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Evaluation of the recognition ability of molecularly imprinted materials by surface plasmon resonance (SPR) spectroscopy☆

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Abstract

Polysulfone with an oligopeptide derivative of glutamyl residues (PSf-$E_{5.8}$) was evaluated as a candidate material for molecular recognition. PSf-$E_{5.8}$ was prepared from the *N*-carboxyanhydride of γ-benzyl-L-glutamate (Glu(OBzl)-NCA) initiated by aminomethylated polysulfone (PSf). Films with molecular recognition sites with preference to adenosine (As) were prepared from PSf-$E_{5.8}$ by an alternative molecular imprinting method using 9-ethyladenine (9-EA) as a print molecule. The molecular recognition phenomena were studied by surface plasmon resonance (SPR) spectroscopy. The apparent affinity constant of the molecular recognition sites toward As was dependent on the imprinting condition and was increased from 1.30×10^4 to 1.60×10^4 mol^{-1} dm^3 with the decrease in the molecular imprinting ratio from 1.0 to 0.25. From the present study, it was shown that SPR is a convenient and facile method to detect molecular recognition interactions.

Keywords: Adenosine; 9-Ethyladenine; Guanosine; Molecular imprinting; Molecular recognition; Surface plasmon resonance (SPR)

1. Introduction

Molecular imprinting, which was first proposed by Wulff and Sarhan in 1972 [1], is now a well-known method for preparing molecular recognition sites by applying a simple radical polymerization [2–7]. Molecularly imprinted polymers have been applied to stationary phase in chromatography [8,9], catalysis [10], membranes [6,11], sensors [4,7,12–14], and so forth. Since 1994, the authors' research group has proposed an alternative molecular imprinting method in which polymeric materials are directly converted into molecular recognition materials [15]. By applying this, polymeric materials such as oligopeptide derivatives [16,17], natural polymer derivatives [16,18], and synthetic polymers [16,19–24] were converted into molecular recognition materials, membranes and sensors. Attention was focused on molecularly imprinted materials from oligopeptide derivatives. These derivatives were attached to cross-linked chloromethylated or aminomethylated polystyrene resins used in solid phase peptide synthesis and adopted as materials forming molecular recognition sites. In those cases, each molecular recognition site is constructed from a single strand of oligopeptide in the presence of print molecule [25]. If each site is prepared from multi oligopeptide derivatives, its molecular recognition

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ability will be enhanced compared with that from a single strand oligopeptide derivative. Accordingly, a novel material for molecular recognition sites was synthesized from aminomethylated polysulfone (PSf) and their recognition ability was studied by using SPR spectroscopy, in connection with a potential sensor device. In the present study, the nucleic acid component adenosine was adopted as a model target molecule, since recognition of nucleic acid components is of interest and importance in connection with biosensors, drug therapy, genetic engineering, and so forth. Pioneering studies on recognition and transport of nucleic acid components have been investigated by using molecularly imprinted membranes [26–29]. To this end, recognition sites towards adenine were constructed by adopting 9-ethyladenine (9-EA) as a print molecule. The recognition of adenosine/guanosine (As/Gs) was studied as a model mixture.

2. Experimental

2.1. Materials

PSf, having a degree of substitution (DS) of 0.9, was prepared by the modification of polysulfone Udel P-3500 as reported previously [30]. Following similar procedures for the present study, commercial polysulfone was lithiated with 1.0 mol equivalents of *n*-butyllithium, quenched with benzonitrile, then reduced with sodium borohydride to give PSf with DS 0.9.

γ-Benzyl-L-glutamate was purchased from Peptide Institute, Inc. (Japan) and used without further purification. Chloroform and *N*,*N*-dimethylformamide (DMF) were purified by standard methods [31]. Triphosgene, ethanol, 1-octanethiol, and sodium azide were used without purification. The print molecule, 9-EA was purchased from Sigma Chemical Co. and used without further purification. The substrates, adenosine (As) and guanosine (Gs) were purchased from Seikagaku Co. Distilled water was employed.

2.2. Preparation of polysulfone with oligopeptide side chain (PSf-$E_{5.8}$)

The synthetic scheme is shown in Fig. 1. Glu(OBzl)-NCA was prepared from triphosgene and the corresponding amino acid according to the method described in the literature [32,33], mp 89.7–90.0 °C. The structure was confirmed by ^{1}H NMR. The polymer PSf-$E_{5.8}$ was obtained by polymerization of 1.10 g of Glu(OBzl)-NCA solution in 30 cm^3 of chloroform with 0.66 g of PSf as the initiator. The polymerization was conducted at ambient temperature over 1 week. The product was recovered by precipitating the chloroform solution into ethanol, and then the precipitate was purified by dissolving it in DMF and re-precipitating it with ethanol. The average degree of polymerization of Glu(OBzl)-NCA was determined to be 5.8 by ^{1}H NMR spectroscopy recorded with a Varian Gemini 200 NMR Spectrometer, using a 50 g dm^{-3} dimethylsulfoxide-d_6 (DMSO-d_6) solution with tetramethylsilane (TMS) as the internal standard. The ^{1}H NMR spectrum of PSf-$E_{5.8}$ is shown in Fig. 2. The subscript of 5.8 in the polymer name denotes the degree of polymerization for the oligopeptide side chain.

2.3. Construction of molecular recognition sites

The molecularly imprinted films having molecular recognition sites toward As were prepared as follows: a gold-deposited glass plate was immersed in a 1.0×10^{-5} mol^{-1} dm^3 solution of 1-octanethiol in ethanol for 30 min at ambient temperature prior to the molecular imprinting. The film was prepared by spin-casting a 2.5 g dm^{-3} DMF solution of PSf-$E_{5.8}$ onto the pre-treated gold-deposited glass plate. The rotation speed for spin casting was 5000 rpm. A prescribed amount of the print molecule 9-EA was dissolved in the spincasting DMF solution for the preparation of molecularly imprinted films. 9-EA was omitted for the preparation of control films.

2.4. Evaluation of molecular recognition ability of molecularly imprinted materials

The molecular recognition of the prepared films toward the target molecule As was evaluated by SPR spectroscopy. The change in the incident angle ($\Delta\theta$) responding to the addition of substrates was recorded on the SPR apparatus (SPR670S, Nippon Laser & Electronics Laboratory). During the measurement, 0.02 wt.% NaN_3 aqueous buffer was passed over the molecularly imprinted material surface at 5 mm^3 min^{-1}. The flow was periodically replaced

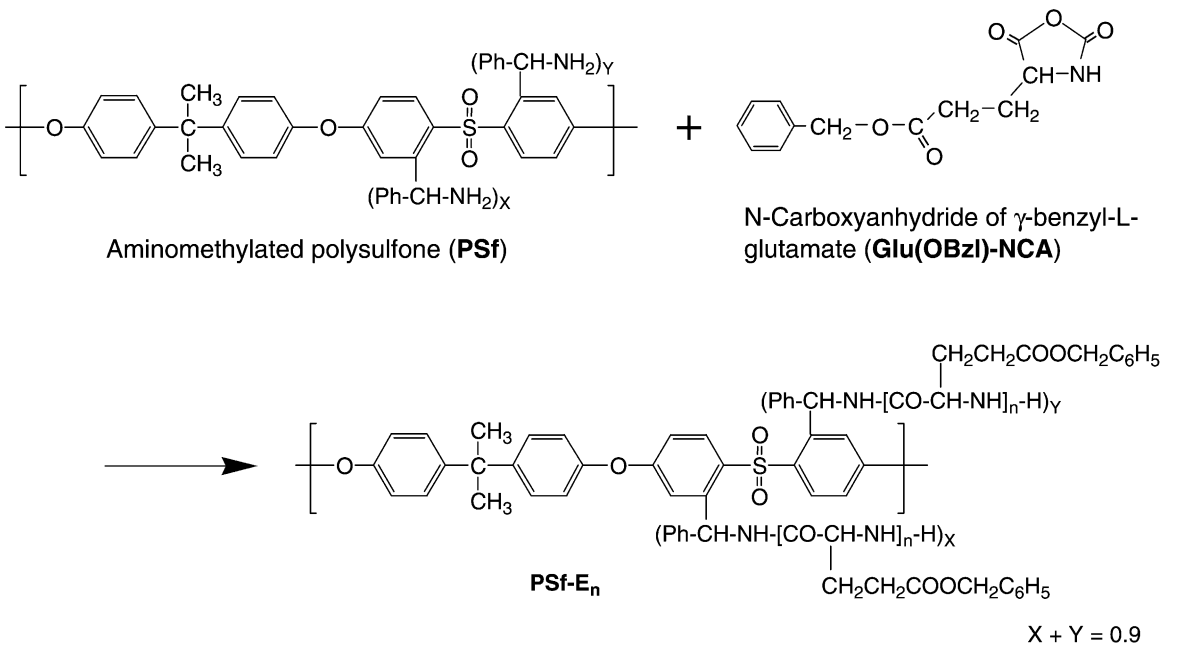

Fig. 1. Synthetic scheme of polysulfone bearing oligopeptide derivatives.

with solutions of the same buffer containing As or Gs. The experiment was carried out at 27 °C.

3. Results and discussion

In the present study, adsorption phenomena of molecularly imprinted materials were studied by using SPR spectroscopy. SPR is an optical method to detect changes in the refractive index of the medium close to the gold surface [34]. The relationship between reflected intensity and the angle of incidence gives a minimum reflected intensity, corresponding to the excitation of surface plasmons at the gold–solution interface. The value of the incidence angle giving the minimum reflected intensity (θ) shifts with changes in the refractive index of the interfacial region close to the gold surface [35,36]. The shift in θ ($\Delta\theta$) is proportional to the amount of adsorbed substrate at the surface. Using $\Delta\theta$, an apparent adsorption isotherm of a given target molecule can be drawn. In the present study, 9-EA was adopted as a print molecule.

Before studying the molecular recognition ability of 9-EA imprinted PSf-$E_{5.8}$ by SPR, a control film of non-imprinted material (coated on a gold-deposited glass plate in the absence of 9-EA) was measured. $\Delta\theta$ cannot be directly converted into the concentration of a given substrate adsorbed in the spin-cast film, even though the value of $\Delta\theta$ is proportional to the amount of the adsorbed substrate. In the present study, it can be regarded that the multiplication of the substrate concentration adsorbed in the molecularly imprinted material ($[\text{substrate}]_m$) by the factor f gives the $\Delta\theta$ ($\Delta\theta = f[\text{substrate}]_m$, f is the factor converting $[\text{substrate}]_m$ into $\Delta\theta$). The apparent adsorption isotherms of As and Gs can be obtained by plotting the observed $\Delta\theta$ as a function of the substrate concentration and are shown together in Fig. 3. Both apparent adsorption isotherms for As and Gs are superimposed and are not distinguishable. Also, both are straight lines passing through the origin, implying As and Gs were non-specifically adsorbed in PSf-$E_{5.8}$. In this case, $\Delta\theta$ for the substrate As and Gs, which was non-specifically adsorbed in the control non-imprinted material, can be represented by the following equation:

$$\Delta\theta = f[\text{substrate}]_m = fk_{A,app}[\text{substrate}]$$

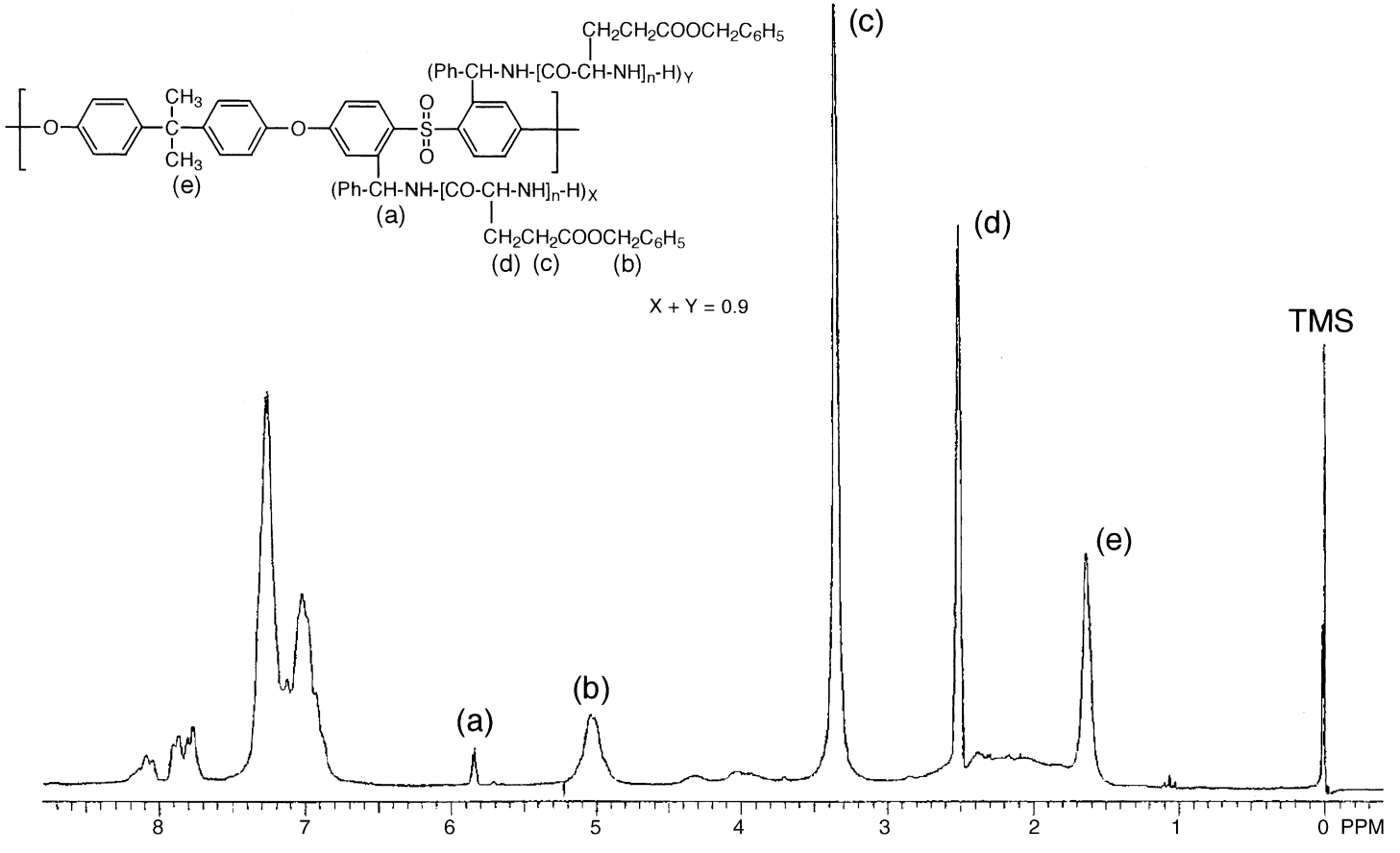

Fig. 2. ^{1}H NMR spectrum of polysulfone bearing oligopeptide derivative (PSF-$E_{5.8}$).

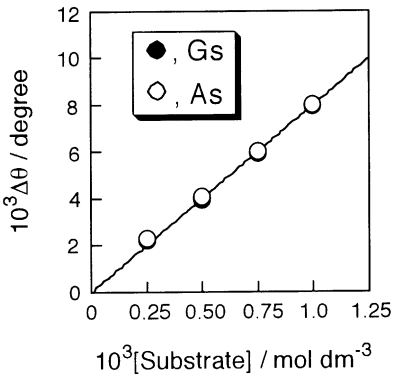

Fig. 3. Adsorption isotherms (superimposed) of As and Gs on the control non-imprinted film PSf-$E_{5.8}$.

where $k_{A,app}$ denotes the apparent adsorption constant, and [substrate] is the concentration of the substrate in the buffer.

The apparent adsorption isotherms for 9-EA imprinted PSf-$E_{5.8}$ are shown in Figs. 4(a)–6(a). For the molecular imprinting condition, the molar ratio of the amount of 9-EA to that of oligopeptide derivative in PSf-$E_{5.8}$, was 0.25 for Fig. 4, 0.50 for Fig. 5, and 1.0 for Fig. 6, respectively. All three types of 9-EA imprinted films gave similar adsorption phenomena; that is, the adsorption isotherms of Gs are all straight lines passing through the origin like those for non-imprinted material shown in Fig. 3. This indicates that there is no specific recognition site toward Gs in 9-EA imprinted PSf-$E_{5.8}$. In this case, $\Delta\theta$ for Gs adsorbed in the molecularly imprinted material can be represented by the following equation:

$$\Delta\theta = f[\mathrm{Gs}]_m = fk_{A,app}[\mathrm{Gs}]$$

where $[\mathrm{Gs}]_m$ is the concentration of Gs adsorbed in the molecularly imprinted material and [Gs] denotes the concentration of Gs in the buffer.

In contrast to this, dual adsorption isotherms were observed for As in 9-EA imprinted PSf-$E_{5.8}$, consisting of non-specific adsorption and an adsorption on an

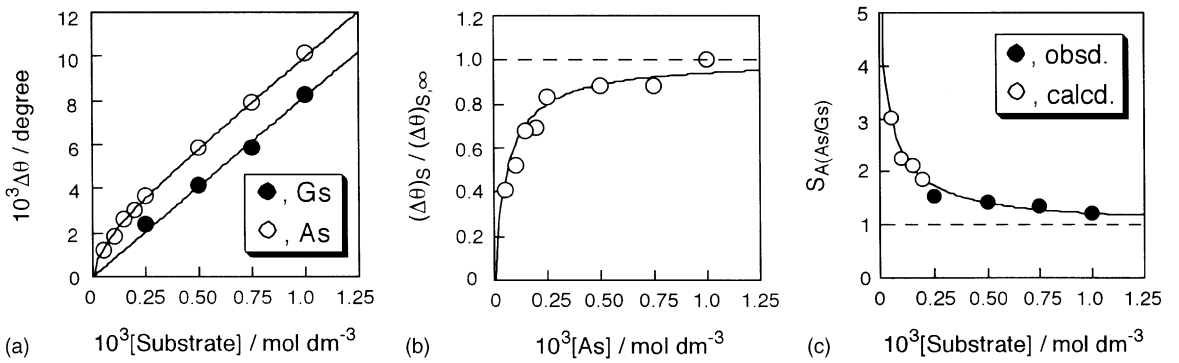

Fig. 4. Adsorption isotherms of As and Gs and adsorption selectivity of the imprinted PSf-$E_{5.8}$ (9-EA/$E_{5.8}$ = 0.25).

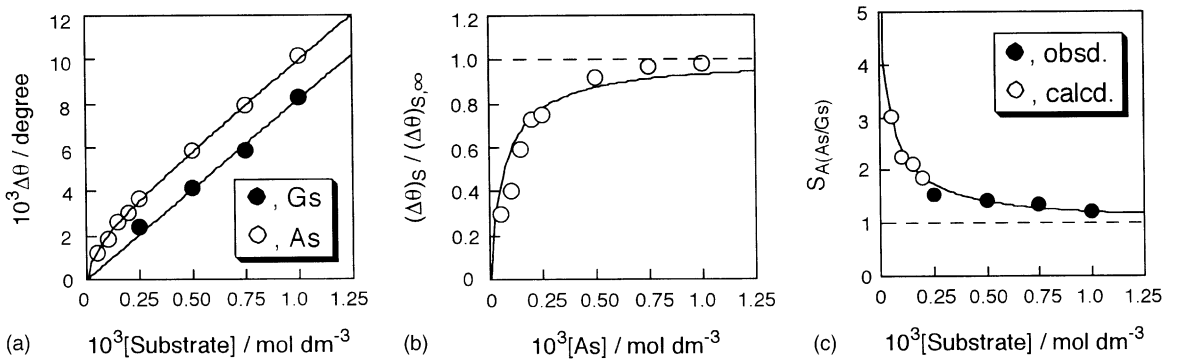

Fig. 5. Adsorption isotherms of As and Gs and adsorption selectivity of the imprinted PSf-$E_{5.8}$ (9-EA/$E_{5.8}$ = 0.50).

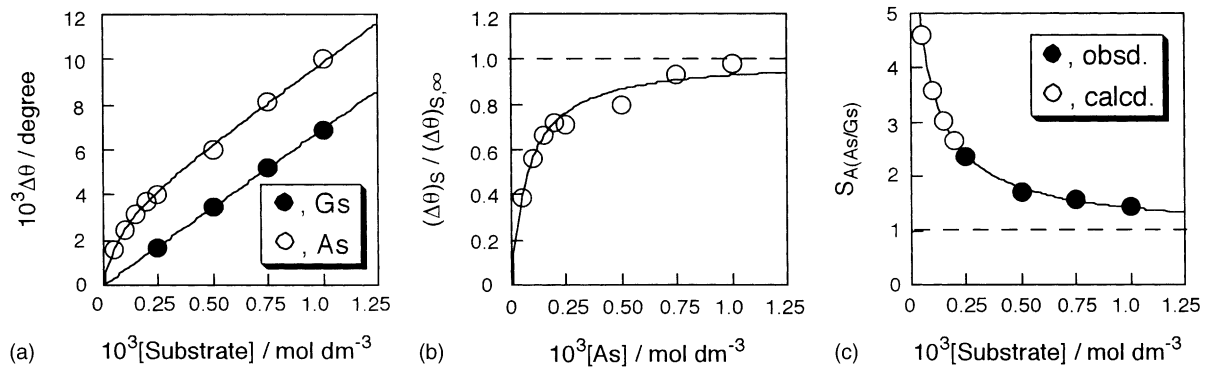

Fig. 6. Adsorption isotherms of As and Gs and adsorption selectivity of the imprinted PSf-$E_{5.8}$ (9-EA/$E_{5.8}$ = 1.0).

As specific recognition site. The concentration of As adsorbed in the molecularly imprinted material can be represented by the following equation:

$$\Delta\theta = f[\mathrm{As}]_\mathrm{m} = f\left\{k_{\mathrm{A,app}}[\mathrm{As}] + \frac{K_{\mathrm{S,app}}[\mathrm{site}]_0[\mathrm{As}]}{1 + K_{\mathrm{S,app}}[\mathrm{As}]}\right\}$$

where $[\mathrm{As}]_\mathrm{m}$ is the total concentration of adsorbed As in the molecularly imprinted material, $K_{\mathrm{S,app}}$ denotes the apparent affinity constant between As and the molecular recognition site toward As, $[\mathrm{site}]_0$ is the concentration of molecular recognition site in the molecularly imprinted material, and [As] is the concentration of As in the buffer.

The apparent affinity constant between As and the formed molecular recognition site were determined by the following procedure; the difference in the shift ($(\Delta\theta)_\mathrm{S}$) between that for As and Gs at a given concentration, which corresponds to the apparent amount of As adsorbed on the As recognition site was obtained. In the case that there was no experimental $\Delta\theta$ value for Gs at a given concentration below 0.5×10^{-3} mol^{-1} dm^3, the difference between the $\Delta\theta$ for As and the extended straight line for Gs was adopted as $(\Delta\theta)_\mathrm{S}$. $(\Delta\theta)_\mathrm{S}$ can be correlated with the adsorption equation by the following equation:

$$(\Delta\theta)_\mathrm{S} = f\left\{\frac{K_{\mathrm{S,app}}[\mathrm{site}]_0[\mathrm{As}]}{1 + K_{\mathrm{S,app}}[\mathrm{As}]}\right\}$$

A relative shift in θ, the ratio of $(\Delta\theta)_\mathrm{S}$ to $(\Delta\theta)_\mathrm{S}$ corresponding to the infinite concentration, $(\Delta\theta)_{\mathrm{S},\infty}$ ($=f[\mathrm{site}]_0$), was plotted as a function of As concentration. $(\Delta\theta)_\mathrm{S}/(\Delta\theta)_{\mathrm{S},\infty}$ is correlated with the following equation:

$$\frac{(\Delta\theta)_\mathrm{S}}{(\Delta\theta)_{\mathrm{S},\infty}} = \frac{K_{\mathrm{S,app}}[\mathrm{As}]}{1 + K_{\mathrm{S,app}}[\mathrm{As}]}$$

From the relationship between the ratio $(\Delta\theta)_\mathrm{S}/(\Delta\theta)_{\mathrm{S},\infty}$ and As concentration, as shown in Figs. 4(b)–6(b), the apparent affinity constant for each molecularly imprinted material was determined. The determined apparent affinity constants, $K_{\mathrm{S,app}}$, are summarized in Table 1. Those three values are over 1.0×10^4 mol^{-1} dm^3. In a previous study [16], the molecular recognition site toward As was constructed from tetrapeptide derivative, of which sequence was H-Asp(OcHex)-Ile-Asp(OcHex)-Glu(OBzl)-O-CH_2-(DIDE), in the presence of 9-EA. The affinity constant between the molecular recognition site from DIDE and As was determined to be 2.4×10^3 mol^{-1} dm^3. The apparent affinity constant for PSf-$E_{5.8}$ was 5.4–6.8 times higher than that for DIDE, depending on the imprinting condition. In the case of DIDE, a tetrapeptide derivative of DIDE was attached to a cross-linked chloromethylated polystyrene resin for solid phase peptide synthesis. DIDE derivatives could not interact

Table 1
Apparent affinity constant of molecular recognition site toward As

9-EA/$E_{5.8}$[a]	$K_{\mathrm{S,app}}$ (mol^{-1} dm^3)
0.25	1.60×10^4
0.50	1.40×10^4
1.0	1.30×10^4

[a] Molar ratio for molecular imprinting condition.

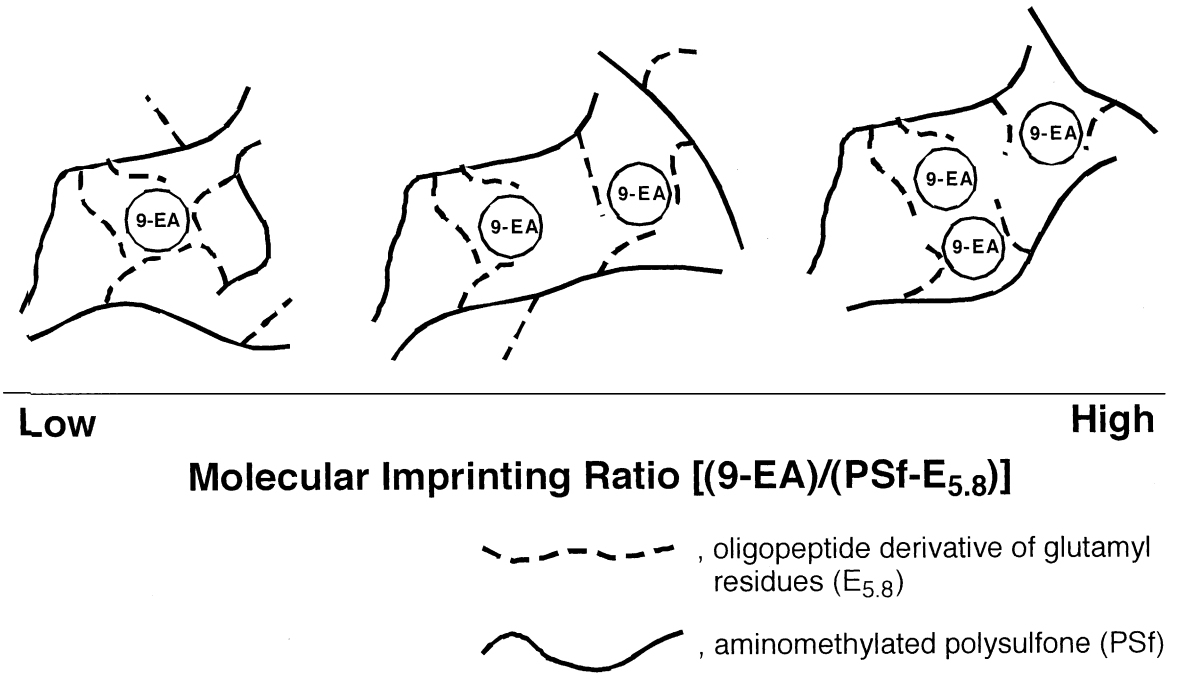

Fig. 7. Schematic image of the relationship between molecular imprinting ratio and interaction mode.

with the print molecule cooperatively and in addition the molecular recognition site was constructed from a single strand of DIDE. In contrast, the present oligopeptide derivative was attached to PSf having no cross-linking so that PSf-$E_{5.8}$ could be dissolved in appropriate solvents. As a result, the oligopeptide derivatives in PSf-$E_{5.8}$ interact with 9-EA cooperatively. From this, the molecular recognition site in the present study was thought to be constructed from multiple oligopeptide derivatives, not from a single strand of it. As can be seen in Table 1, the apparent affinity constant was dependent on the molecular imprinting condition, increasing with decreasing molecular imprinting ratio. This can be explained as follows: multiple oligopeptide derivatives, consisting of glutamyl residues (PSf-$E_{5.8}$), cooperatively interacted with the print molecule 9-EA in DMF solution during the molecular imprinting process. The relationship between molecular imprinting condition and interaction mode between 9-EA and PSf-$E_{5.8}$ are schematically shown in Fig. 7. At the low molecular imprinting condition, more oligopeptide derivatives interacted with one print molecule, resulting in a higher apparent affinity constant. As a result, the apparent affinity constant gave higher values with the decrease in the molecular imprinting ratio as summarized in Table 1.

In Figs. 4(c)–6(c), the calculated adsorption selectivity between As and Gs are given because the experiment for selective adsorption from As/Gs mixture cannot be conducted by SPR spectroscopy. The calculated adsorption selectivity, $S_{A(As/Gs)}$, can be represented by the following equation:

$$S_{A(As/Gs)} = \frac{\Delta\theta_{As}}{\Delta\theta_{Gs}}$$

Closed circles in every Figure were calculated by using observed $\Delta\theta$ values for As and Gs. Open circles were obtained by using $\Delta\theta$ values for As and extrapolated straight line for Gs. As is observed for the adsorption selectivity profile for materials having a specific recognition site, adsorption selectivity toward As increased with the decrease in substrate concentration.

Compared with usual adsorption experiments for the evaluation of molecularly imprinted materials [16–19,21,22,25], SPR spectroscopy provides a rapid and facile evaluation method. From another viewpoint, the combination of molecularly imprinted material and SPR spectroscopy is expected to lead to an

analytical method for the detection of a given target molecule.

4. Conclusions

Polysulfone having oligopeptide derivative of PSf-$E_{5.8}$ were prepared from the *N*-carboxanhydride of Glu(OBzl) initiated by PSf. Molecular recognition sites toward As were prepared from PSf-$E_{5.8}$ by an alternative molecular imprinting by using 9-EA as a print molecule. The molecular recognition phenomena were studied by SPR spectroscopy. The apparent affinity constant was dependent on the molecular imprinting condition and was increased from 1.30×10^4 to $1.60 \times 10^4\,\mathrm{mol^{-1}\,dm^3}$ with the decrease in the molecular imprinting ratio from 1.0 to 0.25. From this, it was deduced that multiple oligopeptide residues were involved in the formation of a molecular recognition site from the print molecule. The present study demonstrates that SPR is a facile method for the detection of molecular recognition interactions rapidly and conveniently.

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