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Binding analysis of peptides that recognize preferentially *cis*-azobenzene groups of synthetic polymers‡

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Peptides identified by the PD method against a film surface composed of azobenzene-containing synthetic polymers were characterized by QCM measurements. Among the four peptides analyzed, the c16 peptide with the sequence Trp-His-Thr-Leu-Pro-Asn-Ala showed the highest binding affinity to the films rich in *cis*-azobenzene groups. SPR served to determine the association/dissociation constants of the c16 peptide against the *trans*- and *cis*-azobenzene groups. The binding constants were estimated to be 1.3×10^5 and 1.4×10^6/M, respectively, indicating a high specificity of the c16 peptide for *cis*-azobenzene conformer. Then mutants of the c16 peptide were synthesized and characterized to gain information on the structural requirements for the *cis*-form specificity. An Ala-scan clearly revealed that all the amino acid residues of the c16 peptide were essential for the specificity. More importantly, the results with peptides of inverted sequence and containing Gly insertions confirmed that the specificity is strictly derived from the primary sequence of the c16 peptide. Copyright © 2010 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: azobenzene; peptide ligands; recognition; *cis*/*trans* conformers; PD

Introduction

The development of combinatorial biotechnologies such as genetically engineered PD and CSD provides a highly efficacious method to identify biological ligands such as peptides and proteins that recognize target molecules based on affinity selection processes [1]. The target could be material surfaces composed of inorganic compounds such as metals [2,3], semiconductors [4,5], metal oxides [6,7], as well as organic compounds such as carbon nanotubes [8,9], carbon nanohorns [10], fullerenes [11], and synthetic polymers [12–19]. The identified peptides recognize their target specifically in a non-covalent manner based on electrostatic, hydrogen bonding, $\pi-\pi$ stacking, van der Waals, and/or hydrophobic interactions. In general, the specific binding of peptides to the target contributes to lower the Gibbs free energy of adsorption, and provides a thermodynamic driving force that is utilized in diverse applications such as catalysts for the preparation of inorganic nanoparticles [20–23], adsorbents for patterning [3], surface modifiers [13,21,24–26], and modifiers of phages [5]/proteins [27] used for their assemblies.

After the successful targeting of chlorine-doped polypyrrole [13] by the PD method, there is increasing interest in the selection of polymer-binding peptides and their utilization in surface-functionalization of polymeric scaffolds. In our previous studies, we have identified by the PD method 7-mer peptides that specifically bind to the film surfaces composed of isotactic [14] or syndiotactic poly(methyl methacrylate) [15], syndiotactic polystyrene [16], and poly(L-lactide) [19]. In these studies, quantitative binding analysis was performed to better understand the binding specificity of the peptides, in terms of the affinity strength and kinetics. The peptides successfully recognized the nanoscale arrangements of functional groups of the polymers, which were dependent on polymeric stereoregularties, amphiphilicities, and porosities. These results revealed that short peptides have the potential to discriminate slight differences in polymer surfaces with an extremely simple chemical structure. Based on these findings, our interest moved to the recognition of small organic molecules introduced into synthetic polymers as pendant groups.

In our previous study [18], the PD method of 7-mer random peptide libraries was applied to the film surfaces composed of azobenzene-containing synthetic polymers under visible light, to indentify peptides that recognize in differentiated manner the *cis*- and *trans*-azobenzene group. Although under visible light the azobenzene groups are preferentially in the *trans*-form in the bulk films, ELISA experiments with the isolated phage clones suggested that all the identified peptides preferentially recognize the *cis*-azobenzene on the film surfaces. Considering the fact that phage clones were eluted from the film surfaces under acidic

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Abbreviations used: *CSD, cell-surface display; EtOAc, ethyl acetate; HBTU, O-(benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate; HEMA, 2-hydroxyethyl methacrylate; K_a, the binding constant; k_1, the association rate constant; k_{-1}, the dissociation rate constant; NMP, N-methylpyrrolidone; PBS, phosphate buffered saline; PD, phage display; QCM, quartz crystal microbalance; RU, resonance unit; SPR, surface plasmon resonance.*

conditions for successful biopanning processes [28], the clones with affinity for the *cis*-forms might be more readily eluted than those with affinity for the *trans*-forms. Moreover, although it is difficult to evaluate the exact *trans*/*cis* ratio of azobenzene groups, the *cis* conformer content on the outermost surfaces of the film might be increased when coated with aqueous phage solution. Among the sequences identified, the binding property of the c16 peptide (H-Trp-His-Thr-Leu-Pro-Asn-Ala-NH_2), which consists of the proposed motif for the *cis*-form specificity, was analyzed by QCM measurements. The results clearly confirmed that the binding affinity of the c16 peptide to films rich in *cis*-azobenzene was greater than that to the *trans*-azobenzene rich films, and that binding could reversibly be changed by alternate irradiation with UV and visible light.

In this work, the binding analysis was extended to other identified peptides and the binding of the c16 peptide was characterized in more details by QCM and SPR measurements of related peptide analogs (Figure 1). The QCM results indicated that the c16 peptide contained the essential sequence responsible for the specific binding affinity to the *cis*-azobenzene rich films. The SPR measurements indicated that the K_a value of the c16 peptide for the *cis*-azobenzene was tenfold that for the films rich in *trans*-azobenzene.

Materials and Methods

Chemicals

4-Hydroxyazobenzene, 2-chloroethanol, acryloyl chloride, and HEMA were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). N^{α}-Fmoc-N^{ε}-biotin-L-lysine (Fmoc-Lys(biotin)-OH) and NovaSyn TGR resin were obtained from Nova Biochem (Läufelfingen, Switzerland). HBTU, HOBt, DIEA and the other amino acids such as Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)OH, Fmoc-Leu-OH, Fmoc-Met-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Thr(tBu)-OH, and Fmoc-Tyr(tBu)-OH were purchased from Watanabe Chemical Industries (Hiroshima, Japan). Solvents and other reagents of synthesis grade were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan), and were used without further purification.

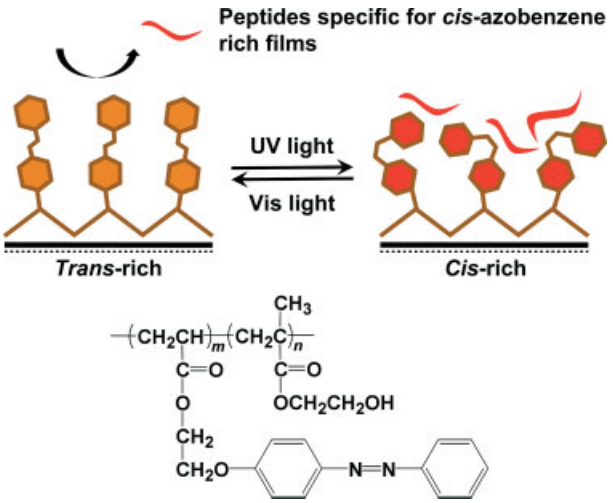

Figure 1. Schematic representation of the peptides specific for *cis*-form azobenzene groups on the polymer-film surfaces. The ratio of *m*/*n* was determined to be 0.49 by ^{1}H NMR.

Synthesis of 2-(4-Phenylazophenoxy)ethanol

4-Hydroxyazobenzene (19.8 g, 0.1 mol), potassium carbonate (13.8 g, 0.1 mol), and potassium iodide (0.6 g, 3.6 mmol) were dissolved by stirring in *n*-butanol (60 ml) in a flask under N_2 protection. After adding 2-chloroethanol (6.75 ml, 0.1 mol), the flask was heated in an oil bath (110 °C) for 7 h. The product was filtered and washed twice by water, and then the aqueous phase was extracted twice with chloroform. Solvent was removed in vacuum and the resulting crude product was purified by chromatography on silica gel column (hexane/EtOAc = 1/1, v/v). Yield: 8.4 g, 35%; ESI-MS (*m*/*z*): 242.5 [M] (calcd. 242.27), 243.6 $[M+H]^+$, 265.4 $[M+Na]^+$, 369.4 $[M+I]^-$; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.34–6.79 (*m*, 9H, ArH), 4.20 (*t*, 2H) 4.07 (*t*, 2H), 2.14 (*s*, 1H).

Synthesis of 2-(4-Phenylazophenoxy)ethyl Acrylate

Acryloyl chloride (2.24 g, 25 mmol) in chloroform (6.6 ml) was dropped into a chloroform (66 ml) solution containing 2-(4-phenylazophenoxy)ethanol (6 g, 25 mmol) and triethylamine (3 g, 25 mmol) at 0 °C under N_2 protection. After 2 h, the mixture was warmed to room temperature and stirred overnight. The product was filtered off and washed twice by water. The aqueous phase was then extracted twice with chloroform and dried over magnesium sulfate. After evaporation of the solvent, the product was purified by chromatography on silica gel column (hexane/EtOAc = 4/1, v/v) and recrystallized twice from hexane. Yield: 5.3 g, 72%; ESI-MS (*m*/*z*): 296.5 [M] (calcd. 296.32), 319.5 $[M+Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.95–6.80 (*m*, 9H, ArH), 6.49, 6.21 and 5.92 (*m*, 3H), 4.59 (*t*, 2H), 4.33 (*t*, 2H).

Synthesis of Poly[2-(4-phenylazophenoxy)ethyl Acrylate-*co*-HEMA]

2-(4-Phenylazophenoxy)ethyl acrylate (1.05 g, 3.6 mmol) and HEMA (0.38 ml, 3.6 mmol) were copolymerized in the presence of a free radical initiator 2,2′-azobisisobutyronitrile (11.7 mg, 71.0 μmol) in DMF (14.7 ml) at 60 °C for 24 h. The resulting copolymer was purified twice by reprecipitation with diethyl ether and dried under vacuum overnight. The M_n (36 000) and M_w/M_n (1.7) values were determined by gel permeation chromatography using a DMF (containing 10 mM LiBr) as solvent (M_n is the number-average molecular weight and M_w is the weight-average molecular weight). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.85 (4H), 7.48 (3H), 6.95 (2H), 4.50–3.68 (12H), and 0.88–1.45 (6H).

Preparation of Polymer Films

The films of poly[2-(4-phenylazophenoxy)ethyl acrylate-*co*-HEMA] (~20 nm thickness) were prepared by spin-coating the copolymer in chloroform (17 mg/ml) at 2000 rpm for 1 min on the 27 MHz QCM sensor tips (Affinix Q, Initiam) for QCM measurements or on gold-coated glass slides (SIA Kit Au, Biacore) for SPR measurements. The gold surface of QCM and SPR sensor chips were cleaned with 30% H_2O_2/H_2SO_4 (1/3, v/v) 'piranha solution' for 30 s, followed by rinsing with Milli-Q water several times before spin-coating. (*Caution!* Piranha solution should be handled with extreme care, and only small volumes should be prepared at one time.) Then, the films were irradiated by UV light using a hand-held UV lamp

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(SLUV-4, K.K. Iuchi Seieido) at 365 nm for 4 min in a darkroom to prepare the *cis*-rich films. On the other hand, the *trans*-rich films were obtained by keeping the films in a bright room after spin-coating.

Peptide Synthesis

Peptides were synthesized using standard Fmoc chemistry with NovaSyn TGR resin (300 mg, amino group 0.25 mmol/g) to give the peptides with free N- and amidated C-termini. Fmoc amino acids were activated with 0.45 M HBTU/HOBt in DMF and 0.9 M DIEA in NMP, and were coupled in threefold molar excess to the resin. To remove the Fmoc groups, 20% piperidine in NMP was used. The coupling reactions were repeated sequentially. After the synthesis, the resins were dried for 4 h and treated with a cleavage solution containing 2.5% Milli-Q water and 2.5% Triisopropylsilane in TFA at room temperature for 1 h. After filtration, the crude peptide products were obtained by precipitation with cooled diethyl ether and the subsequent evaporation in vacuum overnight, and were then purified by high-performance liquid chromatography (ELITE LaChrom, Hitachi Hitechnologies) with a Cosmosil 5C18-AR-300 column (Nacalai Tesque) at a flow rate of 6 ml/min using a linear gradient from 1 to 80% acetonitrile in water containing 0.1% TFA. After freeze-drying, the peptides were characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (Bruker AutoFLEX mass spectrometer).

QCM Analysis

The peptides were biotinylated with Fmoc-Lys(biotin)-OH at the C-termini and used for QCM measurements. The peptides (1 μM in PBS, pH 7.4) were first incubated with the polymer films on the QCM sensor chips for 1 h at room temperature. After gentle rinsing the film surfaces with Milli-Q, the chips were dried with nitrogen gas and were setup into the QCM cell containing 7 ml PBS. The buffer solution was maintained at 25 °C with stirring and the frequency changes were recorded continuously over time by a 27 MHz QCM apparatus (Affinix Q, Initiam). After the baseline became stable, 3.5 μl of streptavidin solution (100 μM in PBS) was added into the cell to give a final concentration of streptavidin of 50 nM. Each experiment was carried out 3–10 times. The frequency decreases ($-\Delta F$, Hz) of the QCM was calculated as follows: $-\Delta F = F_{0\,s} - F_{60\,s}$, where $F_{0\,s}$ and $F_{60\,s}$ is the resonant frequency before and after the binding of streptavidin for 60 s, respectively.

SPR Analysis

SPR measurements were performed with a dual channel BIAcore X (BIAcore AB, Uppsala, Sweden) and the data were collected using Biacore X Control Software ver. 2.3. The gold-coated glass slides, which had been coated with the polymer films, were setup according to the Biaevaluation handbook. A buffer of 10 mM HEPES containing 150 mM NaCl (HBS-N, pH 7.4, Biacore) was flowed at a rate of 20 μl/min at 25 °C. After the baseline became stable, the peptides dissolved in HBS-N at appropriate concentrations were applied to the polymer film for 3 min (association), and then dissociated into HBS-N buffer under the same conditions for 15 min (dissociation). The resulting sensorgrams at four different concentrations were fitted by local fitting analysis for RU_{max} and global fitting analysis for k_1 (1/Ms) and k_{-1} (1/s) using Biaevaluation ver. 4.1 (assuming a 1 : 1 Langmuir binding model). Finally, K_a was estimated by the following equation: $K_a = k_1/k_{-1}$ (1/M). The baseline drift was maintained at less than 0.5 (RU/min) during the SPR measurements.

Results and Discussion

Binding Analysis of Identified Peptides

In our previous study [18], 12 phage clones that displayed the 7-mer peptides possibly specific for *cis*-azobenzene groups were isolated by the PD method. Among the peptides, in the first instance the c16 peptide with the sequence H-Trp-His-Thr-Leu-Pro-Asn-Ala-NH_2 was analyzed as it was consistent with the sequence motif, Trp-His-Thr-(hydrophobic)-Pro-Asn-Ala, proposed for the *cis*-azobenzene specificity. The binding affinity of the biotinylated c16 peptide was analyzed by QCM measurements monitoring the subsequent binding of streptavidin. In this work, the c05 (H-*Trp*-Pro-*Thr*-Pro-*Pro*-Tyr-*Ala*-NH_2, four amino acid residues are identical to the c16 peptide), c04 (H-Met-*His*-Gln-Gly-Ser-*Asn*-Thr-NH_2 with two amino acid residues identical to the c16 peptide), and c11 (H-His-Leu-His-Tyr-Ala-Leu-Pro-NH_2 with no sequence similarity to the c16 peptide; negative control) peptides were analyzed for the binding affinities of their biotinylated derivatives to the polymer films by QCM measurements.

Figure 2 shows the frequency changes of the QCM chips after the binding of streptavidin to the polymer films precoated with each biotinylated peptide under visible and UV light to prepare *trans*- and *cis*-azobenzene rich films, respectively. The ratios of $-\Delta F$ values under UV and visible light ($-\Delta F_{UV}/-\Delta F_{Vis}$) were estimated as index of the *cis*-form specificity, as shown in the inset of Figure 2. As expected, the binding affinity and specificity of the c16 peptide for the *cis*-azobenzene rich film were the highest among the four peptides analyzed. Although ELISA experiments of all phage clones suggested a specificity for the *cis*-form [18], the c05 peptide considerably lost specificity. Although the c04 peptide almost maintained its specificity for the *cis*-form, the binding affinity was

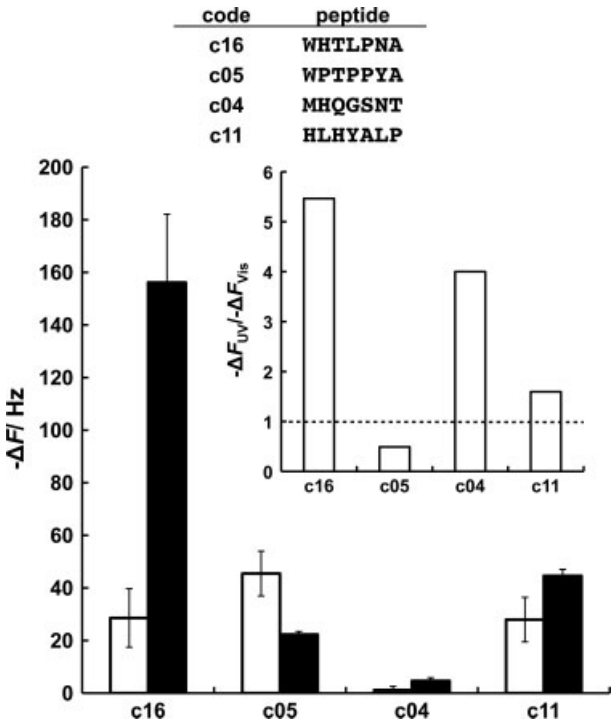

Figure 2. Frequency changes of streptavidin at 50 nM for polymer films precoated with the C-terminally amidated c16, c05, c04, and c11 peptides at 1 μM for 1 h under visible (white) and UV light (black). The inset shows the ratios of the frequency changes under UV light to that under visible light.

drastically decreased. This observation suggests that the K_a of the c04 peptide for the *cis*-form rich films was relatively small compared to that of the c16 peptide. The low specificity of the c11 peptide was consistent with the previous ELISA.

The different results between the ELISA experiments and the present QCM analysis are possibly derived from the different molecular environment of the peptides. In the case of phage clones, 3–5 copies of the peptides are fused onto the N-terminus of the pIII coat proteins via a Gly-Gly-Gly-Ser linker. Through this, the peptides on the phage surface might form an adequate conformation for recognition of the *cis*-form rich films. In other words, the c05 and c04 peptides freed from the phage surfaces seem to lose their binding affinity and/or specificity. As a consequence, it was found from the QCM measurements that the c16 peptide exhibits the greatest affinity and specificity for the *cis*-azobenzene rich films among the four peptides previously identified by the PD method [18].

K_a Analysis of the c16 Peptide

SPR measurements were performed to estimate kinetic parameters of the c16 peptide. Figure 3 shows typical SPR sensorgrams for the *trans*-azobenzene-rich films composed of both the association and dissociation processes. The sensorgrams were suitably fitted to determine the kinetic parameters. The Chi-squared values (an index of fitting reliability) were 7.06 and 1.64 for the *trans*- and *cis*-azobenzene rich films, respectively. The values of less than ten are considered to be acceptable according to the Biaevaluation handbook.

The derived K_a value of the c16 peptide for the *trans*-azobenzene was 1.3×10^5/M and for the *cis*-azobenzene 1.4×10^6/M, which is tenfold greater than the former value (Table 1). The significant increase in the K_a value was mainly due to 20-fold greater k_1 (37/Ms and 760/Ms, respectively), since the former k_{-1} was half of the latter (2.8×10^{-4}/s and 5.5×10^{-4}/s, respectively). In our previous studies [19,29], the K_a values of 7-mer peptides for each polymer target have been estimated similarly by SPR measurements, and were in the range between 10^4 and 10^5/M. Therefore, K_a value of the c16 peptide for the *cis*-azobenzene-rich films is relatively large among polymer-binding peptides.

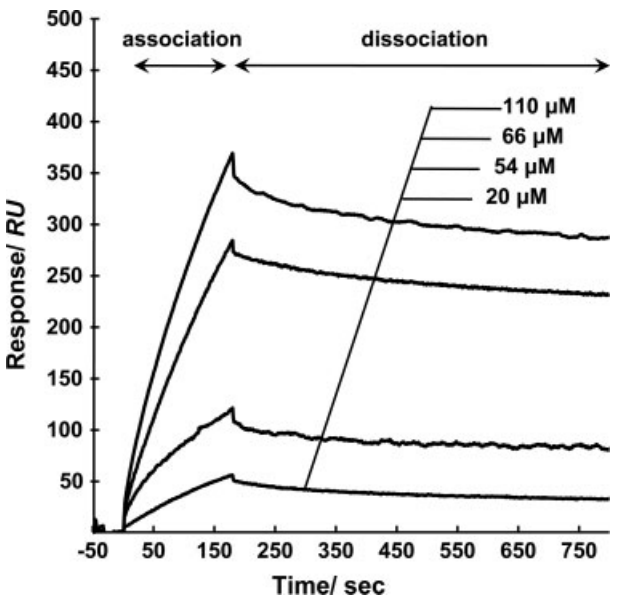

Figure 3. SPR sensorgrams of the c16 peptide with *trans*-azobenzene rich polymer-film surfaces.

Table 1. SPR kinetic parameters for the c16 peptide

Polymer film	k_1 (Ms)	k_{-1} (10^{-4}/s)	K_a (10^5/M)
Under visible light	37	2.8	1.3
Under UV light	760	5.5	14

Detailed Binding Analysis of the c16 Peptide

For a better insight into the importance of the primary amino acid sequence of the c16 peptide, each amino acid was stepwise replaced with alanine (Ala-scan). The affinities of the mutant peptides were analyzed by the QCM measurements under the same conditions. Figure 4 shows the frequency changes of the QCM chips after the binding of streptavidin to the polymer films precoated with each biotinylated mutant peptide under visible and UV light. The inset shows the ratios of $-\Delta F$ values ($-\Delta F_{UV}/-\Delta F_{Vis}$) as the specificity index. Any replacement resulted in the extreme decrease in the amounts of streptavidin bound to the film, particularly after the irradiation with UV light. Therefore, the specificity was obviously decreased, except for W1A. These observations strongly suggest that all amino acids of the c16 peptide are essential for the binding to *cis*-azobenzene. In the case of W1A, the specificity seems to be retained as the amounts

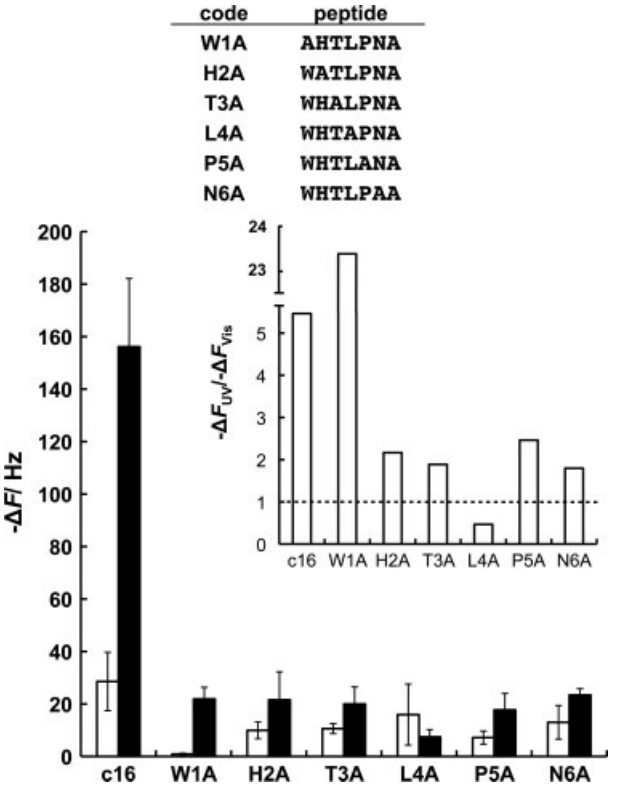

Figure 4. Frequency changes of streptavidin at 50 nM for polymer films precoated with the mutant peptides of W1A, H2A, T3A, L4A, P5A, and N6A at 1 μM for 1 h under visible (white) and UV light (black). The inset shows the ratios of the frequency changes under UV light to that under visible light. The peptides are C-terminally amidated.

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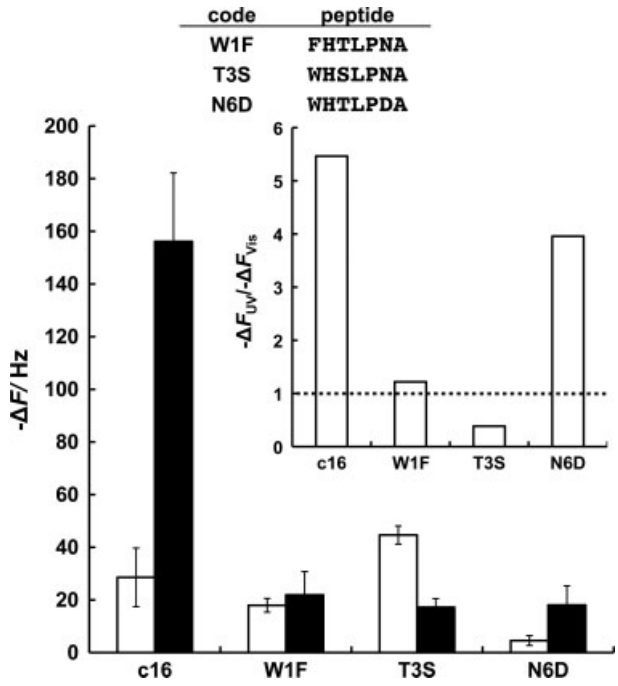

Figure 5. Effects of changes of W1, T3, and N6 of the c16 peptide to F, S, and D on the binding to the polymer films. The binding activity was assayed as described in Figure 4. The inset shows the ratios of the frequency changes under UV light to that under visible light. The peptides are C-terminally amidated.

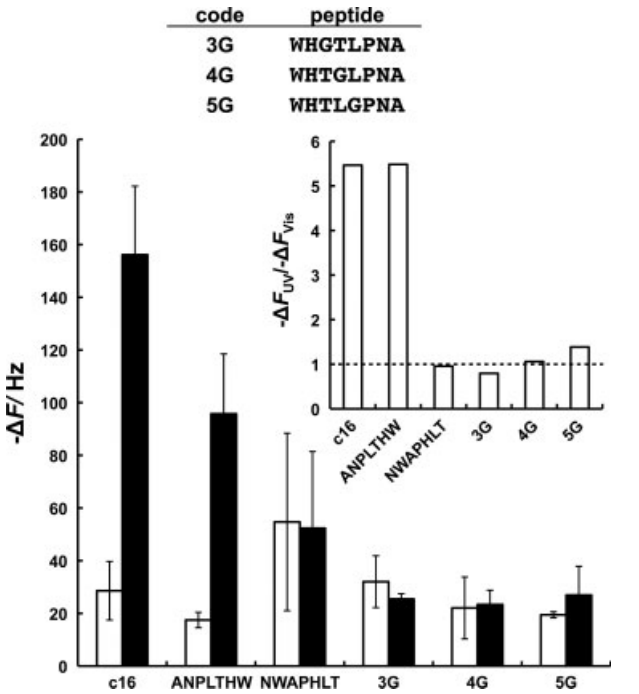

Figure 6. Effects of insertion of a G after H2-T3, T3-L4, and L4-P5 of the c16 peptide on the binding to the polymer films. The binding activity was assayed as described in Figure 4. The inset shows the ratios of the frequency changes under UV light to that under visible light. The peptides are C-terminally amidated.

of streptavidin bound to the *trans*-azobenzene rich films is also drastically decreased.

To further investigate the importance of amino acid residues involved in interactions with the azobenzene groups, the C-terminal Trp was replaced with Phe (W1F). If the aromatic group of Trp is essential for the binding to azobenzene groups based on $\pi - \pi$ interactions, W1F is supposed to behave in a manner similar to the c16 peptide. However, W1F completely lost its specificity for the *cis*-azobenzene (Figure 5). This observation clearly suggests that the indole ring of Trp rather than the benzene ring is essential for binding. Next, we replaced His with Phe (H2F). Unfortunately, H2F could not be dissolved in any buffer employed. We then replaced Thr in position 3, which could interact with azobenzene groups via hydrogen bonding interactions, with Ser (T3S) to investigate the importance of its hydroxyl groups (Figure 5). In contrast to our expectation, T3S showed binding to the *trans*-form rather than to the *cis*-form. It is difficult to rationalize this result; however, the lack of a methyl group in the middle position of the c16 peptide obviously changes the binding and specificity of the c16 peptide. Furthermore, Asn as additional candidate for hydrogen bonding interactions with azobenzene groups, was replaced with Asp (N6D) and the specificity was similarly lost (Figure 5). All these observations strongly indicate the essential requirement of each amino acid residue of the c16 peptide not only for efficient interactions with *cis*-azobenzene groups but also for adopting the suitable peptide conformation required for the interactions.

As all the amino acid residues of the c16 peptide were found to be important, we focused on the order of the residues. Interestingly, the inverted sequence of the c16 peptide (ANPLTHW) also showed binding affinity and specificity for the *cis*-azobenzene almost identical to those of the parent c16 peptide. However, the shuffled c16 peptide (NWAPHLT) does not show any specificity confirming the importance of the intact sequence. To investigate whether the essential motif of the c16 peptide consists of smaller domains in spatial neighborhood, Gly was used as a linker and inserted into the middle three positions of the c16 peptide. All the peptides with these insertions (3G, 4G, and 5G) lost the *cis*-form specificity (Figure 6). Therefore, the specificity has to derive from the adequate conformation induced by the primary sequence of the c16 peptide. It is worthy to note that the c16 peptide did not form any secondary structure in aqueous solution (data not shown).

Conclusion

The peptides identified by the PD method against the film surface of azobenzene-containing copolymers were characterized by QCM and SPR measurements. Among the four peptides identified, the c16 peptide, which was fully consistent with our previous hypothesis of the essential motif specific for *cis*-azobenzene groups [18], showed the highest binding affinity and specificity for the *cis*-azobenzene-rich films. The association/dissociation processes of the c16 peptide were quantified by SPR experiments to estimate the kinetic parameters. The c16 peptide showed a tenfold greater K_a for the *cis*-azobenzene than for the *trans*-form, due to greater k_1. Various mutants of the c16 peptide were characterized to gain structural information for the *cis*-form specificity. Each of the amino acid residues in the c16 peptide was found to be essential, and could not be replaced even by amino acids with a similar structure. Moreover, insertion of Gly into the c16 peptide revealed the importance of the continuous sequence of the c16 peptide for the specificity. These promising results on the c16 peptide are likely to give important information

about the recognition of photoresponsive azobenzene polymers for appropriate biological applications in the near future.

References

1 Sarikaya M, Tamerler C, Schwartz DT, Baneyx FO. Materials assembly and formation using engineered polypeptides. *Annu. Rev. Mater. Res.* 2004; **34**: 373–408.
2 Brown S. Metal-recognition by repeating polypeptides. *Nat. Biotechnol.* 1997; **15**: 269–272.
3 Naik RR, Stringer SJ, Agarwal G, Jones SE, Stone MO. Biomimetic synthesis and patterning of silver nanoparticles. *Nat. Mater.* 2002; **1**: 169–172.
4 Whaley SR, English DS, Hu EL, Barbara PF, Belcher AM. Selection of peptides with semiconductor binding specificity for directed nanocrystal assembly. *Nature* 2000; **405**: 665–668.
5 Lee SW, Mao CB, Flynn CE, Belcher AM. Ordering of quantum dots using genetically engineered viruses. *Science* 2002; **296**: 892–895.
6 Sano KI, Shiba K. A hexapeptide motif that electrostatically binds to the surface of titanium. *J. Am. Chem. Soc.* 2003; **125**: 14234–14235.
7 Hayashi T, Sano K, Shiba K, Kumashiro Y, Iwahori K, Yamashita I, Hara M. Mechanism underlying specificity of proteins targeting inorganic materials. *Nano Lett.* 2006; **6**: 515–519.
8 Wang SQ, Humphreys ES, Chung SY, Delduco DF, Lustig SR, Wang H, Parker KN, Rizzo NW, Subramoney S, Chiang YM, Jagota A. Peptides with selective affinity for carbon nanotubes. *Nat. Mater.* 2003; **2**: 196–200.
9 Li XJ, Chen W, Zhan QW, Dai LM, Sowards L, Pender M, Naik RR. Direct measurements of interactions between polypeptides and carbon nanotubes. *J. Phys. Chem. B* 2006; **110**: 12621–12625.
10 Kase D, Kulp JL, Yudasaka M, Evans JS, Iijima S, Shiba K. Affinity selection of peptide phage libraries against single-wall carbon nanohorns identifies a peptide aptamer with conformational variability. *Langmuir* 2004; **20**: 8939–8941.
11 Morita Y, Ohsugi T, Iwasa Y, Tamiya E. A screening of phage displayed peptides for the recognition of fullerene (C60). *J. Mol. Catal. B: Enzym.* 2004; **28**: 185–190.
12 Adey NB, Mataragnon AH, Rider JE, Carter JM, Kay BK. Characterization of phage that bind plastic from phage-displayed random peptide libraries. *Gene* 1995; **156**: 27–31.
13 Sanghvi AB, Miller KPH, Belcher AM, Schmidt CE. Biomaterials functionalization using a novel peptide that selectively binds to a conducting polymer. *Nat. Mater.* 2005; **4**: 496–502.
14 Serizawa T, Sawada T, Matsuno H, Matsubara T, Sato T. A peptide motif recognizing a polymer stereoregularity. *J. Am. Chem. Soc.* 2005; **127**: 13780–13781.
15 Serizawa T, Sawada T, Kitayama T. Peptide motifs that recognize differences in polymer-film surfaces. *Angew. Chem. Int. Ed.* 2007; **46**: 723–726.
16 Serizawa T, Techawanitchai P, Matsuno H. Isolation of peptides that can recognize syndiotactic polystyrene. *Chembiochem* 2007; **8**: 989–993.
17 Serizawa T, Iida K, Matsuno H, Kurita K. Cellulose-binding heptapeptides identified by phage display methods. *Chem. Lett.* 2007; **36**: 988–989.
18 Chen J, Serizawa T, Komiyama M. Peptides recognize photoresponsive targets. *Angew. Chem. Int. Ed.* 2009; **48**: 2917–2920.
19 Matsuno H, Sekine J, Yajima H, Serizawa T. Biological selection of peptides for poly(L-lactide) substrates. *Langmuir* 2008; **24**: 6399–6403.
20 Sano KI, Sasaki H, Shiba K. Specificity and biomineralization activities of Ti-binding peptide-1 (TBP-1). *Langmuir* 2005; **21**: 3090–3095.
21 Slocik JM, Stone MO, Naik RR. Synthesis of gold nanoparticles using multifunctional peptides. *Small* 2005; **1**: 1048–1052.
22 Ahmad G, Dickerson MB, Church BC, Cai Y, Jones SE, Naik RR, King JS, Summers CJ, Kroger N, Sandhage KH. Rapid, room-temperature formation of crystalline calcium molybdate phosphor microparticles via peptide-induced precipitation. *Adv. Mater.* 2006; **18**: 1759–1763.
23 Slocik JM, Naik RR. Biologically programmed synthesis of bimetallic nanostructures. *Adv. Mater.* 2006; **18**: 1988–1992.
24 Pender MJ, Sowards LA, Hartgerink JD, Stone MO, Naik RR. Peptide-mediated formation of single-wall carbon nanotube composites. *Nano Lett.* 2006; **6**: 40–44.
25 Sano K, Sasaki H, Shiba K. Utilization of the pleiotropy of a peptidic aptamer to fabricate heterogeneous nanodot-containing multilayer nanostructures. *J. Am. Chem. Soc.* 2006; **128**: 1717–1722.
26 Matsui T, Matsukawa N, Iwahori K, Sano KI, Shiba K, Yamashita I. Realizing a two-dimensional ordered array of ferritin molecules directly on a solid surface utilizing carbonaceous material affinity peptides. *Langmuir* 2007; **23**: 1615–1618.
27 Sano K, Ajima K, Iwahori K, Yudasaka M, Iijima S, Yamashita I, Shiba K. Endowing a ferritin-like cage protein with high affinity and selectivity for certain inorganic materials. *Small* 2005; **1**: 826–832.
28 Roberts BL, Markland W, Siranosian K, Saxena MJ, Guterman SK, Ladner RC. Protease inhibitor display M13-phage-selection of high-affinity neutrophil elastase inhibitors. *Gene* 1992; **121**: 9–15.
29 Serizawa T, Sawada T, Matsuno H. Highly specific affinities of short peptides against synthetic polymers. *Langmuir* 2007; **23**: 11127–11133.

