Enantioselective Electrodialysis of *N*-α-Acetyltryptophans through Molecularly Imprinted Polymeric Membranes

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Molecularly imprinted polymeric membranes, bearing tetrapeptide derivative H-Asp(OcHex)-Ile-Asp(OcHex)-Glu(OBzl)-CH_2-, recognized N-α-acetyl-L-tryptophan from racemic N-α-acetyltryptophans. Electrodialysis of racemic N-α-acetyltryptophans gave permselectivity toward L-isomer, reflecting adsorption selectivity.

It is an interesting subject to develop the membranes showing optical resolution.[1] Excepting optical activity, optically active substances, such as racemic amino acids, give same physicochemical properties. It is required to introduce chiral micro environment into molecular recognition site in the membrane so that the membrane may show optical resolution. Molecular imprinting technique might be one of the promising and easy ways to introduce chiral recognition site into synthetic membranes.[2] Such an pioneering approach has been done for the transport of nucleic acid component.[3] In this case, the synthetic polymeric membrane was prepared by radical polymerization in the presence of template molecules.

In the present paper, tetrapeptide derivative, which is expected to render a chiral recognition site, H-Asp(OcHex)-Ile-Asp(OcHex)-Glu(OBzl)-CH_2-, was adopted as a recognition site for optical resolution. This led us to the alternative molecular imprinting technique.[3] The concept of our alternative molecular imprinting is depicted in Figure 1. That is, "molecular memory" of the template is introduced in the membrane simultaneously while the polymeric membrane is prepared from polymer solution, in other words, membrane preparation with simultaneous molecular imprinting. In the present paper, we would like to report the enantioselective electrodialysis of N-α-acetyltryptophans through molecularly imprinted polymeric membranes.

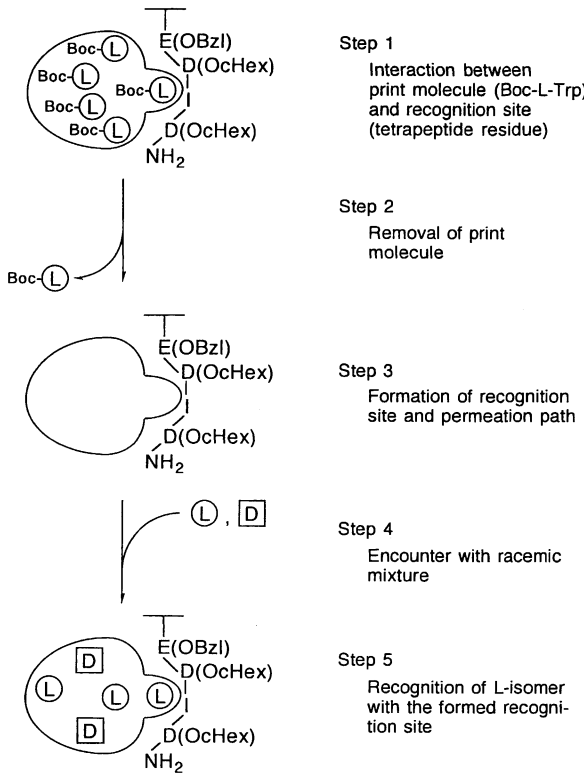
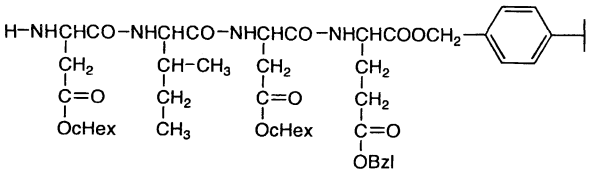


Figure 1. Concept of membrane preparation with simultaneous molecular imprinting.

The polymeric membrane was prepared[4] from tetrahydrofuran solution containing the polystyrene resin bearing tetrapeptide derivative (DIDE-Resin)[5] and copolymer of acrylonitrile and styrene (AS)[6], because DIDE-Resin did not form self-stand membrane by itself.



DIDE-Resin

Figure 2 (a) shows the dependence of adsorption selectivity[7] on the imprinting conditions. The amount of N-α-acetyltryptophans adsorbed in the membrane was given in relative one, which was converted to that of DIDE derivative basis, which was found in the membrane, for convenience of the following discussion. Each amount of isomer adsorbed in the membrane was increased linearly with the increase in the molecular imprinting ratio. Even though molecular imprinting conditions were changed, L-isomer was always incorporated in the membrane in preference to D-isomer, an excess of around 0.8 DIDE derivative over D-isomer adsorbed in the membrane. Below the mole ratio of print molecule to tetrapeptide derivative of 1, most of N-α-acetyltryptophan adsorbed was L-isomer. From the above it can be concluded that the presence of Boc-L-Trp in the membrane preparation process might be effective not only for the formation of non-selective cavity but also for that of molecular recognition site. Namely, Boc-L-Trp in the membrane preparation process can work as a porogen and as a print molecule to shape molecular recognition site. The adsorption selectivity toward N-α-acetyl-L-tryptophan is plotted against the membrane preparation condition and shown in Figure 2 (b). The adsorption selectivity increased with the decrease in (Boc-L-Trp)/(DIDE) ratio and reached 6 at the mole ratio of 0.5. The adsorption selectivity toward L-isomer was increased with the reduction of non-specific cavity by the decrease in mole ratio of print molecule to tetrapeptide derivative in the membrane preparation process.

Enantioselective permeation of racemic N-α-acetyltryptophan mixture was carried out by using concentration difference as a driving force for permeation.[8] Enantioselective permeation was observed. Against adsorption selectivity, D-isomer was preferentially permeated like permeation of racemic tryptophan previously reported.[3] In this case, the separation factor toward L-isomer was calculated to be 0.86.[9] This might be due to the suppression of permeability of L-isomer by the relatively high affinity to the membrane.

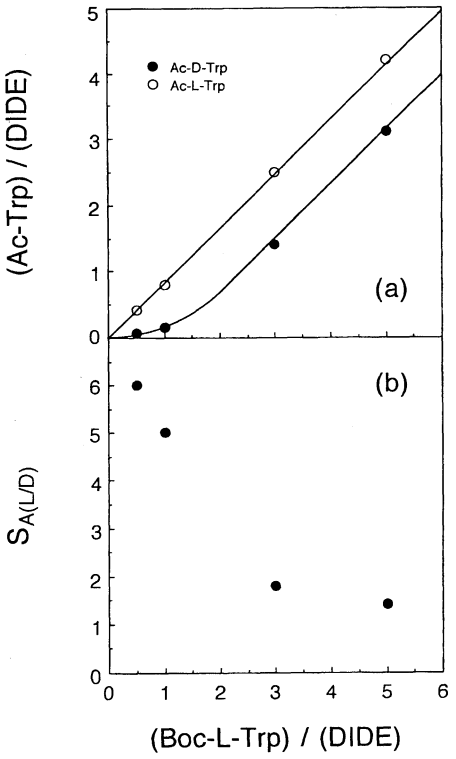


Figure 2. Effect of the membrane preparation conditions on adsorption of N-α-acetyltryptophans.

It is an interesting and crucial subject to permeate the isomer, which is selectively adsorbed in the membrane, in the present study, L-isomer. Electrodialysis was adopted as a membrane transport system to realize such a permselectivity.[10] The effect of applied potential difference on enantioselective electrodialysis of N-α-acetyl-L-tryptophans is given in Figure 3. In the electrodialysis, no induction period for permeation was observed. The amount of each N-α-acetyltryptophan permeated through the membrane was proportional to electrodialysis time. The total flux was linearly proportional to the applied potential difference ΔE. Over 15.0V of ΔE, the enantioselective permeation was not observed, that is, separation factor was determined to be unity. However, the separation factor increased with the decrease in ΔE, and below 2.5V of ΔE, separation factor toward L-isomer, N-α-acetyl-L-tryptophan, reached 6, which was equal to adsorption selectivity.

The present results suggest that the electrodialysis of racemic N-α-acetyltryptophans through the molecularly imprinted polymeric membranes, which was prepared by the technique of membrane preparation with simultaneous molecular imprinting, attained the permselectivity reflecting its adsorption selectivity.

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References and Notes

1 M. Yoshikawa, J. Izumi, T. Kitao, S. Koya, and S. Sakamoto, *J. Membrane Sci.*, **108**, 171 (1995); and Refs. 2-10 therein.
2 G. Wulff, *Angew. Chem., Int. Ed. Engl.*, **34**, 1812 (1995).
3 S. A. Piletskii, I. Y. Dubei, D. M. Fedoryak, and V. P. Kukhar, *Biopolim. Kletka*, **6**, 55 (1990).
4 The prescribed amount of the print molecule Boc-L-Trp, the amount of which being 0.5 to 5.0 times that of the tetrapeptide derivative in the resin, was dissolved in 2 cm^3 of THF with 0.010 g of DIDE-resin (DIDE content, 0.028 mmol / g-Resin). Then 0.190 g of AS was dissolved in the previous THF solution. The THF solution thus prepared was poured onto a flat laboratory dish and the solvent allowed to evaporate. The print molecule was extracted by methanol till the print molecule could scarcely detected by UV analysis.
5 DIDE-Resin was prepared by Merrifield's technique of solid phase peptide synthesis previously described in Ref. 1.
6 Weight fraction of acrylonitrile unit was 0.33 and produced by Ube Cycon, Ltd.
7 Adsorption selectivity toward L-isomer $S_{A(L/D)}$ is defined as $S_{A(L/D)} = ((AA)_L / (AA)_D) / (C_L/C_D)$. In this equation, $(AA)_i$ and C_i are the amount of N-α-acetyltryptophan in the membrane and the concentration in the solution after equilibrium was reached, respectively. The quantitative analyses of N-α-acetyltryptophans were carried out by using HPLC equipped with a CROWNPAK CR(+) column (Daicel Chemical Ind., Ltd.).
8 A 50 vol% aqueous ethanol solution, containing D- and L-isomer of N-α-acetyltryptophans and NaN_3 (as a fungicide), was placed in the one side of the chamber, while a 50 vol% aqueous ethanol solution containing NaN_3 was placed in the other side. Each concentration of N-α-acetyltryptophan was fixed to be 1.0 mmol dm^{-3}. The permeation experiment was done at 40 °C with stirring.
9 The separation factor $\alpha_{(L/D)}$ is defined as the flux ratio J_L/J_D divided by the concentration ratio C_L/C_D in the feed side.
10 A 50 vol% aqueous ethanol solution of racemic N-α-acetyltryptophans was placed in the both chambers of the permeation cell. Each concentration of N-α-acetyltryptophan was fixed to be 1.0 mmol dm^{-3}. The electrodialysis was carried out with prescribed applied voltage between platinum black electrodes at the constant temperature of 40 °C with stirring.

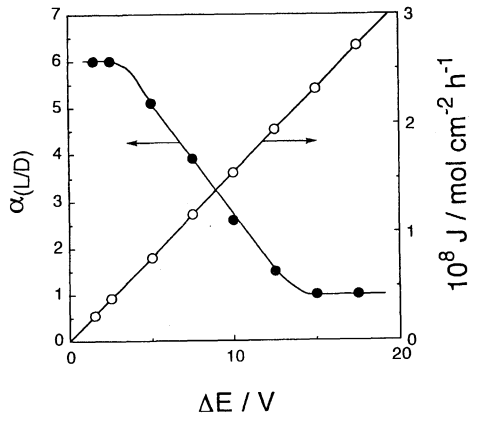

Figure 3. Influence of ΔE on enantioselective electrodialysis ((Boc-L-Trp) / (DIDE) = 0.5).