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A selection of short peptides that interact with a porphyrin as a small target by immobilized phage display

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Porphyrin H_2TMpyP binding peptides were selected from a phage displayed pentapeptide library that was immobilized onto a solid phase and, as a result, a binding motif that binds to the porphyrin in the same way as a motif obtained by our previous combinatorial chemistry was revealed.

Two methods, combinatorial chemistry and phage display, are used to obtain novel interactions between polypeptides or proteins and desired target molecules. Combinatorial chemistry can select functional molecules from complete libraries of molecules of five or fewer amino acids.[1] On the other hand, phage display can deal with huge libraries and use cDNA libraries for a selection, so that this method is suitable for high molecular weight molecules such as proteins, although some deviation of the libraries occurs because of codon usage by the host cells.[2,3] From such properties, combinatorial chemistry has been used to obtain small functional peptides,[4] while phage display has been applied to epitope mapping of proteins or antibody selection *in vitro*.[5,6]

However, binding motifs obtained *via* the two methods do not always correspond with each other.[7] For example, Maruyama *et al.* specified two epitopes of human galectin-3 *via* a λ phage display system, but the epitopes were not similar to the motifs selected by combinatorial chemistry.[8] The discrepancy may be due to the difference of the selection procedures, *i.e.* an immobilized library and a free target are used for combinatorial chemistry, while a free library and an immobilized target have been used for phage display so far.[9] Here we have carried out a peptide selection against a porphyrin [5,10,15,20-tetrakis(*N*-methylpyridinium-4-yl)-21*H*,23*H*-porphine; H_2TMpyP] which was a small target molecule of our combinatorial chemistry study reported previously,[4] using a new phage display selection method with an immobilized library and the free target porphyrin as for the combinatorial chemistry selection.

A random pentapeptide library displayed on a phage was constructed[10] and used for peptide selection with H_2TMpyP. The library can contain up to 20^5 ($= 3.2 \times 10^6$) individual peptides with different sequences. The phage library was immobilized on nitrocellulose filters as follows, instead of immobilization of the target (the commonly used method for a selection using a phage display system).[9] The recombinant phages were incubated with an *Escherichia coli* TG1 (*supE*) fresh culture and spread on 2×YT agar plates.[11] The spread phages on the plates were transferred onto nitrocellulose filters. The filters were blocked with 1% bovine serum albumin in PBT buffer[12] for 2 min and washed with PBT buffer for 2 min. Then the filters were incubated with 10 μM H_2TMpyP in 2×SSC buffer[12] for 12 h and washed with 2×SSC for 4 min. Porphyrin binding phages on the filters were detected *via* emission of the porphyrin at 656 nm. As a result, four plaques with very strong signals, corresponding to the four phages shown in Fig. 1, were specified by the assay with H_2TMpyP from over 1×10^7 plaques. The four plaques were amplified and their phagemids were sequenced by the dideoxy method. The translated amino acid sequences of the introduced random region at the N-terminus of the pIII coat protein of the four phages were His-Ala-Ser-Tyr-Ser (HASYS), Arg-Ala-Ser-Ser-Leu (RASSL), Arg-Leu-Tyr-Val-Arg (RLYVR) and Leu-Pro-Tyr-Ala-Thr (LPYAT). Every phage displayed a peptide with aromatic amino acids or Arg on the pIII protein. Equal amounts of phage particles of the four positive phages were blotted on a nitrocellulose filter and incubated with H_2TMpyP. The HASYS phage that displayed aromatic His and Tyr was the tightest binder of all, as shown in Fig. 1, with a K_a of about 10^5 M^{-1}.

We obtained previously a porphyrin binding peptide Tyr-Ala-Gly-Tyr (YAGY) that interacted with H_2TMpyP by a sandwiching interaction from a combinatorial library.[4] Molecular modeling was carried out to anticipate the interaction between the HASYS phage and H_2TMpyP. A crystal structural study of the N-terminus domain of the pIII protein[13] suggested that the structure of an expressed peptide in a fusion protein was not influenced by the pIII coat protein and was flexible. Thus we carried out the molecular modeling of an N-terminus tridecapeptide of the pIII protein Ala-Ala-Gln-Leu-Ala-His-Ala-Ser-Tyr-Ser-Ala-Ala-Ala containing the HASYS sequence. 120 conformations of the tridecapeptide–H_2TMpyP complex were generated after a simulated annealing from 0 to 1000 K. Although the two molecules were dissociated during the annealing in about half of the conformations, H_2TMpyP was stacked in the space between the side chains of His and Tyr in all the remaining conformations. The energies of these complexes were minimized and over two thirds of the minimized structures corresponded to the structure shown in Fig. 2. As a result, a cognate sandwiching[14,15] interaction, as in the case of YAGY, was suggested to be a suitable way for the HASYS peptide to interact with H_2TMpyP. Many natural proteins use a His residue to interact with a porphyrin,[16,17] however, the imidazole ring of the His stands perpendicular to the porphyrin ring. In this case, the vertical conformation was suggested to be unstable by molecular modeling, probably because the porphyrin H_2TMpyP used as the target molecule did

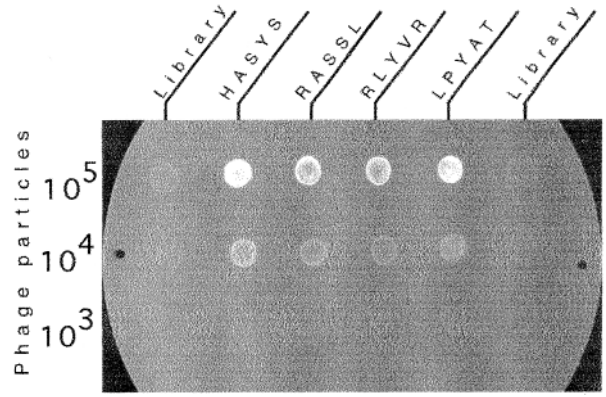

Fig. 1 Binding of the positive phages to H_2TMpyP visualized by fluorescence of the H_2TMpyP. After the selection, equal amounts of phage particles of the four positive phages (HASYS, RASSL, RLYVR, LPYAT) were blotted on a nitrocellulose filter and incubated with H_2TMpyP. Library is the phage before the selection.

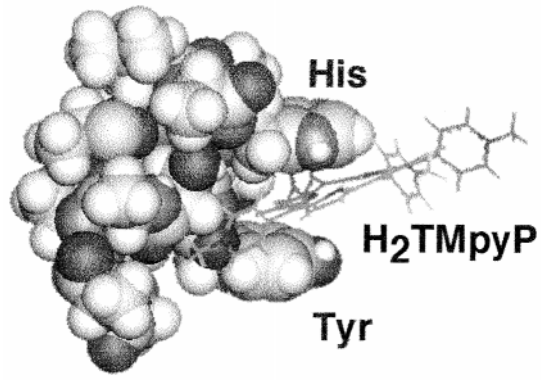

Fig. 2 Proposed structure of the HASYS–H_2TMpyP complex drawn with a molecular modeling calculation using QUANTA97/CHARMm23.2. The energy of the complex was minimized by the adopted basis set Newton–Raphson method. All calculations were performed on a Silicon Graphics Indigo2 workstation running IRIX 5.3.

not have a metal in its center. Consequently, the HASYS peptide should interact with H_2TMpyP by means of a sandwiching interaction in the same manner as the H_2TMpyP–YAGY interaction, as shown in Fig. 2. The YAGY peptide was selected from a biased combinatorial library in which two aromatic amino acids were inserted into positions *i* and *i*+3 or *i*+4.[4] The fact that the same sandwiching interaction was revealed from a completely random library suggests that the stacking between aromatic amino acids and a porphyrin is the most profitable manner for a short peptide to interact with a porphyrin.

In a few cases of selection, only with a protein as the target molecule, common motifs were revealed from the two methods, combinatorial chemistry and phage display. Bianchi *et al.* obtained a very similar consensus from a phage library and a combinatorial library for a binding motif to monoclonal immunoglobulin,[18] and many streptavidin binding phages selected from a phage library with 36 random amino acids[19] contained a similar sequence to one of the consensus sequences picked from a combinatorial pentapeptide library.[1] In both the cases, the target molecule was very large and the obtained interaction was very strong. When a small target is used, as in the present study, such a strong binding with enough energy for anchoring a phage to the target molecule cannot be expected because the available interactions are restricted. So, a high background may hinder simple and efficient selection by the normal phage display system with an immobilized small target.[8] Consequently our system, which binds free H_2TMpyP (MW 1364) to a solid support *via* the interaction between a porphyrin and a phage, is superior to a system that binds a free phage with a 10^4 times greater mass than the porphyrin by the same interaction.

In summary, we have succeeded in obtaining the same peptide motif that binds to a small target H_2TMpyP from both combinatorial chemistry and phage display with an immobilized phage library. Our results suggest that the immobilized phage display system is a promising method for identifying functional short peptides that interact with a small target.

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