Reconfirmation of Recognition Site in Composites of Imprinted β-Cyclodextrin Polymer/Solid Support as Stationary Phase of HPLC

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β-Cyclodextrin (β-CyD) was imprinted to a tripeptide H–Phe–Lys–Phe–NH_2 on the surface of modified silica-gels, alumina, and Poly-HEMA [poly(hydroxyethyl methacrylate)]. In spite of notable differences in surface charges and other physicochemical properties of these solid supports, all the imprinted β-CyD polymers showed notable imprinting effects, confirming the overwhelming roles of ordered assemblies of β-CyD for the molecular recognition.

Preparation of artificial receptors, which recognize large guest molecules in water, has been attracting interest. Our group has been developing a new strategy for preparation of these synthetic antibodies with the use of CyDs as functional monomers for molecular imprinting.[1–3] The imprinting effects are significantly high. For example, closely related decapeptides, angiotensin I and [Val^5]-angiotensin I, were clearly distinguished by the β-CyD polymers imprinted to either of them, although both peptides are different from each other only in the fifth amino acid (Ile vs. Val).[4] It was proposed that several CyD molecules are immobilized complementarily to the nonpolar and bulky groups of the template during the imprinting process.

In most of these studies, the imprinting of β-CyD was achieved on the surface of silica-gel bearing vinyl groups, and the resultant polymer/silica-gel composites were used as the stationary phase of HPLC analysis. Accordingly, in addition to the interactions of templates (and guests) with the imprinted β-CyD polymers, their interactions with the silica-gel, if any, could affect the results. In order to obtain a still clearer picture of the molecular imprinting, we here have carried out the imprinting of β-CyD on four different solid supports, and quantitatively compared the imprinting effects of the corresponding polymer/support composites. Both electrostatic effects and hydrophobic effects are assessed by using a tripeptide H–Phe–Lys–Phe–NH_2, which has two positive charges (on the Lys and the N-terminus) and two nonpolar sites (two Phe), as the template.

The methods of preparation of four kinds of solid supports for the imprinting are shown in Figure 1. In (A), silica-gel (Nucleosil 300–10 from MACHEREY-NAGEL, Germany; grain size 10 μm, pore size 30 nm in diameter, and specific surface area 100 $m^2 g^{-1}$) was directly treated with 3-acrylamidopropyltriethoxysilane at 50 °C (Silica-1). In (B), the same silica-gel was first treated with 3-aminopropyltriethoxysilane and triethylamine at 140 °C, and then with acryloyl chloride and triethylamine in dry dichloromethane (Silica-2). In (C), acrylamido groups were introduced onto the surface of alumina (from Nacalai; grain size 40–50 μm, pore size 2–3 nm in diameter, and specific surface area 150 $m^2 g^{-1}$) in virtually the same way as (A).[5] For the modification of poly-HEMA, the beads bearing *N*-hydroxysuccinimide esters (Shodex BIOACT gel NHS; NHS concentration = 0.25 mmol/g) reacted with *N*-acryloyl-1,2-diaminoethane in water containing 0.2 M $NaHCO_3$ and 0.25 M Na_2SO_4. In the typical molecular imprinting, mono-6-(*N*-acrylamido)-6-deoxy-β-CyD bearing an acrylamido group in the primary hydroxy side (30 μmol), *N*,*N*′-methylenebisacrylamide (MBAAm) as a crosslinking agent (180 μmol), and H–Phe–Lys–Phe–NH_2 as template molecule (15 μmol) were dissolved in 50 mM of tris(hydroxymethyl)aminomethane (Tris) buffer solution (pH 8.0, 5 mL), and then vinylated silica-gel (600 mg) was dispersed. The polymerization was started by adding potassium persulfate (11 μmol, 3 mg) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED: 20 μmol, 3 μL) as an initiator-system under nitrogen at 35 °C for 20 h. The solid part was washed with water and subsequently with methanol.

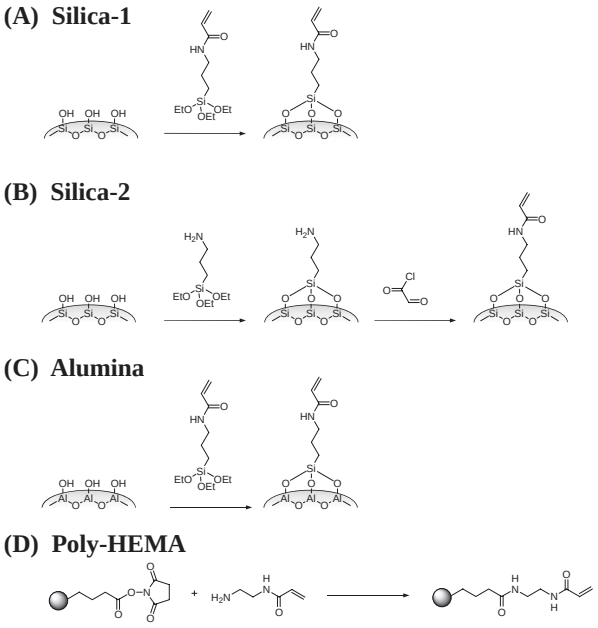

Figure 1. Preparation of various supports bearing vinyl groups for the β-CyD molecular imprinting.

The amounts of vinyl groups introduced onto the surface of the supports were determined by thermogravimetry analyses (RIGAKU, Thermo Plus 2 TG8120). The experiments were carried out under air from 50 to 700 °C at a heating rate of 20 K/min

Table 1. Zeta potential of each support

Support	Zeta potential/mV
Silica-gel (unmodified)	−45.5
Silica-1	−40.4
Silica-2	−28.3
Alumina (unmodified)	14.7
Alumina bearing vinyl groups	3.4

Table 2. Imprinting effects α for the imprinted β-CyD polymers prepared on the surface of various solid supports using H–Phe–Lys–Phe–NH_2 template[a]

Guest	α (k)			
	Silica-1	Silica-2	Alumina	Poly-HEMA
H–Arg–Phe–NH_2	1.2 (17.8)	1.1 (5.6)	1.1 (1.2)	0.6 (0.6)
H–Lys–Phe–Lys–OH	1.1 (13.3)	1.1 (2.0)	1.0 (3.0)	0.3 (0.5)
H–Phe–Lys–Phe–NH_2	1.7 (49.5)	1.4 (15.6)	1.3 (5.6)	1.1 (3.6)
H–Phe–Lys–Phe–Lys–NH_2	—[b]	1.1 (47.3)	1.2 (17.5)	0.8 (1.3)
H–Phe–Lys–Phe–Lys–Lys–NH_2	—[b]	—[b]	1.0 (32.8)	0.7 (1.0)

[a]The capacity factors k are shown in parentheses. [b]Not detected.

and the difference in weight between 240 and 700 °C was taken as the organic compound bound to the support. The amounts of vinyl groups in Silica-1, Silica-2, and vinyl group-bearing alumina, prepared as depicted in Figure 1, were determined to be 0.16, 0.31, and 0.20 mmol/g, respectively. The vinyl content in Silica-2, prepared by the method in (B), is about 2 times as large as that in Silica-1, showing more efficient coverage of the silica-gel surface by the organic moiety. Zeta potentials of the modified supports were measured under the conditions that the imprinting was achieved (Table 1).[6] As expected from the partial dissociation of Si–OH, the silica-gel itself is negatively charged, and the negative charge decreases with increasing coverage of the surface (Silica-2 > Silica-1). On the other hand, the surface of alumina is positively charged, and the positive charges are decreased upon the introduction of vinyl groups to the surface.

These composites formed from imprinted β-CyD polymers and solid support were packed in a stainless steel column tube (50 mm × 4.6 mm i.d.), and retention behavior of various oligopeptides was monitored at 260 nm with the eluent flow rate of 1.0 mL min^{-1}. The capacity factor (k) was calculated as $(t - t_0)/t_0$, where t and t_0 are the retention times of guest and acetone (void marker). The imprinting effects were evaluated by α value defined by k_{imp}/k_{non}. Here, the subscripts imp and non refer to imprinted polymer and nonimprinted polymer (the polymer prepared in the absence of the template molecule), respectively.

The imprinting efficiencies are summarized in Table 2. The imprinted β-CyD polymers prepared on both of the two modified silica-gels (Silica-1 and Silica-2) showed considerably larger α values (larger imprinting effects) towards the template H–Phe–Lys–Phe–NH_2. The bindings of other oligopeptides were less affected by the imprinting to this tripeptide. These two modified silica-gels are considerably different from each other in the amounts of vinyl groups introduced (0.16 mmol/g vs. 0.31 mmol/g). Furthermore, their zeta potentials are notably different from each other (Table 1). Apparently, the present molecular imprinting effect is primarily ascribed to the formation of ordered assemblies of β-CyD molecules in which each β-CyD takes a complementary position to nonpolar and bulky group of the template. Direct interactions of the template with the negative charges of the silica-gels or with the hydroxy groups on their surface, if any, are not essential for the present imprinting. Consistently, the imprinting was also successful when alumina was used as the support, although the imprinting effects were smaller than those obtained by using the silica-gel supports. Note that the surface of alumina is positively charged under the conditions used for the imprinting and the guest-binding (Table 1). The imprinting of β-CyD on neutral support poly-HEMA was also efficient.[7]

In conclusion, molecular imprinted β-CyD polymers showed satisfactory imprinting effects irrespective of the solid supports used. This finding confirms that formation of ordered assemblies of β-CyD is the origin of the molecular imprinting as proposed before. Further promising possibilities of β-CyD imprinting for the developments of artificial antibodies are indicated.

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References and Notes

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6 In pH 8.0 Tris-HCl buffer, solid supports were dispersed at 1 wt % concentrations, and these mixtures were diluted to 1/8 (for the silica-gels 1 and 2) and 1/16 (for the alumina support). Under these conditions, these solid supports were satisfactorily dispersed.
7 In this case, reproducibility of the α value was rather poor, probably because the support is partially decomposed by the radicals formed during the imprinting.

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