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Peptide Screening from a Phage Display Library for Benzaldehyde Recognition

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Peptides are promising probes for sensor development, while peptide screening for volatile organic compound (VOC) binding remains a challenge. In this study, the peptides binding to benzaldehyde were screened from a phage display library. The peptide sequences showing the highest binding activity were NPAATMA, SIFPVSR, and MPRLPPA. The binding in the gas phase was also confirmed using a candidate peptide-immobilized ZnO nanowire structure. The screened peptides could prove to be useful for benzaldehyde sensor development.

Keywords: Peptide probe | VOC | Benzaldehyde

Over the past few years, there has been a rapid increase in interest toward volatile organic compounds (VOCs) in various fields, such as environmental monitoring, food quality control, chemical warfare, explosive detection, and health assessment.[1–4] For instance, the correlation between benzaldehyde (C_6H_5CHO), one of the VOCs, and lung cancer,[5] food taste and flavor,[6] and cell culture conditions[7] has recently been revealed. These VOCs, including benzaldehyde, were conventionally measured by gas chromatography mass spectrometry (GC-MS)-based techniques. However, in order to meet the expansion of VOC measurement demands in various situations, alternative and simpler sensors to replace or complement GC-MS are being investigated.[8–10]

It is generally known that the design of a VOC binding probe is key for new VOC sensor development.[11–13] Although pattern recognition based-VOC sensors using multiple probes have been reported,[14–16] VOC recognition probe development for target molecule recognition is challenging and important for the rationalization of selective VOC detection. Several VOC sensors that use affinity biomolecular probes such as antibodies, olfactory receptors, and peptides have also been reported.[17–19] Among these target-recognition biological molecular probes, short peptides have several advantages, which include their high stability, simplicity of library development from a combination series of 20 natural amino acids, process of chemical synthesis, and ease of quality control.[20,21] Furthermore, as peptide molecules are smaller than other target-recognition proteins such as antibodies and receptors, the problem of Debye length can be overcome with field-effect transistors (FET) that enable rapid, sensitive and label-free detection.[16,22] Using small recognition molecules, it enables a binding reaction to occur within the Debye length and thus sensitive measurement is possible.[19,23] However, the reports on peptide probes for VOC binding have been very limited so far due to the difficulty of probe peptide design.[11,19,24–31]

In addition to the screening of VOC binding probes, solid material functionalization by the screened molecule is also an important task for rationalization of VOC sensor and capture device fabrication. Herein, we investigate the peptide functionalization of glass and ZnO nanowire surfaces for benzaldehyde binding in aqueous and gas phase, respectively. ZnO is currently receiving increasing attention due to the diverse applicability in the synthesis of various nanostructures (e.g., particulates and wires).[32–35] In particular, nanowire structures with a high surface-to-volume ratio have made it a contender for chemical and biological sensors.

In this study, screening of a benzaldehyde recognition peptide probe was demonstrated using a phage display library. Benzaldehyde is an organic compound consisting of a benzene ring with a formyl substituent. As the formyl group is relatively unstable, in the first screening step, the carboxylic group is displayed on a glass slide through cross-linking between a side carboxylic group in terephthalic acid and an amine group precoated on the surface of the glass slide (Supplementary information). In order to identify benzaldehyde-binding peptides, the Ph.D.-7 Phage Display Peptide Library with a complexity of 2.8×10^9 (New England Biolabs, Beverly, MA) was used for biopanning. The library solution was dropped on the surface-modified cover glass for 1 h. After washing with 50 mM Tris-buffered saline, pH 7.5 (TBS) 5 times, the bound phages were eluted using a 0.3% (v/v) TBS solution containing benzaldehyde. After each round of panning, the numbers of eluted and amplified phages, counted as PFUs, were measured using agar plates containing X-gal/IPTG/tetracycline to set the same number of input phages in each round. A freshly prepared surface-modified slide glass was used for each round. Six rounds of biopanning were repeated, followed by the cloning and DNA sequencing of the phages.

As a result, 23 peptide sequences were identified from 27 sequenced plaques as benzaldehyde-binding candidates (Supplementary Table 1). Among 27 plaques, four sequences (MPRLPPA, ADARYKS, HWNTVVS, and NPAATMA) were identified from two plaques. As NPAAPMA was similar to NPAATMA, it is also listed in Table 1. In addition, because both SIFPVSR and SILPVTR were identified from one plaque and showed a similar amino acid sequence, they are also listed. As a total, 7 peptide sequences were identified as the candidate peptides for benzaldehyde binding (Table 1). These peptide sequences displayed hydrophobicity ranging from −1.486 to 0.7 and isoelectric points from 5.52 to 9.50. The values were diverse. However, although all sequences have some hydrophobic amino acids such as alanine (A), leucine (L), valine (V),

Table 1. List of the 7 phage clones with the peptide sequences displayed on their surface

Peptide[1]	Binding frequency	Isoelectric point[3]	GRAVY[4]
MPRLPPA	2/27	9.50	−0.257
ADARYKS	2/27	8.63	−1.486
HWNTVVS	2/27	6.74	−0.100
NPAATMA	2/27	5.52	0.214
NPAAPMA	1/27	5.52	0.086
SIFPVSR[2]	1/27	9.47	0.543
SILPVTR[2]	1/27	9.47	0.700

[1]Multiple isolated peptides from 27 sequenced phage clones are listed. [2]As SIFPVSR and SILPVTR revealed some consensus amino acids within their sequences, these two peptides are also listed. [3 and 4]The isoelectric point and the grand average of the hydropathy value (GRAVY) analyzed using the ProtParam tool of ExPASy (http://web.expasy.org/protparam/).

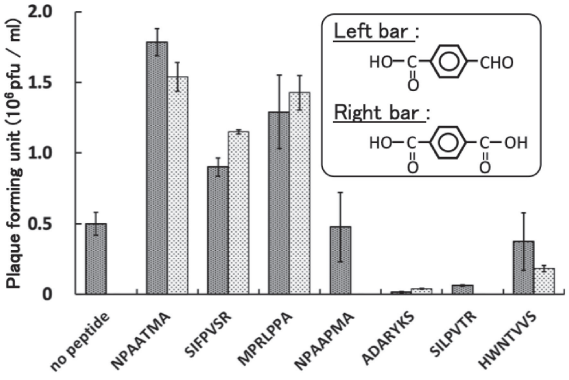

Figure 1. Evaluation of benzaldehyde-binding peptide expressed on screened phages on solid surface modified with terephthalaldehyde acid (left bar) and terephthalic acid (right bar). The higher plaque forming unit (pfu) indicates a stronger binding affinity.

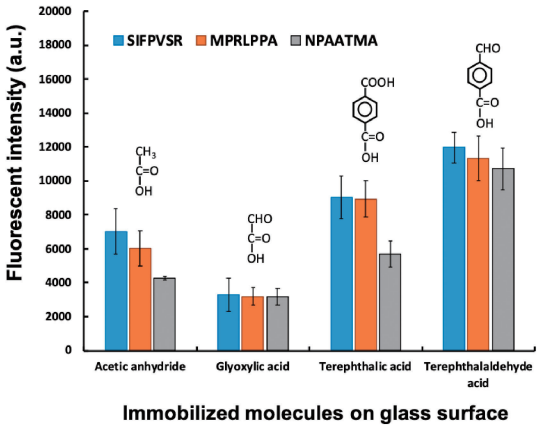

Figure 2. Evaluation of selectivity of benzaldehyde-binding peptides using FITC-tagged peptide candidates. Binding assay was conducted between each FITC-tagged peptide (SIFPVSR, MPRLPPA, and NPAATMA) and different molecules (acetic anhydride, glyoxylic acid, terephthalic acid, and terephthalaldehyde acid) immobilized on a glass surface. Utilized immobilized molecular structures are inserted in this figure.

phenylalanine (F), and proline (P), the total hydrophobicity of peptides was not so high. This seems to be sensible because the benzaldehyde molecule has a hydrophobic benzene ring and a slightly hydrophilic aldehyde group. These observations indicate that the optimization of reaction solution including salt concentration and pH may be important for sensor development in order to properly express the peptide-benzaldehyde interaction. In addition, interestingly, many candidate peptides included an amino acid, proline, which can cause the twist conformation or have an angled shape. As the molecular size of benzaldehyde (0.53 nm and 0.66 nm)[36] is relatively smaller than the peptide sequence (approximately 2.5 nm by 7 amino acids), the curved structure may be responsible for the molecular recognition and for the binding at multiple sites in the peptide molecule.[37–39] In fact, the importance of the amino acid proline in one peptide was previously reported for another small molecule (naphthalene) recognition,[39] but further structural and kinetic analyses should be attained towards understanding of the binding mechanisms.

Using candidate peptide displaying phages, the binding to glass surface modified with the benzaldehyde analogs, terephthalic acid and terephthalaldehyde acid, was evaluated. Each peptide expressing phage (1.0×10^9) in TBS was dropped on the surface-modified glass slide for 1 h. The surface was washed with TBS, eluted with 0.2 M glycine-HCl (pH 2.2), and immediately neutralized with 1 M Tris-buffer at pH 9.3. Using the titer count of the obtained elute, the binding affinity was evaluated. The results indicated that NPAATMA, MPRLPPA, and SIFPVSR showed stronger binding affinity for both molecules than the control phage (no peptide expression) (Figure 1). From this result, these three peptides (NPAATMA, MPRLPPA, and SIFPVSR) were examined for the following experiments.

For selectivity evaluation of candidate peptides, the binding between the glass surface modified with benzaldehyde analogs and each fluorescent tagged peptide was measured. The glass surface modification procedure based on the cross-linking between carboxylic and amine groups was nearly similar as shown above except for the surface molecular species including glyoxylic acid (CHOCOOH), acetic anhydride ($(CH_3CO)_2O$), terephthalic acid ($C_6H_4(CO_2H)_2$), and terephthalaldehyde acid ($CHOC_6H_4(CO_2H)$). All FITC-tagged peptides (purity >80%) were purchased from Sigma-Aldrich, Tokyo, Japan. The peptide was dissolved in TBS (10 μM peptide solution) and was immediately dropped onto glass surfaces modified with different molecules. After incubation for 1 h in 25 °C, the glass was washed by dipping in TBS followed by ultrapure water. The fluorescent intensities of peptide dropped circular area were evaluated using a fluorescent image scanner (Typhoon FLA9500). All three peptides showed high-binding activity to benzene-containing molecules and especially to terephthalaldehyde acid (Figure 2). As all peptides showed higher affinity to terephthalaldehyde acid than glyoxylic acid, all peptides seem to have an affinity toward the benzene structure. This is interesting because benzene contamination in the environment is a serious issue due to its toxicity, volatility and solubility in water, and sensor development for benzene detection is currently needed.[40] If the peptide probe can recognize the difference between

molecular benzene and its analogues, these peptide probes might be useful for benzene detection sensor development.

In addition, from the observation of higher affinity to terephthalaldehyde acid than terephthalic acid, all peptides showed higher affinity to the aldehyde group than to the carboxylic group. From these results, both benzene and aldehyde molecular structures seem to attribute to the binding with all three peptides. Interestingly, although NPAATMA showed weaker affinity to terephthalaldehyde acid and terephthalic acid than that of SIFPVSR and MPRLPPA, the selectivity was remarkable. The fluorescent intensity of NPAATMA from terephthalaldehyde acid was 1.88 times that from terephthalic acid, while those of SIFPVSR and MPRLPPA were 1.32 and 1.27 times, respectively. Therefore, while SIFPVSR and MPRLPPA seem to be useful for benzaldehyde capture, NPAATMA may be applicable for benzaldehyde sensing due to its superior selectivity. It should be noted that the results using phage-displayed peptides and chemically synthesized peptides were slightly different. For example, in the experiment using phages, the binding activities of NPAATMA to terephthalaldehyde and terephthalic acid were similar level (Figure 1), while the chemically synthesized peptide showed weaker binding affinity to only terephthalic acid (Figure 2). The result suggests the binding of NPAATMA to terephthalic acid is supplemented by the phage-derived side effects such as the presence of linker amino acid on phage and the increase of structural rigidity by displaying on phage rather than free peptide.

After confirmation of peptide binding to benzaldehyde under aqueous conditions, the binding was also performed in the gas phase using the ZnO nanowire structure (Figure S1). The peptide function expression including target molecular binding in the gas phase remains challenging, although several studies have been reported.[11,27] Using GS-MS analysis, we found that SIFPVSR and MPRLPPA peptides showed enhanced benzaldehyde-binding efficiency on the ZnO nanowire structure compared to only ZnO or peptide-modified ZnO with AAAA and NPAATMA (Figure 3). The benzaldehyde capture efficiencies of SIFPVSR and MPRLPPA were 6.9 and 3.5 times higher than that of bare ZnO nanowire, respectively. As the isoelectric points of SIFPVSR and MPRLPPA are 9.47 and 9.50, these peptides are relatively anionic peptides, whereas those of NPAATMA and AAAA are 5.52 and 5.61. In the gas phase for benzaldehyde capture on a ZnO nanowire structure, these charge-based interactions may play an important role in binding.

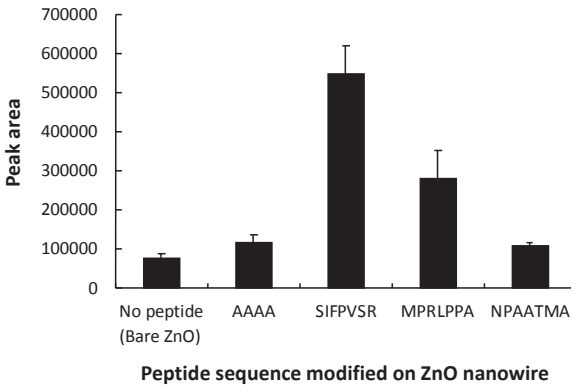

Figure 3. Evaluation of the benzaldehyde binding property onto a ZnO nanowire structure in the gas phase. The benzaldehyde capture efficiency was evaluated from the area of GC-MS chromatogram with selected-ion monitoring mode.

In this study, some benzaldehyde-binding peptides were screened from a phage display library using a biopanning process onto a benzaldehyde analog-modified glass surface. The binding mechanisms seem to be related to hydrophobic and electrostatic interactions and to the presence of proline amino acid. As the binding property was confirmed onto a solid surface, the molecular probes would be applicable for various types sensors such as SPR, FET, and QCM.[41,42] Some of the screened peptides, including SIFPVSR and MPRLPPA, revealed benzaldehyde capture enhancement on the ZnO nanowire structure in the gas phase. VOCs can be used as biomarkers contained in the exhaled breath from cancer patients for diagnoses purposes and for monitoring the treatment of the disease.[43,44] As the screened peptide also demonstrated a benzaldehyde-binding property in gas phases, these peptide probes might be utilized for benzaldehyde sensor development in gas phase in the future.

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