Optical Resolution of Racemic Amino Acids through DNA-Poly(4-vinylbenzyl)trimethylammonium Polyion Complex Membranes

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ABSTRACT: Novel polyion complex membranes were prepared from DNA-Na and poly(4-vinylbenzyl)trimethylammonium chloride (PVBTMAC). The newly prepared DNA-PVBTMA polyion complex membranes showed a chiral separation ability toward racemic lysine (Lys) mixtures. The membranes transported D-Lys in preference to the corresponding L-isomer of Lys. The permselectivity toward the D-isomer reached 1.26. From transport experiments and adsorption studies, it was revealed that the permselectivity was mainly determined by diffusivity selectivity.
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      Polyion Complex / Separation /

DNA molecules carry 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 one of promising natural polymers to give durable films.[2,3] Since the appearance of those reports,[2,3] DNA has been intensively studied in connection with optical devices,[4–7] electric[8,9] or ion[10–12] conductivity, ion permeation,[13] capture of metal ions[14] or endcrine disruptors[14,15] chiral separation,[16–18] and gas separation.[19] Among many applications of DNA molecules, the authors focused their attention on separation membranes from DNA since membranes will play an important role in environmental and energy related processes.[20,21] Chiral separation is an indispensable method in industries dealing with pharmaceuticals, agricultural chemicals, perfumes, and so forth. There are a couple of ways, such as asymmetric synthesis and resolution of racemates, to obtain optically pure enantiomers. Though asymmetric synthesis of pure enantiomers has been developed, its development is still slow. The resolution of racemates is still main methods for the production of enantiomerically pure compounds in industry. Among chiral separation technologies, such as diastereomer crystallization, preferential crystallization, chemically kinetic resolution, enzymatic kinetic resolution, chromatography, and membrane separation, the last process with membranes is perceived as economically and ecologically competitive to other conventional chiral separation methods to separate a pair of enantiomers. Membrane separation technology can be carried out continuously under mild conditions and process scale-up is relatively easy. To this end, the authors has reported chiral separation of racemic amino acids with novel membranes from polyion complex, which were prepared from DNA sodium salt (DNA-Na) and polydiallyldimethylammonium chloride (PDADMAC), because DNA is considered as a polyanion with a huge molecular weight.[18] In the present study, poly(4-vinylbenzyl)trimethylammonium chloride (PVBTMAC) was adopted as a corresponding counter polycation for polyion complex formation. Novel polyion complex membranes were prepared from DNA-Na and PVBTMAC and chiral separation of racemic amino acids through those polyion complex membranes were studied.

EXPERIMENTAL

Materials

Fiberlike purified DNA sodium salt (DNA-Na) of 93% from salmon milt (Nippon Chemical Feed Co.) was used. (4-Vinylbenzyl)trimethylammonium chloride (VBTMAC) was kindly provided by AGC SEIMI CHEMICAL CO., LTD. and used without further purification. 2,2′-Azobis[2-methyl-N-(2-hydroxyethyl)-propionamide] (AMHP), D-glutamic acid (D-Glu), L-glutamic acid (L-Glu), D-lysine (D-Lys), L-lysine (L-Lys), D-phenylalanine (D-Phe), L-phenylalanine (L-Phe), NaBr, KCl and NaN$_3$ (fungicide) were used without further purification. Water purified with an ultrapure water system (Simpli Lab, Millipore S. A., Molsheim, France) was used.

Poly(4-vinylbenzyl)trimethylammonium chloride (PVBTMAC) was synthesized as follows: into a 270 g of purified H$_2$O, 30.000 g (1.42×10^{-1} mol) of

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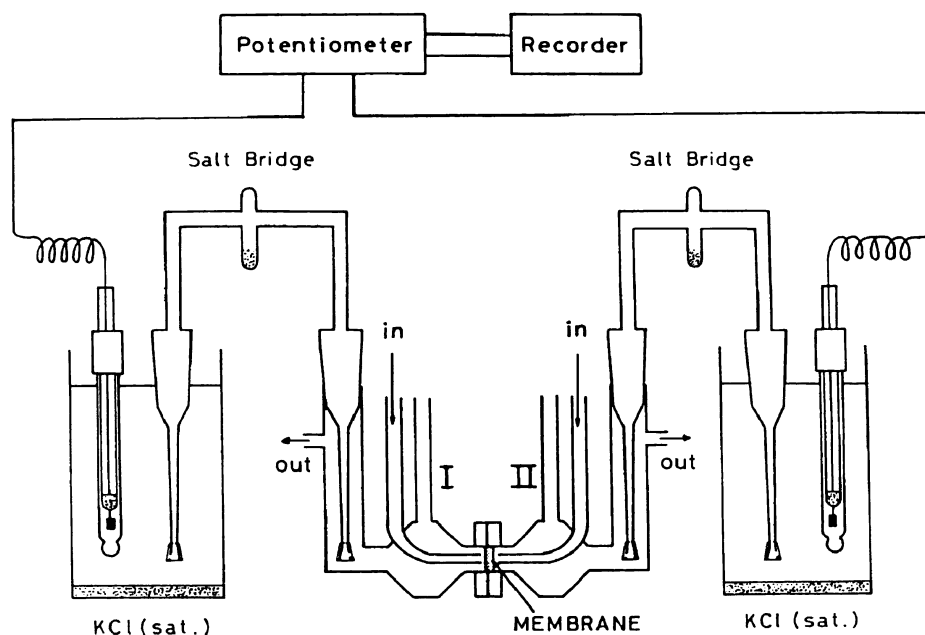
Figure 1. Schematic diagram of the cell used for the measurement of membrane potentials.

VBTMAC and $0.204\,g$ $(7.07 \times 10^{-4}\,mol)$ of radical initiator AMHP were dissolved. The reaction mixture was stirred at $70\,°C$ under a nitrogen atmosphere for $21\,h$. And then the reaction mixture was heated at $80\,°C$ for an additional $23\,h$. Aqueous PVBTMAC solution, of which concentration was nominally 10%, was obtained. The polymer solution thus obtained was diluted in purified H_2O to the final polymer concentration of *ca.* 1.0%.

$$\left[CH_2 - CH \right]_n$$

PVBTMAC
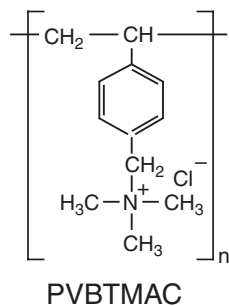
Membrane Preparation

In the present study, the composition of the base pairs of DNA-Na was not studied. Here, 662.0, the average value of base pairs of DNA-Na was adopted as an average molecular weight of the constitutional repeating unit (base pair). Polyion complex membranes were prepared from DNA-Na and PVBTMAC. DNA-PVBTMA(60/40) was prepared as follows: $10.000\,g$ of 1.2% DNA-Na aqueous solution $(1.81 \times 10^{-4}\,mol$ of base pairs) and $5.118\,g$ of 1.0% aqueous PVBTMAC solution $(2.42 \times 10^{-4}\,mol$ of constitutional repeating units) were added to a $200.0\,g$ of $20\,wt.\%$ 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 $0.20\,MPa$. The retentate was thoroughly washed with pure H_2O at the operation pressure of $0.20\,MPa$ so that the NaBr concentration in the filtrate (the permeate) could reach $2.0 \times 10^{-3}\,mol\,dm^{-3}$, which was confirmed by conductimetry. DNA-PVBTMA(50/50) membrane was prepared from $10.000\,g$ of 1.2% DNA-Na aqueous solution and $7.678\,g$ of 1.0% aqueous PVBTMAC solution $(3.63 \times 10^{-4}\,mol$ of constitutional repeating units) and DNA-PVBTMA(40/60) from $10.000\,g$ of 1.2% DNA-Na aqueous solution and $11.516\,g$ of 1.0% aqueous PVBTMAC solution $(5.44 \times 10^{-4}\,mol$ of constitutional repeating units). The polyion complex membrane thus obtained could not be removed from the regenerated cellulose UF membrane.

Membrane Potential Measurement

The membrane potential was measured by using the standard laboratory set-up.[22] The schematic diagram of the apparatus used for measuring membrane potentials is shown in Figure 1. The electromotive force which across between the bulk solution was conducted by saturated KCl bridge 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[23]

Table I. Membrane potential and transport numbers for K^+ and Cl^- in DNA-PVBTMA polyion complex membranes

	membrane potential/mV		transport number	
	$0.2\,mol\,dm^{-3}$ side	$0.1\,mol\,dm^{-3}$ side	$t(K^+)$	$t(Cl^-)$
DNA-PVBTMA (60/40)	0	0.50	0.514	0.486
DNA-PVBTMA (50/50)	0.07	0	0.498	0.502
DNA-PVBTMA (40/60)	4.60	0	0.371	0.629

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

where C_I and C_{II} represent the concentration 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 by following the procedures described in the previous paper.[24] All experiments were carried out at 40 °C.

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

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

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

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

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

RESULTS AND DISCUSSION

Characterization of Polyion Complex Membranes

Before studying chiral separation of racemic amino acids with those DNA-PVBTMA polyion complex membranes thus prepared, the membrane potentials for those three types of membranes were measured to characterize them. Using observed membrane potentials, transport numbers of K^+ and Cl^- in the polyion complex membranes were determined and those calculated values are summarized in Table I together with the corresponding membrane potentials. The transport numbers of K^+ and Cl^- in aqueous solution were reported to be around 0.4905 and 0.5095, respectively.[25] The transport number of K^+ for DNA-PVBTMA(60/40) membrane was determined to be 0.514, which was slightly higher than that in bulk aqueous solution of 0.4905. From this, it was revealed that the amount of fixed anionic charge site was more than that of cationic charged one. This coincides with

the fact that DNA-PVBTMA(60/40) nominally consisted of 1.81×10^{-4} mol of base pairs and 2.42×10^{-4} mol of PVBTMAC constitutional repeating units. The transport number of K^+ and Cl^- for DNA-PVBTMA(50/50) membrane were close to those in aqueous solution. This leads to the conclusion that DNA-PVBTMA(50/50) membrane was electrically neutralized, which can be supported by the composition of polyion complex membrane. On the other hand, the transport number of K^+ for DNA-PVBTMA(40/60) membrane was determined to be 0.371, which was much more lower than that in aqueous solution. This revealed that there was excess amount of cationic charges in the DNA-PVBTMA(40/60) membrane. This also coincides with the membrane preparation process that it nominally consisted of 1.81×10^{-4} mol of base pairs and 5.44×10^{-4} mol of PVBTMAC constitutional repeating units.

Chiral Separation of Racemic Amino Acids with Polyion Complex Membranes

In the present study, three types of racemic amino acids were adopted as model racemic mixtures, such as racemic glutamic acids (Glu's), which have very polar anionic side chains, racemic lysines (Lys's) with very polar cationic side chains, and racemic phenylalanines (Phe's), having aromatic side chains. Time transport curves for those three types of racemic amino acids through DNA-PVBTMA(60/40) membrane are shown in Figure 2, those through DNA-PVBTMA(50/50) membrane in Figure 3, and those through DNA-PVBTMA(40/60) in Figure 4, respectively.

D-Lys was transported in preference to the corresponding L-isomer through negatively charged DNA-PVBTMA(60/40) and positively charged DNA-PVBTMA(40/60) membranes, while chiral separation of racemic Lys's through the electrically neutral DNA-PVBTMA(50/50) membrane was hardly observed. The electrically neutral DNA-PDADMA polyion complex membrane transported the L-isomer of tryptophan or phenylalanine in preference to the corresponding D-isomer in the previous study.[18] As for other racemic amino acids, such as racemic Glu's and Phe's, optical resolution was hardly observed in the present study. Transport experiments for three
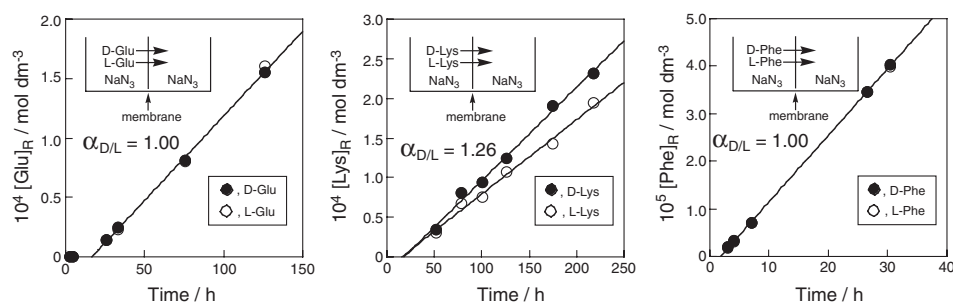
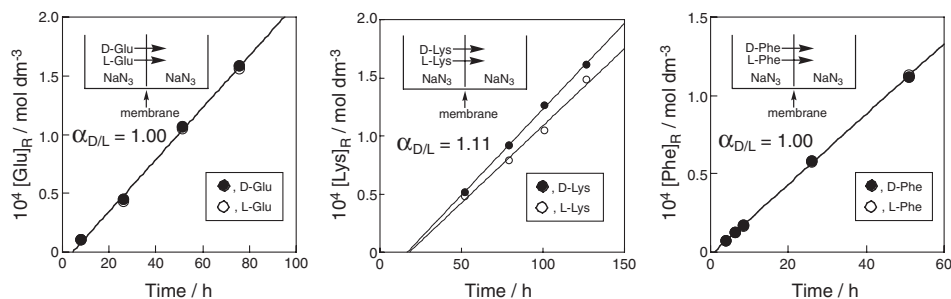**Figure 2.** Time-transport curves of three types of racemic amino acids through DNA-PVBTMA(60/40) membranes at 40 °C.
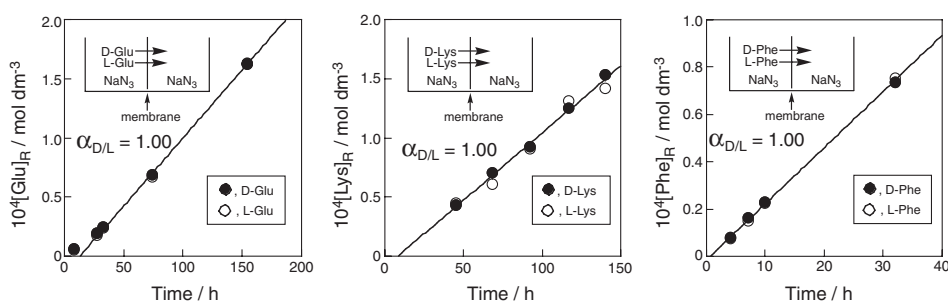
Figure 3. Time-transport curves of three types of racemic amino acids through DNA-PVBTMA(50/50) membranes at 40 °C.

Figure 4. Time-transport curves of three types of racemic amino acids through DNA-PVBTMA(40/60) membranes at 40 °C.

types of racemic amino acids through those three types of polyion complex membranes are summarized in Table II. Permselectivities toward D-Lys for DNA-PVBTMA(60/40) and DNA-PVBTMA(40/60) membranes were determined to be 1.26 and 1.11, respectively.

In order to elucidate the mechanism for the expression of chiral separation, adsorption selectivities of those three types of membranes toward racemic amino acids were studied. The results of adsorption experiments are summarized in Table III. In the table, the amount of amino acid adsorbed in the membrane is given as a relative amount to total charged site or excess ionic site in each membrane. In the present study, the surface of those three types of membranes seemed to be slightly swollen compared to adsorption selec-

tivity experiments of DNA-PDADMA polyion complex membranes.[18] Among those data, L-Lys was remarkably adsorbed in DNA-PVBTMA(60/40) and DNA-PVBTMA(40/60) membranes in preference to D-Lys, and those adsorption selectivities were determined to be 0.33 and 0.39, respectively. Those adsorption selectivities are converted to be 3.03 and 2.56, respectively, if they are given toward L-isomer ($S_{A(L/D)}$). In the previous study, DNA-PDADMA polyion complex membrane also showed adsorption selectivity toward the L-isomer,[18] and those values were reported to be 1.24 for L-tryptophan and 1.17 for L-Phe, respectively. Compared to adsorption selectivities of L-Lys for DNA-PVBTMA(60/40) and DNA-PVBTMA(40/60) membranes, other adsorption selectivities were close to unity. In the present study,

Table II. Transport of racemic amino acids through DNA-PVBTMA polyion complex membranes

Membrane	Amino Acid	$10^8 J_D/\text{mol cm}^{-2}\,\text{h}^{-1}$	$10^8 J_L/\text{mol cm}^{-2}\,\text{h}^{-1}$	$\alpha_{D/L}$
DNA-PVBTMA(60/40)	Phe	1.87	1.87	1.00
DNA-PVBTMA(60/40)	Glu	1.90	1.90	1.00
DNA-PVBTMA(60/40)	Lys	1.57	1.25	1.26
DNA-PVBTMA(50/50)	Phe	3.17	3.17	1.00
DNA-PVBTMA(50/50)	Glu	1.47	1.47	1.00
DNA-PVBTMA(50/50)	Lys	1.51	1.51	1.00
DNA-PVBTMA(40/60)	Phe	3.00	3.00	1.00
DNA-PVBTMA(40/60)	Glu	2.95	2.95	1.00
DNA-PVBTMA(40/60)	Lys	1.97	1.77	1.11

Table III. Adsorption selectivities of DNA-PVBTMA polyion complex membranes toward racemic amino acids

Membrane	Amino Acid	10^2(D-AA) (anionic site)	10^2(L-AA) (anionic site)	10^2(D-AA) (cationic site)	10^2(L-AA) (cationic site)	10^2(D-AA) (excess ionic site)	10^2(L-AA) (excess ionic site)	$S_{A(D/L)}$
DNA-PVBTMA(60/40)	Glu	0.090	0.075	0.135	0.113	0.270	0.226	1.19
DNA-PVBTMA(60/40)	Lys	0.488	1.395	0.733	2.093	1.465	4.185	0.33
DNA-PVBTMA(60/40)	Phe	0.231	0.218	0.347	0.327	0.693	0.654	1.06
DNA-PVBTMA(50/50)	Glu	0.785	0.862	0.785	0.862	—	—	0.91
DNA-PVBTMA(50/50)	Lys	0.519	0.595	0.519	0.595	—	—	0.87
DNA-PVBTMA(50/50)	Phe	0.085	0.094	0.085	0.094	—	—	0.90
DNA-PVBTMA(40/60)	Glu	0.314	0.291	0.209	0.194	0.627	0.582	1.08
DNA-PVBTMA(40/60)	Lys	0.617	1.519	0.411	1.012	1.234	3.037	0.39
DNA-PVBTMA(40/60)	Phe	0.199	0.217	0.133	0.145	0.398	0.435	0.91

L-Lys, which is thought to be positively charged under experimental conditions, was preferentially incorporated into two types of DNA-polyion complex membranes, such as that with excess anionic charge site and that positively charged. If Coulombic interaction were the dominant factor for chiral recognition, L-Lys would not be incorporated into DNA-PVBTMA(40/60) membrane, which was positively charged. However, L-Lys was observed to be selectively incorporated into DNA-PVBTMA(40/60) membrane. This revealed that Coulombic interaction between L-Lys and charged site in a polyion complex membrane might not be the dominant factor for the present adsorption selectivity. The interaction between amino acids and 2-deoxyribose and that between amino acids and bases are enumerated as the interactions for the chiral recognition. At least, it can be said that similar site works as a chiral recognition in negatively charged DNA-PVBTMA and positively charged DNA-PVBTMA polyion complex membranes to discriminate L-Lys and the corresponding D-Lys. However, at the moment, we cannot specify the precise interaction mode for chiral recognition.

The diffusivity selectivity ($S_{D(D/L)} = D_D/D_L$, where D_D and D_L are the diffusion coefficients of the D-isomer and the L-isomer, rerspectively) for each reacemic mixtures can be determined using permselectivity ($\alpha_{D/L}$) and adsorption selectivity ($S_{A(D/L)}$). The estimated diffusivity selectivities are summarized in Table IV together with permselectivities and adsorption selectivities. Results summarized in Table IV revealed that the chiral separation was mainly governed by the diffusivity selectivity toward the target molecule. Contrary to adsorption selectivity, D-Lys,

Table IV. Results of chiral separation with DNA-PVBTMA polyion complex membranes

Membrane	Amino Acid	$\alpha_{D/L}$	$S_{A(D/L)}$	$S_{D(D/L)}$
DNA-PVBTMA(60/40)	Glu	1.00	1.19	0.84
DNA-PVBTMA(60/40)	Lys	1.26	0.33	3.82
DNA-PVBTMA(60/40)	Phe	1.00	1.06	0.94
DNA-PVBTMA(50/50)	Glu	1.00	0.91	1.10
DNA-PVBTMA(50/50)	Lys	1.00	0.87	1.15
DNA-PVBTMA(50/50)	Phe	1.00	0.90	1.11
DNA-PVBTMA(40/60)	Glu	1.00	1.08	0.93
DNA-PVBTMA(40/60)	Lys	1.11	0.39	2.85
DNA-PVBTMA(40/60)	Phe	1.00	0.91	1.10

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

which was not preferentially incorporated into the membrane, was selectively transported through DNA-PVBTMA(60/40) and DNA-PVBTMA(40/60) membranes. In other words, retarded transport of preferentially adsorbed permeant was observed. This might be due to relatively strong interaction between the preferentially incorporated enantiomer and the membrane. Such phenomena were often observed in chiral separation.[24,26–29]

In the future, it is necessary to suppress the swelling of polyion complex membranes to enhance their permselectivity. Such an approach is currently under investigation in our laboratory.

CONCLUSIONS

Novel polyion complex membranes were prepared from DNA-Na and poly(4-vinylbenzyl)trimethylammonium chloride (PVBTMAC). The newly prepared DNA-PVBTMA polyion complex membranes showed a chiral separation ability toward racemic lysine (Lys) mixtures. The membranes transported D-Lys in preference to the corresponding L-Lys. The permselectivity toward the D-isomer reached 1.26. From transport experiments and adsorption studies, it was revealed that the permselectivity was mainly governed by diffusivity selectivity. The present results suggest that polyion complex membranes, consisting of DNA as a polyanion, have potential to separate racemic enantiomers. The present results indicate the great possibility for practical applications.

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