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A Novel Peptide Array Using a Phage Display System for Protein Detection

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A novel peptide array for the protein detection system is described, based on a phage display system. The binding strength and specificity of the antibodies against immobilized phage-displayed peptides were the same as against freed peptides. Therefore, phage-displayed peptides can be used for ligands of a protein-detecting peptide array.

Protein-detecting microarrays are becoming increasingly popular detection and analysis tools for investigating protein interactions and protein profiling. In recent post-genome research, information based on genomics will be applied to numerous fields including proteomics, which will be one of the most important fields in bioscience and biotechnology. One of the most effective tools in this area is DNA microarray technology that can analyze mRNA transcript levels expressed under various conditions.[1] However, because proteins have specific 3D structures, functions, interactions, and post translational modifications, it is needed to be analyzed directly in order to obtain such information.

Protein microarray technology is expected to be a powerful high throughput tool for direct analyses of protein structures, functions, and interactions. Since the pioneering demonstration of the feasibility of immobilizing proteins on glass slides, protein microarrays have become a significant research area in biotechnology.[2] However, due to the complicated structures and interactions of proteins compared to DNAs or RNAs, various difficulties still remain as barriers for construction of useful protein microarrays. We have constructed designed peptide libraries with suitable secondary structures such as β-loop[3] and α-helix[4] and applied them as capture agents for protein-detecting microarrays, because peptides have the following advantages: (i) peptides are more easily designed, synthesized to form suitable conformations, and introduce functions than proteins, especially antibodies; (ii) peptides with secondary structures can mimic proteins; (iii) peptides can be immobilized at defined sites through their functional groups. Microarrays using hundreds of peptides can be used to detect and identify the proteins. In contrast, recent developments in biotechnology have lead to easy construction of peptide libraries displayed on phage surfaces with much higher diversity (ca. 10^9) than synthetic methods.[5] Because plural peptides can be displayed on different coat proteins, specific functionalization can be achieved. Furthermore, phages can be amplified easily by infection of host cells. Therefore, phage-displayed peptides thought to be good candidates for the construction of novel peptide microarrays with higher diversities.[6] However, quantitative interaction analyses between immobilized phage-displayed peptides and analytes have not been performed.

In this paper, we constructed a phage-based peptide microarray, and the interactions between the peptides and analytes (proteins) were quantitatively investigated. Three kinds of phages displaying different tag-peptides were genetically constructed as models. Tag-peptides are normally used to detect and quantify the tag-modified protein by using antibodies. The peptides displayed on phages that immobilize onto substrates can be appropriately characterized (Figure 1a).

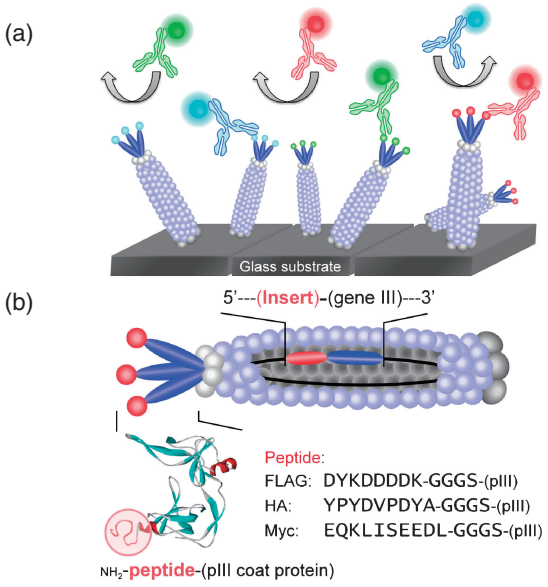

Figure 1. Schematic illustrations of (a) detection of tag-peptides using antibodies and (b) construction of tag-peptide displaying phages. (a) Anti-tag-peptide antibodies were modified with fluorescence molecules for detection. Antibodies specifically bind to tag-peptides of the same color. (b) Tag-peptides were fused to the pIII coat protein.

FLAG, HA, and Myc peptides (sequences are shown in Table 1 and Figure 1b) were genetically fused to minor coat proteins of M13 bacteriophage via Gly–Gly–Gly–Ser as a spacer using a Ph.D. Peptide Display Cloning System (New England Biolabs, Inc., Figure 1b), and the fusion was checked by conventional DNA sequencing. To determine suitable substrates for the construction of phage-based peptide microarrays, two different glass slides modified with positively charged amino group in different density (high and low amino groups are 2.0×10^{-9} and 0.4×10^{-9} mol cm^{-2}, respectively) (Matsunami Glass) were used, because the phage surface (especially major coat proteins) is negatively charged.[8] FLAG, HA, Myc, and nondisplayed wild type (WT) phages were immobilized with several concentrations, and quantified by Alexa Fluor®680 conjugated anti-M13 phage antibodies to estimate apparent affinity constants (K_{app}) and maximum binding amounts (A_{max}) against glass slide surfaces (Figure S1 and Table S1[9]). K_{app}

Table 1. Affinity constants of antibodies against tag-peptides

Antibody	Peptide sequence	$K_a/10^6\,M^{-1}$	
		Phage-displayed[a]	Peptide[b]
FLAG	DYKDDDDK	190	150
HA	YPYDVPDYA	1100	340
Myc	EQKLISEEDL	6.9	17

[a]K_a against tag-peptides displayed on the phages, which were immobilized onto a glass substrate. [b]K_a against free peptides in solution.[7]

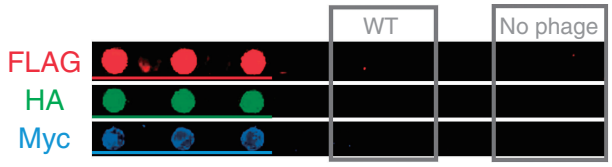

Figure 2. A fluorescence image of various phage-immobilized glass slides detected by anti-tag-peptide antibodies. anti- (top) FLAG, (middle) HA, and (bottom) Myc antibodies were labeled with fluorescence molecules and used to detect the each tag-peptide displayed on the phages that immobilized on the glass slide.

values of various phages against both high and low density surfaces ranged from 10^8–$10^9\,M^{-1}$, demonstrating that the displayed peptide did not affect the binding strength. In contrast, though A_{max} values against high density surfaces were the same within the experimental errors, whereas those values against low surfaces were much different. The A_{max} values against low surfaces were dependent on the hydrophobicity index values of displaying tag-peptides (Table S1[9]), suggesting that the displayed peptides interact with the hydrophobic area between amino groups of the substrate via hydrophobic interactions. This indicates that amino-modified surfaces with high densities are suitable to immobilize phage-displayed peptides for construction of the microarray.

To detect the phage-displayed peptides, anti-tag-peptide antibodies were modified with fluorescent molecules and applied to the phage-immobilized glass slides with high density amino groups ([phage] = 2 nM, Figure 2). All the antibodies specifically bound to tag-peptide-displaying phages and did not bind to WT phages and amino-modified glass surfaces at all. These results suggested that antibodies can recognize small peptides displayed on phages immobilized on glass substrates. To understand these affinities in a more quantitative manner, the dependences of the relative binding amount of bound antibodies to the antibody concentration was obtained (Figure S2[9]). The relative amounts of bound antibodies saturated at a certain level for all antibodies. Assuming Langmurian binding, the affinity constants (K_a) were obtained and shown in Table 1. The K_a values were comparable to the K_a against free tag-peptides in solution,[7] and the order of the values were the same. Therefore, it was demonstrated that the immobilized phage-displayed peptides adequately interact with applied proteins.

For the construction of protein-detecting microarrays, immobilized ligands (in this case, phage-displayed peptides) must preferentially interact with the appropriate protein even if the protein is mixed with a number of other proteins. To confirm

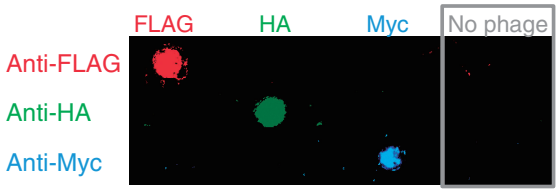

Figure 3. A fluorescence image of various phage-immobilized surfaces detected after mixing with three different anti-tag-peptide antibodies.

selective interaction, three kinds of anti-tag-peptide antibodies were mixed and applied to each tag-peptide displaying phage-immobilized glass surfaces (Figure 3). The peptides were selectively recognized by antibodies in the antibody-mixed solution. Furthermore, the signal/noise ratio of the quantified fluorescent intensities were larger than 5 in the minimum case, demonstrating sufficient selectivity (Figure S3[9]). The phage-displayed peptides clearly recognized the protein, which exists in the mixture of protein solutions.

In conclusion, we demonstrated that phage-displayed peptides immobilized onto glass reasonably well interact with the proper protein, if any, mixtures. The K_a values of antibodies against the phage-displayed and -immobilized peptides were almost the same in those of against free peptides. Therefore, peptide microarrays using the phage display will be useful and effective tools for the construction of protein characterization systems. Detailed detection and analysis of protein interactions using peptides displayed on phages with a variety sequences, secondary structures, and higher diversities will be reported in the near future.

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References and Notes

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9 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

