks is plotted to reduce the base-line shift caused probably by the LB of the sample. The peak intensity gradually decreased with increasing temperature until 40 °C, followed by a steep decrease. Finally, the CD peaks completely disappeared at 55 °C. CD peaks appeared again when the sample film was cooled, indicating reversibility of the anisotropic structure of the phenoxy groups. The results indicated that the anisotropic structure of the phenoxy groups was significant at a lower temperature. As temperature increased, the phenoxy groups became more free. Eventually, the phenoxy groups were suddenly free from the anisotropic structure over 50 °C, indicating a steep increase in its molecular motion over 50 °C. The change in structure of the side-chain moieties in NON6-PLG probably induced the endothermic peak apeparing in Figure 4. A plausible structure that can explain the macroscopic anisotropy of phenoxy groups is a laminar assembly (such as a monolayer
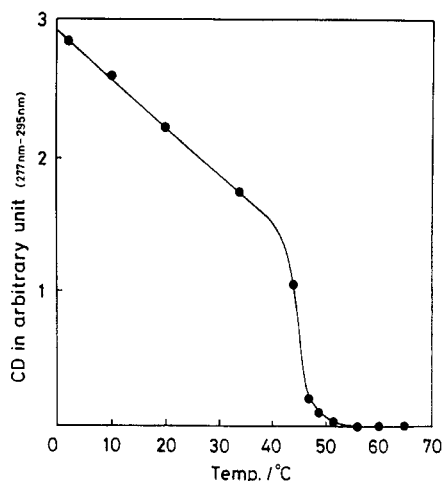
Figure 6. Temperature dependence of the peak intensity for the CD signal due to the phenoxy groups.
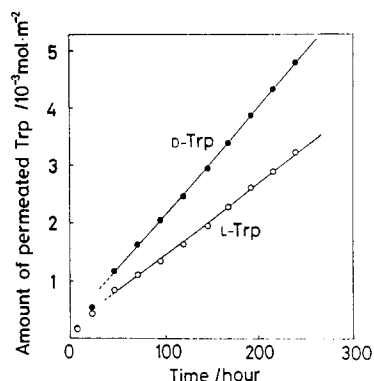

Figure 7. Permeation behavior of D- and L-Trp from a racemic mixture through NON6-PLG membrane at 40 °C. Initial concentration of DL-Trp on the donor side was 4.9 mmol/L.

of amphiphilic substances) of pendant chains along the backbone's long axis caused by phase separation between hydrophilic and hydrophobic portions. Further study on the microstructure of NON6-PLG is now in progress.

Permeation Behavior of Amino Acid Isomers through NON6-PLG Membrane. The permeation behavior of optical isomers from racemic Trp and Tyr through NON6-PLG membranes at 40 °C was evaluated. As shown in Figure 7, rather low selectivity and faster permeations of D and L isomers were observed during the initial 50 h than after 50 h. After 50 h, the permeation rates of the D and L isomers became steady. While the reason for the change in permeation rate and selectivity on time course of permeation has not been established, a similar behavior was also observed in the permeations of Trp at different temperatures and the permeation of Tyr, as will be mentioned later in this article.

In the steady state (after 50 h), preferential permeation of D-Trp over L-Trp was observed. The ratio of permeation constant of the D isomer to that of the L isomer determined from the slope of the straight lines in Figure 7 was calculated to be 1.5, indicating that D isomers permeated 1.5 times faster than L isomers. The preferential permeation held for over 250 h. The permeation behavior of D- and L-Tyr from a racemic mixture at 40 °C is shown in Figure 8. As before, slightly faster permeations of D and L isomers were also found during the initial 100 h. Moreover, a considerable difference in permeation rate between D and L isomers was observed. The difference in permeation rate became even greater after 100 h.
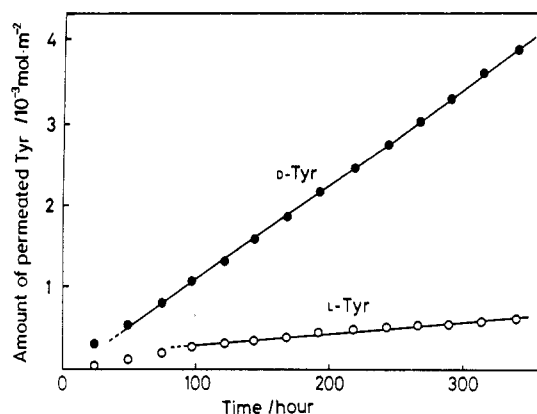
As seen from Figure 9, liquid chromatograms of Tyr

Figure 8. Permeation behavior of D- and L-Tyr from a racemic mixture through NON6-PLG membrane at 40 °C. Initial concentration of DL-Tyr on the donor side was 2.0 mmol/L.
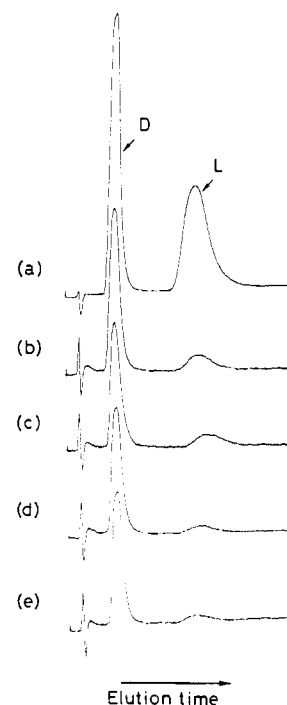

Figure 9. Liquid chromatogram of Tyr permeated through NON6-PLG membrane: (a) racemic Tyr: (b–e) Tyr collected from the acceptor side during 49–75, 75–97, 122–144, and 144–168 h, respectively. Column: Chiralpak-WH; eluent: 0.5 mmol/L aqueous CuSO₄; detector: UV at 275 nm.

on the acceptor side clearly revealed the change in the permselectivity; i.e., the amount of L isomer on the acceptor side decreased after 100 h in comparison with that before 100 h. Such changes in permeation rate and selectivity observed in the permeation of Trp and Tyr isomers seem related to some structural alterations of the membrane induced by absorption of amino acids on the membrane. The value of over 8.4 for the ratio of the permeation rate of D isomer to that of L isomer obtained demonstrates the marked excellence in permselectivity of the NON6-PLG membrane to optical isomers of Tyr. The differential permeation between D and L isomers held for over 300 h without any change in selectivity and permeation rate.

The effect of temperature on permselectivity of the NON6-PLG membrane was evaluated by examining the permeation behavior of Trp at a lower temperature (34 °C) (Figure 10). Surprisingly, in contrast to the result (Figure 7) obtained at 40 °C, no permeation of L isomers occurred at 34 °C during a time period of over 700 h, resulting in a complete resolution of the optical isomers
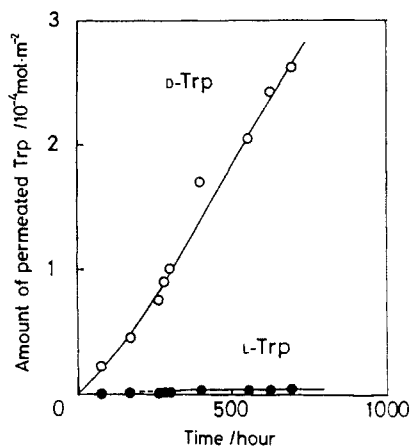
Figure 10. Permeation behavior of D- and L-Trp from a racemic mixture through NON6-PLG membrane at 34 °C. Initial concentration of DL-Trp on the donor side was 4.9 mmol/L.

of Trp, even though the permeation rates of the isomers markedly dropped at 34 °C compared with that at 40 °C.

A complete optical resolution of Trp isomers was also attained when the permeation temperature was lowered to 1 °C. Increasing selectivity with decreasing temperature is often observed in various chemical processes, including a variety of chemical reactions. However, the rise in permselectivity at a lower temperature might involve other important factors such as the change in side-chain conformation and in hydrophilic–hydrophobic balance in the membrane with varying temperature. To our knowledge, this is the first successful optical resolution by means of a membrane separation process using synthetic polymeric membranes.

Conclusion

Enantioselective permeations of Trp and Tyr isomers through membranes based on α-helical poly(amino acids) having amphiphilic side chains were observed. Poly-(amino acid) backbones were aligned along their long axes and were well ordered in the film. Further, amiphiphilic pendant chains were shown to have anisotropy, at least partly, in a membrane below 50 °C. This result indicates that not only the α-helical backbone but also the pendant chain structure are well ordered in the film. Thus, it is considered that chiral recognition based on helical poly(amino acid) for Tyr and Trp is aided by such ordered structure of backbone and pendant chains,

resulting in differential behavior of chiral amino acid isomers through the membrane (partition or diffusion). The temperature dependence of the permselectivity is probably related with the change in the ordered structure of the pendant chain portion. Moreover, the change in hydrophilic–hydrophobic balance, which modifies interaction between amino acid and the membrane at varying temperatures, is another influential factor on permselectivity to amino acid isomers. Thus, membranes based on α-helical poly(amino acids) with amphiphilic pendant chains are expected to have the capability of separating a variety of optical isomers, not only Trp and Tyr, by regulating environmental conditions such as temperature and/or regulating pendant chain structure. Further, the performance of the membrane remained constantly high for over 700 h, suggesting that NON6-PLG is a durable membrane good for practical use.

References and Notes

(1) (a) Newcomb, M.; Helgeson, R. C.; Cram, D. J. *J. Am. Chem. Soc.* **1974,** *96,* 7367. (b) Newcomb, M.; Toner, J. L.; Helgeson, R. C.; Cram, D. J. *Ibid.* **1979,** *101,* 4941.

(2) (a) Mukerjee, P. *J. Pharm. Sci.* **1971,** *60,* 1528. (b) Stelmaszek, J. *J. Membr. Sci.* **1977,** *2,* 197. (c) Li, N. N. *AIChE J.* **1971,** *17,* 459. (d) Li, N. N. *Ind. Eng. Chem. Process Des. Dev.* **1971,** *10,* 215. (e) Alessi, P.; Canepa, B.; Kikic, I. *Can. J. Chem. Eng.* **1979,** *57,* 54.

(3) (a) Colonna, C.; Molinari, H.; Banfi, S. *Tetrahedron* **1983,** *39,* 1635. (b) Banfi, S. *Tetrahedron* **1984,** *40,* 5207.

(4) Cho, I.; Kim, G. *J. Org. Chem.* **1988,** *53,* 5187.

(5) Ueoka, R.; Murakami, Y. *J. Chem. Soc., Perkin Trans. 2* **1983,** *219.*

(6) (a) Uematsu, I.; Uematsu, Y. *Adv. Polym. Sci.* **1984,** *No. 59,* 37. (b) Tachibana, T.; Oda, E. *Bull. Chem. Soc. Jpn.* **1973,** *46,* 2583. (c) Watanabe, J.; Sasaki, S.; Uematsu, I. *Polym. J.* **1977,** *9,* 337.

(7) Byler, D. M.; Susi, H. *Biopolymers* **1986,** *25,* 469.

(8) (a) Saeva, F. D.; Wysocki, J. J. *J. Am. Chem. Soc.* **1971,** *93,* 5928. (b) Iizawa, E.; Yang, J. T. *Mol. Cryst. Liq. Cryst.* **1974,** *29,* 27. (c) Uematsu, I.; Watanabe, J. *Liquid Crystalline Polymers*; Imura, K., Asada, T., Abe, A., Eds.; Shiguma Shuppan Co.: Tokyo, 1988; p 66 (in Japanese).

(9) (a) Norden, B. *J. Phys. Chem.* **1977,** *81,* 151. (b) Jensen, H. P.; Schellman, J. A.; Troxell, T. *Appl. Spectrosc.* **1978,** *32,* 192. (c) Shindo, Y.; Ohmi, Y. *J. Am. Chem. Soc.* **1985,** *107,* 91. (d) Shindo, Y.; Nakagawa, M. *Appl. Spectrosc.* **1985,** *39,* 860.

Registry No. (±)-Trp, 54-12-6; DL-tyrosine, 556-03-6.