Molecularly Imprinted Resins for Chiral Separation of Amino Acids

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Abstract
An enatioselective surface-imprinted polymer for an amino acid was prepared by the molecularly imprinted technique. The long-chain quaternary ammonium chloride was found to serve the dual function of an emulsifier and host molecule. Chiral recognition ability and ligand specificity of the imprinted polymer were demonstrated by several batchwise tests. The surface-imprinted polymer could recognize the chirality of N-protected glutamic acid; therefore it preferentially adsorbed the corresponding enantiomer that was imprinted in the preparation. The pH and buffer concentration in the aqueous solution are the key factors enhancing enantioselectivity. The high interfacial activity of the functional molecule and the low swelling property of the imprinted polymer were important in ensuring high imprinting effect.

1 Introduction
Molecular imprinting is a promising technique for preparing separation materials, which show a high selectivity to a target substrate.[1-3] The surface-imprinted polymer is easily prepared by a water-in-oil emulsion polymerization. This method is characterized by allowing these molecules to self-assemble at the oil-water interface to form recognition sites on the polymer surface. This approach has been successfully applied to the preparation of selective adsorbents for the mutual separation of metal ions [4-7] and the optical resolution of amino acids,[8,9] which can recognize their corresponding templates in an aqueous buffer solution.

To effectively provide selective binding sites on the polymer surface, a stable emulsion should be formed prior to polymerization. In previous studies, a surfactant (emulsion stabilizer), which does not interact with the template molecule in principle, was required to maintain the emulsion stability. However, the existence of the emulsion stabilizer at the oil-water interface inhibits the self-assembly process, because the complexation between the template and the functional host molecule takes place at the interface. If the functional host molecule acts as the emulsifier, an emulsion stabilizer is not required. Moreover, the use of such a molecule serving this dual function of emulsifier and host simplifies the surface imprinting system. On the basis of our previous knowledge, a cationic surfactant, benzyltrimethyl-n-tetradecylammonium chloride (Zephiramine; Zeph, Fig. 1) was selected as the candidate for the bi-functional molecule.

In the present study, we demonstrated the potential of using the bi-functional molecule, Zeph, for the preparation of surface imprinted polymers. An enantioselective polymer for an amino acid derivative, N-
benzyloxycarbonyl-glutamic acid (Z-Glu) was prepared. Their rebinding properties for four kinds of amino acid derivatives in aqueous media were evaluated from adsorption selectivity, and the chiral recognition mechanism was discussed.

2 Experimental

2.1 Materials

![Functional host molecule (Zeph)](image)

N-Protected amino acid derivatives were purchased from Novabiochem (Darmstadt, Germany). Each enantiomer of glutamic acid (Glu) and Zeph was obtained from Tokyo Chemical Industry (Tokyo, Japan). Divinylbenzene (DVB) from Wako Pure Chemical Industries (Osaka, Japan) was used after treatment with silica gel to remove the inhibitor. Other reagents were of commercially available HPLC or analytical grades.

2.2 Surface imprinting method

A surface imprinted polymer was prepared by the previously described protocol. An aqueous solution (15 cm$^3$) containing 0.01 M (M = mol dm$^{-3}$) template molecules and 0.1 M tris(hydroxymethyl)aminomethane (Tris) was added to the DVB solution. The mixture was sonicated for 4 min to provide a water-in-oil (W/O) emulsion. After the addition of 0.18 g (0.7 mmol) 2,2'-azobis(2,4-dimethylvaleronitrile) as the initiator, polymerization was carried out at 328 K for 2 h under a nitrogen flow. The obtained bulk polymer was dried in vacuo and ground into particles of an appropriate size (ca. 30 mm). The polymer particles were then washed 4 times with an aqueous solution (500 cm$^3$) containing 0.1 M NaCl and 0.01 M Tris, followed by distilled water (500 cm$^3$). Finally, the particles were dried under vacuum and stored at ambient temperature until use.

2.3 Rebinding studies

A typical procedure of the guest rebinding experiments was as follows: A dry polymer (0.1 g) was incubated with an aqueous amino acid derivative solution (5 ml) for 4 hours at 298 K. After filtration with a syringe driven filter unit (Millex-LG, Millipore), the concentrations of free amino acid derivatives in the filtrate...
were determined by a HPLC system (LC-10AT VP, Shimadzu) with a C\textsubscript{18} reversed phase column and an UV-VIS detector (SPD-10A VP, Shimadzu). The total amount of substrates bound to the polymer was calculated by the differences in the concentration between the initial and the residual substrates.

3 Results and Discussion

3.1 Performance of molecularly imprinted resins for chiral separation

In the present study, water-in-oil emulsions were prepared without an emulsion stabilizer, expecting that Zeph also acted as the emulsifier. The amount of Zeph released from the imprinted polymer in removing the template molecules was analyzed to evaluate the degree of immobilization of Zeph into the polymer matrix. Little release of Zeph was observed (<5\%). The high fixation may be achieved by the π-π stacking between Zeph and the matrix DVB and the entanglement of the polymer chains and the hydrophobic moieties of Zeph.

The effect of pH on the rebinding of Z-D- and L-Glu was investigated for the surface imprinted polymers prepared with each enantiomer of Z-Glu as the template (Fig. 2). The adsorption behavior of Z-D,L-Glu was sensitive to pH, and the imprinted polymers provided high selectivity toward their corresponding template enantiomer in a mid pH range. This pH dependency could be explained by the degree of interaction between Z-Glu and the imprinted polymer.

![Fig. 2 Rebinding characteristics of Z-L-Glu imprinted polymer, and Z-D-Glu imprinted as a function of pH.](image)

In an alkaline buffer, Z-Glu exists as a di-anionic species and would preferentially adsorb on the polymer through two-point ionic interaction with the selective binding sites. In a lower pH range, Z-Glu was deduced to adsorb through one-point interaction with the nonspecific binding sites, because the g-carboxyl group of Z-Glu is neutralized. When the control polymer was used in this analysis, selective adsorption was not observed for Z-D, and L-Glu. It was demonstrated that chiral recognition was successfully achieved by the surface imprinting method using the bi-functional molecule, Zeph.

3.2 Substrate selectivity of surface imprinted resin

In several cases, molecularly imprinted polymers can distinguish a specific structure from ones that resemble it, even though the structural difference is very slight. For instance, the presence or absence of a single methyl or methylene group can be crucial for recognition. To determine the recognition ability for the surface
imprinted polymer in the present work, rebinding studies were conducted using several structural analogues of Z-Glu, *i.e.* Z-Asp, Z-Gln and a free amino acid, Glu. No enantioselectivity was observed on the adsorption of these guests, although the Z-L-Glu imprinted polymer preferentially adsorbed Z-L-Glu (Fig. 2). Nonspecific adsorption must be due to the ionic interaction between the charged carboxyl group of the guest molecules and the cationic quaternary ammonium group randomly existing on the polymer matrix. Below pH 7, no adsorption of Glu was observed, because most of Glu exists as the zwitterion, and the polymer-Glu interaction would be inhibited by the electrostatic repulsion. The decrease in the Glu uptake over pH 10 might be due to the swelling and the micro-structural changes of the polymer.

### 3.3 Mechanism of chiral separation

It is expected that, prior to polymerization, a template-Zeph complex is formed at the oil-water interface through the two-point ionic interaction between negatively charged carboxyl groups of Z-Glu, and the cationic ammonium group of Zeph. After the removal of the template, the selective binding sites, which contain two functional groups at predetermined positions, should be formed on the polymer matrix. Based on computational modeling, it has been explained that the complementary micro-pocket to the hydrophobic moieties of the template is created in the binding sites by the surface imprinting technique [9]. According to this, we can deduce that the imprinting operation provides the pocket that is able to accommodate the hydrophobic Z group of the template molecule (Fig. 3).

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**References**