Directional Affinity of Short Peptides for Synthetic Polymers

Takaaki Date,[†] Keiji Tanaka,[‡] Toshihiko Nagamura,[‡] and Takeshi Serizawa*[,§,||]

Graduate School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8654, Japan, Graduate School of Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan, Research Center for Advanced Science and Technology (RCAST), The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan, and Precursory Research for Embryonic Science and Technology, and Japan Science and Technology Agency (JST), 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

Received December 19, 2007
Revised Manuscript Received April 30, 2008

Recently developed combinatorial biotechnologies such as phage display (PD) and cell-surface display methods have revealed that short peptides with specific affinities for artificial materials could be isolated from diverse peptide libraries based on affinity selection processes.[1] Material surfaces composed of inorganic (metals,[2] semiconductors,[3] and metal oxides[4]) and organic compounds (carbon nanotubes,[5] carbon nanohorns,[6] fullerenes,[7] and synthetic polymers[8]) were applied to these selection methods. Such novel material-binding peptides are receiving much attention as peptidyl nanomaterials because of their potential applications: catalysts for the preparation of inorganic nanoparticles,[9] adsorbents for patterning,[2c] surface modifiers,[8c,9a,10] and modifiers of phages[3b]/proteins[11] used for assembly. To determine the intrinsic functions of peptide motifs after adequate chemical and biological modifications, it is important to determine whether a N or C terminus of the peptide is suitable for conjugation with the desired foreign molecules.

Our previous attempts of the PD method using M13 bacteriophages displaying 7-mer random peptide libraries on pIII coated proteins successfully identified a c02 peptide composed of the Glu-Leu-Trp-Arg-Pro-Thr-Arg sequence that was specifically bound to film surfaces prepared from a target isotactic poly(methyl methacrylate) (it-PMMA) rather than a structurally similar reference, syndiotactic (st) PMMA.[8d] In situ binding analysis of the c02 peptide from its aqueous solution using surface plasmon resonance (SPR) measurements demonstrated that the affinity constant (K_a) of the c02 peptide for it-PMMA (2.8×10^5 M^{-1}) was more than 40 times greater than that for st-PMMA (6.8×10^3 M^{-1}) and that a C-terminal 4-mer peptide composed of the Arg-Pro-Thr-Arg sequence still showed a similarly high K_a ratio between it- and st-PMMAs.[12] The observations suggested that the specificity of the c02 peptide was likely determined by the C terminal 4-mer sequence. Therefore, the c02 peptide is considered to be an adequate candidate to further demonstrate the directional affinity for polymer surfaces.

Surface force measurements using atomic force microscopic (AFM) setups are potential methods to quantify the adhesion forces between the cantilever tip coated with the peptides and the material surfaces.[4d,5b,8c] In this case, the peptides were biotinylated using its glutamic acid derivative and were immobilized on the surface of the tip through avidin−biotin strong interactions. Since each peptide terminus is exposed from the tip by introducing biotin into the opposite terminus, directional peptide affinities can be analyzed. Due to steric hindrance, the immobilized terminus would interact with the target materials with difficulty. However, up to now, there has been no research quantitatively demonstrating any difference in affinity based on the direction of peptide immobilization, despite this being a crucial parameter for the further conjugation of peptide motifs.

* To whom all correspondence should be addressed: RCAST, The University of Tokyo. Tel&Fax: +81-3-5452-5224. E-mail: t-serizawa@bionano.rcast.u-tokyo.ac.jp.
[†] Graduate School of Engineering, The University of Tokyo.
[‡] Kyushu University.
[§] RCAST, The University of Tokyo.
[||] JST.

(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) Brown, S. *Nat. Biotechnol.* **1997**, *15*, 269. (b) Brown, S.; Sarikaya, M.; Johnson, E. *J. Mol. Biol.* **2000**, *299*, 725. (c) Naik, R. R.; Stinger, S. J.; Agarwal, G.; Jones, S. E.; Stone, M. O. *Nat. Mater.* **2002**, *1*, 169.
(3) (a) Whaley, S. R.; English, D. S.; Hu, E. L.; Barbara, P. F.; Belcher, A. M. *Nature* **2000**, *405*, 665. (b) Lee, S.-W.; Mao, C.; Flynn, C. E.; Belcher, A. M. *Science* **2002**, *296*, 892.
(4) (a) Brown, S. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 8651. (b) Barbas, C. F., III; Rosenblum, J. S.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6385. (c) Sano, K.-I.; Shiba, K. *J. Am. Chem. Soc.* **2003**, *125*, 14234. (d) Hayashi, T.; Sano, K.-I.; Shiba, K.; Kumashiro, Y.; Iwahori, K.; Yamashita, I.; Hara, M. *Nano Lett.* **2006**, *6*, 515.
(5) (a) 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. (b) Li, X.; Chen, W.; Zhan, Q.; Dai, L.; Sowards, L.; Pender, M.; Naik, R. R. *J. Phys. Chem. B* **2006**, *110*, 12621.
(6) Kase, D.; Kulp, J. L., III; Yudasaka, M.; Evans, J. S.; Iijima, S.; Shiba, K. *Langmuir* **2004**, *20*, 8939.
(7) Morita, Y.; Ohsugi, T.; Iwasa, Y.; Tamiya, E. *J. Mol. Catal. B: Enzym.* **2004**, *28*, 185.
(8) (a) Adey, N. B.; Mataragnon, A. H.; Rider, J. E.; Carter, J. M.; Kay, B. K. *Gene* **1995**, *156*, 27. (b) Berglund, J.; Lindbladh, C.; Nicholls, I. A.; Mosbach, K. *Anal. Commun.* **1998**, *35*, 3. (c) Sanghvi, A. B.; Miller, K. P-H.; Belcher, A. M.; Schmidt, C. E. *Nat. Mater.* **2005**, *4*, 496. (d) Serizawa, T.; Sawada, T.; Matsuno, H.; Matsubara, T.; Sato, T. *J. Am. Chem. Soc.* **2005**, *127*, 13780. (e) Serizawa, T.; Sawada, T.; Kitayama, T. *Angew. Chem., Int. Ed.* **2007**, *46*, 723. (f) Serizawa, T.; Techawanitchai, P.; Matsuno, H. *ChemBioChem* **2007**, *8*, 989. (g) Serizawa, T.; Iida, K.; Matsuno, H.; Kurita, K. *Chem. Lett.* **2007**, *36*, 988.
(9) (a) Slocik, J. M.; Stone, M. O.; Naik, R. R. *Small* **2005**, *1*, 1048. (b) Sano, K.-I.; Sasaki, H.; Shiba, K. *Langmuir* **2005**, *21*, 3090. (c) Ahmad, G.; Dickerson, M. B.; Church, B. C.; Cai, Y.; Jones, S. E.; Naik, R. R.; King, J. S.; Summers, C. J.; Kröger, N.; Sandhage, K. H. *Adv. Mater.* **2006**, *18*, 1759. (d) Slocik, J. M.; Naik, R. R. *Adv. Mater.* **2006**, *18*, 1988.
(10) (a) Pender, M. J.; Sowards, L. A.; Hartgerink, J. D.; Stone, M. O.; Naik, R. R. *Nano Lett.* **2006**, *6*, 40. (b) Sano, K.-I.; Sasaki, H.; Shiba, K. *J. Am. Chem. Soc.* **2006**, *128*, 1717. (c) Matsui, T.; Matsukawa, N.; Iwahori, K.; Sano, K.-I.; Shiba, K.; Yamashita, I. *Langmuir* **2007**, *23*, 1615.
(11) Sano, K.-I.; Ajima, K.; Iwahori, K.; Yudasaka, M.; Iijima, S.; Yamashita, I.; Shiba, K. *Small* **2005**, *1*, 826.
(12) Serizawa, T.; Sawada, T.; Matsuno, H. *Langmuir* **2007**, *23*, 11127.

10.1021/cm703603f CCC: $40.75
Published on Web 06/18/2008

Scheme 1. Biotinylated Derivatives of the C02 Peptide

X_1-ELWRPTR-X_2

B-c02: X_1 = biotinylated E, X_2 = amide

c02-B: X_1 = free, X_2 = biotinylated E

In this study, the adhesion forces of the c02 peptide immobilized onto the AFM cantilever tip through the biotinylated N or C termini (Scheme 1) against PMMA surfaces were quantified by AFM force measurements to experimentally demonstrate the directional affinities of the c02 peptide (Figure 1). Alternatively, the K_a of the biotin-derivative peptides, which can be regarded as typical compounds conjugated with the corresponding peptide motif, for PMMA surfaces were analyzed by SPR measurements to further validate the AFM measurements. The present study was designed to demonstrate that material-binding peptides with extremely short sequences, similar to structurally regular proteins, have a preferential orientation for their interactions with artificial materials.

It-PMMA (M_n = 23 200, M_w/M_n = 1.3, *mm*:*mr*:*rr* = 97:3:0) and st-PMMA (M_n = 28 200, M_w/M_n = 1.3, *mm*:*mr*:*rr* = 0:11:89) were previously synthesized[12] and were used for all experiments. B-c02 and c02-B, of which the N and C terminus of the c02 peptide are biotinylated, respectively, with a free N terminus and an amidated C terminus, were prepared by solid-phase peptide synthesis using standard Fmoc-based procedures.[13] Adhesion force measurements using the AFM setup (SPI-3700, Seiko Instruments) were performed by modifying the AFM tip in accordance with conventional methods previously reported.[14] Biacore X was used for SPR measurements, following our previous study.[12] Experimental details are summarized in Supporing Information.

A typical force−displacement curve obtained from combining the cantilever tip coated with B-c02 and the target it-PMMA surface is shown in the inset of Figure 2. The adhesion force was observed from changes in the deflection of the tip. The force measurement consisted of an approach trace, followed by a retraction trace of the tip interacting with the it-PMMA. Initially, the surface was approached by the piezo element. After contact, the piezo compressed the tip against the sample, up to a certain displacement. During the subsequent retraction, the tip remained in contact with the it-PMMA until the retracting force exceeded the interfacial adhesion force, which corresponded to the affinity between the immobilized B-c02 and the it-PMMA. In this way, it was found that the adhesion force measurement using the AFM setup could successfully quantify the affinity between the immobilized peptide and the polymer surface. It is noted that a single tip coated with the c02 peptide detected a similar adhesion force within an experimental error of at least 10 times the force measurement. Therefore, it was confirmed that the c02 peptide was stably immobilized without denaturation during the force measurements. The resulting adhesion forces ranged on the order of ~7.5 pN nm^{-2}, which are comparable to the forces obtained from a polymer-binding peptide using a similar AFM setup.[8c] In the next step, the effects of the peptide species and the polymer surfaces were evaluated as follows.

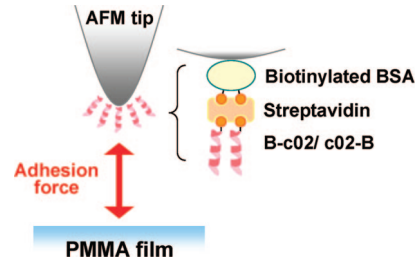

Figure 1. Schematic representation of the adhesion force measurements.

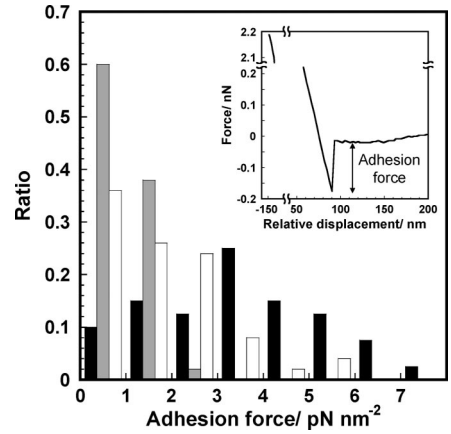

Figure 2. Histograms of the adhesion forces for combinations of B-c02/it-PMMA (black), c02-B/it-PMMA (gray), and B-c02/st-PMMA (white). The inset shows a typical force−displacement curve for the retraction trace obtained from a cantilever tip coated with B-c02 against it-PMMA.

Table 1. AFM Adhesion Forces and SPR Kinetic Parameters

peptide/polymer	mean adhesion force[a] (pN nm^{-2})	k_1 (M^{-1} s^{-1})	k_{-1} (10^{-3} s^{-1})	K_a (10^3 M^{-1})
B-c02/it-PMMA	3.8[b]	26	0.03	760
c02-B/it-PMMA	1.0	18	0.23	79
B-c02/st-PMMA	1.6	15	0.13	110

[a] Estimated by fitting to a Poisson distribution. [b] The adhesion force of the tip coated with the outermost streptavidin surface (without peptide) against it-PMMA was smaller than this value (see text).

Histograms of the adhesion force for each combination are shown in Figure 2. Each histogram became an approximately single distribution and was fitted assuming a Poisson distribution. The resulting mean adhesion forces are summarized in Table 1. The adhesion forces of the c02 peptide to the PMMAs differed significantly depending on the immobilizing terminus as well as the adhered polymer. The mean adhesion force of the tip coated with B-c02 against it-PMMA (3.8 pN nm^{-2}) was 4 times greater than a tip coated with c02-B against it-PMMA (1.0 pN nm^{-2}). It is noted that the adhesion force of the tip coated with the outermost streptavidin surface (without peptide) against it-PMMA (0.8 pN nm^{-2}) was significantly smaller than the aforementioned forces (see Figure S1 in Supporing Information), thereby indicating successful detection of peptide−polymer interactions. These observations strongly suggest that the affinity of the c02 peptide directed from the C terminus was greater than that from the N terminus. Furthermore, the tip coated with B-c02 showed a significantly smaller adhesion force against a reference st-PMMA (1.6 pN nm^{-2}) as

(13) Fields, G. B.; Nobel, R. L. *J. Pept. Protein Res.* **1990**, *35*, 161.
(14) Moy, V. T.; Florin, E.-L.; Gaub, H. E. *Colloids Surf.* **1994**, *93*, 343.

compared with it-PMMA, indicating that the specificity of the c02 peptide was maintained even after immobilization onto a solid surface. Unfortunately, the ratio of the adhesion forces for B-c02 between it-PMMA and st-PMMA was smaller than the K_a values (see below). Since the extremely small c02 peptide was immobilized onto the larger streptavidin and since the tip was compressed against the PMMA films, the nonspecific adsorption of streptavidin bodies might underestimate the peptide specificity. Our previous studies suggested that the c02 peptide had a specific motif at the C terminal region.[12] The adhesion force measurement successfully confirmed this significant property. It is noted that the mean adhesion forces were estimated from the original data to be 3.5 ± 1.8 pN nm^{-2}, 0.9 ± 0.5 pN nm^{-2}, and 1.8 ± 1.3 pN nm^{-2} for combinations of B-c02/it-PMMA, c02-B/it-PMMA, and B-c02/st-PMMA, respectively. As a consequence, we can report the first directional affinity characteristic of an extremely short material-binding peptide through adhesion force measurements.

The modification of the c02 peptide with foreign molecules without losing its high affinity is a key process for developing novel peptidyl nanomaterials. The adhesion force measurements described above demonstrated the directional affinity characteristic of the c02 peptide in its solid state. These observations would suggest that modification of the N terminus with foreign molecules does not interfere with c02 affinity, in contrast to the C terminus, possibly due to steric hindrance. Therefore, the affinities of B-c02 and c02-B, which can be regarded as model peptide derivatives conjugated with glutamic acid γ-biotinylated through a mono(propylene glycol) and di(ethylene glycol) spacer, against PMMA surfaces, were analyzed kinetically by SPR measurements using an aqueous peptide solution. The potential of highly sensitive SPR methods for analyzing peptide−material affinities has already been reported in previous studies.[12,15] In fact, the time-dependent affinities of short peptides against polymer film surfaces were successfully detected by SPR measurements (see Figure S2 in Supporing Information).

The kinetic parameters and the K_a of the peptides are also summarized in Table 1. The K_a of B-c02 for it-PMMA was estimated to be 7.6×10^5 M^{-1}, which was slightly greater than that of the original c02 peptide. Since the additional Glu, mono(propylene glycol), di(ethylene glycol), and biotin residues of B-c02 to the c02 peptide might interact with it-PMMA through nonspecific hydrogen bonding and/or van der Waals interactions, the slight increase in K_a would be acceptable. More importantly, the K_a of c02-B for it-PMMA (0.79×10^5 M^{-1}) was 10 times smaller than that of B-c02, even though both peptides contained the same it-PMMA-specific c02 peptide sequence. In terms of kinetic parameters, B-c02 was more rapidly associated with it-PMMA and more slowly dissociated from it-PMMA as compared with c02-B, and thus the former K_a was greater than the latter. These observations strongly indicate that the N terminal modification is suitable for realizing the potential of the c02 peptide. In other words, the C terminal modification interfered with the original affinity of the c02 peptide. On the other hand, the K_a of B-c02 for a reference st-PMMA (1.1×10^5 M^{-1}) was 7 times smaller than for it-PMMA. The K_a ratio of B-c02 between it- and st-PMMAs ($\sim$7) was much smaller than that of the c02 peptide ($\sim$41[12]). It is difficult to reasonably explain this difference, although the additional residues might similarly increase the K_a for st-PMMA through nonspecific interactions. Accordingly, these observations suggest a potential methodology to further modify the c02 peptide with foreign molecules. It was also found that the K_a values analyzed using the aqueous peptide solution were correlated to the adhesion forces. Therefore, adhesion force analysis would be a useful methodology to characterize the directional affinity of material-binding short peptides.

This is the first study to report the fact that extremely short peptides show directional affinity for target artificial materials based on adhesion force and kinetic measurements. The AFM setups successfully quantified the adhesion forces of a cantilever tip coated with peptides against polymer surfaces. SPR measurements demonstrated different kinetic parameters dependent on the N or C terminal peptide modification, as well as the polymer species. In terms of the c02 peptide, the C terminal region had preferential access for the target it-PMMA, and therefore the N terminus was found to be suitable for modification with foreign molecules. Similar analysis of other material-binding peptides and functional modification of the c02 peptide are now in progress.

Acknowledgment. The authors thank Dr. H. Matsuno (University of Tokyo), Dr. K. Akabori, and Dr. Y. Fujii (Kyushu University) for helpful discussion and technical support and Prof. M. Komiyama (University of Tokyo) for help with MALDI-TOF MS measurements. This work was supported by the Fundation for the Promotion of Industrial Science, JST (PRESTO), JSPS (Grants-in-Aid for Scientific Research (B) no. 20350052), and the Global COE Program for Chemistry Innovation.

Supporting Information Available: Experimental details, reference adhesion forces, and SPR sensor-grams (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

CM703603F

(15) (a) Tamerler, C.; Oren, E. E.; Duman, M.; Venkatasubramanian, E.; Sarikaya, M. *Langmuir* **2006**, *22*, 2712. (b) Seker, U. O. S.; Wilson, B.; Dincer, S.; Kim, I. W.; Oren, E. E.; Evans, J. S.; Tamerler, C.; Sarikaya, M. *Langmuir* **2007**, *23*, 7895.