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DESIGN OF NOVEL PORPHYRIN-BINDING PEPTIDES BASED ON ANTIBODY CDR

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Abstract: Novel porphyrin-binding peptides were designed on the basis of an antigen binding site of an anti-heme monoclonal antibody. Synthetic peptides were modified with a pyrene moiety. The spectroscopic measurements revealed that the synthetic peptides bound a porphyrin effectively.

Introduction

Surface loops of proteins play an important role in molecular recognition. For example, antigen binding sites of antibodies are constructed from six loops known as complementarity determining regions (CDRs). The high affinity and specificity of antibodies to antigens are mainly determined by the amino acid sequences and the structures of CDRs.[1] Furthermore, it is known that there are only small repertories of main-chain conformations of CDRs, referred to as 'canonical structures'.[2,3] Consequently, once the sequence and structure of antibodies have been determined, the CDRs can be identified and peptide analogues based on CDR can be generated. This strategy has been used to develop small peptides that are capable of specific antigen binding or inhibition of intermolecular interaction.[4,5] Several studies have shown that synthetic peptides derived from CDR sequences have binding properties similar to the intact antibody.[6-8] In addition, such analysis might be helpful to dissect structure-function relationships in molecular recognition.

Along with this aspect, we attempted to design novel porphyrin-binding peptides based on a CDR sequence of an anti-heme monoclonal antibody. This would be a useful strategy to construct a binding moiety for functional molecules such as porphyrins. Binding properties of peptides for porphyrin were investigated by the spectroscopic measurements. We also examined effects of restriction of peptide conformation and importance of chain length for the porphyrin binding.

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Results and Discussion

Design and synthesis

It has been revealed that the heavy chain CDR2 (CDRH-2) of the anti-heme monoclonal antibody, 2H5, performs the most important role in the recognition of heme.[9] According to the sequential and structural data in the Protein Data Bank (PDB), we modeled the conformation of CDRH-2 of 2H5 as a β-strand-loop-β-strand (β-hairpin; Figure 1a),[10] which corresponded to the canonical structure 2 of H2.[3] According to the above information, several peptides were designed based on CDRH-2 of 2H5 (Figure 1b). These peptides were modified with a pyrene (Py) moiety to detect the porphyrin binding by spectroscopic measurements and to improve binding efficacy. An intramolecular disulfide bond was introduced at several positions to restrict the conformation (**20C4**, **20C6**, **20C8**). The shorter peptide, **12C4**, was designed to examine necessity of the C-terminal tail in the model structure. PyG was prepared as a reference compound which has no peptide chain except glycine. The peptides were synthesized by the solid-phase method using the Fmoc-strategy.[11] After coupling all amino acids, 1-pyreneacetic acid was introduced to the N-terminus. Cyclic peptides were obtained by oxidizing cysteine residues to an intramolecular disulfide. Synthetic peptides were purified with HPLC and identified by matrix assisted laser desorption ionization time-of-flight mass spectrometry[12] and amino acid analysis.

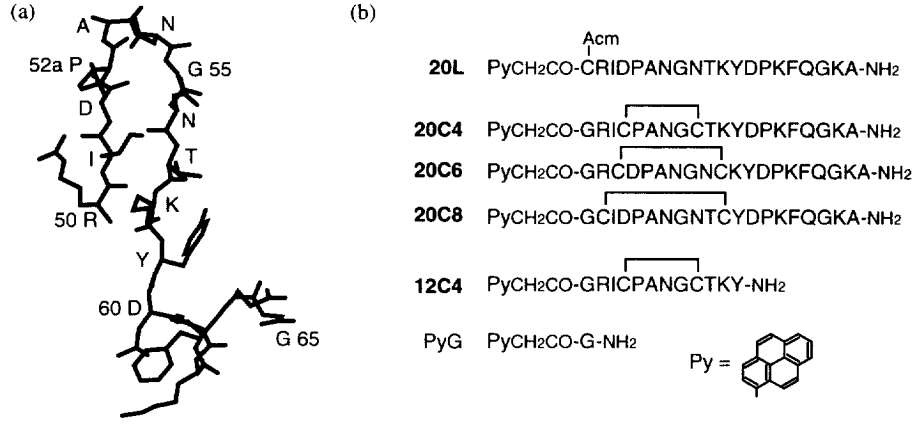

Figure 1 (a) Predicted conformation of CDRH-2 of 2H5 as a β-hairpin. (b) Structure of synthetic peptides derived from CDRH-2.

Spectroscopic study

We used *meso*-tetrakis(4-carboxyphenyl)porphyrin (TCPP) as an "antigen", in this study, to detect the interaction with peptides effectively by means of fluorescence and absorption spectroscopies. By the addition of TCPP, fluorescence intensity of pyrene in the peptides was remarkably decreased in a buffer (pH 7.4). The Stern-Volmer plots of Py-peptides, the inversed fluorescence intensities at 378 nm (I_0/I) vs. TCPP concentrations, deviated upward from a straight line (Figure 2). This should be ascribed to static quenching as well as dynamic quenching because of peptide binding to TCPP.[13] In contrast, the florescence quenching of PyG obeyed a simple Stern-Volmer linear relationship (Figure 2). It means that the peptide chain, but not

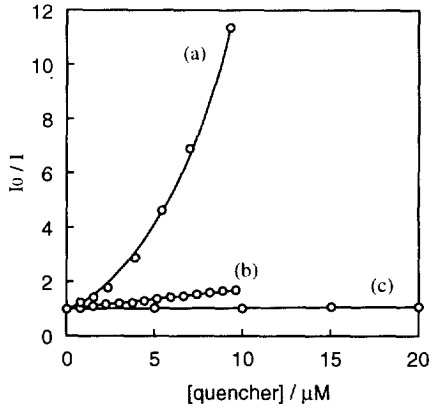

Figure 2 Stern-Volmer plots for the quenching of pyrene fluorescence. (a) Quenching of **12C4** (10 μM) by TCPP. (b) Quenching of PyG (10 μM) by TCPP. (c) Quenching of **12C4** (5 μM) by dinitrophenylacetic acid. λex=268 nm (a), (b) or 345 nm (c), λem=378 nm, in 20 mM Tris-HCl buffer (pH 7.4), at 25 °C.

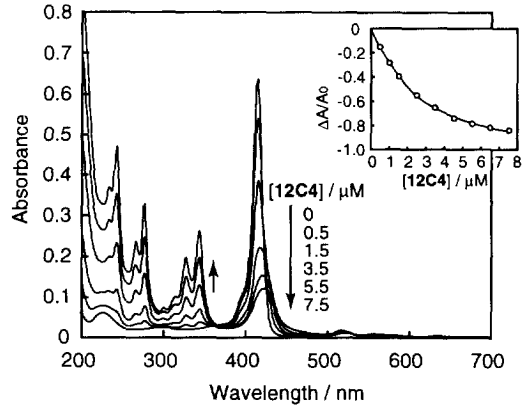

Figure 3 Change of absorption spectra of TCPP (1.5 μM) by the addition of **12C4** in the buffer, at 25 °C. Inset: Plots of absorbance change ($\Delta A/A_0$) vs. concentration of **12C4** at 415 nm. Plots were fitted by an equation of single site binding.

pyrene moiety alone, interacted with TCPP. Furthermore, quenching by dinitrophenylacetic acid, a quencher for pyrene, also showed linear Stern-Volmer plots. These results indicated that the peptides interacted with the porphyrin with some specificity.

When the peptides were added to the buffer solution of TCPP, remarkable hypochromicity and red shift of the Soret band of TCPP at 415 nm were detected (Figure 3). This change of spectra, however, was not detected by the addition of PyG, supporting that the peptide portion was essential to interact with TCPP. The binding constants (*Ka*) of peptides with TCPP were calculated from the decrease of absorbance at 415 nm using a single site binding equation[14] (Figure 3, Table 1). As shown in Table 1, **20C4** bound TCPP most effectively ($Ka = 4.5 \times 10^6\ M^{-1}$). This implied that the suitable restriction of peptide conformation by a disulfide bond was

Table 1 Binding constants (*Ka*) of peptides with TCPP[1)]

compounds	*Ka* (x 10^{-5} M^{-1})	compounds	*Ka* (x 10^{-5} M^{-1})
20C4	45 ±4	**20L**	4.9±0.1
20C6	7.8 ±0.3	**12C4**	6.8±0.2
20C8	0.37±0.06	PyG	—[2)]

[1)]calculated from absorbance change of TCPP (1.5 μM) at 415 nm in 20 mM Tris-HCl buffer (pH 7.4) at 25 °C
[2)]not detected.

Table 2 Effects of methanol on *Ka* of **12C4** with TCPP[3)]

methanol content (%)	*Ka* (x 10^{-5} M^{-1})	methanol content (%)	*Ka* (x 10^{-5} M^{-1})
0	6.8±0.2	30	0.70 ±0.05
5	4.2±0.2	35	0.15 ±0.04
15	2.6±0.1	40	0.097±0.043
20	1.4±0.1	50	—[4)]

[3)]calculated from absorbance change of TCPP (1.5 μM) at 415 nm in the buffer containing various amounts of methanol at 25 °C
[4)]not detected.

effective to increase its binding ability. Moreover, **12C4** was able to bind TCPP with relatively high affinity, suggesting that residues of N-terminus played a main role for the porphyrin binding. The most inefficient binding of the peptide, **20C8**, was possibly due to the loss of amino acids (R and K) important to the binding.

The titration of TCPP with the peptides was also carried out in the buffer containing various amounts of methanol. With increasing percentage volume of methanol, both the spectral change of TCPP and the binding constant of peptides with TCPP were decreased, and no spectral change was observed in 50% methanol (Table 2). These results suggested that hydrophobic interactions also contributed to the TCPP-binding to the peptides.

In conclusion, it was elucidated that the synthetic peptides derived from the CDR of the anti-heme monoclonal antibody and modified with the pyrene moiety bound TCPP with a high affinity ($Ka = 10^4$~10^6 M^{-1}). The affinity to TCPP was improved by the restriction of the conformation according to the model structure of CDR. Furthermore, it was suggested that the N-terminal sequence played an important role in the peptide-TCPP interaction. Although the conformational information should be clarified, these findings indicate that CDR-derived peptide design is applicable to develop the peptides that recognize a specific molecule.

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