Enhanced Adsorption of Protein Fused with Polymeric Material-binding Peptide

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A model protein was fused with the ELWRPTR peptide, which binds specifically to the isotactic poly(methyl methacrylate) (it-PMMA) film surface, and its adsorption on PMMA film surfaces was then investigated by surface plasmon resonance measurements. We found that the adsorption constant of the fusion protein for the it-PMMA film surface was 75-fold greater than that of native protein.

The surface modification of polymeric materials with functional proteins is useful for biomedical applications. Among the various methods,[1] noncovalent immobilization by physical adsorption is the simplest; however, the proteins may desorb readily from the material surfaces. In general, with respect to chemically inert polymers, reactive groups suitable for covalent protein immobilization must be first introduced as coupling sites by methods such as grafting, photooxidation, or plasma.[2]

In the past decade, various inorganic/organic material-binding peptides (MBPs) have been identified by combinatorial biotechnology.[3] We also reported a heptapeptide (c02: ELWRPTR) that specifically binds to an it-PMMA film surface through hydrogen-bonding interactions.[4] The affinity constant for it-PMMA ($K_a = 3.50 \times 10^5\ M^{-1}$ at 20 °C) was 43-fold greater than that for the reference syndiotactic (st) PMMA.[4a]

MBPs have been used as efficient tools for protein modification. For instance, apoferritin,[5] cytokine,[6] and green fluorescent protein[7] have been fused with adequate MBPs such that the fused proteins were more stably immobilized onto inorganic and carbonaceous materials through noncovalent bonding. Despite the considerable contribution to MBPs identification,[3] research into fusion proteins is still limited. It is, therefore, worthwhile to investigate whether MBPs are useful as fusion partners.

Herein, we report the extremely enhanced adsorption of a model protein onto the it-PMMA film surface after fusion with the c02 peptide (Figure 1). It is known that DnaK419-607 (a blocking peptide fragment; BPF), which is part of the substrate-binding domain of the molecular chaperon DnaK from *E. coli*,[8] behaves like an effective blocking reagent due to good adsorption properties for plastic substrates through its hydrophobic region.[9] Also, an *E. coli* over-expression system for recombinant BPF has already been established.[9] In this study, a BPF derivative (dBPF) fused with a C-terminal RGD, which promotes cell adhesion on material surfaces,[10] was selected as a model protein. Therefore, stable immobilization of dBPF is meaningful. Surface plasmon resonance (SPR) measurements kinetically analyzed protein adsorption. We will demonstrate, for the first time, the potential of MBPs as fusion partners in the polymeric materials field.

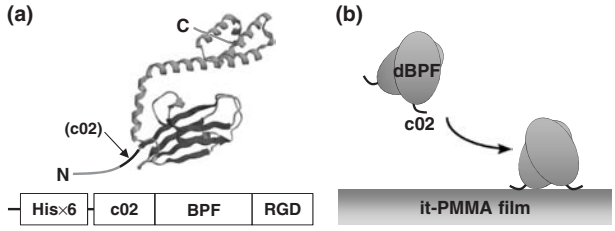

Figure 1. (a) A model and construct of the fusion protein c02–dBPF. A BPF (DnaK419-607) region was schematically drawn referring the 3D structural data for the substrate-binding domain (DnaK389-607: 1DKZ). The fused peptide chains at the N- and C-terminals of BPF were additionally depicted. (b) A schematic illustration of c02 peptide-dependent protein adsorption on the it-PMMA film surface.

Chloroform solutions of commercially available it-PMMA ($M_n = 19000$, $M_w/M_n = 1.10$, *mm*:*mr*:*rr* = 96:2:2) and atactic (at) PMMA ($M_n = 22000$, $M_w/M_n = 1.03$, *mm*:*mr*:*rr* = 5:36:59) were prepared, and PMMA films of approximately 10-nm thickness were fabricated by spin-coating onto gold-coated glass slides. dBPF (22.4 kDa as a monomer) and the N-terminal fusion protein (c02–dBPF, 23.3 kDa as a monomer) were overexpressed in *E. coli* as His-tagged proteins and were purified as a homodimer using Ni–NTA columns. SPR measurements were performed with a BIAcore X (GE Healthcare) in 10 mM HEPES buffer (pH 7.4) containing 150 mM NaCl at 20 °C. The experimental details are summarized in the Supporting Information.[11]

Figure 2 shows typical examples of SPR sensorgrams of the adsorption of c02–dBPF and dBPF for the it-PMMA film surfaces. It is obvious that the amount of c02–dBPF adsorbed was greater than dBPF at similar protein concentrations. Assuming a Langmuir adsorption model, the adsorption rate constant k_1 ($M^{-1}\ s^{-1}$) and desorption rate constant k_{-1} (s^{-1}) were estimated by plotting the observed rate constant k_{obs} as a function of the protein concentration and fitting by the following equation: $k_{obs} = k_1C + k_{-1}$, where C was the concentration of the proteins (Figure 3). Finally, the adsorption constant K_a was calculated by the following equation: $K_a = k_1/k_{-1}$ (M^{-1}). The obtained parameters are summarized in Table 1.

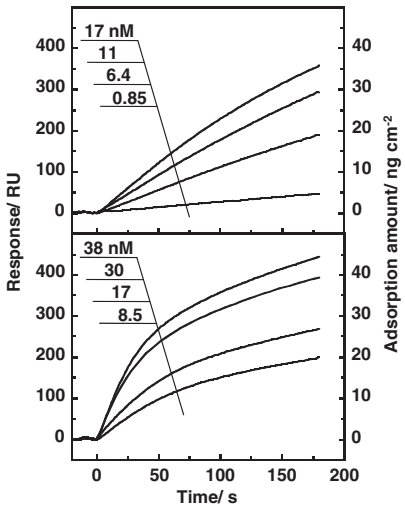

Figure 2. SPR sensorgrams of: (top) c02–dBPF and (bottom) dBPF for it-PMMA at the dimer concentrations indicated.

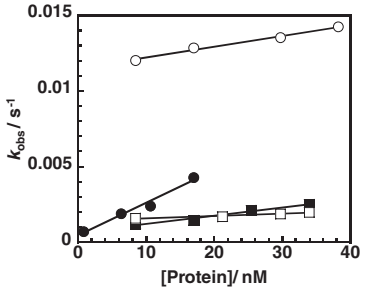

Figure 3. Plots of the k_{obs} of c02–dBPF (●) and dBPF (○) for it-PMMA, c02–dBPF (■) and dBPF (□) for at-PMMA, respectively, as a function of the dimer concentration.

The K_a value of c02–dBPF for the it-PMMA film surface was determined to be $4.9 \times 10^8\ M^{-1}$ and was 75-fold greater than that of dBPF. In fact, the peptide fusion resulted in a threefold increase in k_1 values and a 26-fold decrease in k_{-1} values for the it-PMMA film surface, suggesting that the fusion suppressed protein desorption rather than promoted protein adsorption. In previous studies, we estimated the K_a values of various proteins with different molecular weights and isoelectric points to be 10^4–$10^7\ M^{-1}$ for the it-PMMA film surface,[12] and the K_a value of dBPF was ranged within previous results. Therefore, the aforementioned value of c02–dBPF was considered to be significantly greater. Shiba and co-workers reported the dissociation constant K_d ($=K_a^{-1}$) of L-apoferritin fused with the Ti-binding hexapeptide to be 3.82 nM for a Ti surface.[5a] Similar to the modified L-apoferritin, c02–dBPF showed a high affinity for its designed target.

The K_a value of c02–dBPF for the reference at-PMMA film surface was 7.5-fold greater than that of dBPF. Since the c02 peptide nonspecifically adsorbs onto a st-PMMA film surface,[4a] the affinity for the at-PMMA film surface was somewhat promoted. Furthermore, comparing the adsorption of dBPF for PMMA film surfaces, the K_a value for the at-PMMA film surface was slightly greater than that for the it-PMMA film surface. This observation is consistent with our previous studies on protein adsorption.[12b] Therefore, the fused peptide obviously promoted the affinity of dBPF for the it-PMMA film surface.

Table 1. Adsorption constants and kinetic parameters of c02–dBPF and dBPF for PMMA film surfaces

Polymer film	Protein	K_a $/10^8\ M^{-1}$	k_1 $/10^4\ M^{-1}\ s^{-1}$	k_{-1} $/10^{-4}\ s^{-1}$
it-PMMA	c02–dBPF	4.9	21	4.3
	dBPF	0.065	7.1	110
at-PMMA	c02–dBPF	0.82	5.4	6.6
	dBPF	0.11	1.5	14

In conclusion, we investigated the adsorption of a protein fused with the it-PMMA-binding c02 peptide for PMMA film surfaces by SPR measurements. The fusion protein showed an extremely high affinity for it-PMMA film surfaces, thus indicating that the c02 peptide functioned as a specific immobilization platform for the it-PMMA film surface. Accordingly, it was confirmed that MBPs are useful as fusion partners, even in the polymeric materials field. We believe that this methodology will be useful for polymer-surface modification with functional proteins in biomedical applications.

References and Notes

1 K. Tomizaki, K. Usui, H. Mihara, *ChemBioChem* **2005**, *6*, 782.
2 a) Z. Ma, Z. Mao, C. Gao, *Colloids Surf., B* **2007**, *60*, 137. b) B. Kasemo, *Surf. Sci.* **2002**, *500*, 656.
3 a) M. Sarikaya, C. Tamerler, D. T. Schwartz, F. Baneyx, *Annu. Rev. Mater. Res.* **2004**, *34*, 373. b) F. Baneyx, D. T. Schwartz, *Curr. Opin. Biotechnol.* **2007**, *18*, 312. c) M. B. Dickerson, K. H. Sandhage, R. R. Naik, *Chem. Rev.* **2008**, *108*, 4935.
4 a) T. Serizawa, T. Sawada, H. Matsuno, *Langmuir* **2007**, *23*, 11127. b) T. Serizawa, T. Sawada, H. Matsuno, T. Matsubara, T. Sato, *J. Am. Chem. Soc.* **2005**, *127*, 13780.
5 a) K. Sano, K. Ajima, K. Iwahori, M. Yudasaka, S. Iijima, I. Yamashita, K. Shiba, *Small* **2005**, *1*, 826. b) T. Matsui, N. Matsukawa, K. Iwahori, K.-I. Sano, K. Shiba, I. Yamashita, *Langmuir* **2007**, *23*, 1615.
6 K. Kashiwagi, T. Tsuji, K. Shiba, *Biomaterials* **2009**, *30*, 1166.
7 T. J. Park, S. Y. Lee, S. J. Lee, J. P. Park, K. S. Yang, K.-B. Lee, S. Ko, J. B. Park, T. Kim, S. K. Kim, Y. B. Shin, B. H. Chung, S.-J. Ku, D. H. Kim, I. S. Choi, *Anal. Chem.* **2006**, *78*, 7197.
8 a) X. Zhu, X. Zhao, W. F. Burkholder, A. Gragerov, C. M. Ogata, M. E. Gottesman, W. A. Hendrickson, *Science* **1996**, *272*, 1606. b) N. Tanaka, S. Nakao, H. Wadai, S. Ikeda, J. Chatellier, S. Kunugi, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15398.
9 a) T. Kuroita, A. Sogabe, Y. Takarada, N. Tanaka, PCT Int. Appl. WO 2005/003155, **2005**. b) http://www.toyobo.co.jp/e/seihin/xr/enzyme/pdf_files/293_296BPF_301.pdf
10 Patent in preparation.
11 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
12 a) T. Serizawa, Y. Nagasaka, H. Matsuno, M. Shimoyama, K. Kurita, *Bioconjugate Chem.* **2007**, *18*, 355. b) H. Matsuno, Y. Nagasaka, K. Kurita, T. Serizawa, *Chem. Mater.* **2007**, *19*, 2174.

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