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Effect of solvent composition on chiral recognition ability of molecularly imprinted DIDE derivatives

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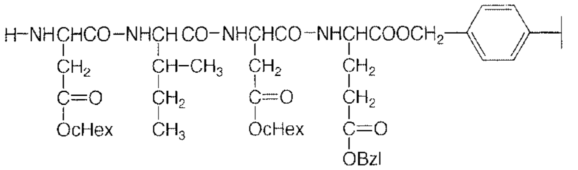
In the present study, molecularly imprinted materials from the tetrapeptide derivative, H–Asp(OcHex)–Ile–Asp(OcHex)–Glu(OBzl)–CH_2–, were adopted as samples to study the effect of the polarity of the environment on the chiral (molecular) recognition ability. The optimum composition, with the best chiral recognition, is a 50 vol% aqueous ethanol solution.

Introduction

The molecular imprinting technique, which was first proposed by Wulff and Sarhan,[1] is regarded as one of the most facile ways to introduce molecular recognition sites into polymeric materials.[2–11] Molecularly imprinted polymers are prepared by polymerization of functional monomers (which are polymerizable monomers bearing functional groups that form covalent or non-covalent bonds with the print molecules) and cross-linkers in the presence of print molecules. The removal of the print molecules yields polymeric materials containing complementary binding sites, that is the print molecule fits into a cavity in which the position and alignment of the functional groups are optimally set for the recognition of the print molecule or its analogues. Molecularly imprinted polymeric materials can also be constructed directly from polymers containing functional groups which interact with the print molecule.[12] By adopting this alternative molecular imprinting technique, oligopeptide derivatives,[13–16] derivatives of natural polymers[17] and entirely non-chiral synthetic polymers[18,19] were converted into chiral recognition or molecular recognition[20] materials. In this case, the stability of the chiral recognition sites formed in the molecular recognition materials is greatly dependent on the nature of the polymer itself (which forms chiral recognition sites) and the solution which is in contact with the molecularly imprinted polymers. The effect of solvent composition on chiral recognition ability was investigated using the tetrapeptide derivative, H–Asp(OcHex)–Ile–Asp(OcHex)–Glu(OBzl)–CH_2– (see structure below), as a candidate for molecularly imprinted materials.



Experimental

Materials

Protected amino acids, Boc-L-Glu(OBzl), Boc-L-Asp(OcHex) and Boc-L-Ile, were kindly provided by Kyowa Hakko Kogyo. Chloromethylated polystyrene resin (Cl-resin) (1% divinylbenzene), the Cl content of which was 0.78 meq g^{-1}, and dicyclohexylcarbodiimide were purchased from Peptide Institute (Osaka, Japan) and were used without further purification. Dichloromethane,[21] trifluoroacetic acid,[21] diisopropylethylamine[22] and propan-2–ol[21] were purified by the usual methods. The copolymer from acrylonitrile and styrene (AS), with a weight fraction of the acrylonitrile unit of 0.33, was kindly supplied by Ube Cycon. Ac-D-Trp, Ac-L-Trp, tetrahydrofuran, sodium azide and ethanol were used without further purification. Distilled water was employed.

Preparation of membrane materials

The membrane materials (DIDE derivatives) were prepared by the solid phase peptide synthesis technique[23–29] as previously reported.[13] The presence or absence of free amino groups (deprotection/coupling) was monitored by the qualitative ninhydrin (Kaiser) test.[30]

From the hydrolysis of polystyrene resin thus obtained and the derivatization with phenylisothiocyanate[31] or (dimethylamino)azobenzenesulfonyl chloride,[32] the concentration of tetrapeptide derivative thus introduced into chloromethylated polystyrene resin was determined to be 0.28 mmol g^{-1} DIDE-resin.

The present DIDE-resin was originally prepared for the chiral recognition of α-amino acids or α-amino acid derivatives.[13–16] In those cases, chiral recognition was performed in aqueous solution or similar. To prevent the structural deformation of the recognition site and to retain the molecular memory in the membrane, the protective groups of the side chain carboxyl moieties were not removed in the present study.

Preparation of molecularly imprinted polymeric membranes

The polymeric membranes studied in the present paper were prepared from a tetrahydrofuran solution. A copolymer of acrylonitrile and styrene (AS), with a weight fraction of the acrylonitrile unit of 0.33, was adopted as a membrane matrix because DIDE-resin does not form self-standing membranes by itself. Boc-L-Trp was adopted as a print molecule.

The molecularly imprinted polymeric membrane was prepared from DIDE-resin and AS, with a mole ratio of print molecule to DIDE derivative of 3.0 as previously reported.[13] In the present study, approximately 93% of added print molecule was recovered from the membrane. The thickness of the membrane thus obtained was 140–150 μm.

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Determination of solvent polarity of aqueous ethanol solution

An evaluation of the solvent polarity values of various mixtures of aqueous ethanol solution was carried out in terms of Dimroth's solvent polarity value [E_T(25 °C)].[33] Dimroth's solvent polarity values were measured as described previously, using 1-octadecyl-3,3-dimethyl-6′-nitrospiro(indoline-2,2′-2*H*-benzopyran) as the indicator.[34,35] A 250 W xenon lamp was used as the light source. The sample solution was illuminated with UV light using a Corning colour filter no. 7-54. Absorption spectra were measured with a Shimadzu UV-1600 UV-visible spectrophotometer.

Adsorption of racemic mixtures to the membranes

The molecularly imprinted polymeric membranes from DIDE-resin were immersed in the racemic Ac-Trp solution (concentration, 1.0 mmol dm^{-3}) and the membrane was allowed to equilibrate at 40 °C. A series of mixtures of ethanol with water, pure water and pure ethanol were used as solvents. Except with pure ethanol as solvent, 0.02 wt.% of sodium azide was added as a fungicide. Aliquots of the solution at the initial stage and after equilibrium had been reached were used for quantitative estimation by HPLC [Jasco PU 1580, equipped with a UV detector (Jasco UV 1570)] employing a Chiralpak MA(+) column [50 mm × 4.6 mm (id)] (Daicel Chemical Ind.) and aqueous copper sulfate as eluent.

The amount of amino acid in the supernatant subtracted from the amount initially in the solution gave the amount of Ac-Trp adsorbed by the membrane.

The adsorption selectivity $S_{A(L/D)}$ is defined as $S_{A(L/D)}$ = [(Ac-L-Trp)/(Ac-D-Trp)]/([Ac-L-Trp]/[Ac-D-Trp])where (Ac-L-Trp) and (Ac-D-Trp) are the amounts of Ac-Trp adsorbed by the membrane, and [Ac-L-Trp] and [Ac-D-Trp] denote the concentrations in the solution after equilibrium had been reached.

Results and discussion

So far, the chiral recognition ability of molecularly imprinted membranes has been investigated in a 50 vol% aqueous ethanol solution where the racemic solution can easily penetrate into small pores in the membrane.[12–20] It is expected that the chiral recognition ability may greatly depend on the composition of the aqueous ethanol solution, *i.e.* the polarity of the solution.

It is expected that an increase in the water content of an aqueous ethanol solution will lead to an increase in the polarity of the solvent. The empirical solvent polarities of aqueous ethanol solutions up to a composition of 30 vol% of water have been evaluated.[36] We determined Dimroth's solvent polarities of various aqueous ethanol solutions. The determined solvent polarity values are summarized in Table 1. In the present study, only solutions containing up to 40 vol% of water were determined due to the solubility of the indicator in the solution. Even though the present study could not cover the whole concentration range, the solvent polarity tended to increase with an increase in the water content in the solution as expected. The measured polarity of an 80 vol% ethanol–water mixture did not coincide with the reported value.[37] We believe that this might be due to the different indicators used.

The adsorption selectivities of racemic Ac-Trp mixtures were investigated in a series of aqueous ethanol mixtures, such as pure water, 25 vol% aqueous ethanol solution, 50 vol% aqueous ethanol solution, 75 vol% aqueous ethanol solution and pure ethanol. The results are given in Fig. 1 as a function of the ethanol concentration in the racemic mixture solution. The amount of Ac-Trp adsorbed by the membrane is given as a relative value (relative to that of the DIDE derivative found in the membrane). As can be seen in the figure, the adsorption selectivity towards Ac-L-Trp gave a maximum value at 50 vol% aqueous ethanol concentration. It decreased with an increase or decrease in the ethanol concentration in the racemic mixture solution. From previous studies,[13] chiral recognition of enantiomers of molecularly imprinted membranes bearing the DIDE derivative as a recognition site occurs *via* the interaction between the carboxyl moiety of Ac-L-Trp and an amino group in the DIDE derivative residue and *via* the absolute configuration of the side chain of the indole moiety in Ac-L-Trp. In the present case, the substrate might be surrounded by tetrapeptide residues and AS copolymer, which is the membrane matrix. A tentative structure for the interior of the molecularly imprinted polymeric membranes is shown in Fig. 2. It should also be made clear that the presence of the print molecule during the membrane preparation process is indispensable in the introduction of chiral recognition sites around DIDE derivative residues.[16] The adsorption selectivity towards the L-isomer decreased from 1.9 to 1.1 with decreasing ethanol concentration from 50 vol% to 0 vol% (pure water). This can be explained as follows. The increase in water content in the solution leads to an enhancement of the ionization of the carboxyl moiety of Ac-Trp and an amino group in the DIDE derivative residue. In other

Table 1 Dimroth's solvent polarity values [E_T(25 °C)] of aqueous ethanol solutions

Ethanol–water[a]	$\lambda_{\max}^{\text{Vis}}$	E_T(25 °C)/kcal mol^{-1}
100/0	548.7	51.9
90/10	543.2	53.9
80/20	539.8	55.2
80/20		53.6[b]
75/25	538.3	55.8
70/30	536.1	56.6
60/40	533.7	57.5
0/100		63.1[b]

[a] vol%/vol%. [b] From ref. 37.

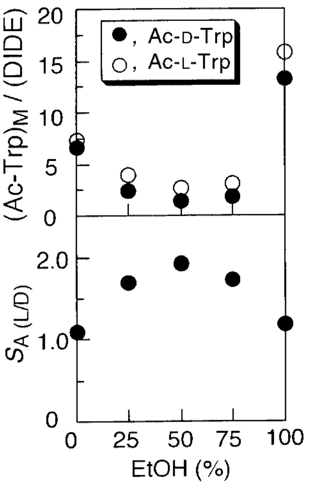

Fig. 1 Effect of ethanol–water content on selective adsorption of racemic Ac-Trp (first adsorption experiment).

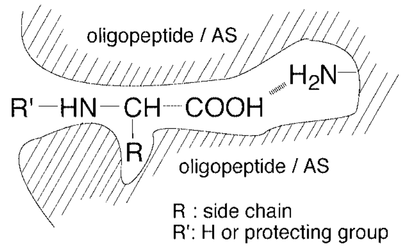

Fig. 2 Tentative structure of the interior of the molecularly imprinted DIDE membrane.

words, the increase in water content in the solution leads to an increase in the electrostatic attraction force, which is an important interaction but is non-directional. As a result, chiral recognition decreases with increasing water content and adsorption selectivity is only slightly observed in pure water solution.

It is expected that the highly directional hydrogen bond between the carboxyl moiety of Ac-Trp and an amino group of the DIDE derivative residue will be enhanced with an increase in ethanol concentration of the racemic mixture solution; in other words, the adsorption selectivity may be expected to increase monotonically with an increase in ethanol concentration. Against this expectation, the adsorption selectivity towards Ac-L-Trp was decreased from 1.9 to 1.2 with an increase in ethanol concentration from 50 vol% to pure ethanol. This result might be due to the deformation of molecular memory, which was formed by the presence of the print molecule, Boc-L-Trp, during the membrane preparation process. To this end, the following experiments were carried out. The five membranes which were in contact with various solutions were immersed again in a 50 vol% aqueous ethanol solution containing racemic 1 mmol dm^{-3} Ac-Trp. If the hypothesis is valid, the adsorption selectivities of the membranes which were immersed in 75 and 100 vol.% aqueous ethanol solution in the first experiment will not give a value of 1.9, which was observed at 50 vol% ethanol concentration, because structural deformation would have occurred partly in the first experiment. The results for these second experiments are given in Fig. 3. As shown in the figure, the membranes which were in contact with 75 vol% and pure ethanol solution in the first adsorption experiment did not give an adsorption selectivity of 1.9, as observed at 50 vol% ethanol concentration in the first adsorption experiment. From this, it was confirmed that structural deformation of the chiral recognition sites occurred in the membrane at and above 75 vol% ethanol in the solution. The membrane matrix AS does not dissolve in ethanol. However, the protective groups of the side chain carboxyl moieties in peptide synthesis, the cyclohexyl and benzyl ester groups, have a tendency to move in ethanol or to be swollen in ethanol-rich aqueous solution. As a result, molecular memory might be lost at and above 75 vol% ethanol in the solution. On the other hand, the adsorption selectivity of membranes which were in contact with pure water or 25 vol% aqueous ethanol solution in the first experiment was the same (1.9) in the second experiment as that of the membrane which was in contact with 50 vol% aqueous ethanol solution. This leads to the conclusion that molecular memory is retained below 50 vol% aqueous ethanol in the solution.

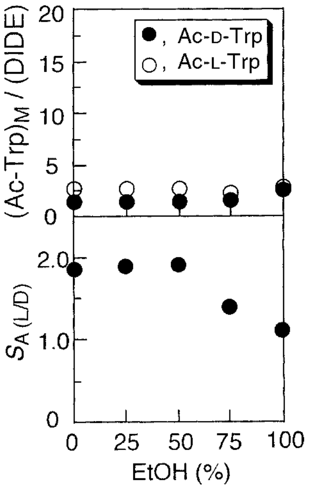

Fig. 3 Selective adsorption of Ac-Trp in a 50 vol% aqueous ethanol solution (second adsorption experiment). (Abscissa of ethanol concentration denotes the solutions in which the membranes were immersed in the first experiment.)

From the present study, it can be seen that the polarity of the environment greatly affects the chiral (molecular) recognition of molecularly imprinted polymeric materials. For chiral recognition sites (prepared from tetrapeptide derivatives by applying an alternative molecular imprinting technique) to be retained, the highest concentration of ethanol was determined to be 50 vol%. The optimum composition (where the best chiral recognition is observed) is a 50 vol% aqueous ethanol solution.

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