Polymer-Recognizing Peptides

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# Peptide Motifs That Recognize Differences in Polymer-Film Surfaces**

*Takeshi Serizawa,* Toshiki Sawada, and Tatsuki Kitayama*

Recently, it has been demonstrated that short peptides recognize and specifically bind to material surfaces on inorganic crystals,[1] semiconductors,[2] metal oxides,[3] and nanocarbons[4] through electrostatic, hydrogen-bonding, and van der Waals interactions, when the surfaces have two- or three-dimensional regular distribution of atoms or functional groups. The peptides were selected from libraries displayed on surfaces of genetically engineered cells or phages.[5] Relationships between material components and peptide sequences have been noticed and applications of these material-binding peptides have also been exemplified.[1–6]

Regular structures on synthetic polymers are potential targets for peptides.[6] For instance, 12-mer peptides that specifically bind to chlorine-doped polypyrrole were reported by selection from phage libraries and were used as surface modifiers to obtain cell-adhesive surfaces.[6c] We also revealed shorter 7-mer peptides that specifically bind to isotactic poly(methyl methacrylate) (it-PMMA, isotactic sequences means that the $C_\alpha$ carbon atoms of adjacent MMA units have the same configuration).[6d] Synthetic polymers have well-defined chemical structures in aqueous solution, a property which should simplify any peptide–polymer interactions. To demonstrate whether peptides generally discriminate between subtle differences in polymer surfaces, polymer targets with distinct nanostructures must be analyzed.

Conventional material surfaces for peptide targets have static surface structures. Although variations in pH value change material charges,[4d] peptide conformations would also alter because of changes in peptide charges. We propose a novel target, variable film surfaces composed of amphiphilic polymers. The surface-accumulation of functional groups on these polymer films can be influenced by the external environment. In cases of syndiotactic (st) PMMA films prepared on glass slides in air (syndiotactic sequences means that the $C_\alpha$ carbon atoms of adjacent MMA units have alternating configurations), hydrophilic ester and hydrophobic alkyl groups accumulated on the glass and air sides, respectively.[7] Therefore, st-PMMA films which have different surfaces are suitable candidates for demonstrating both the potential specificity of peptides towards materials and a novel method controlling peptide–polymer interactions.

Herein, we report the selection of peptide motifs that specifically bind to surfaces on st-PMMA films which were preconditioned in buffered solutions to expose surface ester groups. The peptide motifs are selected from phage libraries displaying 7-mer random peptides (Figure 1). The conditioned st-PMMA films are the so-called target films. Films composed of st-PMMA without conditioning, it-PMMA, and atactic (at) PMMA were used as reference films (atactic sequences means that the configuration of the $C_\alpha$ carbon atoms of adjacent MMA units is random). The resulting peptides showed specificity for the conditioning. This is the first report on the molecular recognition of different material surfaces by short peptides. This study confirms the potentials of peptides to successfully recognize differences in polymer-film surfaces composed of single polymer components.

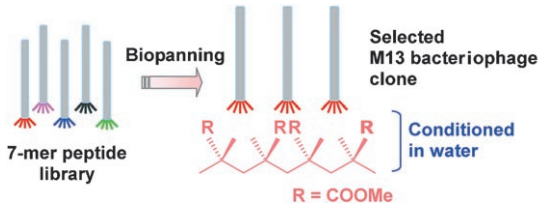


***Figure 1.*** Schematic representation of the selection of st-PMMA binding peptides from phage-displayed peptide libraries (phage pIII coat proteins displaying different peptides are represented by different colors). In the phage display technique, repetitive biopanning processes involve the affinities of phage libraries (or pools) for targets and subsequent elution of bound phages from target surfaces, then phage amplification with *Escherichia coli*, selection of phages displaying target binding peptides, and DNA sequencing of isolated phage clones to determine peptide sequences.

st-PMMA films, approximately 40-nm thick, were spin-coated on glass slides and immersed in Tris-buffered saline (TBS, Tris = tris(hydroxymethyl)aminomethane; pH 7.4) for 15 h (conditioning). A phage library that displays a linear 7-mer peptide with a diversity of approximately $1.28 \times 10^9$

[*] Prof. T. Serizawa
Research Center for Advanced Science and Technology
The University of Tokyo
4-6-1 Komaba, Meguro-ku, Tokyo 153-8904 (Japan)
and
Precursory Research for Embryonic Science and Technology
Japan Science and Technology Agency
4-1-8 Honcho, Kawaguchi, Saitama 332-0012 (Japan)
Fax: (+81) 3-5452-5224
E-mail: t-serizawa@bionano.rcast.u-tokyo.ac.jp
T. Sawada
Research Center for Advanced Science and Technology
The University of Tokyo
4-6-1 Komaba, Meguro-ku, Tokyo 153-8904 (Japan)
and
Division of Materials Science and Engineering
Shibaura Institute of Technology
3-7-5 Toyosu, Koto-ku, Tokyo 135-8548 (Japan)
Prof. T. Kitayama
Graduate School of Engineering Science
Osaka University
1-16 Machikaneyama-cho, Toyonaka 560-0043 (Japan)

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(Ph.D.-7 Phage Display Peptide Library Kit, New England Biolabs, Inc.) in pIII coat proteins was applied to conditioned st-PMMA films. Biopanning cycles were repeated as required, cloning, and DNA sequencing were demonstrated. Binding affinities of phage clones to PMMA films were analyzed by enzyme-linked immunosorbent assays (ELISA) using horseradish-peroxidase-conjugated anti-M13 bacteriophage antibodies.

The amino acid sequences of the peptides that bind to conditioned st-PMMA are shown in Table 1. Frequencies in Table 1 indicate the fraction of the same clone in all the isolated clones. Seventeen clones were identified by 3–8 rounds of biopanning. After four rounds, the diversity of the peptide sequences considerably decreased. The percent appearance of amine-containing amino acids, such as His, Lys, Arg, and Trp, clearly increased compared with those for phage libraries (for comparisons, see Supporting Information, Figure S1). Amine-containing amino acids are essential for peptide interactions with st-PMMA. The frequencies of Pro residues also increased. These observations suggest that phage libraries were directed to specific clones.

***Table 1:*** Amino acid sequences of phage-displayed peptides selected for conditioned st-PMMA films.

| Clone | Frequency[a] | | | | Sequence[b] |
|---|---|---|---|---|---|
| | 3rd | 4th | 5th | 8th | |
| 3-c01 | 8/34 | 10/14 | 14/15 | 30/42 | HPERATL |
| 3-c04 | 9/34 | 3/14 | 1/15 | 10/42 | LPPWQRQ |
| 3-c09 | 4/34 | – | – | – | KPRMPPR |
| 3-c25 | 2/34 | – | – | – | HDHRYPK |
| 4-c01 | – | 1/14 | – | – | HPVHPHR |
| 3-c33 | 1/34 | – | – | 1/42 | HPRWHTP |
| 8-c09 | – | – | – | 1/42 | HPRLGLA |
| 3-c03 | 1/34 | – | – | – | QLKTGLA |
| 3-c17 | 1/34 | – | – | – | GKPMPPM |
| 3-c12 | 1/34 | – | – | – | THLPWQT |
| 3-c22 | 1/34 | – | – | – | HPWWRPS |
| 3-c06 | 1/34 | – | – | – | HALGPSS |
| 3-c14 | 1/34 | – | – | – | HAIYPRH |
| 3-c15 | 1/34 | – | – | – | HKPDANR |
| 3-c18 | 1/34 | – | – | – | AITRSPA |
| 3-c24 | 1/34 | – | – | – | FPGHSGP |
| 3-c26 | 1/34 | – | – | – | NKNYIQH |

[a] Frequencies for each biopanning round. [b] Red: amino acids containing an amino group; blue: amino acids containing a hydroxy group; green: Pro; black: others.

The relative amount of phages bound to the PMMA films is quantified by ELISA. Data from phage clones for target and reference films are shown in Figure 2. Significantly, for many clones, greater amounts bind to target films than to reference films. The amounts bound to reference films were almost the same within experimental error. Although the amounts of some clones bound to nonconditioned st-PMMA films were almost the same as those bound to conditioned st-PMMA films, the apparent affinity constants for the nonconditioned films were smaller than for the conditioned films (see below). These observations suggest that 7-mer peptides displayed on phages can discriminate differences in the conditioning of st-PMMA films, and that these clones do not bind to PMMAs with other stereoregularities.

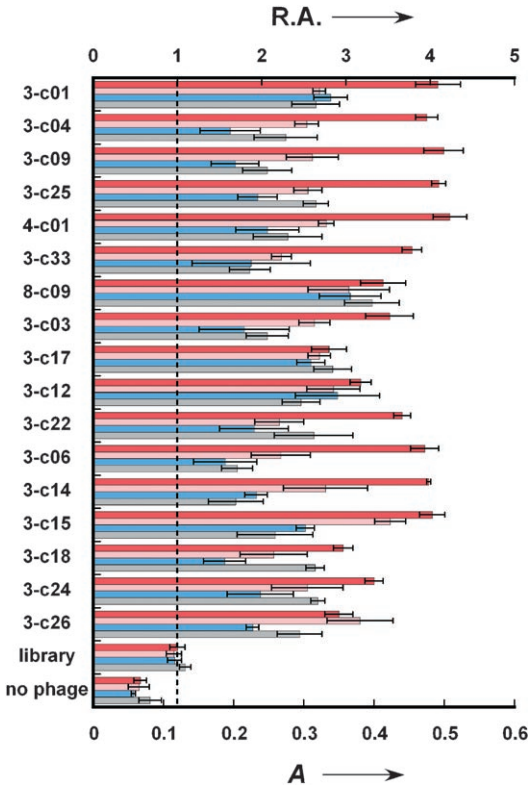


***Figure 2.*** The relative affinity (R.A.) of phage clones selected from the phage-display library for conditioned st-PMMA (red), nonconditioned st-PMMA (pink), nonconditioned it-PMMA (blue), and nonconditioned at-PMMA (gray) films. Absorbance data (*A*) were converted into affinities relative to those of phage libraries for conditioned st-PMMA films (dashed line). The amino acid sequences of each clone are shown in Table 1.

Bound amounts measured against phage concentrations gave the apparent phage–PMMA-film affinity constant $K_{app}$. Figure 3 shows comparisons of the affinity constants to target and reference films (for isotherms and estimations, see Supporting Information, Figure S2). The affinity constants were of the order of $10^{10}\,\text{M}^{-1}$ (high) because of the binding of peptide-displaying phages (similarly shown in our previous study[6d]). Almost all the clones showed specific constants for target films. Clones with different specificities were obtained from biopanning. The affinity constants for the best target clones were more than five-times greater than those for nonconditioned st-PMMA, and were clearly greater than those for nonconditioned it-PMMA films. Note that 3-c01 which has the highest frequencies did not show the highest specificity to target films. This result means that 3-c01 tended to readily amplify with *Escherichia coli*. It was found that the selected phage clones showed greater affinity constants for conditioned st-PMMA films than those for other reference films.

Conditioning of it-PMMA films did not affect the affinity constants, which indicates that conditioning is only essential for amphiphilic target films (see Supporting Information, Table S1). Despite the presence of 75 % *rr* syndiotactic units

  

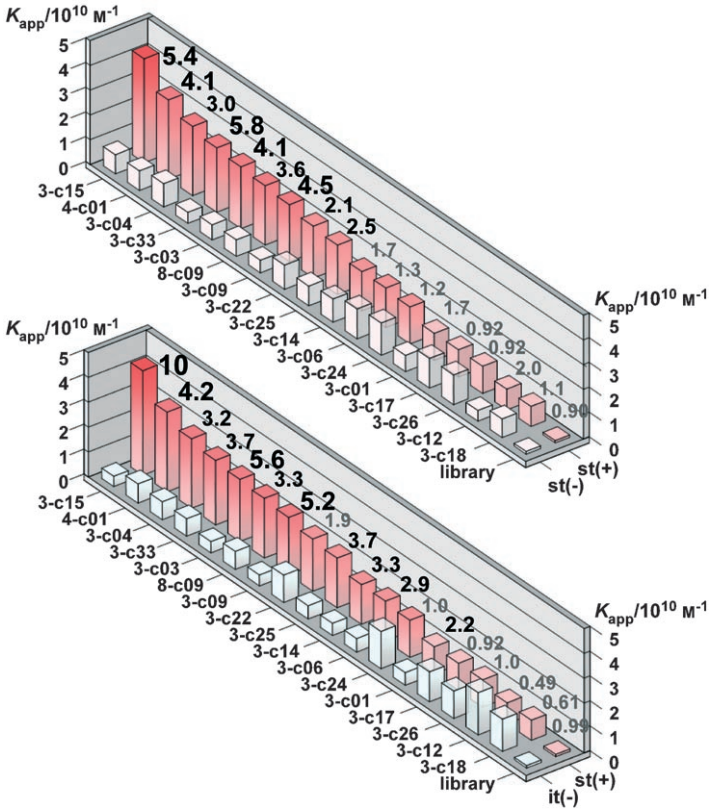


***Figure 3.*** Apparent affinity constants ($K_{app}$) for all the clones to conditioned st-PMMA (st(+)) and nonconditioned st-PMMA (st(−), top) or nonconditioned it-PMMA (it(−), bottom) films. The numbers in the graph indicate the ratio of $K_{app}$ for target films to $K_{app}$ for reference films (the bolder the number, the larger the ratio). The amino acid sequences of each clone are shown in Table 1.

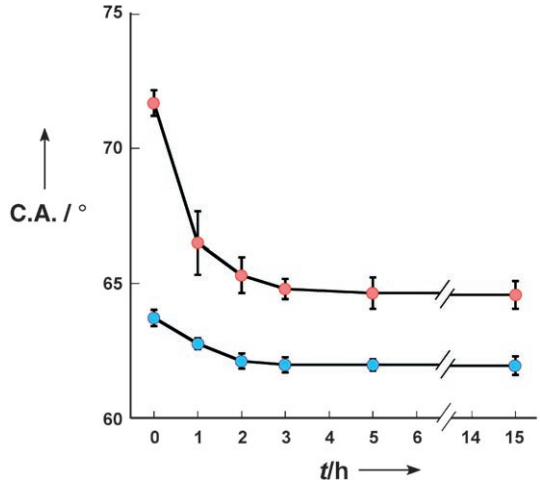


***Figure 4.*** Time dependence of changes in static contact angles (C.A.) of st-PMMA (red circles) and it-PMMA (blue circles) films in TBS.

in conditioned at-PMMA, the affinity constants were clearly smaller than those for target films (also see Supporting Information, Table S1), indicating that peptides recognize syndiotactic units by a means other than certain length criteria. The affinity constants for conditioned at-PMMA were slightly greater than those for conditioned it-PMMA, suggesting that syndiotactic units of at-PMMA might accumulate at surfaces and thereby strengthen peptide interactions. On the other hand, the affinity constants of phage clones for all the reference films were greater than those of the libraries, suggesting that peptides with the potential to interact with PMMA have been selected successfully.

Static contact angles of the st-PMMA films (40-nm thick on glass slides) measured using 3 μL water droplets at ambient temperature decreased with conditioning time and almost saturated after 15 h (Figure 4), suggesting that the hydrophilic ester groups of st-PMMA rather than the hydrophobic alkyl groups were gradually exposed to the water side. The angle changes are small (approximately 7°); however the surface functional groups are clearly changed. The angles for the it-PMMA films hardly decreased, possibly because of the stable conformations already formed at the film surfaces. These observations are supported by a previous study.[7] After conditioning, the difference in angles between st- and it-PMMA films was within 3°. The peptides did not bind to the more hydrophobic nonconditioned st-PMMA films, suggesting that hydrophobic interactions are not essential. In addition, the peptides did not bind to conditioned it-PMMA films with a similar hydrophilicity to conditioned st-PMMA films (see Supporting Information, Table S1). Therefore, peptides would recognize nanoscale arrangements of ester groups, which derive from syndiotactic arrangements.

Studying the homologies for peptides with high affinity constants and selectivities, shows that sequences with amine-containing amino acids adjacent to Pro, such as -His-Lys-Pro- (3-c15), -His-Pro-His-Arg- (4-c01), -His-Pro-Arg-Trp-His- (3-c33), and -Lys-Pro-Arg- (3-c09) seem to be an essential motif for recognizing conditioned st-PMMA. This motif though apparently similar to that for it-PMMA (sequences with hydroxy- and amine-containing amino acids adjacent to Pro)[6d] in fact has significant differences. Increases in the frequencies of Arg and Trp residues are common for both peptides. However, some amine-containing amino acids were typically enriched for st-PMMA, as described above. In it-PMMA binding peptides, it was suggested that hydrogen-bonding interactions between hydroxy protons of Ser, Thr, and Tyr, and ester groups of it-PMMA were significant for it-PMMA recognition. For st-PMMA recognition, amine protons might behave as proton donors to the ester groups of st-PMMA, thus forming hydrogen bonds. As these amino acids are hydrophilic, the peptides did not strongly interact with nonconditioned slightly hydrophobic st-PMMA films, resulting in the recognition of dynamic changes in polymer surfaces. The interactions are enhanced by a kinked structure formed by Pro, to avoid free conformational changes of the peptides.

In conclusion, peptide motifs that recognize differences in polymer-film surfaces were revealed based on selection from peptide-displaying phage libraries directed against appropriately treated st-PMMA films. It was confirmed that peptides have the potential to successfully recognize stereoregularities and slight differences in surface structures of a single-component film. This study is the first to demonstrate that

  

phage clones clearly recognize dynamic changes in polymer-film surface nanostructures. The delicate structural regulation of the target polymer surfaces is an essential factor in controlling peptide–polymer interactions. Recognition details from dynamic polymer surfaces using synthetic peptides freed from phages will be reported in future publications.

## Experimental Section

Materials: St-PMMA ($M_n$ 28200, $M_w/M_n$ 1.26, *mm*/*mr*/*rr* = 0:11:89) (where *mm*, *mr*, and *rr* represent iso-, hetero-, and syndiotacticities, respectively) and it-PMMA ($M_n$ 23200, $M_w/M_n$ 1.26, *mm*/*mr*/*rr* = 97:3:0) were synthesized by conventional living anion polymerization. at-PMMA ($M_p$ 28750, $M_w/M_n$ 1.03, *mm*/*mr*/*rr* = 10:15:75) was purchased from Polymer Laboratories.

Biopanning: A phage solution ($1.2 \times 10^{10}$ pfu/30 μL) was placed onto st-PMMA films and incubated for 1 h at ambient temperature. To remove any unbound phages, the films were rinsed five times with TBS (150 μL) containing Tween-20 (0.1 wt%). Then, the bound phages were eluted by mounting 0.5 M glycine–HCl (20 μL, pH 2.2) onto the films for 15 min at ambient temperature. For the next round of biopanning, the phages were amplified by infecting with *Escherichia coli* strain ER2738.

ELISA: A triplicate ELISA was performed. PMMA films were prepared on glass plates. An aliquot of the phage solution (100 μL) at $5 \times 10^{-11}$ M dissolved in PBS was applied onto the films for 1 h at 20 °C. After that, a horseradish-peroxidase-conjugated anti-M13 bacteriophage antibody (Amersham Biosciences) was applied for 1 h. Then, relative amounts of bound phages were estimated by measuring the absorbance of the products from the substrates (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt) at 405 nm.

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