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Improved gate effect enantioselectivity of phenylalanine-imprinted polymers in water by blending crosslinkers

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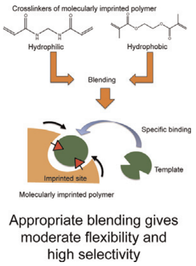
HIGHLIGHTS

- We tried amperometric sensing by gate effect of molecularly imprinted polymer.
- Chiral-selective sensing in water by gate effect was performed successfully.
- The success was realized by blending hydrophobic and hydrophilic crosslinkers.
- The blending effect is probably based on the regulation of flexibility of the site.

GRAPHICAL ABSTRACT



Appropriate blending gives moderate flexibility and high selectivity

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ABSTRACT

In this work, the anodic current at an electrode grafted with a molecularly imprinted polymer (MIP) crosslinked via a combination of hydrophobic ethyleneglycol dimethacrylate (EDMA) and hydrophilic methylene bisacrylamide (MBAA) was found to exhibit enantioselective sensitivity to the phenylalanine template in aqueous solution. An MIP-grafted electrode crosslinked with a 2:1 mixture of EDMA and MBAA was observed to respond to the template with the highest enantioselectivity, such that the change in current induced by the imprinted template was more than four times that induced by the enantiomer of the template. The contact angle of a water droplet on an MIP-coated electrode prepared using the optimal crosslinker blending ratio was also sensitive to the template and again exhibited chiral selectivity. The change in the contact angle induced by the template was more than twice as large as that obtained from the template's enantiomer. Atomic force microscopy showed that the surface of the MIP layer fabricated using a mixture of crosslinkers was rougher than that made with a single crosslinking agent, although there was no apparent correlation between the roughness and the enantioselectivity of the layer. These results indicate that the appropriate combination of crosslinkers enables the chiral-selective gate effect by modulating the flexibility and hydrophilicity of the MIP layer. The approach described herein therefore represents a new means of improving the selectivity of MIPs by blending crosslinkers having different chemical properties.

1. Introduction

Molecularly imprinted polymers (MIPs) are possibly the most readily synthesized artificial receptors capable of discriminating between enantiomers. MIPs can be prepared by the copolymerization of a crosslinking monomer with a functional monomer

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having a high affinity for a specific template [1–4]. Following the removal of the entrapped template, cavities imprinted with the desired steric structure remain in the copolymer matrix, and the resulting MIP can preferentially rebind with the template via these cavities. The selectivity of MIPs is sufficiently high so as to discriminate even between enantiomers [5,6] which have identical chemical and physical properties, such as boiling point, polarity, and solubility. MIPs can be prepared more economically and more rapidly than natural receptors can and also exhibit superior chemical and physical robustness and thus are highly advantageous as recognition elements in selective chemical sensors [7]. The majority of MIPs, however, do not have catalytic properties, and so there is a requirement to develop appropriate methods of using a signal transducer to transform the specific binding event into an electric signal. Many researchers have tried to apply quartz crystal microbalances [8–11] or surface plasmon resonance detectors [12–14] as transducers, such that the binding of the template is detected directly as a consequence of either a change in mass or refractive index of the sensor. Both methodologies, however, require the use of complicated equipment and probes, and so reducing the size of measurement systems incorporating these techniques is difficult. One approach to producing a smaller transducer is to employ a field-effect transistor (FET) device incorporating a nanowire, as demonstrated in a study by Duan et al. in which the surface of a nanowire was modified with a molecular-recognition element [15]. This method, however, requires that the solutions being tested have low ionic concentrations (below approximately 10 mM). Thus it is difficult to use such a device for the analysis of a solute in physiological specimens since these tend to have high ionic concentrations (such as a concentration of 0.15 M in serum).

In contrast, Piletsky et al. [16–18], as well as our own group [19–22], have investigated chemical sensors based on changes in the permeability of a thin MIP layer resulting from its specific interaction with the template, a phenomenon termed the “gate effect” [23], since the template, imprinted site, and pores in the MIP correspond to a key, keyhole, and gate, respectively [19]. The gate effect likely results from morphological changes induced in the MIP as the template molecule inserts into the imprinted site. The change in the permeability of redox species across an MIP layer grafted over an electrode can be readily detected by the resulting current and MIP-grafted electrodes can therefore be used as sensors for various template analytes. Using this technique, we have succeeded in the sensing of theophylline [19], amino acid derivatives [20], glucose [21], and heparin [22] by taking advantage of the gate effect and detecting the faradic current at the surface of the MIP-grafted electrode. The analysis of heparin was found to work especially well such that this analyte could be successfully quantified even in a saline solution simulating physiological fluids [22]. The operation of a sensor employing the gate effect is very simple, requiring only standard electrochemical equipment, and thus it is relatively easy to minimize the dimensions of the overall sensor system.

In a previous study [20], we developed an electrode grafted with poly(ethyleneglycol dimethacrylate (EDMA)-*co*-methacrylic acid (MAA)) imprinted with phenylalanine anilide. The redox current at the MIP-grafted electrode exhibited selectivity for the template molecule that was sufficiently high to allow discrimination between enantiomers of the template when the sensor was employed in non-polar solvents such as dichloromethane, in which both isomers have identical physical and chemical properties. However, this same sensor did not exhibit chiral-specific selectivity when used in polar organic solvents, including dimethylformamide (DMF), acetonitrile, and pyridine. Chirality is often of primary importance when considering isomers in biological systems (especially in the functioning of D-amino acids in the nervous system) [24], and so it is vital for the sensor to be able to discriminate between enantiomers in water solutions rather than in non-polar solvents. Unfortunately, the selectivity of MIPs when applying pure water as the solvent is usually low, due to weak interactions between the functional monomers and the template molecules [25]. To overcome this deficit, the intent of the present study was to demonstrate that chiral-selective sensing using MIPs is made possible by employing a combination of crosslinking monomers.

2. Experimental

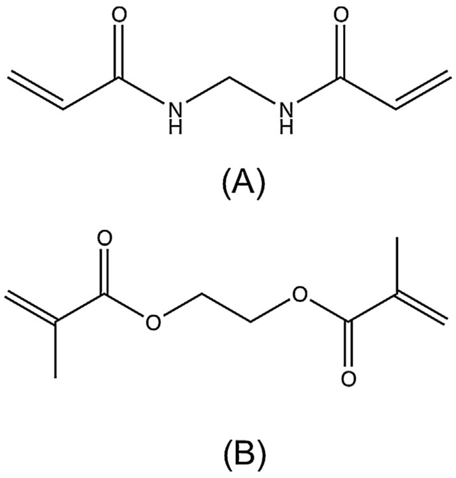
2.1. Chemicals

The precursor 3-aminopropyltrimethoxysilane was purchased from the Tokyo Kasei Co., Ltd. (Tokyo, Japan) (>96%), while toluene (anhydrous, >99.5%), ethanol (99.8%), *N*,*N*-dimethylformamide (DMF) (>99.5%), and potassium hexacyanoferrate(II) (or ferrocyanide) trihydrate (>99.5%) were obtained from the Kanto Chemical Co., Ltd. (Tokyo, Japan) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (water-soluble carbodiimide; WSC) (>98%) was purchased from Dojindo Laboratories (Kumamoto, Japan). L- and D-phenylalanine (Phe) (>99.0%), methacrylic acid (MAA) (>95%), 2-(diethylamino) ethyl methacrylate (DEAEM) (>95%), *N*,*N*-methylenebisacrylamide (MBAA) (>99.0%), ethyleneglycol dimethacrylate (EDMA) (>97%), 4-chloromethyl benzoic acid (>99%), and sodium diethyldithiocarbamate trihydrate (>97%) were purchased from the Wako Pure Chemical Industry Co., Ltd. (Osaka, Japan). Indium-tin oxide (ITO) deposited on glass plates (IN-100, 10 $\Omega\,cm^{-2}$) for use as base electrodes was obtained from the Furuuchi Chemical Co. (Tokyo, Japan).

2.2. Grafting the MIP onto the ITO surface

The diethyldithiocarbamate benzyl groups used as the initiators for radical polymerization were introduced onto the ITO surface serving as the base electrode using a procedure described previously [22].

MAA, DEAEM, and EDMA were distilled under reduced pressure before use. Either L- or D-Phe (49 mg, 0.30 mmol), serving as the templates, were dissolved in 5 mL of distilled water along with DEAEM (166 mg, 0.90 mmol) and MAA (76.6 mg, 0.90 mmol), which acted as the functional monomers. EDMA and MBAA (shown in Scheme 1), acting as the crosslinking monomers



Scheme 1. Structural formulae of crosslinking monomers used in preparation of the MIPs: (A) methylene bisacrylamide (MBAA) and (B) ethyleneglycol dimethacrylate (EDMA).