An Enantioselective Polymer Prepared by the Surface Molecular-Imprinting Technique

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An enantioselective imprinted polymer was prepared by the surface template polymerization technique. An organophosphorus extractant was effective as a functional host molecule for recognizing a certain amino acid. The imprinted polymer exhibited a high template effect toward L-tryptophan methylester compared to its D-isomer, while a reference polymer prepared without an imprinting molecule did not show any selectivity among the enantiomers.

A molecular imprinting technique[1,2] allows polymers to create " tailor-made " recognition sites. The technique requires neither a sophisticated molecular design nor cumbersome multi-step procedures in the preparation. Thus, the technique is conceptually easy to apply to a variety of target molecules. However, it still has some fundamental technical problems yet unresolved, i.e., inapplicability to water-soluble organic substances which are important in the biological field, and the slow rebinding kinetics arising from the inner diffusion of imprint molecules toward the recognition sites which are formed deep in the polymer matrix.

Recently, to overcome these problems, we have proposed a novel molecular imprinting technique, which is called " Surface template polymerization "[3-5]. The solid polymer, which is molecular-imprinted at its internal cavity surface, was prepared by polymerizing a water-in-oil (W/O) emulsion consisting of a water-soluble imprint molecule, a functional host molecule (which is aimed to interact with the imprint molecule), an emulsion stabilizer and a cross-linking agent. In this novel technique, an aqueous-organic interface in W/O emulsions is utilized as the recognition field for a target molecule. The target molecule forms a complex with the functional host molecule, and the orientation of the functional host molecule is fixed at the oil-water interface. This leaves, after polymerization, the recognition sites at the inner cavity surface of the imprinted bulk polymer.

The complex between the functional host molecule and the target molecule should not be too much hydrophobic nor hydrophilic, because otherwise the complex would not be placed at the oil-water interface. In other words, a functional host molecule should be amphiphilic just like a surfactant molecule in order to yield a high template effect for the target molecule. The matrix-forming agent cross-links the organic (oil) phase and stabilizes the water pool or an imprinted water cavity after polymerization. The bulk polymer obtained is ground to particles for interaction with target molecules in solution. The particle has a number of micropores in the matrix, and the recognition sites formed on the surfaces of the micropores are stable enough to be maintained. This technique also realizes a rapid and reversible complexation of target molecules on the imprinted polymer.

In this paper we demonstrate for the first time an enantioselective template effect in surface-imprinted polymers. Here, we used phenylphosphonic acid monododecyl ester (n-DDP, **1**), L-tryptophan methylester (L-TrpOMe, **2**), dioleyl-*L*-glutamate-δ-gluconolactone amide ($2C_{18}\Delta^9GE$) and divinylbenzene (DVB), as a functional host molecule, an imprint molecule, an emulsion stabilizer and a cross-linking agent, respectively. Figure 1 schematically illustrates the strategy of the preparation of the L-TrpOMe-selective polymer.

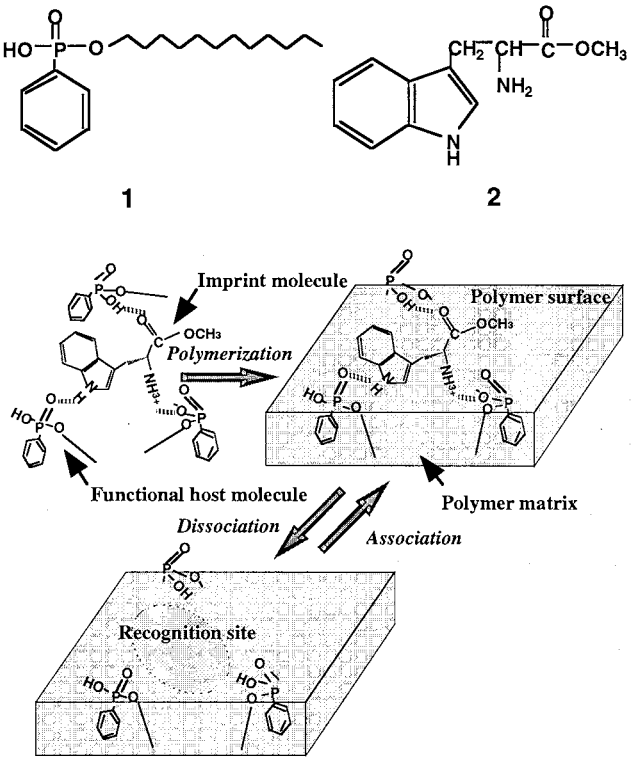

Figure 1. Schematic illustration of surface template polymerization.

For the preparation of enantioselective polymers (L-TrpOMe-imprinted polymers), a 40 cm^3 of DVB containing 60 mol/m^3 n-DDP and 5 mol/m^3 $2C_{18}\Delta^9GE$ was mixed with 20 cm^3 toluene. A 30 cm^3 of an aqueous solution containing 10 mol/m^3 L-TrpOMe, the pH of which was adjusted to 4.5 with 100 mol/m^3 phosphate buffer, was then added to the organic phase. The mixture was sonicated for 4 min to obtain stable W/O emulsions. After the addition of 0.36 g (1.4×10^{-3} mol) of the powder initiator (2,2'-azobis (2,4'-dimethylvaleronitrile), 0.01 wt.% for DVB), the mixture was polymerized at 55 °C for 2 h under a flow of nitrogen. The obtained bulk polymer was dried under vacuum and ground into particles of an appropriate size. The particles were washed with 1000 mol/m^3 hydrochloric acid to remove the imprinted L-TrpOMe and then filtered off. This procedure was repeated several times until the imprint molecule can not be detected in the filtrate with a UV spectrometer. Finally, the polymer was dried *in vacuo* for several days. An unimprinted polymer as a reference was similarly prepared without imprinting L-TrpOMe. The adsorption experiment of D- or L-TrpOMe was conducted in a batchwise method. A 0.05 g of the dry polymer

was added to 5 cm^3 of aqueous solution containing 0.5 mol/m^3 D-, or L-TrpOMe in a test tube. The pH was adjusted to a desired value between 1.5 and 7.5 by using 100 mol/m^3 phosphate buffer. The mixture was shaken in a thermostated water bath at 35 °C for 24 h. The amounts of TrpOMe on the polymers were evaluated by the residual concentration of TrpOMe in the filtrated aqueous solutions. The concentration was measured by high performance liquid chromatography (TSKgel ODS-80T_S column, 4.6 X 250 mm). The elution (acetonitrile/acetate buffer (pH 4.5) = 1/4, v/v) was spectorophotometrically monitored at 278 nm and 1.0 ml/min.

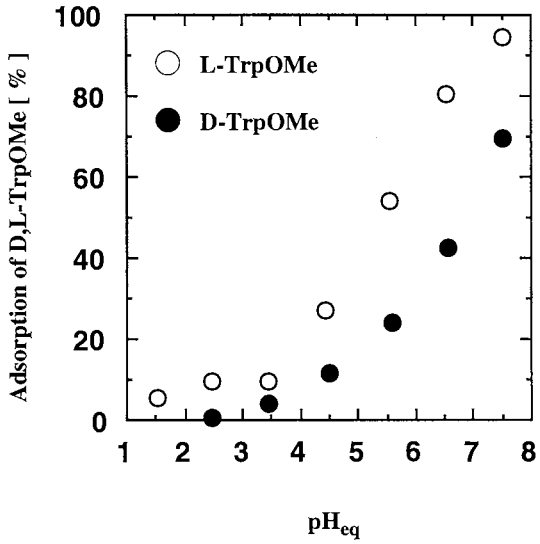

Figure 2. The pH dependence of the adsorption of D- or L-TrpOMe on the L-TrpOMe-imprinted polymer.

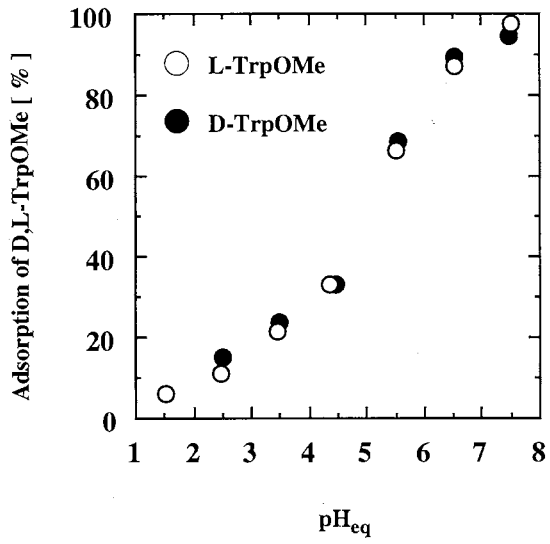

Figure 3. The pH dependence of the adsorption of D- or L-TrpOMe on the unimprinted polymer.

Figures 2 and 3 show the pH dependence of the adsorption of D- and L-TrpOMe on the L-TrpOMe-imprinted and unimprinted polymer. The percent adsorption increased with increasing pH in the solution. This result means that the proton-dissociation of phosphonic acid groups on the polymers play a predominant role in the binding of the amino acid derivative. The imprinted polymers were expected to process a higher adsorption ability than that of the unimprinted polymers which were prepared without the template molecules. However, the unimprinted polymers exhibited a little higher adsorption property than that of the imprinted polymers. Orientation and location of the functional host molecules were very sensitive to the presence of the guest molecules during the polymerization. This reflects the immobilization ratio of the functional host molecules in the polymer matrix. Actually, the presence of the amino acid esters caused the decease in the immobilization ratio of the functional host molecules. Under these conditions we discussed the template effect of the adsorbents by the enantioselectivity toward the imprinting molecule. The L-TrpOMe-imprinted polymer showed a higher template effect toward L-TrpOMe over D-TrpOMe in a whole pH range studied. On the other hand, the unimprinted polymer similarly prepared except for the absence of L-TrpOMe afforded no evidence of enantioselective adsorption. This should be because the functional host molecules is randomly distributed on the polymer surface. These results demonstrated that the surface molecular imprinting technique is very useful for preparing enantioselective adsorbents for the molecular targets in aqueous solution. The enantioselective recognition in the present study is considered to arise from the following three factors: I) the electrostatic interaction between the phosphonic acid moiety in the functional host molecule and the amino group in the target L-TrpOMe; II) the hydrogen-bonding interactions between the functional host molecule and the substrate as postulated in Figure 1; and III) the hydrophobic interaction between the indole ring and the polymer-matrix. At the extension of this work, we are extending this novel surface-imprinting technique to prepare an enantioselective adsorbent material for a variety of amino acids.

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