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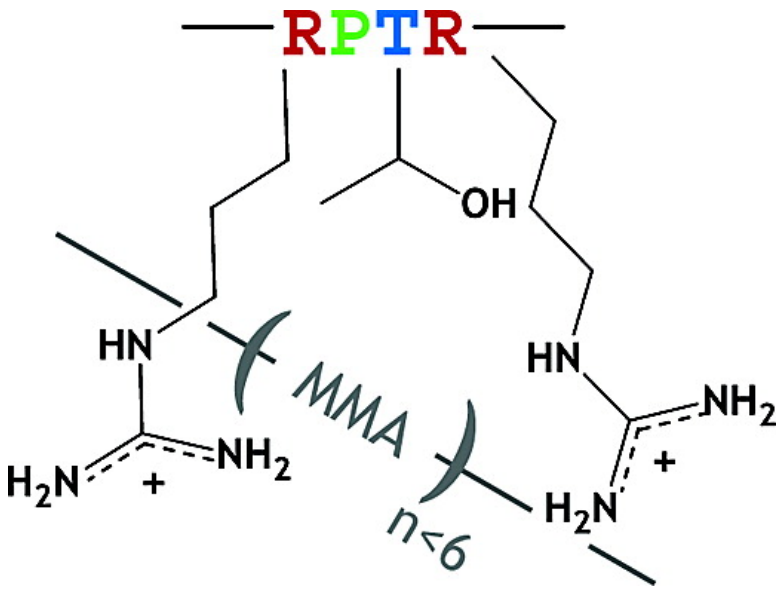

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A Peptide Motif Recognizing a Polymer Stereoregularity

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The targets of proteins (also peptides) are normally biomolecules present in the biological milieu. However, recent studies have revealed that certain novel peptides, which were selected from a peptide library, bound specifically to artificial materials.[1] The selection process was performed by cell-surface display or phage display methods. The specific targets of these peptides were the surfaces of inorganic crystals[1] and nanocarbons.[2] The side chains of the amino acids in these peptides, which have three-dimensional structures that might be regulated, may fit into suitable positions on the crystal surfaces, resulting in specific interactions. Furthermore, the peptides can be used as components for the modification of inorganic crystal surfaces[3] and as reducing catalysts for metal ions.[4] Therefore, the targets of peptides are not necessarily biomolecules.

The possibility that synthetic polymers such as polystyrene[5] and cross-linked polymers of methacrylates[6] can be the targets of peptides was investigated. More recently, a 12-mer peptide that specifically binds to films composed of a conducting polymer, chlorine-doped polypyrrole, was selected from a phage display library.[7] The peptide was chemically conjugated with a well-known cell-adhesive peptide (RGDS), and then the films were coated with the conjugated peptide, resulting in cell-adhesive properties on the films. These observations are significant because peptides have the potential for recognizing the subtle aspects of the delicate chemical structure of polymers. Such polymer-binding peptides could be used in the wide field of polymer science. It is, therefore, attractive to develop novel peptides with selective affinity for polymers with regular nanostructures. Here, we report for the first time a peptide motif that specifically binds to a stereoregular polymer, isotactic (it) poly(methyl methacrylate) (PMMA). This specificity was clearly demonstrated by using reference atactic (at) and syndiotactic (st) PMMAs, which have the same chemical composition as the target but have different stereoregularities. Our results further extend the concept for the molecular recognition of artificial materials by peptides.

A phage library that displays a linear 7-mer peptide with a diversity of $\sim 1.28 \times 10^9$ (Ph.D.-7 Phage Display Peptide Library Kit, New England Biolabs, Inc.) was used. It-PMMA ($M_n = 35\,500$, $M_w/M_n = 1.12$, *mm*:*mr*:*rr* = 98:2:0) (where *mm*, *mr*, and *rr* represent iso-, hetero-, and syndiotacticities, respectively) and st-PMMA ($M_n = 28\,200$, $M_w/M_n = 1.26$, *mm*:*mr*:*rr* = 0:11:89) were synthesized by conventional living anion polymerization. At-PMMA ($M_p = 28\,750$, $M_w/M_n = 1.03$, *mm*:*mr*:*rr* = 10:15:75) was purchased from Polymer Laboratories. The static contact angles of these PMMA films were approximately 60−70°,[8] indicating that the difference in hydrophobicity was not a great factor for polymer recognition. Biopanning is summarized in the Supporting Information. In brief, PMMA films of approximately 20 nm thickness were subjected to biopanning. Enzyme-linked immunosorbent assays (ELISAs) revealed that the phage pool containing some phages with a specific affinity for it-PMMA were obtained after five rounds of biopanning (see Figure S1). The relative affinity tended to increase with an increasing number of biopanning cycles. After cloning and sequencing, nine clones were identified, as shown in Table 1. Some of the clones were observed at high frequencies, more than 6 out of 30 phages.

Table 1. Phage-Displayed Peptides with Selective Affinity for It-PMMA

clone	frequency	sequence[a]	K_{app} (10^{10} M^{-1}) it-PMMA	K_{app} (10^{10} M^{-1}) st-PMMA	ratio[b] (it/st)
c02	6/30	ELWRPTR	10	1.6	6.3
c03	2/30	QLQKYPS	8.5	2.9	2.9
c18	6/30	ARPHLSF	6.2	2.5	2.5
c01	3/30	TLHLSPA	5.4	2.9	1.9
c06	2/30	QTMTYSR	5.0	2.0	2.5
c05	1/30	AAQTSTP	4.9	4.7	1.0
c04	7/30	SSPWMRE	3.5	1.1	3.2
c26	1/30	GIRHTNR	2.4	0.40	6.0
c10	2/30	NLQEFLF	0.69	0.76	0.9
library	-	-	0.32	0.29	1.1

[a] R (Arg) and P (Pro) are shown in red and green, respectively. S (Ser), T (Thr), and Y (Tyr) are shown in blue. [b] Ratio between K_{app} for it-PMMA and that for st-PMMA.

In most clones, Ser, Thr, Tyr, and/or Arg with H-donor side chains (hydroxyl and guanidium groups), which may interact with the ester groups of PMMA via hydrogen bonding, were included. In addition, the Pro, which induces a kink in peptides, was also included, possibly forming a rigid structure. The frequencies of these amino acids were not explained by those intrinsically expressed by the phage. The importance of these amino acids as well as their sequences is discussed later.

The relative affinity of the phage clones for the PMMA films was analyzed by ELISA, as shown in Figure 1. The affinity of all clones for it-PMMA was significantly greater than their affinity for at- or st-PMMAs. For some clones, almost 2-fold more bound to it-PMMA, as compared to at- and st-PMMAs. The affinity of the phage library was independent of the polymer stereoregularity, and the affinity for it-PMMA was much smaller than that of all clones. These observations indicate that selected clones preferentially bound to the target it-PMMA. On the other hand, slightly greater amounts of the clones bound to the reference PMMAs as compared to the phage library binding to the PMMAs. This is due to the fact that the selected peptides were constructed such that

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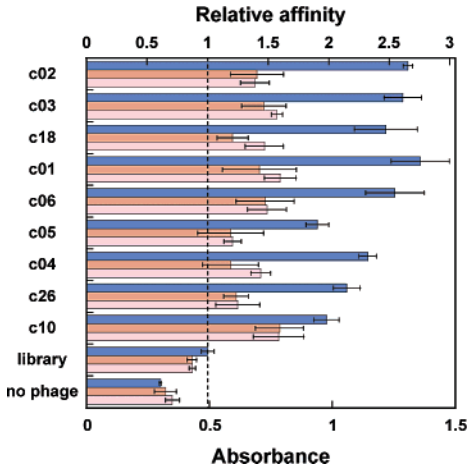

Figure 1. The affinity of phage clones selected from the phage display library for it-PMMA. The blue, red, and pink bars indicate affinity for it-, at-, and st-PMMAs, respectively. The absorbance data were converted to affinity relative to that of a phage library for it-PMMA.

their entire structure has affinity for PMMA (hydrophobicity, hydrogen bonding groups), and thus bound nonspecifically to the references.

To quantitatively understand the binding affinity, the dependence of the phage clone concentration against the relative affinity was obtained for the target it-PMMA and the reference st-PMMA. The dependence was saturated to a certain level of affinity in all cases (see Figure S2). Assuming a Langmuirian-type affinity (although the affinity was analyzed by the binding of the anti-M13 antibody), the curves were fitted to a Langmuirian equation, and then the apparent affinity constants (K_{app}) were obtained. All of the data were fitted to the equation with coefficients of variation ranging 0.965−0.997, indicating the reliability of the fit. The resulting K_{app} and the ratios between K_{app} against it-PMMA and that against st-PMMA, which correspond to the selectivity, are summarized in Table 1. It was found that the frequency does not simply determine the binding affinity. The apparent binding constants ranged in the order of 10^{10} M^{-1}. These constants were too great to be the constants of the peptides only. Since the whole phage nonspecifically adsorbed onto the PMMA films, greater constants might be obtained. Accordingly, we did not discuss the absolute values of the constants.

There are significant trends of the amino acids with respect to affinity and selectivity. The K_{app} against specific it-PMMA was relatively greater, when greater amounts of amino acids with H-donor side chains were positioned adjacent to the Pro, especially for c02 and c03. We hypothesize from this observation that structurally rigid peptides with suitably positioned H-donor groups at the kink will bind strongly to it-PMMA. Although the affinity of c06 without the Pro was medium, this must be due to the rich sequence of H-donor amino acids. On the other hand, the selectivity tended to increase upon increasing the number of Arg residues. In particular, the presence of −Arg−Xxx−Thr−(Xxx)−Arg−, observed for c02 and c26, showed greater selectivity. Accordingly, c02 containing the −Arg−Pro−Thr−Arg− sequence, which was essential for both affinity and selectivity, was the best motif that bound specifically to the target it-PMMA. In fact, the highly sensitive quartz crystal microbalance (QCM) analysis demonstrated that the chemically synthesized c02 peptide preferentially bound to it-PMMA (see Figure S3).

Selected peptides have the potential for recognizing the absolute position of the α-methyl groups or ester groups in two adjacent units of PMMA. Considering the enrichment of amino acids with H-donor side chains, the peptides should recognize the ester groups.

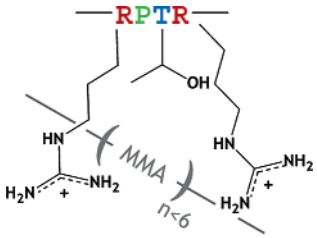

Figure 2. Schematic illustration of the binding of the peptide motif (−RPTR−) with selective affinity for it-PMMA.

The lower affinity for the references with slightly different structures from the target suggests that the selected peptides bound to a single it-PMMA on the film surface, but did not cross-bind to plural it-PMMA chains or to separated units in a single it-PMMA. Since the present peptides recognized this stereoregularity and since the fully extended motif −Arg−Pro−Thr−Arg− has almost the same length as six units of fully extended PMMA, the present peptides seemed to recognize the adjacent 2−6 unit numbers of it-PMMA, as schematically illustrated in Figure 2. We believe this interpretation, even though it-PMMA formed a helical structure on the film surface.[9]

The 7-mer peptides that specifically bind to a stereoregular polymer, it-PMMA, were selected from the phage display library. The motif −Arg−Pro−Thr−Arg− was essential for the affinity and selectivity for it-PMMA. This is the first report on the recognition of polymer stereoregularity using short peptides. It is necessary to further investigate the mechanism responsible for this recognition. Peptide motifs with selective affinity for st-PMMA as well as other structurally regular polymers will be reported soon. Furthermore, polymer-binding peptides have the potential to act as polymerization catalysts, peptide-surfactants, surface-modifiers, and so on. Such versatile applications of peptides will be also reported.

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Supporting Information Available: Biopanning, ELISA of phage pools, dependence of the phage concentration against the relative affinity, and QCM analysis of binding of peptides to it-PMMA. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Sarikaya, M.; Tamerler, C.; Jen, A. K.-Y.; Schulten, K.; Baneyx, F. *Nat. Mater.* **2003**, *2*, 577. (b) Sarikaya, M.; Tamerler, C.; Schwartz, D. T.; Baneyx, F. *Annu. Rev. Mater. Res.* **2004**, *34*, 373.
(2) (a) Zhu, J.; Kase, D.; Shiba, K.; Kasuya, D.; Yudasaka, M.; Iijima, S. *Nano Lett.* **2003**, *3*, 1033. (b) Wang, S.; Humphreys, E. S.; Chung, S.-Y.; Delduco, D. F.; Lustig, S. R.; Wang, H.; Parker, K. N.; Rizzo, N. W.; Subramoney, S.; Chiang, Y.-M.; Jagota, A. *Nat. Mater.* **2003**, *2*, 196. (c) Kase, D.; Kulp, J. L., III; Yudasaka, M.; Evans, J. S.; Iijima, S.; Shiba, K. *Langmuir* **2004**, *20*, 8939. (d) Morita, Y.; Ohsugi, T.; Iwasa, Y.; Tamiya, E. *J. Mol. Catal. B: Enzymatic* **2004**, *28*, 185.
(3) (a) Whaley, S. R.; English, D. S.; Hu, E. L.; Barbara, P. F.; Belcher, A. M. *Nature* **2000**, *405*, 665. (b) Nygaard, S.; Wendelbo, R.; Brown, S. *Adv. Mater.* **2002**, *14*, 1853. (c) Sano, K.-I.; Shiba, K. *J. Am. Chem. Soc.* **2003**, *125*, 14234.
(4) (a) Naik, R. R.; Brott, L. L.; Clarson, S. J.; Stone, M. O. *J. Nanosci. Nanotechnol.* **2002**, *2*, 95. (b) Brown, S.; Sarikaya, M.; Johnson, E. *J. Mol. Biol.* **2000**, *299*, 725. (c) Naik, R. R.; Stringer, S. J.; Agarwal, G.; Jones, S. E.; Stone, M. O. *Nat. Mater.* **2002**, *1*, 169. (d) Yu, L.; Banerjee, I. A.; Matsui, H. *J. Am. Chem. Soc.* **2003**, *125*, 14837.
(5) Adey, N. B.; Mataragnon, A. H.; Rider, J. E.; Carter, J. M.; Kay, B. K. *Gene* **1995**, *156*, 27.
(6) Berglund, J.; Lindbladh, C.; Nicholls, I. A.; Mosbach, K. *Anal. Commun.* **1998**, *35*, 3.
(7) Sanghvi, A. B.; Miller, K. P−H; Belcher, A. M.; Schmidt, C. E. *Nat. Mater.* **2005**, *4*, 496.
(8) Tretinnikov, O. N. *Langmuir* **1997**, *13*, 2988.
(9) Kusanagi, H.; Chatani, Y.; Tadokoro, H. *Polymer* **1994**, *35*, 2028.

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