Sandwiching Interaction of Peptides with a Porphyrin

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Designed oligopeptides were able to bind a porphyrin, 5, 10, 15, 20-tetrakis (N-methylpyridinium-4-yl)-21H, 23H-porphine; H_2TMpyP with sandwiching interaction, and in this binding a position of aromatic residues and secondary structures of the peptides played an important role. The importance of the sandwiching interaction of the peptides was also indicated in the selection (combinatorial chemistry) of peptides binding H_2TMpyP from a large number of peptide sequences.

Porphyrin derivatives are characteristic for their redox potentials and photo-reactive pigments. For example, myoglobin plays a remarkable role for an oxgen carrier owing to the bound porphyrin molecule.[1] Cytochrome c' has also important biological roles as electron carriers.[2] Both myoglobin and cytochrome c' stabilize the porphyrin ring by docking into a hydrophobic pocket of the proteins, which is often formed by aromatic residues. Thus, the interaction between the porphyrin and proteins (peptide domains) is one of the key reactions in biochemistry.

Here, we used 5,10,15,20-tetrakis(N-methylpyridinium-4-yl)-21H,23H-porphine (H_2TMpyP) which is a typical cationic porphyrin derivative[3,4] and ethidium bromide (EB) as a reference as shown in Figure 1 to investigate the interaction between these compounds and peptides. We also attempted to obtain H_2TMpyP-binding oligopeptides with an approach of combinatorial chemistry, because the method is considered to be powerful when one selects lead compounds from a large number of molecules.[5,6]

In order to design a peptide library, at first we synthesized 11 peptides including one or two tryptophan residues providing sandwiching interaction with target molecules between two aromatic indole side chains;[7,8] KAAAAWAAAAAAAAK (L,*i*+0), KAAAAWWAAAAAAAK (L,*i*+1), KAAAAWAWAAAAAAK (L,*i*+2), KAAAAWAAWAAAAAK (L,*i*+3), KAAAAWAAAWAAAAK (L,*i*+4), KAAAWAAAAAWAAAAK (L,*i*+5), KAAWAAAAAAWAAAAK (L,*i*+6), KWW (S,*i*+1), KWAW (S,*i*+2), KWAAW (S,*i*+3), and KWAAAW (S,*i*+4). All oligopeptides were synthesized on a Fmoc solid support synthesis as described previously.[9,10] These oligopeptides have two tryptophan residues at different positions between *i* and *i*+n; n from 0 to 6 for the alanine-rich longer oligopeptides (L peptides) or from 1 to 4 for the shorter oligopeptides (S peptides), although the *i*+0 peptide includes only one tryptophan residue.

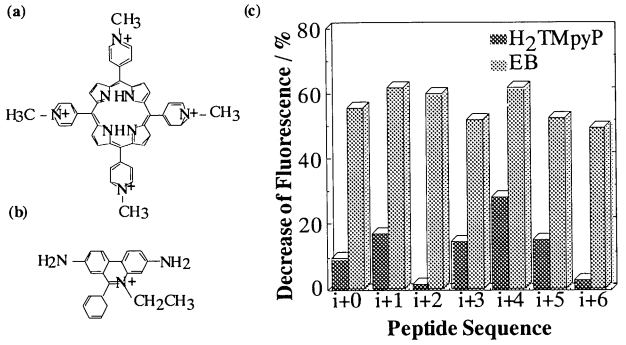

Figure 1. Chemical structures of (A) H_2TMpyP and (B) EB. (C) Fluorescence quenching percentage of the alanine-rich long oligonucleotides by adding 200 μM H_2TMpyP or EB to a buffer containing 10 mM NaCl, 10 mM Na_2HPO_4, and 1 mM Na_2EDTA, pH 7.0 at 20 °C. Excitation wavelength was 278 nm. Fluorescence at 350 nm attributed to tryptophan decreased with bound drugs. Tryptophan concentration of the oligopeptides was 20 μM. Abbreviations were described in the text.

Circular dichroism (CD) spectrum for (L,*i*+4) peptide showed two negative peaks at 222 and 208 nm due to forming α-helical structure. The CD intensity at 222 nm decreased with H_2TMpyP addition (about 10 %), although that at 208 nm did not change. Induced CD of H_2TMpyP was also observed around 420 nm, suggesting there is sandwiching interaction of two indole rings of the peptide with H_2TMpyP, although the induced CD intensity was much smaller than that of α-helical signals. These results indicate that the sandwiching interaction disturb slightly the α-helical structure of the peptide. It was also confirmed with CD spectra that other L peptides formed α-helical conformation while the S peptides showed random-coil spectra (data not shown). For the detail investigation about binding properties of H_2TMpyP to these oligopeptides, fluorescence quenching of tryptophan was measured.

Fluorescence quenching of these oligopeptides by adding H_2TMpyP or EB is shown in Figure 1C. Emission quenching by EB was almost 60% regardless of their tryptophan positions and even the number of the tryptophan residues, while the extent of the fluorescence decrements by H_2TMpyP was quite different among the oligopeptides probably due to the sandwiching interaction. The result suggested that smaller molecule, EB could bind to tryptophan residue mainly by van der Waals interaction regardless of secondary structures of the peptides and the position of aromatic residues. On the other hand, there seems to be a optimizing distance between two tryptophan residues in order to interact with H_2TMpyP. Quenching for L peptides such as (L,*i*+4) peptide was 2-3 times larger than other peptides. When (L,*i*+4) peptide forms an α-helical conformation, two tryptophan side chains face in the distance of 5.4 Å, and the peptide can bind to H_2TMpyP with a sandwiching manner as shown in Figure 2. The result that quenching percentage for (L,*i*+1) peptide was larger than that of (L,*i*+0) peptide would be due to the neighboring two tryptophan side chains which can also bind to H_2TMpyP by the sandwiching manner, which was confirmed by a computer modeling. However, changes of peptide helicity of (L,*i*+4) peptide in the presence of either H_2TMpyP or EB could not detect with CD spectra, suggesting the interaction is not so large. On the other hand, maximum quenching for S peptides by H_2TMpyP was observed at (S,*i*+2)

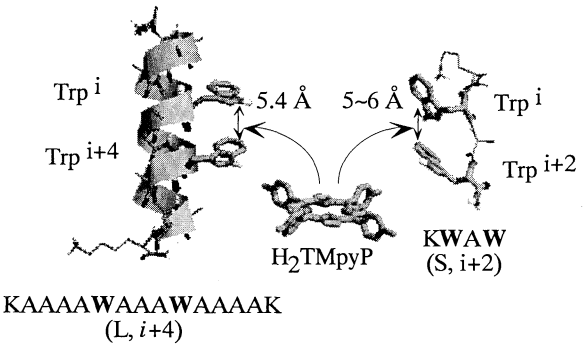

Figure 2. Proposed sandwiching interactions between H_2TMpyP and (L, i+4) or (S, i+2) peptide.

(about 31%). Short peptides are also possible to interact with H_2TMpyP with a sandwiching manner orienting two indole rings of tryptophans to the same face. H_2TMpyP would favor to put between two tryptophan residues rather than EB with van der Waals interaction. The potential for the sandwiching interaction at short oligopeptides is decided by the distance of two indole rings (5~6 Å) (Figure 2). Thus, H_2TMpyP-binding peptides can be designed with considerations about the position of aromatic residues and the secondary structure of the peptide.

Next, a peptide library of Aro-X-X-X-Aro in which Aro represents an aromatic side chain of F, Y, or W, and X is one of 19 natural amino acids except C was constructed to make the sandwiching interaction with H_2TMpyP between two aromatic side chains.[11] H_2TMpyP binding ability to theoretically 62000 kinds of peptide sequences was examined. One of the H_2TMpyP motifs suggested by sequences of selected beads was YAGY (or YAGF) motif. The motif possesses two aromatic residues at i and i+3 positions. Glycine residue at i+2 and a hydrophobic residue of alanine at i+1 position in this motif are typical sequences in a typeII β-turn structure.[12,13] When the motif forms a β-turn structure, two aromatic residues are able to orient in the same side, and the sandwiching interaction with H_2TMpyP between the two tyrosine residues are possible. This report indicates the importance for the sandwiching interaction and the efficiency of combinatorial chemistry in order to obtain low-molecular peptides recognizing a target molecule.

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References and Notes

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