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Multiple hydrogen bonding-based fluorescent imprinted polymers for cyclobarbital prepared with 2,6-bis(acrylamido)pyridine

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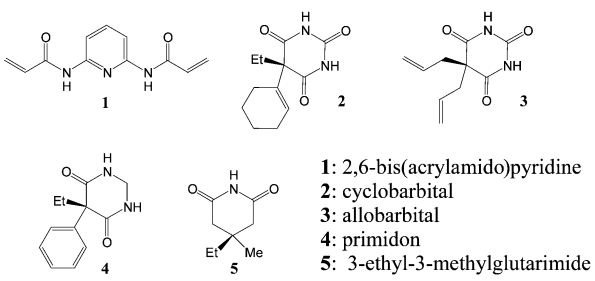
A cyclobarbital-imprinted polymer was prepared using a fluorescent functional monomer, 2,6-bis(acrylamido)pyridine, and the polymer showed not only selective binding of cyclobarbital but also enhancement of fluorescence intensity, suggesting that the polymer could be utilized as a selective fluorescence probe.

Molecular imprinting has attracted attention as a template polymerization technique that can form artificial receptors/antibodies for target molecules.[1] Many researchers have designed and prepared molecularly imprinted polymers for a wide range of target compounds in life science, pharmaceutical science, environmental science, and biotechnology such as nucleotide bases, drugs, sugars, steroids, pesticides and so on. Recently, not only molecular recognition functions but also secondary signal functions due to the binding events have been introduced to molecularly imprinted polymers in order to make selective sensing materials for chemosensors.[2] Fluorescent monomers in particular have been intensively investigated and molecularly imprinted polymers that change their fluorescence intensity due to binding have been reported.[3]

Previously, we have reported on imprinted polymers using 2,6-bis(acrylamido)pyridine **1** as a functional monomer that was capable of forming multiple hydrogen bonding with barbiturates[4] and 5-fluorouracil.[5] Since **1** shows strong fluorescence, we investigated whether or not **1** works as a signalling monomer for molecular imprinting of cyclobarbital **2**; changing its fluorescence intensity due to the binding events of **2**.

The functional monomer **1** was prepared according to the method reported previously.[6] Cyclobarbital-imprinted polymers were prepared as follows: **2** (0.5 mmol) and **1** (1.0 mmol) were dissolved in chloroform (11 mL), and ethylene glycol dimethacrylate (20 mmol) was added as a crosslinking agent and then 2,2′-azobis(2,4-dimethyl valeronitrile) (0.25 mmol) was added as an initiator of radical polymerization. The polymerization mixture was purged with nitrogen gas, sealed, then heated in a water bath at 45 °C for 12 h, followed by heating at 60 °C for 3 h. The resulting polymers were ground and wet-sieved with water and methanol through a 63 μm mesh filter. The materials (> 32 μm, < 63 μm) were packed into stainless steel columns (150 mm × 4.6 mm i.d.). The packed polymer particles were washed with methanol for 24 h to remove the template **2**.



The prepared polymer was evaluated chromatographically by comparison of the retention time of **2** and structurally related compounds such as allobarbital **3**, primidon **4** and 3-ethyl-3-methylglutarimide **5** (Table 1). The imprinted polymer showed the strongest binding for **2**, while **3** was not bound strongly, meaning that the polymer could recognize the difference in the substituent at the 5 position, *i.e.* distinction between 5-cyclohexenyl-5-ethyl and 5,5-diallyl groups. The structurally related **4** and **5** were poorly retained because **4** can not form three-point hydrogen bonding with **1** although a 1 : 2 complex may be formed, and **5** can only form a 1 : 1 complex with **1** although triple hydrogen bonding may occur. Therefore, it was proved that this imprinted polymer possessed recognition ability for the substituents at the 5 position of pyrimidine-2,4,6-trione. Since the corresponding blank polymer prepared without **2** showed almost no affinity (data not shown), the selectivity must have been introduced during the imprinting process.

The dry polymer particles (2.0 mg in $CDCl_3$) were incubated with varying amounts of **2**, **3**, **4** and **5**. After 6 h-incubation at 25 °C, the polymer suspensions were transferred to a quartz cell and fluorescence of the suspensions was measured with an excitation wavelength of 270 nm. When **2** was added to the imprinted polymer suspensions, fluorescence at around 380 nm increased with the concentration of **2** added (Fig. 1). Fluorescence intensities at an emission wavelength of 380 nm are plotted as a function of initial concentrations of **2**, **3**, **4** and **5** in Fig. 2. The largest fluorescence enhancement was observed when **2** was added, and **3** also affected the enhancement although less so than **2**. Almost no enhancement was observed

Table 1 Capacity factors of cyclobarbital and reference compounds in the cyclobarbital-imprinted polymer

Capacity factor[a]			
2	**3**	**4**	**5**
2.64	0.72	0.09	0.43

[a] Eluent: methanol (1mL min^{-1}), sample size: 10 μL (1 mM), detection: 300 nm. The capacity factors (k') were calculated using the equation, $k' = (t_R - t_0)/t_0$, where t_R is the retention time of samples and t_0 is the time taken to elute void maker (acetone).

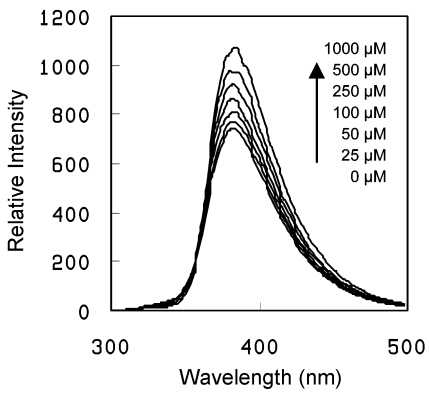


Fig. 1 Fluorescence spectral changes of cyclobarbital-imprinted polymer with the addition of **2** (0–1 mM) (λ_{ex}: 270 nm).

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on addition of the structurally related **4** or **5**. These results are consistent with the chromatographic tests, suggesting that the fluorescence enhancement could be due to formation of hydrogen bonding in the binding sites consisting of **2** residues. Because **4**, which showed poorer binding ability to the polymer (Table 1) due to only two-point hydrogen bonding, affected the fluorescence enhancement less than **2**, stronger binding due to the three point hydrogen bonding formation appears to lead to greater fluorescence enhancement. In the case of **5**, only a 1 : 1 complex may be formed with **1**, resulting in the lower response compared to **2** because of the weakness of the binding.

After incubation of the imprinted polymer with **2**, the supernatants were transferred to sample tubes and aliquots were analyzed by HPLC using the same system as for the chromatographic tests (Table 1) but with chloroform as the carrier solution rather than methanol to quantify the concentrations of unbound **2**. Amounts of **2** bound were obtained by subtracting the corresponding unbound amounts of **2** from the initial **2**. The binding isotherm obtained was saturable, meaning that only a finite number of binding sites exist, and a similar profile was given to the fluorescent response (Fig. 3). The obtained results confirmed that the fluorescence response is dependent upon the binding of **2** to the imprinted cavity.

As can be seen, fluorescence of the polymer was enhanced when **2** was bound and the degree of enhancement was influenced by the binding affinity. The fluorescence enhancement could be mainly caused by increasing the rigidity of **1** residues due to the formation of multiple hydrogen bonding. Further investigation of the mechanism should be addressed in order to develop more sensitive fluorescent monomers that have less fluorescence free forms and more fluorescence when target compounds are bound.

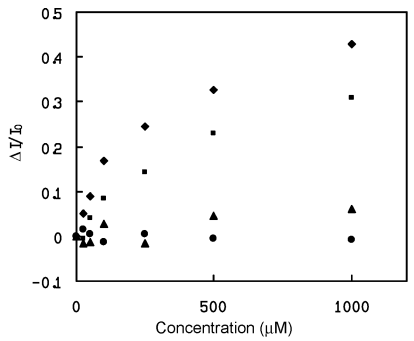

Fig. 2 Fluorescent response of cyclobarbital-imprinted polymer in the presence of various concentrations of **2** (◆) and reference compounds **3** (■), **4** (●) and **5** (▲). λ_{ex}: 270 nm, λ_{em}: 380 nm.

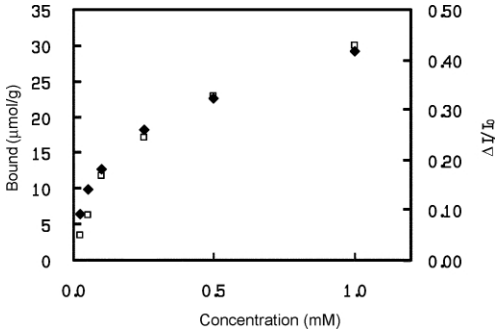

Fig. 3 Binding isotherm of **2** for the imprinted polymers (◆) and fluorescent response of imprinted polymer in the presence of **2** (□). (λ_{ex}: 270 nm, λ_{em}: 380 nm).

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