

DEVELOPMENT OF AN AMPEROMETRIC SENSOR FOR CHIRAL RECOGNITION IN ORGANIC SOLVENT USING THE GATE EFFECT OF MOLECULARLY IMPRINTED POLYMER

Shin-ichi Sekine¹, Yuta Watanabe¹, Yasuo Yoshimi^{1*}, Koji Hattori², Kiyotaka Sakai²

¹Shibura Institute of Technology, 3-7-5 Toyosu, Koto-ku, Tokyo, 135-8548, Japan

² Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo, 169-8555, Japan

1. INTRODUCTION

Biosensors using enzymes or antibodies are unusable in organic solvents. Therefore, cumbersome and costly chromatographic methods have been mainly used for the analysis in organic solvent. Recently, molecularly imprinted polymers (MIPs) have been expected as the substitutes for the enzymes and the antibodies. MIPs have specific binding sites suitable for target molecules (templates) [1, 2]. MIPs have some advantages compared to the enzymes and the antibodies, which are robustness, low cost, easiness for preparation. In addition, MIPs can work in organic solvent. However, there is no established methodology for transducing specific binding events of MIPs with its template toward electric signal. Therefore, we focused attention on “gate effect”. Gate effect is a change of solute permeability of MIP membrane by specific interaction with the template [3]. We have reported that the diffusivity of the redox marker in the thin layer of MIP immobilized on an electrode changed in the presence of the template previously [4]. The gate effect was observed as the change in the current of the redox marker. The result indicated that MIP with gate effect has a function of transducer as well as that of recognitive element of a chemical sensor. In this study, we estimated the chiral recognition in organic solvent electrochemically using the gate effect of MIP.

2. EXPERIMENTAL

Surface of indium-tin (ITO) electrode was methacrylated by 3-methacryloxypropyltrimethoxysilane. The methacrylated electrode was soaked in the mixed solution of D- or L-phenylalanine anilide (PAA; template), ethylene glycol dimethacrylate, methacrylic acid, a polymerization initiator and toluene. Polymerization was allowed to occur by heating at 60°C for 12 h. The electrode was ultrasonicated in methanol/acetic acid to extract the template, and to remove weakly adsorbed polymer (The electrode imprinted with D- and L- PAA is referred to as DIP and LIP-ITO, respectively). Cyclic voltammetry of ferrocene at the MIP-grafted electrode was performed in solvent of chloroform, dichloromethane, pyridine, dimethylformamide (DMF) or acetonitrile. The anodic current in the presence and absence of guest (D- or L-PAA) were compared.

3. RESULTS

Relative changes in the maximum anodic current by 5 mM guest and the factor of chiral selectivity

defined by (1) were shown in Table 1 for the each solvent of the test solution.

$$\alpha = \left| \frac{\text{Change in the current by template}}{\text{Change in the current by enantiomer}} \right| \quad (1)$$

The anodic currents in apolar solvent (chloroform or dichloromethane) changed significantly in the presence of the template, whereas those were hardly sensitive to the enantiomer of the template. However, the anodic currents in polar solvent (pyridine, DMF or acetonitrile) were hardly sensitive to both the template and the enantiomer. These results suggest that the gate effect with chiral discrimination occurs in apolar solvents only. The template would interact with the carboxyl group of the binding site in the MIP through the hydrogen bonds mainly [6]. However, the interactions are weakened in polar solvents which interact with the polar groups in the template and the MIP. The result shows that the gate effect is applicable for sensing in apolar solvent rather than in polar one.

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Table 1 Effect of solvent on the change in the maximum anodic current of ferrocene by guest

Solvent of test solution	Dielectric constant ϵ_r [-]	Dipole moment μ [D]	Relative change in the maximum anodic current of ferrocene by 5 mM guest [%] (n=3)					
			DIP-ITO			LIP-ITO		
			D-PAA (template)	L-PAA	α	D-PAA	L-PAA (template)	α
Chloroform	4.8 ^b	1.04	-14.20 ± 2.25	-1.50 ± 3.50	9.47	-5.00 ± 0.15	-17.10 ± 7.65	3.42
Dichloromethane	7.8 ^a	1.62	-11.20 ± 0.80	-6.80 ± 2.60	1.65	+3.00 ± 3.60	-14.50 ± 6.90	4.83
Pyridine	12.3 ^c	2.15	-1.90 ± 3.44	-2.90 ± 4.56	0.66	-1.80 ± 2.25	-2.10 ± 1.94	1.16
DMF	36.7 ^b	3.86	+6.00 ± 2.00	+6.00 ± 2.00	1.00	+5.00 ± 1.90	+5.00 ± 1.50	1.00
Acetonitrile	37.5 ^b	3.93	+3.00 ± 3.47	+5.00 ± 3.96	0.60	+2.00 ± 2.60	-2.00 ± 2.99	1.00

The values of the dielectric constant and dipole moment were cited from Reference [5].

^a 10 °C, ^b 20 °C, ^c 25 °C, α is the ratio of the relative change in the maximum anodic current by template to that by enantiomer.