Importance of the Position of Vinyl Group on β-Cyclodextrin for the Effective Imprinting of Amino Acid Derivatives and Oligopeptides in Water

Tomo Osawa,[†] Kazumi Shirasaka,[†] Takahiko Matsui,[†] Shinji Yoshihara,[†] Tomohiro Akiyama,[†] Takayuki Hishiya,[†] Hiroyuki Asanuma,*[,†,‡,§] and Makoto Komiyama*[,†]

Research Center for Advanced Science and Technology, The University of Tokyo, Komaba, Meguro-ku, Tokyo 153-8904, Japan, and Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency (JST), Kawaguchi, Saitama 332-0012, Japan

Received January 11, 2006; Revised Manuscript Received February 6, 2006

ABSTRACT: Two kinds of vinyl monomers of β-cyclodextrin (β-CyD) that tether a vinyl group on either wider rim of the truncated cone or its smaller rim were synthesized and applied to the imprinting toward amino acid derivatives and oligopeptides in water. Mono-3-(*N*-acrylamido)-3-deoxy-altro-β-cyclodextrin (3-AAm-CyD) showed a remarkable imprinting effect for the enantioselective recognition of protected amino acids such as *N*-benzyloxycarbonyltyrosine (Z-Tyr). However, the imprinted polymer from mono-6-(*N*-acrylamido)-6-deoxy-β-cyclodextrin (6-AAm-CyD) hardly showed enantioselectivity. According to NOESY analysis on the preorganized β-CyD/Z-Tyr complex in D_2O, the aromatic moieties of Z-Tyr were included into the cavity of β-CyD from its wider rim. Since the vinyl group of 3-AAm-CyD protruded toward the template and was polymerized there, the detailed shape of the template was precisely copied on the polymer by the imprinting. In the case of 6-AAm-CyD, however, the shape of template could not be well transcribed because its vinyl group was located at opposite side of the cavity, and thus copolymerization occurred far from the template molecule. On the other hand, the imprinted polymers from both β-CyD vinyl monomers were effective for the recognition of sequences of tetrapeptides composed of two glycines and two phenylalanines, although the selectivity itself was not remarkable. In these polymers, even the β-CyD residues of 6-AAm-CyD were immobilized complementarily to the phenyl rings and bound them.

Introduction

Since "molecular imprinting" is a comprehensive concept and easy to carry out, numerous strategies for versatile applications have been proposed and examined.[1] Although fundamental concepts have been already realized and several polymers are now proceeding to practical applications, the molecular imprinting technique is still growing ahead. As the kinds of target molecules are extended, new strategies are being required. One of recent interests in molecular recognition is to realize the functions of natural antibody by artificial systems: recognition of biomolecules such as steroids, amino acids, oligopeptides, and proteins in water.[1e] To preorganize functional monomers complementarily to the template in water, hydrogen bondings are unfavorable since they are easily destroyed in water due to competition with the solvent molecules.[2] Accordingly, conventional functional monomers such as acrylic acid and 4-vinylpyridine, which are preferentially used for the imprinting in chloroform, are not available. Functional monomers that can interact with templates in aqueous media are crucially required. In the previous study, we have solved this problem by use of a vinyl monomer of cyclodextrin (CyD), a cyclic oligomer of glucose unit, because it can bind hydrophobic guest in its cavity in water.[3,4] Furthermore, silica gel support was successfully coated with molecularly imprinted CyD copolymer, obtained by using *N*,*N*′-methylenebis(acrylamide) (MBAAm) as a cross-linker, as shown by Scheme 1.[5] By packing the silica gel composite into stainless steel column and applying it to a stationary phase of high-performance liquid chromatography, antibiotics and amino acid derivatives were efficiently discriminated.

One of the most crucial points for effective imprinting is the design of functional monomers. CyD has two kinds of hydroxyl groups to which vinyl group can be tethered: one is primary OH at the smaller side of pail-shaped CyD, and the other is secondary OH at the wider side. The position of vinyl group on the CyD monomer should enormously affect the imprinting because it governs the distance between the template and polymerization site (guest molecules usually take a specific orientation in CyD inclusion complexes).[6] Here, we synthesize two kinds of β-CyD vinyl monomer that tethers a vinyl group on the smaller rim of β-CyD (primary OH side) or its wider rim (secondary OH side), as shown in Figure 1. These β-CyD monomers are used for the imprinting to amino acid derivatives and oligonucleotides in water, and their imprinting efficiencies are quantitatively compared. Furthermore, these results are interpreted in terms of the structures of preorganized β-CyD complexes determined by NMR spectroscopy.

Experimental Section

Materials. β-Cyclodextrin (β-CyD), 6-*O*-α-D-glucosyl-β-cyclodextrin (G1-β-CyD), *N*,*N*′-methylenebis(acrylamide) (MBAAm), and other reagents were from Tokyo Kasei Co. Silica gel used as a support was obtained from MACHEREY-NAGEL from Germany (Nucleosil 300-10: grain size 10 μm, pore size 30 nm in diameter, and specific surface area 100 $m^2\ g^{-1}$) and was dried at 140 °C for 1 day before use. Protected amino acids and oligopeptides as

[†] The University of Tokyo.
[‡] Japan Science and Technology Agency.
[§] Current address: Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan.
* To whom correspondence should be addressed. E-mail: asanuma@mol.nagoya-u.ac.jp; komiyama@mkomi.rcast.u-tokyo.ac.jp.

10.1021/ma060064f CCC: $33.50
Published on Web 03/02/2006

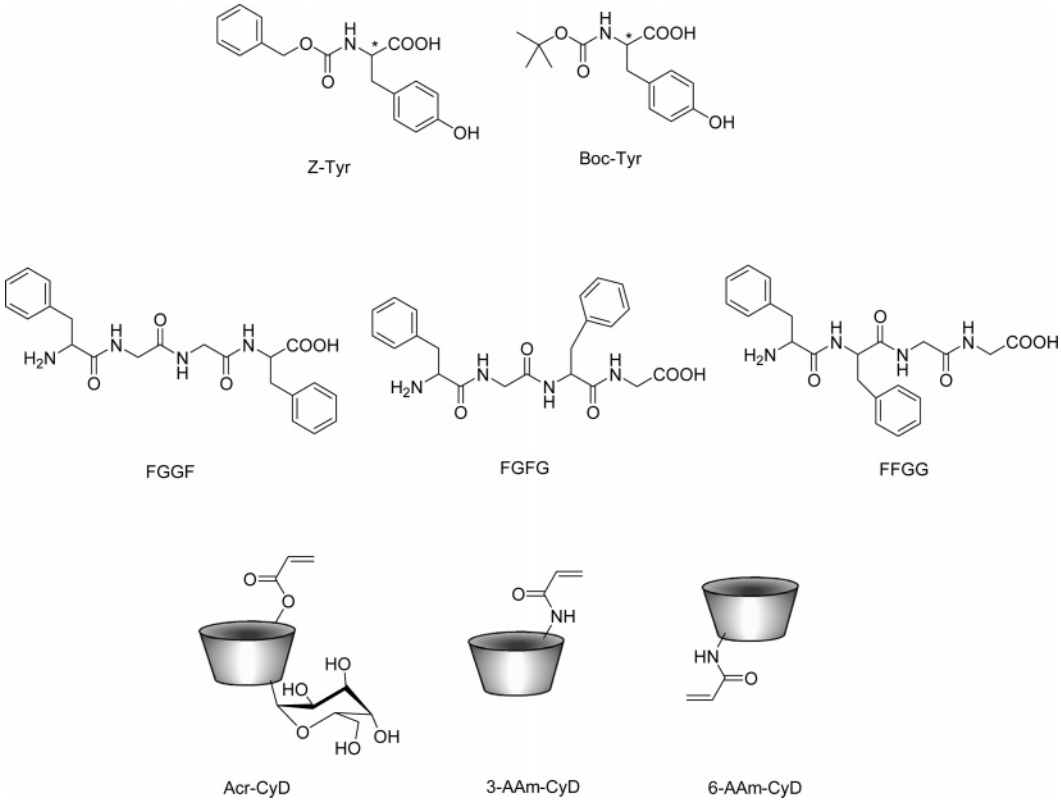
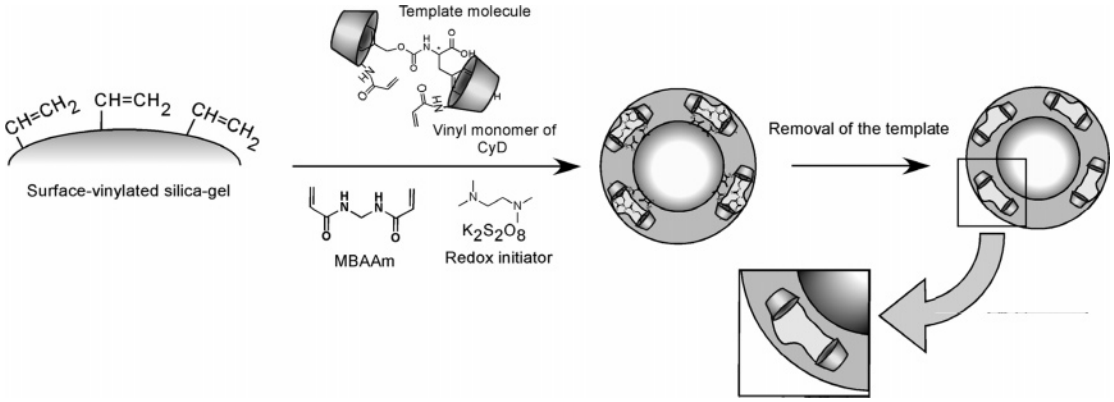


Figure 1. Templates, guests, and vinyl monomers used in the present study.

Scheme 1. Schematic Procedure of the Immobilization of the Imprinted Polymer on the Silica Gel Support



templates and guests (listed in Figure 1) were obtained from Wako Pure Chemical Industries Co. (Japan) and BACHEM AG (Switzerland) and were used without further purification.

The vinyl monomer of β-CyD that tethers the acryloyl group at the wider rim of truncated cone (Acr-CyD, see Figure 1) was synthesized by the ester-exchange reaction of *m*-nitrophenyl acrylate with G1-β-CyD according to the previous reports.[3c,7]

Synthesis of Mono-3-(*N*-acrylamido)-3-deoxy-altro-β-cyclodextrin (3-AAm-CyD). This monomer was synthesized through mono-3-amino-3-deoxy-altro-β-cyclodextrin as follows.[8] β-CyD (100 g) was dried under vacuum at 45 °C and dissolved in dry dimethylformamide (DMF, 500 mL). Then sodium hydride (3.5 g) was added, and resulting mixture was stirred overnight, followed by dropwise addition of tosyl chloride solution (16.8 g in DMF). After 30 min of stirring at room temperature, water was added to terminate the reaction. The reaction mixture was poured into acetone, and white precipitates were collected. Obtained solid was dissolved into water, and monosubstituted tosyl-β-CyD was separated by polystyrene column chromatography (HP-20) with 20−25% aqueous methanol solution as eluent. Yield: 36% (41 g).

Tosyl-β-CyD thus obtained (16.8 g) was dissolved in aqueous NH_3 (28%) solution (300 mL), and the reaction mixture was kept for 24 h at room temperature. Then the solvent was evaporated, and the resulting crude solid was purified by cation-exchange resin (DIAION PK208) with 3% aqueous NH_3 solution as eluent to obtain mono-3-amino-3-deoxy-altro-β-CyD. Yield: 58% (8.5 g).

The DMF solution (10.6 mL) of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphonate (HBTu, 1.8 g), acrylic acid (0.33 mL), and *N*,*N*-diisopropylethylamine (1.6 mL) was added to the DMF solution (53 mL) of mono-3-amino-3-deoxy-altro-β-CyD (5.3 g), and the resulting mixture was stirred for 24 h at room temperature. The solvent was evaporated, and crude oily product was dissolved in a small amount of water and poured into acetone for the precipitation. The white solid was collected and was further subjected to column chromatography on Sephadex G-15 (5 × 200 cm) to purify the mono-3-(*N*-acrylamido)-3-deoxy-β-CyD. Yield: 29% (1.6 g): MALDI-TOF MS (positive mode): obsd 1211.0 (calcd for $[M + Na^+]$: 1210.4). ^1H NMR [D_2O, 500 MHz]: δ = 6.20 (m, 2H), 5.73 (d, 1H, J = 11 Hz), 5.1−4.9 (m, 7H), 4.25 (m, 1H), 4.15 (bs, 1H), 4.0−3.5 (m, 40 H).

Synthesis of Mono-6-(*N*-acrylamido)-6-deoxy-β-cyclodextrin (6-AAm-CyD). A β-CyD vinyl monomer tethering vinyl group at the smaller rim of truncated cone (6-AAm-CyD) was synthesized through mono-6-amino-6-deoxy-β-cyclodextrin.[9] Into dry pyridine

(500 mL), β-CyD (50 g), dried under vacuum at 45 °C, was dissolved, and tosyl chloride (8.4 g) was added dropwise at 0 °C. After stirring for 30 min on ice bath, water was added to terminate the reaction. The reaction mixture was poured into acetone, and white precipitate was collected. The precipitate was dissolved in water, and monotosyl-β-CyD was separated by polystyrene column chromatography (HP-20) with aqueous methanol solution as eluent. Yield: 25% (14.1 g).

Tosyl-β-CyD thus obtained (14.1 g) was dissolved in water (700 mL) at 80 °C and NaN_3 (11.4 g) was added, followed by stirring for 5 h at room temperature. The solvent was evaporated, and the concentrated reaction mixture was poured into acetone. The crude white powder of monoazide of β-CyD (N_3-β-CyD; 12.6 g) was dissolved in DMF (350 mL) together with triphenylphosphine (6.3 g), and aqueous ammonia (28%, 40 mL) was added. After stirring for 4 h, the solvent was evaporated, and the concentrated reaction mixture was poured into acetone to give a white powder of crude mono-6-amino-6-deoxy-β-CyD (NH_2-β-CyD), which was further purified by cation-exchange resin (DIAION PK208) with 3% aqueous NH_3 solution as eluent. Yield: 55% (6.8 g).

The final acrylation was performed as follows: NH_2-β-CyD (6.8 g) and $NaHCO_3$ (5.0 g) were dissolved in water (500 mL), and pH of the solution was adjusted to around 11 with NaOH. To this solution was added acryloyl chloride (0.9 mL) dropwise on ice bath with vigorous stirring, followed by neutralization with aqueous HCl. To hydrolyze partially esterified β-CyD, aqueous ammonia was added, and the mixture was stirred at room temperature for 30 min. The solution was neutralized again with aqueous HCl, and the water was removed by evaporation. The obtained solid was dissolved in water and eluted through anion-exchange resin (DIAION WA30) and then cation-exchange resin (DIAION PK208) to remove the salts. The obtained solution was concentrated by evaporation and was further subjected to column chromatography on Sephadex G-15 (5 × 200 cm). Yield of mono-6-(*N*-acrylamido)-6-deoxy-β-CyD: 9.3% (670 mg): MALDI-TOF MS (positive mode): obsd 1211.0 (calcd for $[M + Na^+]$: 1210.4). ^{1}H NMR [D_2O, 500 MHz]: δ = 6.24 (dd, 1H, J = 10.4, 16.9 Hz), 6.12 (d, 1H, J = 16.9 Hz), 5.71 (d, 1H, J = 10.4 Hz), 5.1−4.9 (m, 7H), 4.0−3.2 (m, 40 H), 3.36 (t, 1H, J = 9.2 Hz), 3.24 (dd, 1H, J = 9.2, 13.8 Hz).

Introduction of Vinyl Groups on Silica Gel Surface. Vinylsilane was introduced on the surface of silica gel according to the following procedure:[5] dried silica gel (10 g) was dispersed in dry toluene−pyridine solution (10/1 in volume; 110 mL), followed by dropwise addition of trichlorovinylsilane (250 μL, 2.0 mmol) under nitrogen. After the dispersion was stirred for 16 h at 50 °C, the silica gel was collected and washed successively with chloroform, methanol, and water. Finally, the modified silica gel was dried under vacuum and used for the immobilization of the imprinted polymer. According to the titration with $KMnO_4$, about 70 μmol of vinyl group was incorporated to 1 g of silica gel. The coverage with vinylsilane was kept below this value, since further incorporation made the surface too hydrophobic for the imprinting.

Immobilization of the Imprinted β-CyD Polymer on the Modified Silica Gel. A vinyl monomer of β-CyD (60 or 20 μmol), *N*,*N*′-methylenebis(acrylamide) (MBAAm) as a cross-linking agent (360 or 120 μmol), and template molecule (30 or 10 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.[10] After stirring the dispersion for a few minutes, the polymerization was started at 35 °C by adding potassium persulfate (7 μmol, 3 mg) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED: 20 μmol, 3 μL) as an initiator system under nitrogen. After 1 h, the solid part was collected and washed with large amount of water and subsequently with methanol to remove the template and unreacted monomers. Sufficient removal of the template was confirmed by analyzing the supernatant and the waste solvent. Thus, the obtained composite of polymer and silica gel was directly packed into the column (vide infra).

Measurement of the Retention Time of Guest by HPLC. The silica gel coated with β-CyD polymer was packed in a stainless steel column tube (50 mm × 4.6 mm i.d, purchased from GL Science). The retention behavior of guest was monitored with an HPLC system (JASCO). As an eluent, 50 mM Tris buffer solution (pH 8.0) was used at a flow rate of 0.25 or 0.5 mL min^{-1}. 20 μL of 1 mM aqueous guest solution was injected to the HPLC, and eluted guest was detected by UV absorption at 260 nm. Retention behavior of the guest was estimated from the capacity factor k, which was calculated according to the following equation:

$$k = (t_1 - t_0)/t_0 \quad (1)$$

where t_1 and t_0 are the retention times of guest and acetone (as void marker), respectively. To evaluate the magnitude of imprinting effect, "imprinting-induced promotion of binding" (abbreviated as IPB) was defined as follows:[11]

$$\text{IPB (\%)} = (k_{imp} - k_{non})/k_{non} \times 100 \quad (2)$$

Here, k_{imp} and k_{non} are capacity factors of the imprinted and nonimprinted polymers, respectively. The IPB values reflect the efficiency of the imprinting more correctly than do the k_{imp}'s themselves, since the difference in the intrinsic binding capacity of various guests toward β-CyD residues is normalized.

NMR Measurement. NMR spectra of the complex between β-CyD and Z-L-Tyr in D_2O were measured on an Avance-600 spectrometer (600 MHz, Bruker) at a probe temperature 280 K. Solution conditions were as follows: [β-CyD] = 10 mM, [Z-L-Tyr] = 5 mM in 50 mM phosphate buffer (pD = 8.0). Here we did not use Tris buffer to avoid the overlap of its proton signal with those from CyD. 2D NOESY spectra were recorded with a mixing time of 600 ms. For the structural analysis of the β-CyD complex, ROESY was always adopted. In this study, we used NOESY because distinct and consistent signals could be observed. TOCSY and COSY spectra were also measured to assign the proton signals.

Results

1. Molecular Imprinting toward Protected Amino Acids. *Imprinting of Acr-CyD or 3-AAm-CyD as Functional Monomer*. Molecular imprinting was carried out according to Scheme 1 (see Experimental Section for details). Silica gel surface was coated with imprinted or nonimprinted β-CyD polymers for the stationary phase of HPLC, and retention behaviors were estimated from the capacity factor k. To evaluate the imprinting efficiency, the IPB value (promotion of binding by the imprinting) was also obtained from eq 2. First, protected amino acids were used as templates to examine the discriminating activity on either the chirality of template or the kind of its protecting group. As functional monomers, Acr-CyD and 3-AAm-CyD, which tether a vinyl group on the secondary OH side, were used (see Figure 1). Each monomer has the following features: 3-AAm-CyD has a vinyl group at the C-3 position of β-CyD, but the saccharide bearing the vinyl group is changed from glucose to altrose during the synthesis. On the other hand, Acr-CyD does not suffer from such isomerization of glucose but is a mixture of C-2- or C-3-substituted compounds.

When Acr-CyD was copolymerized with MBAAm on the silica gel in the absence of template, the derivatives of D-tyrosine were retained to the column slightly more strongly than their L-enantiomers (see the column "nonimp" in Table 1A). This comes from the intrinsic enantioselectivity of β-CyD.[3c] All the guests were bound to the column with comparable affinity. When *N*-benzyloxycarbonyl-L-tyrosine (Z-L-Tyr) was used as template for imprinting, however, capacity factor k for Z-L-Tyr increased from 3.6 to 5.1. The IPB value was 42. In contrast, k values of this polymer for other guests did not notably increase (IPB is only 8 for the binding of both Z-D-Tyr and *N*-(*tert*-butoxycarbonyl)-D-tyrosine (Boc-D-Tyr)). The binding to Boc-L-Tyr was even suppressed by the imprinting (IPB = −3). As

Table 1. Capacitiy Factors and IPB Values for the Polymers of (A) Acr-CyD and (B) 3-AAm-CyD Molecularly Imprinted toward Protected Tyrosines[a,b]

(A) Functional Monomer = Acr-CyD

	capacity factor *k*				
guest	Z-L-Tyr-imp[c]	Z-D-Tyr-imp	Boc-L-Tyr-imp	Boc-D-Tyr-imp	nonimp[d]
Z−L-Tyr	**5.1 (42)**[e]	5.0 (39)	4.1 (14)	3.5 (−3)	3.6
Z−D-Tyr	4.0 (8)	**5.5 (49)**	3.8 (3)	3.7 (0)	3.7
Boc-L-Tyr	3.2 (−3)	4.1 (24)	**4.5 (36)**	3.6 (9)	3.3
Boc-D-Tyr	4.1 (8)	4.8 (26)	4.3 (13)	**4.5 (18)**	3.8

(B) Functional Monomer = 3-AAm-CyD

	capacity factor *k*				
guest	Z-L-Tyr-imp[c]	Z-D-Tyr-imp	Boc-L-Tyr-imp	Boc-D-Tyr-imp	nonimp[d]
Z-L-Tyr	**1.05 (25)**[e]	0.86 (3)	1.18 (40)	0.86 (3)	0.84
Z-D-Tyr	0.96 (6)	**1.08 (19)**	1.24 (37)	1.00 (11)	0.90
Boc-L-Tyr	0.41 (8)	0.38 (0)	**0.68 (79)**	0.38 (0)	0.38
Boc-D-Tyr	0.46 (0)	0.46 (1)	0.69 (52)	**0.54 (19)**	0.46

[a] Amount of the β-CyD monomer for the polymerization was 60 μmol. [b] Flow rate of the eluent was 0.25 mL min^{-1}. [c] Imprinted polymer synthesized in the presence of a template. [d] Nonimprinted polymer synthesized in the absence of a template. [e] IPB values estimated from eq 2 are presented in parentheses.

Table 2. Capacity Factors toward Protected Amino Acids for Nonimprinted Polymers from 6-AAm-CyD and Acr-CyD[a,b]

	capacity factor *k*	
guest	6-AAm-CyD	Acr-CyD
Z-L-Tyr	3.02	1.19
Z-D-Tyr	3.02	1.17
Boc-L-Tyr	3.58	1.11
Boc-D-Tyr	3.49	1.30

[a] Amount of the β-CyD monomer for the polymerization was 20 μmol. [b] Flow rate of the eluent was 0.5 mL min^{-1}.

Table 3. Imprinting of 6-AAm-CyD toward Protected Tyrosines[a,b]

	capacity factor *k*		
guest	Z-L-Tyr-imp[c]	Z-D-Tyr-imp	nonimp[d]
Z-L-Tyr	**4.4 (46)**[e]	3.6 (20)	3.0
Z-D-Tyr	4.0 (33)	3.4 (13)	3.0
Boc-L-Tyr	4.6 (31)	4.1 (17)	3.5
Boc-D-Tyr	4.5 (29)	4.0 (14)	3.5

[a] Note that the amount of 6-AAm-CyD for the polymerization (20 μmol) was one-third of the value used in Table 1. [b] Flow rate of the eluent was 0.5 mL min^{-1}. [c] Imprinted polymer synthesized in the presence of a template. [d] Nonimprinted polymer synthesized in the absence of a template. [e] Parentheses indicate IPB values estimated from the eq 2.

Table 4. Discrimination of Tetrapeptides by Imprinted Polymers from (A) 3-AAm-CyD and (B) 6-AAm-CyD[a,b]

(A) Monomer = 3-AAm-CyD

	capacity factor *k*			
guest	FFGG-imp[c]	FGFG-imp	FGGF-imp	nonimp[d]
FFGG	**0.43 (2)**[e]	0.41 (−4)	0.42 (−2)	0.43
FGFG	0.86 (−2)	**0.96 (8)**	0.91 (2)	0.89
FGGF	0.95 (−5)	0.98 (−3)	**1.12 (11)**	1.01

(B) Monomer = 6-AAm-CyD

	capacity factor *k*			
guest	FFGG-imp[c]	FGFG-imp	FGGF-imp	nonimp[d]
FFGG	**0.67 (12)**[e]	0.65 (8)	0.69 (15)	0.60
FGFG	1.64 (8)	**1.72 (13)**	1.83 (20)	1.52
FGGF	1.65 (2)	1.73 (7)	**1.98 (23)**	1.61

[a] Amounts of 3-AAm-CyD and 6-AAm-CyD for the polymerization were 60 and 20 μmol, respectively. [b] Flow rate of the eluent was 0.5 mL min^{-1}. [c] Imprinted polymer synthesized in the presence of a template. [d] Nonimprinted polymer synthesized in the absence of a template. [e] Parentheses indicate IPB values estimated from eq 2.

a result, Z-L-Tyr was retained most strongly. As expected, the binding to Z-D-Tyr was the strongest when the column was prepared with Z-D-Tyr as a template. For all the compounds investigated, the template used for the imprinting was most strongly bound by the column (these combinations are designated by bold characters in Table 1 (and also in Tables 3 and 4)). Consistently, IPB was the highest under these combinations.

With 3-AAm-CyD as the functional monomer, capacity factors for all the tested guests were smaller than the values with Acr-CyD (compare (B) with (A) in Table 1).[12] However, imprinting behavior was similar to that observed for Acr-CyD. The HPLC columns of Z-L-Tyr-imp and Z-D-Tyr-imp most strongly bound Z-L-Tyr and Z-D-Tyr, respectively.[13] The IPB values were sufficiently large only when the imprinting template was used as the guest. The enantioselectivity of the imprinted polymer was inversed when the chirality of template was reversed (D vs L).

In the imprinting with Boc-L-Tyr and Boc-D-Tyr, however, the *k* values for these Boc-Tyr derivatives were smaller than the values for Z-L-Tyr and Z-D-Tyr, since 3-AAm-CyD polymers intrinsically favor Z-Tyr over Boc-Tyr (see "nonimp" in Table 1B). Even in these cases, the imprinting effects were evident in IPB values. For Boc-L-Tyr-imp, Boc-L-Tyr(IPB = 79) > Boc-D-Tyr(52) > Z-L-Tyr(40) > Z-D-Tyr (37), whereas Boc-D-Tyr(19) > Z-D-Tyr (11) > Z-L-Tyr(3) > Boc-L-Tyr-(0) for Boc-D-Tyr-imp. Thus, molecular imprinting with β-CyD monomer bearing vinyl group on the secondary OH side effectively memorized both the enantioselectivity and the kind of protecting group.

Imprinting of 6-AAm-CyD as Functional Monomer. In 6-AAm-CyD, the acryl group is bound to the primary OH side. The imprinted polymers from this β-CyD monomer bound all the guests very strongly, and their retention times were about 3 times as long as those of the polymer from Acr-CyD (see Table 2). Although the presence of template raised the capacity factors, the guest selectivity was rather poor even under these imprinting conditions (see Table 3). In the imprinting to Z-D-Tyr, for example, the capacity factor of Z-L-Tyr increased much more drastically than that of Z-D-Tyr (middle column). The IPB value on Z-D-Tyr (13) was even smaller than the values on Z-L-Tyr-(20), Boc-L-Tyr(17), and Boc-D-Tyr(14). Even when Z-L-Tyr was used as the template, the binding of Z-L-Tyr was never the strongest among the guests tested (see the left column in Table 3). Thus, the imprinting of 6-AAm-CyD that tethers a

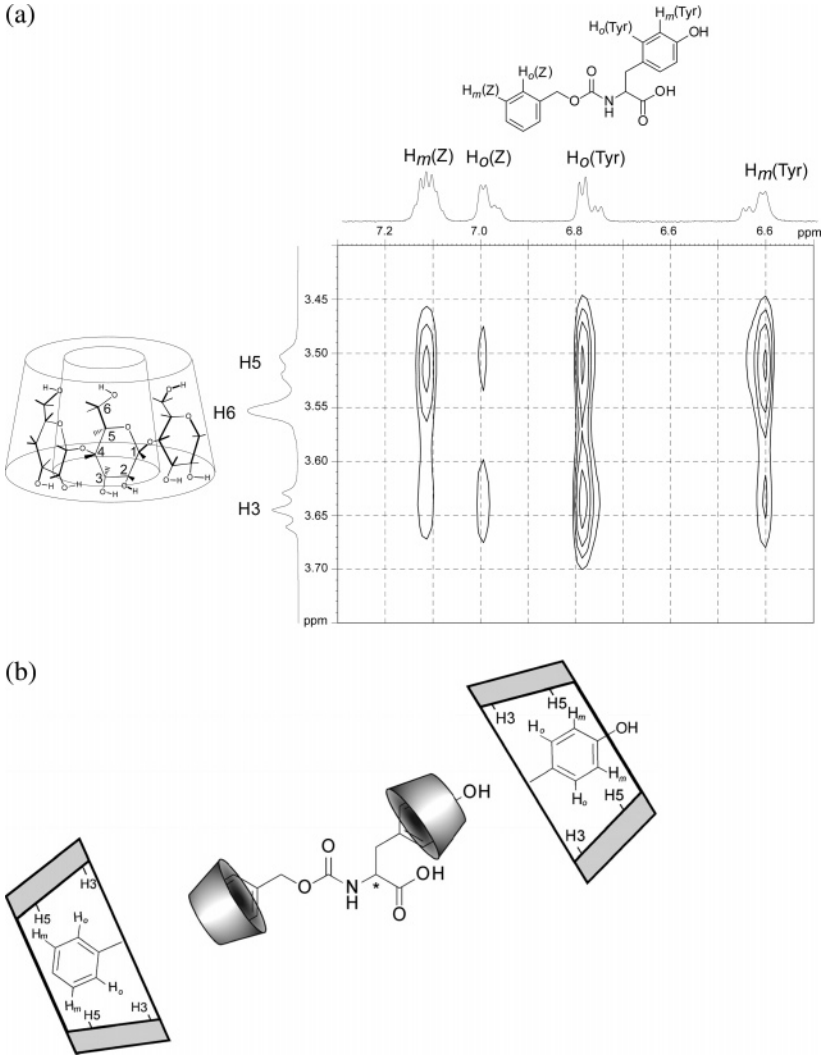


Figure 2. (a) 2D NOESY spectrum (600 MHz) between the aromatic proton signal (F2 axis: 6.5−7.3 ppm) and CyD proton signal (F1 axis: 3.40−3.75 ppm) regions for the complex of Z-L-Tyr and β-CyD in D_2O at 280 K. (b) Its structure expected from NOESY.

vinyl group on the smaller rim could not sufficiently memorize either the enantioselectivity of amino acid derivatives or the kind of their protecting groups.

2. Molecular Imprinting of Tetrapeptides for Recognition of Positions of the Phe Residues. For the discrimination of oligopeptides, recognition of their sequences is important. Here, tetrapeptides composed of two Gly and two Phe were used as templates and were subjected to the imprinting. Since capacity factors of these peptides were entirely different from each other, imprinting could not alter the retention order. However, template peptide always gave the highest IPB value. For example, when 3-AAm-CyD was applied to the imprinting of FGGF, IPB for FGGF was 11, which was the highest among all the tetrapeptides (see Table 4A). On the other hand, the IPB of this FGGF-imprinted polymer for FGFG was only 2, and the binding to FFGG was rather suppressed by the imprinting (IPB value is negative). Interestingly, 6-AAm-CyD, which could not control the enantioselectivity by the imprinting, also showed reasonable imprinting effect. As shown in Table 4B, the highest IPB was observed for the template peptide. All of 6-AAm-CyD, 3-AAm-CyD, and Acr-CyD were available as functional monomers for imprinting of oligopeptides and recognizing the positions of hydrophobic residues.

3. NOESY Spectra of the Complex of β-CyD with Z-L-Tyr. To investigate the preorganized complexes between the amino acid derivatives and the β-CyD vinyl monomers in the imprinting mixtures, the NOESY spectrum of the Z-L-Tyr/β-CyD complex was measured in D_2O. Since β-CyD prefers a hydrophobic moiety, distinct NOE was observed between the aromatic protons of Z-L-Tyr (F2 axis of Figure 2a) and the protons of β-CyD at both 5 and 3 positions (F1 axis) that protrude into the inside of cavity. The signals at 7.1 and 7.0 ppm (H_m(Z) and H_o(Z) in the F2 axis of Figure 2a), which were assigned to the protons on the benzene ring of benzyloxycarbonyl group, exhibited clear NOE with the protons of β-CyD at C-5 and C-3 positions (H5 and H3 in F1 axis of Figure 2a). Significantly, the protons at the meta position with respect to the urethane linkage (H_m(Z) in Figure 2a) gave much stronger NOE signals with the H5 protons on β-CyD than with H3 protons. In the case of H_o(Z), H3 protons on β-CyD gave slightly larger NOE than H5 protons did. These NOEs show that H_m(Z)s were located near the H5, and H_o(Z)s were between the H5 and H3. In other words, β-CyD formed an inclusion complex with the benzene ring from the wider rim, as depicted on the left-hand side of Figure 2b. Similar NOE patterns were observed for the protons on the tyrosine. The meta protons (H_m(Tyr) in Figure 2a) with respect to the carboxylate group showed much stronger NOE with H5 on β-CyD, whereas ortho protons (H_o(Tyr)) gave slightly stronger NOE with H3 than with H5. Thus, it was concluded that these two aromatic rings were included into the β-CyD cavities from the secondary OH side, as illustrated in Figure 2b.

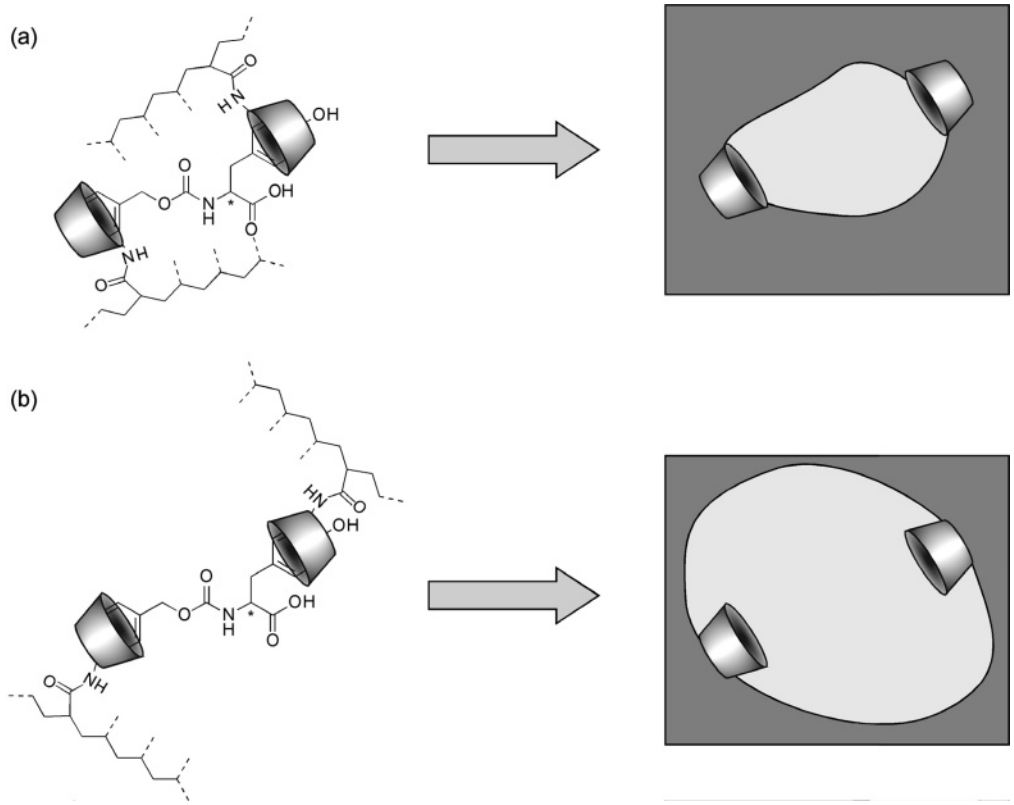


Figure 3. Possible structure of the binding site prepared by the imprinting from (a) 3-AAm-CyD (or Acr-CyD) and (b) 6-AAm-CyD.

Discussion

1. Preorganized Structure of the Complex between β-CyD and Template Molecule. The structure of the guest-binding site, formed by the molecular imprinting, should reflect the preorganized structure between the functional monomer and the template molecule. Since β-CyD has two rims of its truncated cone, there are two possible entries of guest: entries from the smaller rim (the secondary OH side) and from the wider rim (the primary OH side). Thus, there are four possible preorganized structures of β-CyD and Z-Tyr involving two aromatic rings. The NOESY analysis in Figure 2 clearly revealed that the β-CyD/Z-Tyr complex takes one major structure, and both benzene rings of Z-Tyr enter the cavity of β-CyD from the wider rims. Fronza et al. studied on the complex of β-CyD and indomethacin by ^{1}H NMR and molecular modeling study and concluded that entry of β-CyD occurred through its larger rim.[14] Most of the other guests include into the CyD cavity in this fashion.[15] Our results on the β-CyD/Z-Tyr complex are consistent with these results, strongly suggesting that complexation of β-CyD with other amino acid derivatives or oligopeptides also occurs through the inclusion from the wider rim of the truncated cone. Irrespective of the position of the vinyl group and the kind of amino acid derivatives, complexation of a template with β-CyD is expected to occur from the secondary OH side.

2. Effect of the Position of Vinyl Group on the Imprinting Effect. *Imprinting for Discrimination of the Enantiomers of Protected Amino Acids*. Before the polymerization, Z-Tyr or Boc-Tyr is preorganized with β-CyD, as depicted in Figure 2b. In the case of 3-AAm-CyD or Acr-CyD, the vinyl groups protrude toward the template molecule. When a radical initiator (potassium persulfate/TEMED) is added to the mixture of this preorganized complex and cross-linking agent (MBAAm), copolymerization proceeds around the template molecule. Accordingly, the shape of template molecule can be precisely copied on the cross-linked polymer, as illustrated in Figure 3a. In the imprinted polymers, various noncovalent interactions between the template and the polymer backbone are also plausible. Therefore, with the use of 3-AAm-CyD or Acr-CyD, both the chirality of tyrosine and the kind of protecting group are simultaneously imprinted (Table 1). With 6-AAm-CyD, however, the vinyl group is located in the primary OH side and protrudes toward the opposite side of the template. In this case, copolymerization occurs far from the template molecule so that only the positions of β-CyDs are regulated and some vacant space will be formed around the template, as schematically presented in Figure 3b. Consequently, the shape of template molecule will be only coarsely copied to the polymer.

These arguments are consistent with the difference in capacity factors (i.e., retention times). In the case of 3-AAm- or Acr-CyD as the functional monomer, the wider rim of β-CyD is rather crowded with the polymer. Accordingly, access of a guest to the β-CyD from this side is restricted by steric hindrance, and most of the guests should enter from the smaller rim although it is an unfavorable conformation. Consequently, the capacity factor is very small, although the imprinting is precise. For 6-AAm-CyD, however, the polymer chains are primarily located near the smaller rim, and thus the wider side has sufficient space for the guest to enter. Therefore, the capacity factor is much larger than that observed for 3-AAm-CyD or Acr-CyD, but the imprinting is less precise.

Imprinting for Discrimination of Sequences of Tetrapeptides. The imprinting was successful with the use of either 3-AAm-CyD or 6-AAm-CyD, although the selectivity itself was not remarkable.[16] The present imprinting requires to immobilize β-CyDs in the polymer matrix complementarily to the phenyl rings. In contrast with the recognition of the chirality and the kind of protecting group described above, interactions between the template and the polymer backbone are not essential. Thus, both 6-AAm-CyD and 3-AAm-CyD are successfully available as functional monomers.

Conclusions

1. The position of the vinyl group in the β-CyD vinyl monomers significantly affects the imprinting of amino acid derivatives and oligopeptides, which can be explained in terms of the structure of preorganized complex between template and β-CyD.

2. To imprint the chirality of protected amino acids, 3-AAm-CyD or Acr-CyD bearing a vinyl group on the wider rim is much more effective than 6-AAm-CyD.

3. Both 3-AAm-CyD and 6-AAm-CyD (having a vinyl group on the primary OH side) are available to imprint the sequences of tetrapeptides.

Acknowledgment. This work was supported by Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency (JST). Partial support by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan, is also acknowledged.

Supporting Information Available: Figure showing the retention behavior of the enantiomers of Z-Tyr with Z-D-Tyr-imprinted column prepared from 3-AAm-CyD (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Wulff, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1812. (b) Haupt, K.; Mosbach, K. *Trends Biotechnol.* **1998**, *16*, 468. (c) Spivak, D.; Shea, K. J. *J. Org. Chem.* **1999**, *64*, 4627. (d) Sellergren, B. *Angew. Chem., Int. Ed.* **2000**, *39*, 1031. (e) Komiyama, M.; Takeuchi, T.; Mukawa, T.; Asanuma, H. *Molecular Imprinting*; Wiley-VCH: Berlin, 2003, and reference cited therein.

(2) Allender, C. J.; Brain, K. R.; Heard, C. M. *Progress in Medicinal Chemistry*; Elsevier Science: Oxford, 1999; p 235.

(3) (a) Asanuma, H.; Kajiya, K.; Hishiya, T.; Komiyama, M. *Chem. Lett.* **1999**, 665. (b) Asanuma, H.; Hishiya, T.; Komiyama, M. *Adv. Mater.* **2000**, *12*, 1019. (c) Asanuma, H.; Akiyama, T.; Kajiya, K.; Hishiya, T.; Komiyama, M. *Anal. Chim. Acta.* **2001**, *435*, 25.

(4) Piletsky, S. A.; Andersson, H. S.; Nicholls, I. A. *Macromolecules* **1999**, *32*, 633.

(5) (a) Akiyama, T.; Hishiya, T.; Asanuma, H.; Komiyama, M. *J. Inclusion Phenom. Macrocyclic Chem.* **2001**, *41*, 149. (b) Hishiya, T.; Akiyama, T.; Asanuma, H. Komiyama, M. *J. Inclusion Phenom. Macrocyclic Chem.* **2002**, *44*, 365.

(6) (a) Kano, K.; Kitagishi, H.; Tamura, S.; Yamada, A. *J. Am. Chem. Soc.* **2004**, *126*, 15202. (b) Hoshino, T.; Miyauchi, M.; Kawaguchi, Y.; Yamaguchi, H.; Harada, A. *J. Am. Chem. Soc.* **2000**, *122*, 9876.

(7) Harada, A.; Furue, M.; Nozakura, S. *Macromolecules* **1976**, *9*, 701.

(8) (a) Khan, A. R.; Forgo, P.; Stine, K. J.; D'Souza, V. T. *Chem. Rev.* **1998**, *98*, 1977. (b) Rong, D.; D'Souza, V. T. *Tetrahedron Lett.* **1990**, *31*, 4275. (c) Murakami, T.; Harata, K.; Morimoto, S. *Chem. Lett.* **1998**, 553.

(9) Melton, L. D.; Slessor, K. N. *Carbohydr. Res.* **1971**, *18*, 29.

(10) In all the experiments, the molar ratio of β-CyD vinyl monomer, MBAAm, and template was kept constant at 2:6:1.

(11) Hishiya, T.; Shibata, M.; Kakazu, M.; Asanuma, H.; Komiyama, M. *Macromolecules* **1999**, *32*, 2265.

(12) The binding constant of the Z-L-Tyr/Acr-CyD was almost the same as that of Z-L-Tyr/3-AAm-CyD (about 100 M^{-1} at 10 °C) as determined by NMR analyses. Thus, considerably longer retention times for Acr-CyD are probably attributable to the difference in the polymerization reactivity of Acr-CyD and 3-AAm-CyD.

(13) Actual HPLC profiles of the Z-D-Tyr-imprinted polymer were depicted in Figure S1 in the Supporting Information. Imprinting made the profile tailing, probably due to the inhomogeneity of the binding sites, because about half of the CyD would remain uncomplexed during the polymerization as estimated from the binding constant.

(14) Fronza, G.; Mele, A.; Redenti, E.; Ventura, P. *J. Org. Chem.* **1996**, *61*, 909.

(15) (a) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: Berlin, 1978. (b) Szejtli, J. *Cyclodextrins and their Inclusion Complexes*; Akadémiai Kiadó: Budapest, 1982.

(16) Tetrapeptides used here are rather flexible and will not take fixed structure. Therefore, imprinting did not work very well compared with protected amino acids.

MA060064F