Recognition of Solution Structures of Peptides by Molecularly Imprinted Cyclodextrin Polymers

Shi-hui Song,[†] Kazumi Shirasaka,[†] Mami Katayama,[†] Suguru Nagaoka,[†] Shinji Yoshihara,[†] Tomo Osawa,[†] Jun Sumaoka,[†] Hiroyuki Asanuma,*[,‡] and Makoto Komiyama*[,†]

Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan, and Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan

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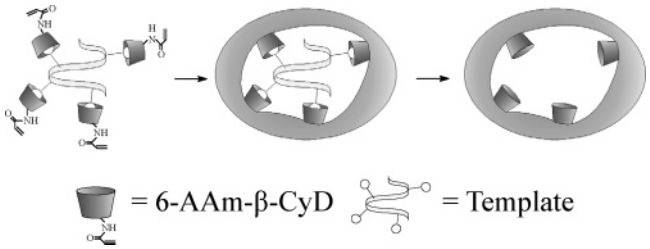
Preparation of artificial receptors that bind peptides in water is one of the most attractive themes because of their potential applications to separation/purification of bioproducts, biosensing, therapy, and others.[1] However, synthetic approaches toward these receptors have been often hampered by difficulty in placing several recognition moieties at predetermined positions in nanometer-scaled space. Thus, the molecular imprinting technique was often employed.[2] Most of previous imprintings were based on the following three strategies: (1) imprinting of the surface structure of protein,[3] (2) imprinting of a short oligopeptide sequence in exposed domain of protein,[4] and (3) use of protein-recognizing biomolecule as functional monomer.[5] These artificial receptors showed selective affinity to the template proteins. However, few of these receptors distinguished dynamic structures of peptides in solutions, although many important characteristics of peptides (biological functions, supramolecular assembling, and others) are primarily governed by these solution structures. There has been no general strategy for the design of desired artificial receptors.

Here we report new types of receptors which strictly recognize solution structures of oligopeptides in water. In the presence of template oligopeptide, a vinyl monomer of β-cyclodextrin (β-CyD) is polymerized in water, and several β-CyD molecules are immobilized complementarily to apolar and bulky groups in the template (Scheme 1).[6,7] Of the oligopeptide templates employed here (shown in Figure 1), angiotensin II (AII) is an octapeptide hormone for blood pressure regulation in the human body. Its precursor decapeptide is angiotensin I (AI), which is converted to the active hormone AII by enzymatic removal of its C-terminal His-Leu. In spite of the apparent similarity of amino acid sequences of these two oligopeptides, the imprinted β-CyD polymers clearly distinguish the difference in their structures in water and separate them from each other. Furthermore, alteration of only one amino acid (isoleucine) in AI and AII to valine (AI→[Val^5]-AI and AII→[Val^5]-AII) is also successfully recognized by the imprinted polymers, since they recognize the solution structure of each of these closely related oligopeptides.

The molecular imprinting of β-CyD was carried out on the surface of silica gel, and the efficiency of molecular imprinting was evaluated in terms of the retention behavior of the polymer/silica gel composites as the stationary phase of HPLC.[7e,8]

* Corresponding authors. E-mail: komiyama@mkomi.rcast.u-tokyo.ac.jp; asanuma@mol.nagoya-u.ac.jp.
[†] The University of Tokyo.
[‡] Nagoya University.

Scheme 1. Schematic View of Molecular Imprinting of β-CyD toward Oligopeptide Template



AI:	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu
AII:	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
[Val^5]-AI:	Asp-Arg-Val-Tyr-Val-His-Pro-Phe-His-Leu
[Val^5]-AII:	Asp-Arg-Val-Tyr-Val-His-Pro-Phe

Figure 1. Primary structures of AI (angiotensin I), AII (angiotensin II), [Val^5]-AI ([Val^5]-angiotensin I), and [Val^5]-AII ([Val^5]-angiotensin II) used in the present study.

Acrylamido groups were introduced to the surface of silica gel, and in the presence of this chemically modified silica gel, mono-6-(*N*-acrylamido)-6-deoxy-β-CyD (6-AAm-β-CyD) and *N*,*N*′-methylenebis(acrylamide) (MBAAm) were polymerized by a radical initiator. In Figure 2A, the imprinting reactions were achieved in pH 8.0 Tris buffer, and the guest-binding activity was assayed also at pH 8.0 (in the same buffer). When AI was the template for the imprinting, the binding of β-CyD polymer to this decapeptide was greatly promoted (compare the chart (i) with (ii) in Figure 2A). The capacity factor k_{imp} of this imprinted polymer for AI was 19.1, which was 2.1 times as large as that (k_{non} = 9.1) of nonimprinted β-CyD polymer obtained in the absence of AI (see Table 1). On the other hand, the capacity factor of this imprinted polymer for the binding of AII (k_{imp} = 4.6) was far smaller than that for the binding of AI. Still more importantly, the imprinting with AI as template increased the binding activity toward AII only slightly (the HPLC patterns of AII are also presented in (i) and (ii) of Figure 2A). These results indicate that the molecular imprinting is never directly associated with the primary structure of template oligopeptide (note that the sequence of AII is exactly the same as the N-terminal octapeptide of AI). Rather, the conformations of oligopeptides in solutions take more crucial roles for the imprinting. Consistently, when the template for the imprinting was AII, the binding to AII was promoted in much greater extent than the binding to AI (see (iii) in Figure 2A). The capacity factor (k_{imp} = 5.4) for the binding of AII was 1.7 times as large as the corresponding k_{non} (3.2), whereas the binding of AI was less affected by the imprinting to AII (k_{imp}/k_{non} = 1.3).[9]

The importance of solution structures of oligopeptides for the imprinting and guest binding has been concretely evidenced by the following experiments. After the assay of guest-binding activity at pH 8.0, the HPLC eluent was changed to pH 3.0 citrate buffer, and the guest-binding property of the AI-imprinted β-CyD polymer, prepared in the pH 8.0 Tris buffer, was analyzed at pH 3.0 (the HPLC chart is presented in (i) of Figure 2B). Quite significantly, the AI-imprinted β-CyD polymer bound AI only poorly at pH 3.0 (capacity factor k_{imp} = 1.8). The retention times of AI and AII were almost the same here, although they were completely separated at pH 8.0. Apparently, the memory of AI template, imprinted to the β-CyD polymer at pH 8.0, disappeared in the assay at pH 3.0. This imprinted

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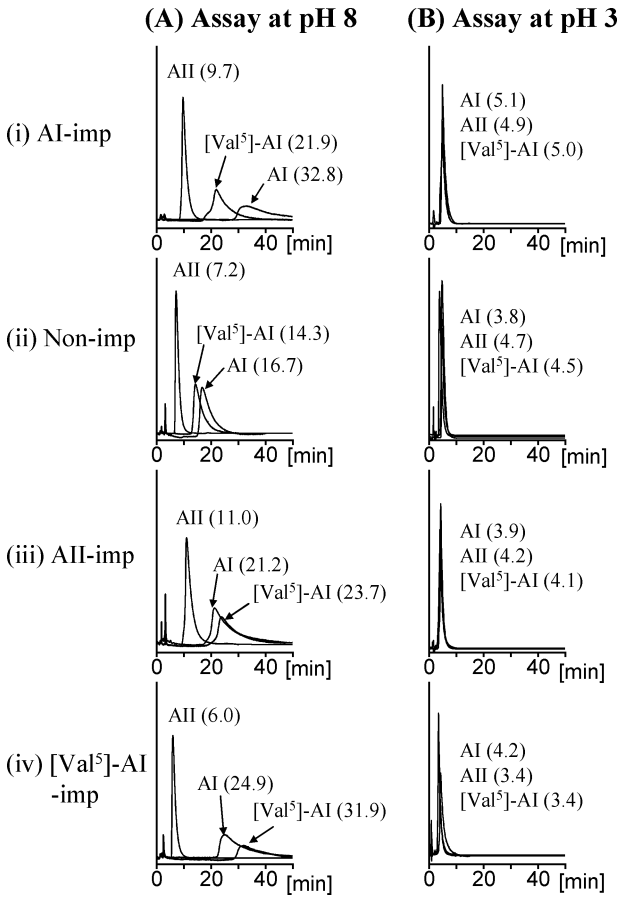

Figure 2. Typical HPLC patterns using (i) AI-imprinted β-CyD polymer, (ii) nonimprinted β-CyD polymer, (iii) AII-imprinted β-CyD polymer, and (iv) [Val^5]-AI-imprinted polymer as stationary phase. The retention times of guest molecules (in min) are presented in parentheses. All the polymers were prepared at pH 8, and the binding activities were assayed at either pH 8 (A) or pH 3 (B). Column length = 50 mm; flow rate = 0.5 mL min^{-1} with Tris buffer at pH 8 and citrate buffer at pH 3.

Table 1. Capacity Factors of AI-Imprinted and Nonimprinted β-CyD Polymers Prepared at pH 8 and Assayed at Either pH 8 or pH 3

	imprinting at pH 8		nonimprinting at pH 8	
guest	assay at pH 8	assay at pH 3	assay at pH 8	assay at pH 3
AI	19.1	1.8	9.1	1.2
AII	4.6	1.8	3.2	1.7
[Val^5]-AI	11.3	1.7	7.7	1.5
[Val^5]-AII	4.0	1.9	3.0	1.8

polymer primarily memorizes the solution structure of AI under the imprinting conditions (pH 8.0). Since this solution structure is notably different from the structure at pH 3.0, the binding at pH 3.0 is never affected by the imprinting at pH 8.0.[10] It is noteworthy that this drastic pH-dependent change of guest-binding activity (between chart (i) in Figure 2A and that in Figure 2B) is completely reversible. When the HPLC eluent was changed back to the pH 8.0 Tris buffer, the remarkable guest-binding activity of the imprinted β-CyD polymer to AI recovered to the original value (data not presented). AII was only poorly bound. In both the imprinting process and the guest-binding assay, the phenyl ring of Phe^8 and the phenol of Tyr^4, as well as other apolar side chains, should primarily interact with β-CyD molecules.[6] According to previous NMR study,[11] the orientations and positions of these groups in AI are considerably different from those in AII. Accordingly, when these β-CyD molecules are immobilized in the imprinted polymers complementarily to AI, the resultant ordered assembly of β-CyD binds AII only poorly. The crucial difference in biological activity between these two oligopeptide (active AII vs inactive AI) is also, at least partially, associated with the difference in their conformations.

As clearly shown by these results, the imprinted β-CyD polymers memorize peptide conformations rather than their primary structures alone. Thus, even closely related oligopeptides can be precisely recognized in terms of difference in solution structures. For example, [Val^5]-AI is identical with AI except for the replacement of Ile^5 of AI with Val^5 (Figure 1). When nonimprinted β-CyD polymer was used as the HPLC stationary phase, these two oligopeptides showed similar retention behavior and were never sufficiently separated from each other (see chart (ii) in Figure 2A). Conventional host−guest interactions of this nonimprinted polymer are insufficient to recognize the alteration from Ile to Val in the decapeptides. However, the AI-imprinted β-CyD polymer bound AI much more strongly than [Val^5]-AI at pH 8.0 and satisfactorily separated them each other ((i) in Figure 2A). The capacity factor k_{imp} for AI was 1.7 times as large as the corresponding value for [Val^5]-AI. In contrast with this successful recognition between AI and [Val^5]-AI, the AI-imprinted β-CyD polymer bound AII and [Val^5]-AII (Ile^5→Val^5 substitute of AII) with comparable affinity (Table 1). Thus, the possibility that the small difference in physicochemical properties between Ile and Val (and slight preference of Ile by β-CyD, if any) gives rise to the significant difference in the binding of AI and [Val^5]-AI is unlikely.[12] The whole conformations of these decapeptides should be notably affected by the amino acid residues which are located at position 5.[13]

All these arguments have been further supported by chart (iv) in Figure 2A, where β-CyD was imprinted to [Val^5]-AI as template at pH 8.0. The [Val^5]-AI-imprinted polymer bound [Val^5]-AI at pH 8.0 more strongly than AI, which was in contrast with the stronger binding of AI by the AI-imprinted polymer (compare (iv) with (i) in Figure 2A). The change of Ile^5 to Val^5 was clearly recognized by the imprinting, even when these two amino acids have similar physicochemical properties (hydrophobicity and others). The binding of AII and [Val^5]-AII was much weaker, as expected. In the binding assay at pH 3.0, both [Val^5]-AI and AI were bound only minimally ((iv) in Figure 2B), exactly as observed in (i) of Figure 2B. The imprintings presented here are far more efficient than the imprintings toward smaller oligopeptides.[7d] When Phe-Phe-Gly-Gly and Phe-Gly-Phe-Gly were used as templates, for example, the imprinting increased the binding activity only by around 10%. These small oligopeptides show rather free conformational fluctuations in water, which are unsuitable for the molecular imprinting. However, the oligopeptides employed here have more concrete structures in water, providing more clear-cut imprinting.

In conclusion, imprinted CyD polymers successfully memorize solution structures of oligopeptides in water. They are useful, for example, for precise analysis, separation, and purification of target peptide hormone from complex mixtures, since the difference in only one amino acid can be strictly recognized. Preparation of CyD-based artificial membrane receptors and other applications are also promising. These attempts are now in progress in our laboratory.

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Supporting Information Available: Capacity factors of AII- and [Val^5]-AI-imprinted β-CyD polymers prepared at pH 8 and assayed at either pH 8 or pH 3 (shown in Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Acrylamido groups were introduced to the surface of silica gel (Nucleosil 300-10 from Macherey-Nagel; specific surface area 100 $m^2\ g^{-1}$) by treating the silica gel with acrylamidopropyltriethoxylsilane in xylene.[7e] According to the titration with $KMnO_4$, about 140 μmol of vinyl groups was introduced to 1 g of the silica gel (this corresponds to $\sim$1 acrylamidopropyl group per 1 nm^2). In the presence of this vinylated silica gel (600 mg) and template oligopeptide (7.5 μmol), 6-AAm-β-CyD (30 μmol) and MBAAm (180 μmol) were polymerized at 35 °C under nitrogen for 20 h by the combination of potassium persulfate (3 mg) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (3 μL). About 50 mg of β-CyD polymer was bound to 1 g of the silica gel, as determined by the weight loss observed upon its combustion under air. There exists 1 β-CyD molecule in $\sim$4 nm^2 of the surface of the silica gel. The polymer/silica gel composite thus obtained was directly packed in a stainless steel column tube (50 mm $\times$ 4.6 mm i.d.), and the retention behavior was monitored at 260 nm with flow rate 0.5 mL min^{-1}. The capacity factor *k* was determined by using acetone as void marker.

(9) Nonimprinted polymer bound AI also more strongly than AII, although the difference is far smaller. The solution structure of AI would be more favorable for nonspecific apolar interactions with the polymer/silica gel composites.

(10) At pH 3, AI should take a stretching structure because of accumulated positive charges.

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