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Molecularly imprinted polyamide membranes for chiral recognition

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SUMMARY

Molecularly imprinted polymeric membranes were prepared from amorphous poly(hexamethylene terephthalamide/isophthalamide). Membranes imprinted by Z-D-Glu recognize the D-isomer, of which absolute configuration is the same as that of the print molecule, in preference to the corresponding L-isomer, and vice versa. The amino acid preferentially adsorbed by the membrane was also selectively permeated by electrodialysis. An optimum separation factor of 2.0 was reached at the applied potential difference of 2.0 V.

INTRODUCTION

Molecular recognition plays an important role in nature, for instance, receptors, enzymes, transporters, and so forth. Developing artificial receptors, in other words, constructing molecular recognition sites artificially, is one of the fascinating and unsolved subjects for chemists. In the last two decades, molecular recognition with synthetic compounds, such as crown ethers (1), cyclodextrins (2), cyclophanes (3) calixarenes (4), molecular clefts (5), and so forth, has been intensively investigated. It will, however, require detailed molecular designs to attain those artificial receptor molecules. The molecular imprinting technique, first proposed by Wulff in 1972 (6), is one of promising and facile methods to impart molecular recognition sites in synthetic polymers (7). In that technique, functional monomers, forming covalent or non-covalent bonds with the print molecule, are radically polymerized so that specific recognition sites toward the target molecule can be introduced into the cross-linked polymers. As mentioned above, the molecular imprinting technique is the most easiest method to generate synthetic macromolecules with molecular recognition ability. However, the molecularly imprinted polymeric materials, prepared by conventional molecular imprinting, are not reusable. In other words, once the molecularly imprinted material, of which recognition site is prepared for the recognition of a given target molecule, falls into disuse, such a material cannot be applicable to the recognition of other molecule, and will do nothing but will be discarded. In addition to this, diversity of molecularly imprinted materials are not expected by applying conventional molecular imprinting technique, even though it was a pioneering methodology. Based on this, the authors' research group has proposed another method

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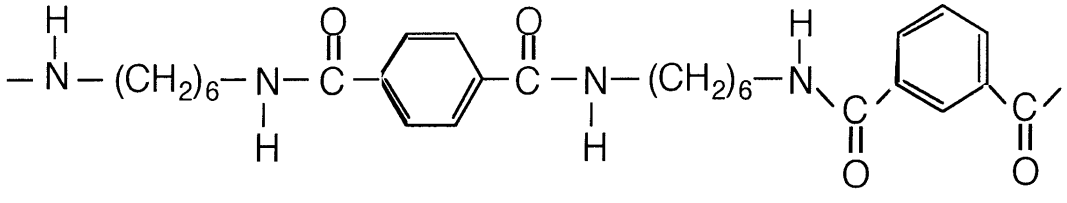
since 1994 (8), which has been named an alternative molecular imprinting technique. Contrary to the conventional molecular imprinting, molecular recognition sites are formed at the same time as the molecular imprinting materials are prepared from the polymer solution. This implies that molecular recognition sites can be introduced in various materials, such as oligopeptide derivatives (9), derivatives of natural polymer (10), and synthetic polymers (11). In addition to this, as expected from the formation mechanism of imprinted materials, once the molecularly imprinted polymer, which was prepared for the recognition of a given target molecule by applying an alternative molecular imprinting technique, falls in disuse, the imprinted polymer can be dissolved and converted into another molecular recognition material by adopting a different target molecule as a print molecule. In other words, molecularly imprinted polymeric materials prepared by our method can be reusable.

It is interesting to investigate whether molecular recognition sites are formed from other synthetic polymers as well as carboxylated polysulfones (11). To this end, the authors adopted amorphous polyamide poly(hexamethylene terephthalamide/isophthal-amide) as a candidate material to form molecular recognition sites. And chiral recognition of racemic amino acid derivatives were investigated.

SELECTION OF CANDIDATE MATERIALS FORMING MOLECULAR RECOGNITION SITES

It is necessary to think about the properties, that candidate materials forming molecular recognition sites by the alternative molecular imprinting technique should be possessed of. First of all, though it is a matter of course, the candidate polymer, which constructs molecular recognition sites, shows solvent resistance toward solvent or solutions, where they are used to recognize target molecules. Otherwise swelling of the materials leads to the structural deformation of recognition site, which is prepared by the presence of a print molecule. Following properties are also required to prevent from structural deformation of generated recognition sites and to retain the "molecular memory" in the molecularly imprinted materials; the glass transition temperatures of candidate polymers should be higher than the operating temperature to keep their glassy state or candidate materials give a high crystallinity, which play an important roll as a physical crosslinking. In the present study, the authors intended to obtain a molecularly imprinted material from an amorphous candidate polymer with relatively high glass transition temperature. Poly(hexamethylene terephthalamide/isophthalamide) (6T/6I = 31.6/68.4, mol/mol) is one of polymers matching with the requirement mentioned above (12). So molecularly imprinted polymeric membranes were prepared from poly(hexamethylene terephthalamide/iso-phthalamide) in the presence of an optical pure Boc-D-Trp or Boc-L-Trp as a print molecule and their chiral recognition ability were investigated.



Poly(hexamethylene terephthalamide/isophthalamide)

EXPERIMENTAL

Preparation of molecularly imprinted polymeric membrane

Each polymeric membrane in the present study was prepared from 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) solution, containing the imprinting components. Poly(hexamethylene terephthalamide/isophthalamide) acts not only as a functional polymer having hydrogen binding sites for amino acid recognition, but also as a membrane matrix like molecularly imprinted cellulose acetate (10) or carboxylated polysulfone (11) membrane. N-α-Benzyloxycarbonyl-D-glutamic acid (Z-D-Glu) or N-α-benzyloxycarbonyl-L-glutamic acid (Z-L-Glu) were adopted as print molecules. Mole ratios of 0.125, 0.188, and 0.250 for print molecule to unit mole of polyamide in the membrane preparation process were studied. A 200 mg quantity of poly(hexamethylene terephthalamide/isophthalamide) and a prescribed amount of print molecule, Z-D-Glu or Z-L-Glu, were dissolved in 3.0 cm^3 of HFIP. The amount of Z-Glu was 28.5 mg for the ratio of 0.125, 42.8 mg for 0.188, and 57.1 mg for 0.250, respectively. The HFIP solution thus prepared was poured into a TeflonPFA 7.5 cm diameter flat laboratory dish, and the solvent was allowed to evaporate at ambient temperature for 24 h. The resulting membrane was further dried at 50 °C for 2 h. After drying, the print molecule was extracted from the resultant membrane by a large volume of methanol until the print molecule was hardly detectable in methanol by UV analysis. In the present study, most of added print molecule was leached from the membranes.

Glass transition temperature of the molecularly imprinted membrane

The glass transition temperature of the molecularly imprinted membrane was studied by a Perkin-Elmer DSC-7. After the print molecule was extracted from the membrane, the membrane was immersed in 50 vol. % aqueous ethanol at 40 °C for 3 days. After equilibrium was attained, the membrane to be measured were blotted with a filter paper to remove extra external liquid and then sealed in aluminum sample pans. The sample was cooled to -55 °C and then heated at 20 °C min^{-1} to 200 °C. Nitrogen at flow rate of 20 cm^3 min^{-1} was used throughout the DSC measurement.

Adsorption selectivity

The molecularly imprinted polymeric membranes were immersed in a 1.0 mmol dm^{-3} racemic glutamic acid (D/L-Glu) solution in 50 vol. % aqueous ethanol, and the mixture was allowed to equilibrate at 40 °C for 336 h. A 0.02 wt. % of 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 amino acid adsorbed by the membrane. Quantitative measurement of aliquots of the solution at the initial stage and after equilibrium were made using a high performance liquid chromatography (HPLC) instrument (JASCO PU 1580) equipped with a UV detector (JASCO UV 1570) and a CHIRALPAK MA(+) column (50 x 4.6 (i.d.) mm) (Daicel Chemical Ind., Ltd.) and aqueous copper sulfate solution as an eluent.

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

$$S_{A(i/j)} = ((i\text{-}AA) / (j\text{-}AA)) / (C_i / C_j)$$

where (i-AA) and C_i are the amount of optical isomer (i) amino acid adsorbed in the membrane and the concentration in the solution on after equilibrium was reached, respectively.

Electrodialysis

A 50 vol. % aqueous ethanol solution of racemic Glu was placed in both chambers of the permeation cell. The concentration of racemic Glu was fixed to be 1.0 mmol dm^{-3}, as with the adsorption experiments. The electrodialysis was carried out at 40 °C with stirring, and a constant applied voltage of 2.0 V between platinum black electrodes (10 mm square; distance between the electrodes, 65 mm). Aliquots were drawn from the permeate side at each sampling time. The amounts of D-Glu and L-Glu that permeated through the membrane (J_i, J_j) were determined on an HPLC instrument described above.

The separation factor $\alpha_{i/j}$ is defined as the ratio J_i / J_j divided by the concentration ratio C_i / C_j.

$$\alpha_{i/j} = (J_i / J_j) / (C_i / C_j)$$

RESULTS AND DISCUSSION

The dependence of adsorption of racemic Glu on imprinting conditions is summarized in Figure 1. The results for Z-D-Glu imprinted poly(hexamethylene terephthalamide/isophthalamide) membranes are shown in Figure 1 (a) and (b), and those for Z-L-Glu imprinted poly(hexamethylene terephthalamide/isophthalamide) ones in Figure 1 (c) and (d), respectively. In Figure 1, the amount of adsorbed Glu by the membranes are given in relatives ones, which were converted to those of a repeating unit. All plots gave straight lines; that is, the adsorbed amounts increase linearly with the increase in the molecular imprinting ratio. These two membranes show adsorption selectivity, in other words, give chiral recognition. The membranes imprinted by Z-D-Glu recognize the D-isomer in preference to corresponding L-isomer, and those imprinted by Z-L-Glu recognize the L-isomer in preference to D-isomer. As observed in cellulose acetate (10) or non-chiral carboxylated polysulfone membrane (11), the membranes imprinted by D-isomer recognize the D-isomer, of which absolute configuration is the same as that of the print molecule, and vice versa. In the Z-D-Glu imprinted membranes, the excess amount of amino acid preferentially adsorbed by the membrane was 0.127 times that of the poly(hexamethylene terephthalamide/isophthalamide) repeating unit in the membrane. In the L-isomer imprinted membranes, that was 0.128 times of the repeating unit in the membrane. The adsorption selectivity ($S_{A(i/j)}$) for Z-D-Glu imprinted membranes increased from 1.7 to 3.2 with the decrease in the molecular imprinting conditions from 0.250 to 0.125 and that for Z-L-Glu imprinted poly(hexamethylene terephthalamide/isophthalamide) membranes from 1.7 to 3.1 with the decrease in the membrane preparation conditions.

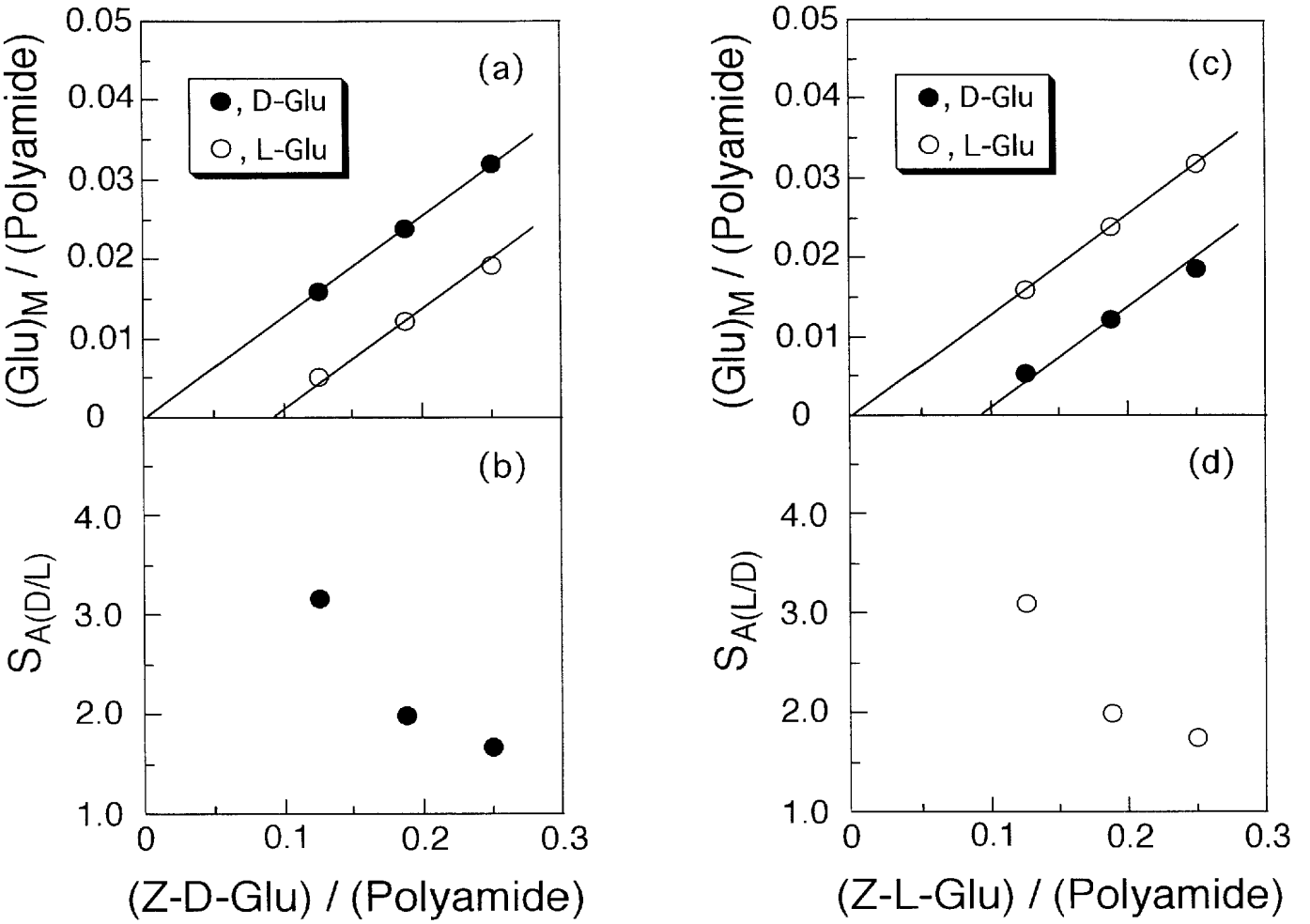
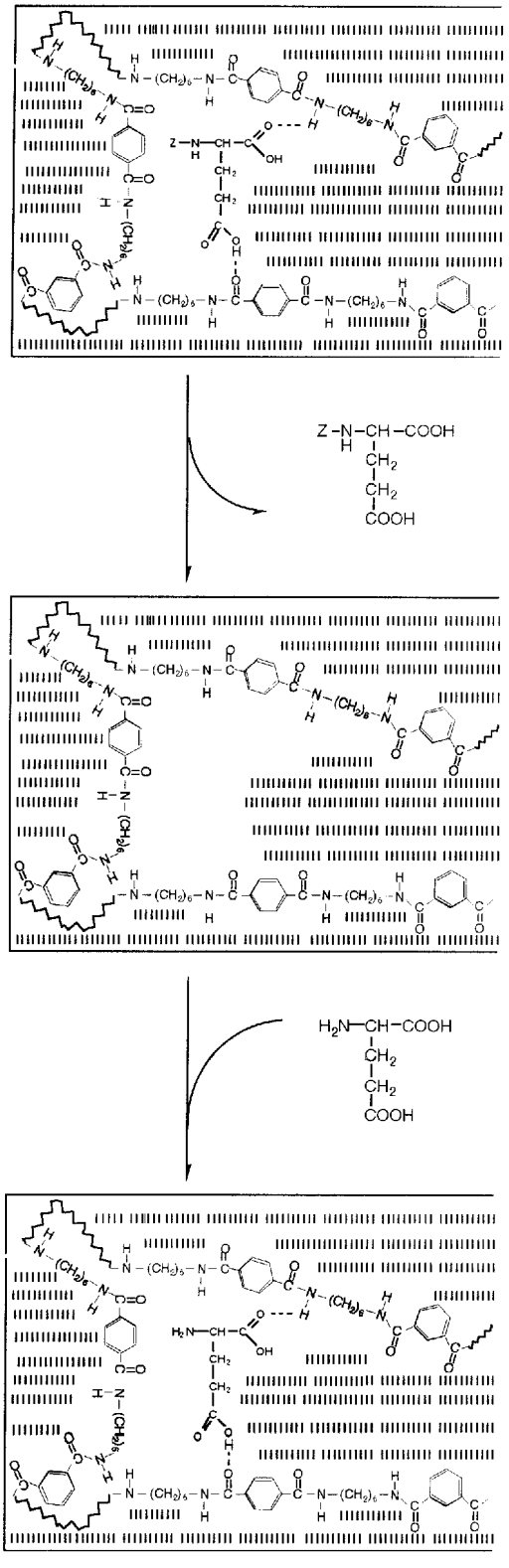


Figure 1 Effect of the molecular imprinting condition on Glu adsorption (a), on adsorption selectivity toward D-Glu (b) of the membrane imprinted by Z-D-Glu and that on Glu adsorption (c), and on adsorption selectivity toward L-Glu (d) of the membrane imprinted by Z-L-Glu.



Step 1

Imprinting during membrane formation process

Step 2

Removal of a print molecule

Step 3

Selective recognition of a target molecule

Z– : $C_6H_5CH_2OCO–$

Figure 2 Tentative scheme of the formation of molecularly imprinted polyamide membrane and its chiral recognition.

From the results given in Figure 1 and those reported previously (8-11), it can be said that the chiral recognition site was induced by the presence of print molecule Z-D-Glu or Z-L-Glu in the membrane preparation process. And the "molecular memory", which was memorized in the membrane preparation process, is retained even in the aqueous ethanol solutions. This is, in addition to adsorption experiments, supported by the fact that the glass transition temperature of the moleclarly imprinted membrane was determined to be 54 °C, which is higher than the operating temperature. In addition to the glass transition temperature of 54 °C, a slight anomaly was observed at 96 °C. This suggests that there is a more rigid segment in the membrane, which is less affceted by the presence of aqueous ethanol in the membrane. A representative scheme for the formation of molecularly imprinted polymeric membranes and the recognition of racemic amino acid mixtures are given in Figure 2. The hydrogen bonding between carboxyl group in Glu and amide moiety in poly(hexamethylene terephthalamide/isophthalamide) and the absolute configuration of side chain of Glu might be the dominant factor to the chiral recognition of racemic Glu.

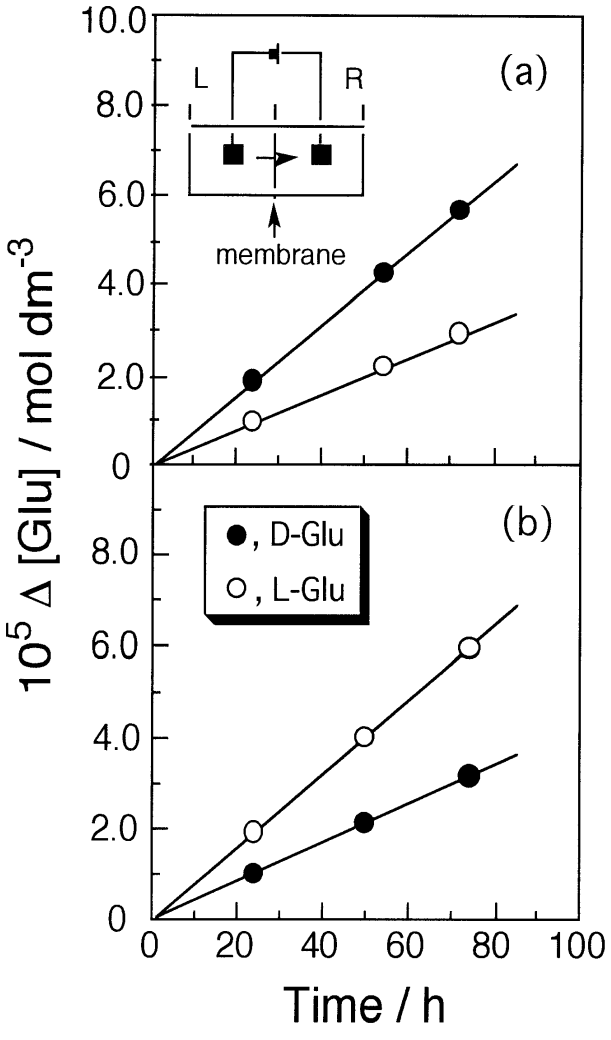


Figure 3 Time-transport curves of D-Glu and L-Glu by electrodialysis at ΔE = 2.0 V through molecularly imprinted polymeric membranes. ((a) (Z-D-Glu) / (Polyamide) = 0.188; (b) (Z-L-Glu) / (Polyamide) = 0.188; $[D\text{-}Glu]_0 = [L\text{-}Glu]_0 = 1.0 \times 10^{-3}$ mol dm^{-3}.)

Enantioselective electrodialysis of racemic Glu with these membranes is a possible application of molecularly imprinted polymeric membranes in the chemical industry. In the present study, the applied potential difference ΔE was fixed to be 2.0 V so that the permselectivity may directly reflect its adsorption selectivity (8-11). The enantioselective electrodialysis experiments were carried out using the imprinted membranes with the imprinting ratio of 0.188, which showed the highest durability among three different imprinted membranes. The time-transport curves of racemic Glu mixtures are shown in Figure 3. From the pH value of racemic Glu solution of 5.4 and the pKa value of Glu (13), the net charge of Glu in the present study was calculated to be -0.93. That is, Glu

was transported to the anode. In Figure 3 (a), the membrane imprinted by Z-D-Glu selectively permeated D-Glu and conversely L-Glu was preferentially permeated through the membrane imprinted by Z-L-Glu as shown in Figure 3 (b). As expected, the pemselectivities for these membranes were determined to be 2.0, which were equal to the adsorption selectivities. The total flux values are 1.65 x 10^{-3} mol cm^{-2} h^{-1} for the Z-D-Glu imprinted membrane and 1.58 x 10^{-3} mol cm^{-2} h^{-1} for the L-isomer imprinted one.

CONCLUSION

Molecularly imprinted polymeric membranes showing optical resolution can be prepared from non-chiral synthetic polyamide poly(hexamethylene terephthalamide/ isophthalamide) by applying by an alternative molecular imprinting technique. The membrane imprinted by D-isomer recognizes D-isomer in preference to the corresponding L-isomer, and vice versa. Electrodialysis of the racemic amino acid showed that permselectivity directly reflects its adsorption selectivity.

ACKNOWLEDGEMENT

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