Molecular Imprinting of Cyclodextrin in Water for the Recognition of Peptides

Hiroyuki Asanuma, Kentaro Kajiya, Takayuki Hishiya, and Makoto Komiyama*
Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, Hongo, Tokyo 113-8656

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Vinyl monomer of 6-O-α-D-glucosyl-β-cyclodextrin was molecularly imprinted to dipeptides in water. The obtained polymers efficiently and selectively bound the template under physiological conditions. This method is applicable to various guests having versatile functional residues.

Mimicking natural receptor is one of the attractive themes for organic chemists. Various artificial receptors for small guests have been synthesized by placing appropriate functional groups complementarily to the target guest molecule.[1] Molecular imprinting technique has been also developed for this purpose.[2]

Recent interests are focusing onto the artificial receptors for nano-sized large molecules. However, their design and synthesis are still difficult because functional groups should be placed at predetermined positions precisely. The methodology we proposed is to connect modules orderly, each of which can bind a part of a large molecule.[3] We chose β-cyclodextrin (β-CyD) as a module and prepared its ordered assembly for steroids by applying molecular imprinting technique: cyclodextrins were crosslinked with diisocyanates in DMSO in the presence of a steroid as a template molecule. The polymer efficiently and selectively bound the target steroid in aqueous media.[3,4] However, only limited templates were applicable to this method because of the following reasons: 1) the molecules having carboxyl or amino groups are not available as templates because they react with isocyanates, 2) in DMSO, adduct formation between the templates and CyDs is weaker than in water. Here, we report a new molecular imprinting method by using a vinyl monomer of CyD. Highly selective dipeptide-receptors are prepared by a radical polymerization of the CyD monomer in the presence of various templates in bulk water.

The vinyl monomer of CyD was synthesized by ester-exchange reaction of *m*-nitrophenyl acrylate with 6-O-α-D-glucosyl-β-CyD (G1-β-CyD) in water.[5] Here, G1-β-CyD was used instead of β-CyD in order to improve the solubility in water and facilitate the column chromatography. The molecularly imprinted polymers were prepared in water by a radical polymerization of the CyD monomer with N,N'-methylenebisacrylamide as crosslinker in the presence of various dipeptides (see Figure 1).[6] After the polymerization, the template molecules and unreacted CyD monomers were removed from the polymer by treatment with acetone, methanol, and water. The obtained polymer was dried and subjected to the binding experiment. Complete removal of the template molecule was confirmed by the ^{13}C-CP/MAS NMR spectroscopy. As a control, non-imprinted CyD polymer was prepared in the same way except for the absence of the template.[6]

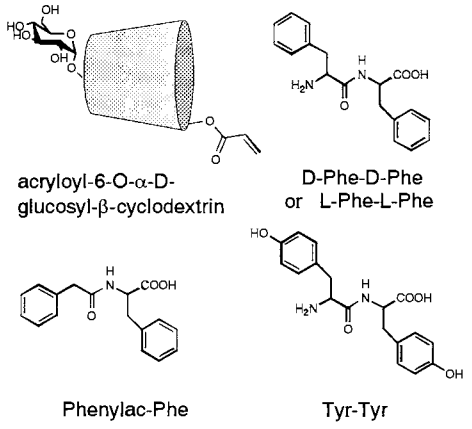

Figure 1. The structures of the vinyl monomer of CyD and the templates used in this study.

The molecularly imprinted polymer, prepared in the presence of D-phenylalanyl-D-phenylalanine (D-Phe-D-Phe), adsorbed 22.4% of the template molecule in water (Table 1: the adsorption conditions are described in Ref.7). In contrast, only 8.2% was bound to the polymer prepared in the absence of the template. Thus, eminent template effect is conclusive. The guest binding by the imprinted CyD polymer was totally reversible so that all the bound guest was easily removed from the polymer by the treatment with methanol. On the following binding experiment, the capacity was essentially identical with the foregoing run. Since the homopolymer of N,N'-methylenebisacrylamide did not show adsorption activity at all, the CyD molecules immobilized onto the polymer are responsible for the guest binding.

Table 1. Binding activities of various dipeptide-imprinted CyD polymers

Template (Substrate)	Adsorption activity [a] imprinted	non-imprinted	Efficiency [b]
D-Phe-D-Phe	0.224	0.082	2.7
Phenylac-Phe	0.114	0.065	1.8
Tyr-Tyr	0.111	0.041	2.7

[a]Molar fraction of the guest bound by the polymer with respect to the total amount of guest. Details for the binding experiments are described in Ref. 7. [b]The ratio of adsorption activity of imprinted polymer to that of non-imprinted polymer.

The binding constant of the imprinted polymer towards D-Phe-D-Phe was determined as 7000 M^{-1} (1 M = 1 mol dm^{-3}) on the basis of Langmuir-type adsorption, whereas that of the non-imprinted polymer was as small as 1200 M^{-1}. Thus the promotion of binding activity towards the guest, induced by the template, is attributed to the modification of binding sites suitable for the templates. The simple increase in the number of binding sites by the imprinting is ruled out.

The enantioselectivity is also improved by the present imprinting. The non-imprinted polymer bound 8.2% of D-Phe-D-Phe whereas it did 6.4% of L-Phe-L-Phe. The selectivity

(D-Phe-D-Phe/L-Phe-L-Phe) was 1.3. When the CyD vinyl monomer was polymerized in the presence of D-Phe-D-Phe, adsorption activity for the D-Phe-D-Phe significantly increased as described above (22.4%). In contrast, this imprinted polymer did not exhibit much affinity towards L-Phe-L-Phe (9.7%). Thus, the selectivity was much improved by the imprinting (from 1.3 to 2.3). Exactly as expected, L-Phe-L-Phe was adsorbed preferentially to D-Phe-D-Phe when L-Phe-L-Phe was used as a template. The selectivity (L-Phe-L-Phe/D-Phe-D-Phe) was improved from 0.77 (=1/1.3) to 1.7.

The increase in the binding activity by the present imprinting was also observed for other dipeptides or modified amino acids (see Table 1). Since G1-β-CyD can form sufficiently stable complex with these template molecules in water, the orientation of the CyD molecules was effectively regulated.[8] It should be noted that these template molecules are not available to our previous imprinting method using diisocyanates as crosslinker.[3]

In conclusion, efficient polymeric receptors for dipeptides were prepared by applying molecular imprinting technique to the polymerization of CyDs. This method is promising for the recognition of various large molecules in water.

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6 Typical procedure of the preparation of molecularly imprinted polymer was as follows: acryloyl G1-β-CyD (0.3 mmol) and D-Phe-D-Phe (0.15 mmol) were dissolved in water (15 ml) at pH 8.0 (5 mM Tris buffer). To this solution, N,N'-methylenebisacrylamide (2.7 mmol) and $K_2S_2O_5$ (35 mg) were added and the mixture was heated at 50 °C for 2 h under O_2-free condition. The white precipitate was collected and washed with acetone, methanol, and water, followed by desiccation. From elemental analysis, the copolymerization ratio of the incorporated G1-β-CyD with respect to the crosslinker was 0.048 for the imprinted polymer, and 0.045 for non-imprinted one.

7 Guest binding experiments were carried out by incubating 128 mg of polymeric receptor with 3 ml of 1.5 mM aqueous guest solution at room temperature for 2 h. During the binding experiment, pH of the guest solution was kept at 8.0 (5 mM Tris buffer). Then the mixture was centrifuged and the concentration of the guest in the liquid phase was analyzed by reversed phase HPLC (Merck LiChrosphere RP-18(e) ODS column). In case of Tyr-Tyr, 64 mg of the polymeric receptors was contacted with 3 ml of 0.15 mM guest solution. Otherwise imprinting effect could not be estimated properly because the amounts of bound Tyr-Tyr were saturated for both imprinted and non-imprinted polymers under the conditions described above.

8 For example, the association constant for the G1-β-CyD and Tyr-Tyr adduct formation was determined as 2.3×10^2 M^{-1} at pH 8.0 (5 mM Tris buffer) at R.T. from fluorescence spectra by assuming 1:1 complex formation. Based on this value, about 80% of Tyr-Tyr in the liquid phase should be bound by G1-β-CyD under the polymerization conditions. In the imprinting reactions, CyD molecules are assumedly bound each other in a stepwise manner, providing the imprinted polymers in which the mutual conformation of two CyD residues is regulated.[3]