Chitin Nanofiber Membranes for Chiral Separation

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Abstract

Nanofiber membranes for chiral separation were prepared from chitin, which is the most abundant natural amino polysaccharide. The membrane showed chiral separation ability by adopting concentration gradient as a driving force for membrane transport. In other words, the chitin nanofiber membrane selectively transported the D-isomer of glutamic acid (Glu), phenylalanine (Phe), and lysine (Lys) from the corresponding racemic amino acid mixtures.

Keywords: Chiral separation, Chitin, Membrane, Nanofiber membrane, Optical resolution

1. Introduction

L-Glutamic acid (L-Glu), which is usually used as a seasoning in Japan, is tasty, while the corresponding D-glutamic acid (D-Glu) gives a different taste. This is one of familiar examples. A lot of optically active compounds show different physiological activities, depending on their mirror image isomers (Voet *et al.*, 1990; McKee *et al.*, 2003). From this, the production of optically pure compounds is an important process in various industries, involving pharmaceuticals, agrochemicals, food additives, fragrances, and so forth. Asymmetric synthesis is thought to be an ultimate way to obtain enantiometrically pure compounds from a standpoint of green chemistry. However, the development of asymmetric synthesis is not so fast. Chiral separations, such as crystallization resolution, kinetic resolution, chromatographic separation, membrane-based separation and so forth, are still mighty and promising methods to obtain optically pure compounds. Among those chiral separation methods, membrane-based separation is ecologically and economically competitive to other chiral separation methods. Membrane-based separation is continuously operated under mild conditions and process scale-up is relatively easy. From above, membrane-based separation can be called "green separation technology" not only in chiral separation but also in separations of other mixtures. Membrane-based separation with separation membranes fabricated from green polymer will surely become a green separation technology.

The authors research group developed membranes from various naturally occurring or 'green polymers', their derivatives, and wastes from food industries as resources for membrane material so that we can construct sustainable environment and society. To this end, the authors' research group developed membranes from various raw materials, such as cellulose acetate for optical resolution (Izumi *et al.*, 1997; Yoshikawa *et al.*, 1999), egg shell membranes for chiral separation (Kondo *et al.*, 2001), agarose for pervaporation (Yoshikawa *et al.*, 2000, 2002), gelatin for vapor permeation (Yoshikawa *et al.*, 2004a), proteins from *Geobacillus thermodenitrificans* DSM465 for vapor permeation (Yoshikawa *et al.*, 2004b) and molecular recognition (Yoshikawa *et al.*, 2006, 2007a), DNA for gas (Matsuura *et al.*, 2006) and chiral separation (Yoshikawa *et al.*, 2007b, 2007c; Iwamoto *et al.*, 2009), chitosan for chiral (Iwamoto *et al.*, 2010a) and vapor permeation (Iwamoto *et al.*, 2010b), optical

resolution with molecularly imprinted nanofiber membranes from cellulose acetate (Sueyoshi *et al*., 2010), and keratin for optical resolution (Sueyoshi *et al*., 2011).

Chitin, which is an abundant green polymer on the earth and is main component of exoskeletons of arthropods, such as crustaceans and insects, is one of promising green polymers as a membrane material. Chitin is a polymer of N-acetyl glucosamine and containing chiral environment, which is expected to discriminate absolute configuration of a given mixture of enantiomers. One of the authors has recently isolated chitin nanofibers from the exoskeletons of crabs and shrimps (Ifuku *et al*., 2009, 2010). The obtained chitin nanofibers have a highly uniform structure of 10-20 nm in width and a high aspect ratio. Furthermore, as chitin nanofibers consist of an antiparallel extended crystalline structure, they have excellent mechanical properties, including a high Young's modulus, high fracture strength and low thermal expansion. Given their characteristic nano-form and excellent physical properties, chitin nanofibers are strong candidates for a membrane for chiral separation. To this end, chitin nanofiber membranes were fabricated and their chiral separation ability was studied adopting racemic mixture of amino acids as model racemates.

2. Experimental Section

2.1 Materials

Chitin powder from crab shells was purchased from Nacalai Tesque. D-Glutamic acid (D-Glu), L-glutamic acid (L-GLu), D-phenylalanine (D-Phe), L-phenylalanine (L-Phe), D-lysine (D-Lys), and L-lysine (L-Lys) were used as received. Water purified with an ultrapure water system (Simpli Lab, Millipores S. A., Molsheim, France) was used.

2.2 Membrane Preparation

Chitin nanofibers were prepared from commercially available chitin powder derived from crab shells according to a previously described procedure (Ifuku *et al*., 2010). Fibrillated chitin nanofibers were dispersed in water at a fiber content of 0.1 wt%. The suspension was vacuum-filtered using a hydrophilic polytetrafluoroethylene membrane filter (Millipore, pore size: 0.2 mm). The obtained chitin nanofiber membrane was hot-pressed at 100 °C for 30 min to obtain a dried sheet. The thickness of the chitin nanofiber membrane thus obtained was determined to be 85 μm.

2.3 Enantioselective Transport

A membrane (area, 3.0 cm^2) was fixed tightly between two chambers of a permeation cell. The volume of each chamber was 40.0 cm^3. An aqueous solution of racemic mixture of amino acid was placed in the left-hand side chamber and an aqueous solution in the right-hand side chamber. Each concentration of racemic amino acid was fixed to be 1.0 x 10^{-3} mol dm^{-3}. All experiments were carried out at 40 °C. The amounts of the D- and L-isomers that transported through the membrane were determined by liquid chromatography (LC) [JASCO PU 1580, equipped with a UV detector (JASCO 1570)] employing a CHIRALPAK MA (+) column (50 mm x 4.6 mm (i.d.)) (Daicel Chemical Ind. Ltd.) for the analyses of racemic Glu's and Phe's, and a CROWNPAK CR (+) column (150 x 4.0 mm (i.d.)) for the measurement of racemic Lys's. An aqueous copper sulfate solution was used as a mobile phase for Glu and Phe analyses, and a perchloric acid solution as eluent for Lys analysis.

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

$$J = Q\delta/At \tag{1}$$

where Q (mol) denotes the amount of transported amino acid, δ (cm) membrane thickness, A (cm^2) the membrane area, and t (h) is the time.

The permselectivity, $\alpha_{i/j}$, is defined as the flux ratio, J_i/J_j, divided by the concentration ratio ([i-AA]/[j-AA]):

$$\alpha_{i/j} = (J_i/J_j)/([i\text{-}AA]/[j\text{-}AA]) \tag{2}$$

The subscripts D and L refer to the D-isomer of amino acid and the L-isomer of amino acid, respectively.

2.4 Adsorption Selectivity

The membrane was immersed in a 1.0 x 10^{-3} mol dm^{-3} racemic amino acid solution and the mixture was allowed to equilibrate at 40 °C. A 0.02 wt. % sodium azide was added as a fungicide. The amount of amino acid in the supernatant subtracted from the amount initially in the solution gave the amount of the amino acid adsorbed by the membrane. Quantitative analyses were done as above.

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

$$S_{A(i/j)} = ((i\text{-}AA)/(j\text{-}AA))/([i\text{-}AA]/[j\text{-}AA]) \tag{3}$$

where (i-AA) and (j-AA) denote the amount of enantiomer of amino acid adsorbed in the membrane, and [i-AA] and [j-AA] are the concentrations in the solution after equilibrium had been reached, respectively.

3. Results and Discussion

Three types of racemic amino acid were adopted as model racemates. Among nineteen types of amino acid, racemic glutamic acids (Glu's) with very polar anionic side chains, racemic phenylalanine (Phe's) having aromatic side chain, and racemic lysine (Lys's) with very polar cationic side chains, were adopted as model racemates in the present study.

Concentration gradient was adopted as a driving force for membrane transport of above amino acids. As an example, time-transport curves of racemic mixture of three types of amino acid are shown in Figure 1. The D-isomer of Phe was transported in preference to the corresponding L-isomer. The permeselectivity toward D-Phe was determined to be 1.16. As for other amino acids, such as Glu and Lys, the D-isomer was preferentially transported through the present chitin nanofiber membrane like Phe. The permselectivies were determined to be 1.04 for racemic Glu's and 1.07 for racemic Lys's, respectively. The permselectivities for the present chitin nanofiber membranes were not so high comparing with membrane performances previously reported (Maier. *et al*., 2007; Xie *et al*., 2008; Higuchi *et al*., 2010).

Adsorption selectivities of those membranes toward three types of racemic amino acid were studied so that the mechanism for the expression of permselectivities could be elucidated. In Table 1, the amounts of amino acids adsorbed in the membrane and adsorption selectivities are given. The L-isomers of Glu and Phe were incorporated into the membrane in preference to the corresponding antipodes, while D-Lys was selectively adsorbed in the membrane. Especially, the adsorption selectivity toward L-Phe reached 2.33. From permselectivity and adsorption selectivity, diffusivity selectivity, $S_{D(i/j)}$, can be determined by equation (4).

$$S_{D(i/j)} = \alpha_{i/j}/S_{A(i/j)} \; (= D_i/D_j) \tag{4}$$

where D_i and D_j are diffusion coefficients of i-isomer and j-isomer, respectively. The diffusivity selectivities for those three types of amino acid are summarized in Table 2 together with flux values and other selectivities, such as permselectivity and adsorption selectivity. As for the transport phenomena of Glu and Phe, the enantiomer, which was less incorporated into the membrane, was quickly diffused within the membrane. In other words, the diffusivity of enantiomer preferentially adsorbed in the membrane was retarded. This retarded diffusion might be due to a relatively strong interaction between the enantiomer selectively adsorbed and the membrane as often observed in chiral separation (Yoshikawa *et al*., 2003, 2007c; Masawaki *et al*., 1992; Aoki *et al*., 1995; Tone *et al*. 1996). On the other hand, the diffusivity selectivity for Lys gave same tendency like adsorption selectivity even though the value of selectivity was not so high.

From the intercept of the steady-state portion of time-transport curve in Figure 1, the time lag, for a given enantiomer is determined (Mulder, M., 1996). Using those time lags, diffusivity selectivity can be estimated by equation (5).

$$S_{D(i/j)} = \theta_j/\theta_i \tag{5}$$

As can be seen in Figure 1, the time lag for the D-isomer was shorter than that for the L-isomer. This leads to the conclusion that the diffusion coefficient for D-amino acid was higher than that for the corresponding L-isomer. As a result, it was qualitatively concluded that the diffusivity selectivity toward D-amino acid was over unity. It was revealed that the D-isomer diffused within the membrane faster than the L-isomer. The diffusivity selectivity qualitatively elucidated from time lag data coincided with that determined from permselectivity and adsorption selectivity.

In our previous study of enantioselective transport of racemic amino acids through molecularly imprinted membranes (Yoshikawa *et al*., 2003), applying an optimum potential difference as the driving force, the permselectivity, reflecting adsorption selectivity, was attained. In other words, applying enantioselective electrodialysis, permselectivity, which corresponds to the adsorption selectivity, was attained. In the present study on optical resolution of racemic Phe's, additional potential difference was applied by electrodialysis to obtain permselevctivity, reflecting adsorption selectivity. Against expectation, permselectivity was hardly observed. This might be due to the fact that enantiomers non-specifically dragged by applied potential difference diffused through the mesh between chitin nanofiber, which was still too large to express permselectivity. Narrowing mesh size between chitin nanofibers or plugging the mesh with some polymeric materials would enhance permselectivity.

4. Conclusions

Nanofiber membranes for chiral separation were prepared from chitin, which is the most abundant natural amino polysaccharide. The membrane showed chiral separation ability by adopting concentration gradient as a driving force for membrane transport. In other words, the chitin nanofiber membrane transported the D-isomer of glutamic acid (Glu), phenylalanine (Phe), and lysine (Lys) from the corresponding racemic amino acid mixtures faster than the corresponding L-isomer.

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Table 1. Adsorption of racemic mixture of amino acids in chitin nanofiber membrane

Amino Acid	(Amino Acid)/mem.		$S_{A(D/L)}$	$S_{A(L/D)}$
	10^5 mol/g-mem.	10^3 mol/CRU[a]		
D-Glu	5.18	9.27	0.96	1.04
L-Glu	5.41	9.68		
D-Phe	1.57	2.81	0.43	2.33
L-Phe	3.66	6.55		
D-Lys	3.01	5.38	1.04	0.96
L-Lys	2.90	5.18		

[a] Constitutional repeating unit of chitin.

Table 2. Results of chiral separation with chitin nanofiber membrane[a]

Amino Acid	J_D	J_L	$\alpha_{D/L}$ $(\alpha_{L/D})$	$S_{A(D/L)}$ $(S_{A(L/D)})$	$S_{D(D/L)}$ $(S_{D(L/D)})$
	mol cm cm^{-2} h^{-1}	mol cm cm^{-2} h^{-1}			
Glu	7.93 x 10^{-10}	7.59 x 10^{-10}	1.04 (0.96)	0.96 (1.04)	1.08 (0.92)
Phe	1.25 x 10^{-9}	1.08 x 10^{-9}	1.16 (0.86)	0.43 (2.33)	2.70 (0.37)
Lys	1.18 x 10^{-9}	1.10 x 10^{-9}	1.07 (0.93)	1.04 (0.96)	1.03 (0.97)

[a] $\alpha_{i/j} = S_{D(i/j)} \times S_{A(i/j)}$ [i = D, j = L or i = L, j = D]
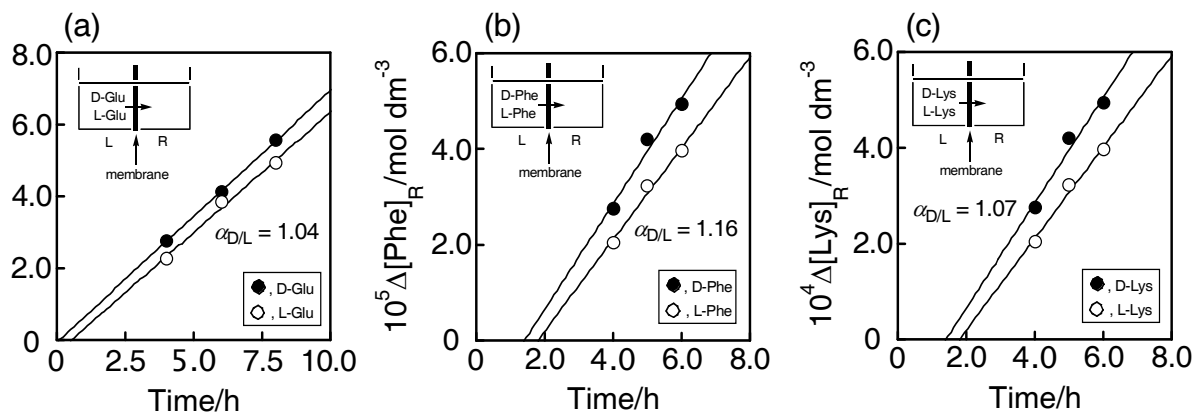
Figure 1. Time-transport curves of racemic mixtures of Glu's (a), Phe's (b), and Lys's (c) through the chitin nanofiber membrane at 40 °C

$([\text{D-AA}]_0 = [\text{L-AA}]_0 = 1.0 \times 10^{-3}$ mol dm^{-3}; AA: Glu, Phe, or Lys.)