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Molecular Imprinting of Cyclodextrins Leading to Synthetic Antibodies

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Abstract

Molecularly imprinted cyclodextrins were applied to HPLC stationary phases for efficient recognition of nano-scaled guests in water. When cholesterol was used as a template molecule, the imprinted polymer selectively retained this guest more strongly and selectively than did non-imprinted polymer. The imprinting effect in the retention behavior was consistent with previously reported physicochemical analyses. Immobilization of the imprinted cyclodextrin polymer on the surface of silica gel in water was also successful. The polymer-silica gel composites obtained had sufficient physical strength, and were useful for HPLC stationary phases. These molecularly imprinted polymers could recognize steroids, amino acid derivatives, dipeptides, and antibiotics. Moreover, we found that the enantio-selectivity toward the template molecules was significantly promoted. This methodology has great potential for the recognition of large guest molecules in water, and can be an alternative to affinity chromatography for the separation of bio-molecules.

Introduction

To date, extensive study on the preparation of artificial receptors has been reported [11]. One of the most interesting hosts is cyclodextrin (CyD) [2], which forms inclusion compounds with hydrophobic guests in water. Modified CyDs also have been synthesized and efficiently accommodate guest molecules through hydrophobic interactions and others (e.g., electrostatic interaction).

In the recognition of nano-scaled guests such as peptides and proteins, however, monomeric CyDs are not sufficiently effective. Selective recognition of these large molecules is very crucial for the biochemical science and biochemical industry. It has been already reported that CyD dimers [3–5] and trimers [6] are effective since two or three CyDs therein cooperatively bind hydrophobic moiety of the guests. The strategy that we have developed recently is to prepare ordered assembly of CyDs by using molecular imprinting technique [7–13]. The procedure is as follows: (1) a template forms inclusion complex with two or more CyDs; (2) the adduct is cross-linked (immobilized) to form the complementary structure to the template; (3) washing out the template (see Figure 1). Thereby selective binding site for the template can be formed and this site efficiently binds the target compound [14–18]. These CyD assemblies have high affinity towards nano-scaled guests in water.

The selectivity of these imprinted CyDs is sufficiently high in aqueous media. In the present study, we apply these molecularly imprinted CyDs to HPLC stationary phases. The stationary phases are prepared in two methods. For hydrophobic templates such as steroids, which are hardly soluble in water, the imprinting is achieved in organic solvents. Cross-linkage with dusocyanate reagent is effective [14, 15, 17]. Imprinting is also achieved in water, where the stronger complex of CyDs is formed with the templates. Furthermore, efficient and robust stationary phases are obtained by molecular imprinting of CyDs on the surface of silica gel [18].

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Experimental

Preparation of HPLC stationary phases in DMSO

Cross-linking of β-CyD in DMSO was done in the same procedure as mentioned previously [14]. After the CyD polymer obtained was finely chopped into pieces by using pestle and mortal, the polymer particles with the size between 23 μm and 65 μm were collected and packed into a stainless steel column. The column was washed with acetonitrile until the baseline was stable, and retention times for various guests were measured by using UV spectrometer.

Preparation of imprinted polymer on the surface of silica gel

For the imprinting on silica gel in water, radical polymerization was employed with methylenebisacrylamide as cross-linker in the presence of templates. Syntheses of CyD monomer (acryloyl CyD) and vinylated silica gel were previously reported [18]. Acryloyl CyD, the cross-linker, and

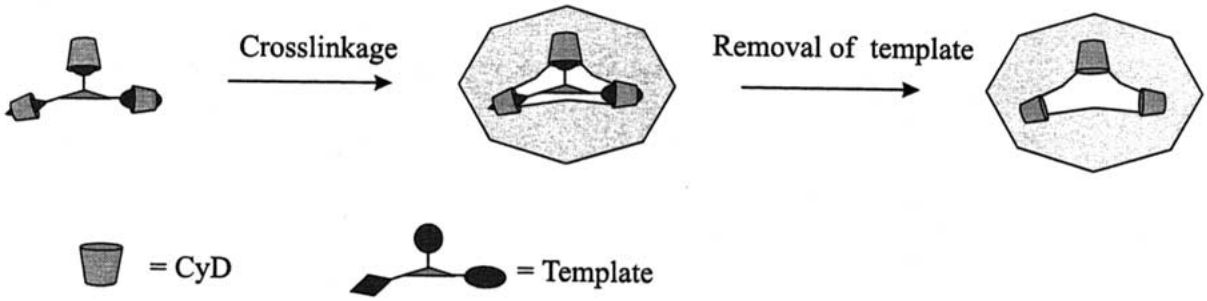

Figure 1. Molecular imprinting of CyDs for the recognition of nano-scale guests.

Table 1. Retention behavior of the cholesterol-imprinted and non-imprinted β-CyD-polymer columns prepared in DMSO

Guest	Retention time/min[a]	
Cholesterol-imp	Non	
Cholesterol	16.8	7.7
Progesterone	7.8	6.9
Pregnenolone	5.7	4.9
Phenol	2.2	2.2
Acetone	1.9	1.9

[a]Eluent: AN/water = 60/40 (v/v).

Table 2. Retention behavior of the antibiotics-imprinted and non-imprinted β-CyD polymers prepared in water

β-CyD polymer	Retention time/min[a]	
	Vancomycin	Cefazolin
Non-imp	10.3	9.3
Vancomycin imp	14.7	9.7
Cefazolin-imp	9.9	11.1

[a]Eluent: 50 mM ammonium formate buffer.

vinylated silica gel were copolymerized by using $K_2S_2O_8$ as radical initiator. The silica gel coated with the imprinted CyD polymer was packed into a stainless steel column, and the template and other chemicals were washed out with methanol. Non-imprinted column was prepared under the same conditions except for the absence of the template. Retention behavior of these polymer-coated silica gel composites was studied by using ammonium formate buffer as eluent.

Results and discussion

Selective promotion of binding by molecular imprinting in DMSO

Table 1 shows the retention behavior of the cholesterol-imprinted and non-imprinted β-CyD polymers prepared in DMSO. The imprinted polymer retains the target (cholesterol) more strongly than the non-imprinted one. When pregnenolone and progesterone are used as the guest compounds, however, promotion of binding by the imprinting (with cholesterol) is much less. These results show that molecular imprinting of CyDs provides guest-selectivity in steroid family. Since the retention time for phenol or acetone is not changed, the imprinting does not alter the number of binding sites, but rather enhances the binding activity of these sites. Assumedly, the orientation of CyD molecules is regulated to bind the target compound cooperatively. On the other hand, progesterone as template did not affect the retention behavior of steroid guests (data not shown). Those remarkable differences in imprinting efficiency are consistent with the previous results obtained by batch adsorption assay [14] and physicochemical analyses. Promotion of retention induced by the imprinting is probably attributed to the accelerated dimerization of CyDs in imprinting mixture, which was clearly evidenced by MALDI-TOFMS study [17].

Recognition of antibodies

Table 2 shows retention behavior of antibiotics-imprinted CyD polymers as HPLC stationary phases. The imprinted polymer was prepared on the surface of silica gel in water as described in Experimental section. When vancomycin is the template, the imprinted polymer retains this template more strongly than does the non-imprinted polymer. On the other hand, the retention time of vancomycin-imprinted polymer for cefazolin is almost identical to that of non-imprinted polymer. The present imprinting provides the polymer with sufficient selectivity toward the template compound.

With cefazolin as templates, similar imprinting effect is observed. Cefazolin-imprinted polymer retains the template molecule more strongly than the non-imprinted one, while this imprinting does not promote the binding toward vancomycin. Two or three CyD molecules are essential for the promotion of binding, since phenethicillin (having only one CyD-binding site) is not an effective template and hardly improves the binding towards this penicillin derivative. These results indicate that this method should be more useful for the targets such as proteins and other biologically important molecules, which bear many hydrophobic sites.

Recognition of chiral dipeptides

The composites of CyD-imprinted polymer with silica gel as stationary phases in water are also applied to the recognition of dipeptides (Table 3). When L-Phe-L-Phe is used as a template, this L-form is more strongly retained by the

Table 3. Retention behavior of the imprinted and non-imprinted CyD polymers toward Phe-Phe

β-CyD polymer	Retention time/min[a]	
	L-Phe-L-Phe	D-Phe-D-Phe
None-imp	6.8	6.8
L-Phe-L-Phe-imp	7.3 (+0.4)	6.9
D-Phe-D-Phe-imp	7.8	8.9 (+1.1)

[a]Eluent: 50 mM ammonium formate buffer.
[b]Parentheses show the difference in retention time (mm) between D-form and L-form.

imprinted polymer while there is no promotion of binding for the D-form. With D-Phe-D-Phe as template, however, retention time for D-Phe-D-Phe is longer than that of L-form. By molecular imprinting of CyDs in water, we can control the affinity toward chiral compounds. This enantio-selective recognition by the imprinted CyD polymers was applicable to various other guests in water (data not shown).

In summary, we have presented here the versatile method to prepare tailor-made receptors for nano-scaled guests by using molecular imprinting. The cross-linkage reaction can be successfully done in organic solvents and in water. This technique provides structure-selective and enantio-selective receptors. Furthermore, the imprinting on the surface of silica gel is promising to prepare artificial HPLC stationary phases for the separation of biofunctional nano-scaled molecules in water.

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