Chiral Separation of Racemic Amino Acids through DNA-Polydiallyldimethylammonium Polyion Complex Membranes

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Summary: Novel polyion complex membrane was prepared from DNA-Na and polydiallyldimethylammonium chloride (PDADMAC). The newly prepared DNA-PDADMA polyion complex membrane showed a chiral separation ability, which was measured by using various racemic amino acids as model racemic mixtures. The membrane transported the L-isomer of Trp or Phe in preference to the corresponding D-isomer and permselectivities toward the L-isomer were determined to be 1.26 for L-Trp and 1.25 for L-Phe, respectively. From adsorption experiments, it was revealed that the permselectivity was mainly governed by adsorption selectivity.

Keywords: chiral separation; DNA; membrane; optical resolution; polyion complex membrane

Introduction

In Hokkaido, Japan, over 1,000 tons of DNA from salmon milt can be produced by suitable extraction process although they are now being abandoned. DNA molecules carry an important biological information on genetics of living things with its double-stranded structure consisting of complementary nucleic acid base pairs.[1] On the other hand, DNA molecules have a huge molecular weight of over 6 millions so that DNA was reported to be promising polymeric materials to give durable films,[2] and they have been studied in connection with optical devices,[3–6] electric[7,8] or ion[9–11] conductivity, ion permeation,[12] capture of metal ions[13] or endcrine disruptors,[13,14] and chiral separation.[15] It is an indispensable research subject to develop potential utilization of natural resources. Among many applications of DNA molecules, the authors focused their attention on separation membranes derived from DNA because membranes will play an important role in environmental and energy related processes.[16,17] Chiral separation of an optically pure enantiomer from a given racemic mixture is one of important membrane separation processes in connection with pharmaceuticals, foods, agricultural chemicals, perfume production, and so forth. The present paper describes the chiral separation of racemic amino acids with the membranes from DNA-polydiallyldimethylammonium polyion complexes.

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Experimental Part

Materials

Fiberlike purified DNA sodium salt (DNA-Na) of 93% from salmon milt (Nippon Chemical Feed Co.) was used. Polydiallyldimethylammonium chloride PAS-

H-5L, of which nominal molecular weight was 40,000, was kindly supplied by Nitto Boseki Co., Ltd. and used without further purification. Water purified with an ultrapure water system (Simpli Lab, Millipore S. A., Molsheim, France) was used. Model racemic permeants, such as D-tryptophan (D-Trp), L-tryptophan (L-Trp), D-phenylalanine (D-Phe), L-phenylalanine (L-Phe), D-tyrosine (D-Tyr), L-tyrosine (L-Tyr), D-glutamic acid (D-Glu), L-glutamic acid (L-Glu), D-lysine (D-Lys), and L-lysine (L-Lys) were purchased from Peptide Institute, Inc. and used without further purification. Sodium azide (fungicide), NaBr, and KCl were used as received.

Membrane Preparation

In the present study, the composition of the base pairs of DNA-Na was not determined. Here, 662.0, the average value of the base pairs of DNA-Na, was adopted as an average molecular weight of the constitutional repeating unit (base pair). Polyion complex of DNA-Na and polydiallyldimethylammonium chloride (PDADMAC) was prepared as follows: 0.255 g (3.852×10^{-4} mol) of DNA-Na was dissolved in 20.995 g of H_2O to obtain 1.20% aqueous DNA solution. 0.105 g (6.494×10^{-4} unit mol) of PDADMAC was dissolved in 10.395 g of H_2O so that 1.00% aqueous PDADMAC solution could be prepared. 20 wt.% aqueous NaBr solution was prepared by dissolving 65.0 g of NaBr into 259 g of H_2O. The aqueous DNA-Na solution and the aqueous PDADMAC solution thus prepared were added to the 20 wt.% aqueous NaBr solution. The mixed solution was stirred for 1 h at ambient temperature. The mixture was poured into the ultrafiltration cell (Model 8400, Amicon). The mixture was filtered with a regenerated cellulose UF membrane (diameter, 76 mm; Amicon) with a nominal molecular weight limit of 1,000 at the operation pressure of 0.2–0.3 MPa. The retentate was thoroughly washed with pure H_2O at the operation pressure of 0.2–0.3 MPa. The thickness of the DNA-PDADMA polyion complex membrane was around 72 μm.

Measurement of Membrane Potential

The membrane potential was measured by using the standard laboratory set-up.[18] The electromotive force which across between the bulk solution was conducted by saturated KCl bridges and calomel electrodes and measured by a potentiometer, Electrometer HE-106 (Hokuto Denko Co.). The bulk solutions of both sides of the membrane were stirred by the flow of themselves.

The anion transport number (t_-) of the membrane was determined by the concentration-membrane potential ε ($E_I - E_{II}$) at 25 °C with an aqueous solution of KCl using[19]

$$\varepsilon = E_I - E_{II} = (2t_- - 1)(RT/F)\ln(C_I/C_{II}) \quad (1)$$

where C_I and C_{II} represent the concentrations of the solution on either side of the membrane (0.200 and 0.100 mol dm^{-3}, respectively), F is the Faraday constant, R is the gas constant, and T means the absolute temperature.

Membrane Performance Study

Enantioselective permeation of racemic amino acids and adsorption selectivity of the membrane toward racemic amino acids were carried out following the procedures described in our previous paper.[20] All experiments were carried out at the constant temperature of 40 °C.

The permselectivity $\alpha_{(L/D)}$ is defined as the flux ratio J_L/J_D divided by the concentration ratio of racemic amino acids [L-AA]/[D-AA]:

$$\alpha_{(L/D)} = (J_L/J_D)/([L-AA]/[D-AA]) \quad (2)$$

The adsorption selectivity $S_{A(L/D)}$ is defined as:

$$S_{A(L/D)} = ((L-AA)/(D-AA))/([L-AA]/[D-AA]) \quad (3)$$

> where (L-AA) and (D-AA) are the amounts of racemic amino acids adsorbed in the membrane and [L-AA] and [D-AA] are the concentrations in the solution after equilibrium had been reached.

Results and Discussion

The membrane potential was determined to be +0.058 mV. From the observed potential, the transport number of Cl^- was determined to be 0.510, while that of K^+ was obtained to be 0.490. The transport numbers for K^+ and Cl^- in aqueous solution were reported to be around 0.4905 and 0.5095, respectively.[21] The transport numbers for K^+ and Cl^- in the membrane determined in the present study were close to those in bulk aqueous solution. From this, it was revealed that the DNA-PDADMA polyion complex membrane prepared in the present study was electrically neutralized.

From the fact that he polyion complex membrane in the present study consisted of PDADMAC and DNA-Na, which was one of natural polymers, it can be expected that the membrane could show an enantioselective permeation of racemic mixtures. To this end, five types of racemic amino acids were adopted as model permeants and enantioselective permeation, where the concentration gradient was adopted as a driving force for membrane transport, was studied. The time-transport curves for five types of racemic amino acids through the present polyion complex membrane are shown in Figure 1. As expected, the L-isomer of Trp and that of Phe were transported in preference to the corresponding D-isomer. The permselectivity toward L-Trp was determined to be 1.26, while that toward L-Phe reached 1.25. On the contrary, selective transport was hardly observed in the transport of racemic Tyr's, Glu's, and Lys's through the membrane. In other words, racemic amino acids with aromatic side chains, such as racemic Trp's and Phe's, enantioselective permeation was attained, while chiral separation was hardly observed in the transport of racemic Tyr's, which is an amino acid having aromatic side chain with polar non-charge group (hydroxyl moiety) and those with very polar side chains, such as Glu and Lys.

It is interesting and indispensable to elucidate the mechanism for the expression of permselectivity. To this end, adsorption selectivity toward each racemic amino acid pairs of the present polyion complex membrane was investigated. The amounts of racemic permeants adsorbed in the membrane and their adsorption selectivities are summarized in Table 1. In this table, the amount of each enantiomer adsorbed in the membrane is given as a relative one, which was converted to an ion complex basis. As for racemic amino acids with aromatic side chains, such as racemic Trp's and Phe's, the L-isomer was incorporated into the polyion complex membrane in preference to the corresponding D-isomer. The following interactions might be enumerated as the interactions for the chiral recognition; Coulombic interaction between amino acids and phosphates, interaction between amino acids and 2-deoxyribose, and that between amino acids and bases. Hydrophobicity of aromatic side chain of Trp and Phe may also contribute to the adsorption selectivity. However, at the moment, we cannot specify the precise interaction mode for chiral recognition. The adsorption selectivities $S_{A(L/D)}$ for racemic Trp's and Phe's were determined to be 1.24 and 1.17, respectively. On the other hand, as for other racemic amino acids, such as racemic Tyr's, Glu's, and Lys's, adsorption selectivity was slightly observed. Using permselectivity ($\alpha_{(L/D)}$) and adsorption slectivity ($S_{A(L/D)}$), we can elucidate the diffusivity selectivity ($S_{D(L/D)} = D_L/D_D$, where D_L and D_D are the diffusion coefficients of the L-isomer and the D-isomer, respectively) for each racemic mixture. The estimated diffusivity selectivities are also summarized in Table 2 together with permselectivities and adsorption selectivities. Results summarized in Table 2 revealed that the chiral separation was mainly governed by the adsorption selectivity toward the target molecule, and the diffusivity selectivity assisted in the chiral separation of racemic amino acids through the DNA-PDADMA polyion complex membrane.

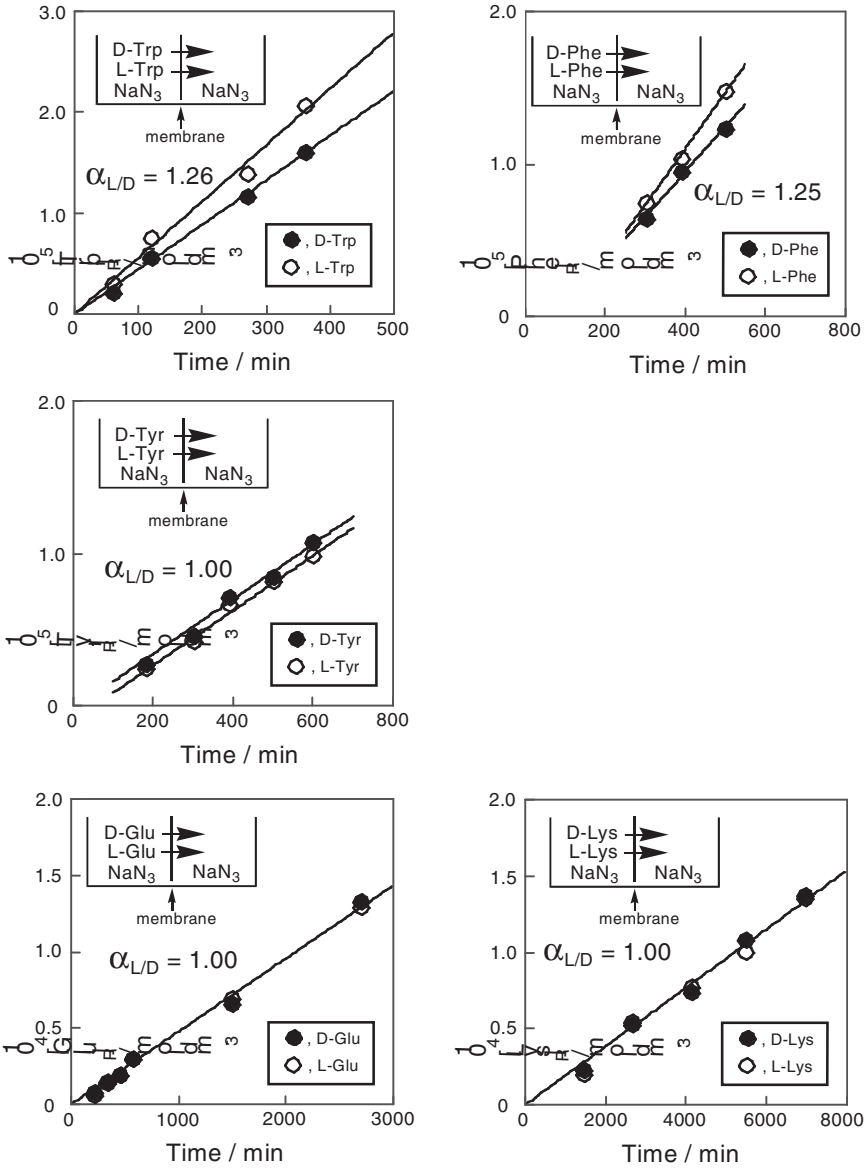

Figure 1.
Time-transport curves of various racemic amino acids throught the DNA-PDADMA polyion complex membranes at 40 °C.

Table 1.
Adsorption selectivity of DNA-PDADMA polyion complex membranes toward racemic amino acid mixtures.

	(L-isomer)/(ion complex)	(D-isomer)/(ion complex)	$S_{A(L/D)}$
Trp	2.50×10^{-3}	2.03×10^{-3}	1.24
Phe	4.71×10^{-3}	4.08×10^{-3}	1.17
Tyr	4.03×10^{-3}	4.10×10^{-3}	0.98
Glu	1.99×10^{-3}	1.91×10^{-3}	1.04
Lys	1.01×10^{-2}	1.00×10^{-2}	1.01

Table 2.
Summary of Chiral separation of polyion complex membrane.

	$\alpha_{L/D}$	$S_{A(L/D)}$	$S_{D(L/D)}$
Trp	1.26	1.24	1.02
Phe	1.25	1.17	1.07
Tyr	1.00	0.98	1.02
Glu	1.00	1.04	0.96
Lys	1.00	1.01	1.00

$S_{D(L/D)} = \alpha_{L/D}/S_{A(L/D)}$.

Conclusions

Novel polyion complex membrane was prepared from DNA-Na and PDADMAC. The newly prepared DNA-PDADMA polyion complex membrane showed chiral separation ability. The membrane transported the L-isomer of Trp or Phe in preference to the corresponding D-isomer and permselectivities toward the L-isomer were determined to be 1.26 for L-Trp and 1.25 for L-Phe, respectively. Adsorption experiments revealed that the permselectivity was mainly governed by adsorption selectivity. The results obtained in the present study suggest that polyion complex membranes consisting of DNA could be applicable to chiral separation.

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