Chiral Assembly of Porphyrins Regulated by Amphiphilic α-Helix Peptides

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Amphiphilic α-helix peptides carrying porphyrin moieties were designed and synthesized. The tetraphenylporphyrins were able to be fixed and arranged with a chiral twist in a three-dimensional structure constructed by the amphiphilic assembly of the α-helices.

Studies on natural proteins applicable for electronic devices have been accumulated and their structural features have been elucidated at the level of atomic resolution, for example, bacteriorhodopsin and bacterial photosynthetic reaction center. Along with these findings, much attention has been focused on construction of three-dimensional (3D) structures of polypeptides containing functional chromophores. Considerable efforts have been devoted to the *de novo* design of polypeptides and proteins with stabilized and pre-defined structures. Ultimate goal of the protein design would be to know the principles in the highly efficient functions of natural proteins and to produce artificial molecules with such functions. As one of the progresses in this field, DeGrado *et al.* demonstrated the excellent modeling of multi-haem-containing proteins such as cytochrome bc_1 with simply designed α-helix peptides and a natural haem, Fe-protoporphyrin IX.[1] We have also investigated the designed peptides which have a protein-like tertiary structure composed of α-helices.[2] On the basis of these studies, we have found that the designed peptides could organize orientation of functional chromophores such as porphyrin, pyrene, Ru-trisbipyridine complex, quinone, viologen, and other photo- and redox-active groups, on the α-helical scaffold. In this study, we investigated chiral assembly of tetraphenylporphyrins which were anchored on side chains of newly designed amphiphilic α-helix peptides. Dependence of the porphyrin assembly on the conformational changes of the peptide was examined.

A 14-peptide was designed to take an amphiphilic α-helix structure, in which a hydrophobic amino acid Leu, charged amino acids Lys and Glu, and non-charged hydrophilic amino acids Ala and Gln were deployed on the helix in a manner similar to coiled-coil proteins (Figure 1).[2] 5-(4-Carboxyphenyl)-10,15,20-tris(4-methylphenyl)porphyrin (Por)[3] was introduced on the side chain of Lys at the 6th position instead of Leu. The porphyrin-peptide was dimerized by the disulfide linkage of Cys residues. The peptides, Por1α and Por2α, were synthesized by the solid-phase method using Fmoc chemistry with carefully selected protecting groups [Cys(Acm) for Por1α, Cys(Trt) for Por2α, Gln(Trt), Glu(*t*Bu), Lys(Boc)[6], and Lys(ClZ)[5,12]].[4] The *N*-hydroxysuccinimide ester of the porphyrin was reacted with the Lys[6] side chain of the partially-protected (ClZ) peptides. The ClZ groups of the Por-introduced peptides were deprotected by trimethylsilyl trifluoromethanesulfonate.[5] The crude products were purified with C4 HPLC to high purity (>95%) in yields of 14% (Por1α) and 11% (Por2α). The peptides were subjected to SDS-PAGE with or without mercaptoethanol to give a single spot at an expected molecular weight (Por1α 2500, Por2α 5000 without mercaptoethanol; Por1α and Por2α 2500 with the reductant), indicating that the two segments were dimerized by the disulfide bond to form Por2α. Furthermore, both the peptides showed a similarity in the apparent molecular weight (10000) judging from gel-filtration chromatography using TSKgel G2000SW (50 mmol dm^{-3} phosphate buffer, pH 7.0) detected at 220 and 420 nm, suggesting that Por1α and Por2α are in tetrameric and dimeric form, respectively, to take a four-α-helix 3D structure in aqueous solution.

The CD spectra at the amide region showed that the peptides take highly α-helical structures (Figure 2). The α-helicity $[\theta]_{222}$ of Por1α in a buffer solution was slightly larger than that of Por2α. The 1α-peptide without the porphyrin could not take an α-helical structure in aqueous solution. Therefore, the stabilization of the α-helix structure of Por1α is ascribed to the peptide assembly by the introduced large hydrophobic moiety of the porphyrin. It appears that Por1α is flexibly assembled to a four-α-helix conformation with higher α-helicity than Por2α. At the Soret band of the porphyrin, surprisingly great CD spectra were observed in aqueous solution, which were shown as split shape ($\Delta[\theta]_{430\text{-}405}$ = 4.0 x 10^6 deg cm^2 $dmol^{-1}$ for Por1α and 2.0 x 10^6 for Por2α). The great chiral twist between the porphyrins[6] indicates that the porphyrin moieties are highly oriented in close positions in the four-helix bundle structure. The intensity of Por1α was 2-times greater than that of Por2α. The porphyrin assembly was further confirmed by the absorption and fluorescence spectra.[7] The absorption spectra of the porphyrins at the Soret band were split at 406 (B_y) and 423 nm (B_x), and both Soret and Q bands were broadened with extremely reduced intensity and red-shifted by 1-14 nm compared with the porphyrin in trifluoroethanol (TFE). These UV-vis characters may be correlated with those of dimeric porphyrins in head-to-tail

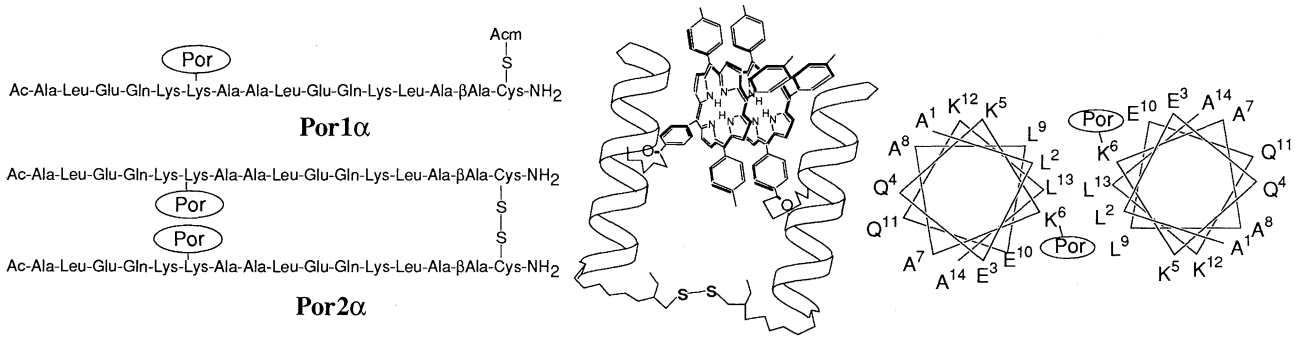

Figure 1. Structure of Por1α and Por2α. Illustration of designed structure of Por2α and helix wheel are shown. One letter symbols of amino acids are A, Ala; E, Glu; K, Lys; L, Leu; Q, Gln.

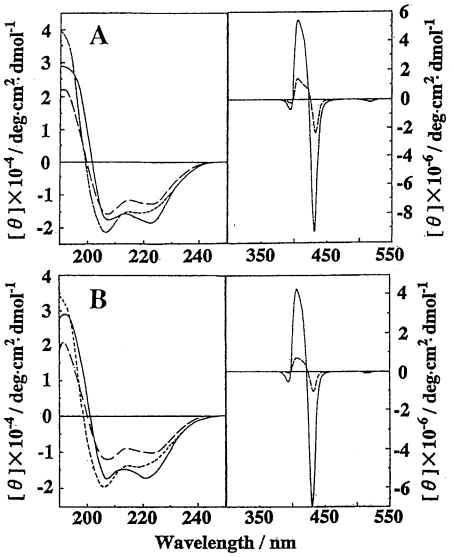

Figure 2. CD spectra of Por1α (A) and Por2α (B) in 20 mmol dm^{-3} TrisHCl buffer (pH 7.4) (– – – –), 40% TFE/buffer (———), and TFE (- - - -). [θ] at amide and porphyrin regions are indicated per amino acid residue and per porphyrin, respectively. [Por]=2.0 x 10^{-6} mol dm^{-3}, 25 °C.

orientation.[8] The fluorescence intensities of the peptides were also reduced in 1/16 compared with the porphyrin in TFE.[7] The split CD appears to be combined spectra at 420 nm (B_x) of -/+ sense and at 400 nm (B_y) of +/-.[6] The relationship between the sense of the exciton interactions and the 3D structure of the porphyrin-peptides is under investigation by NMR and computer modeling studies.

Because the amphiphilic folding of peptides could be regulated by the addition of organic solvent,[2] the spectra were measured at the various content of TFE (Figure 3). At the first stage of the addition (<40%), both the ellipticities at 222 and 430 nm were increased with increasing TFE content, especially at the Soret band, the intensities, surprisingly, enhanced in 4-6 fold ($\Delta[\theta]_{430\text{-}405}$ = >1 x 10^7 deg cm^2 $dmol^{-1}$). With further addition of TFE, the ellipticities gradually decreased and then the split CD spectra disappeared at 70-80% TFE content. The α-helicity $[\theta]_{222}$ behaved similar to the split CD. The maxima of the ellipticities were observed at 30 and 40% TFE, respectively, for Por1α and Por2α in the split CD and the α-helical maxima existed at the same contents. The absorption and the fluorescence spectra were also changed by the TFE titration, though both intensities were increased. At the beginning of the titration (<20%), intensities were gradually increased, then enhanced more steeply until 70-80% TFE, and curves reached to plateaus. In TFE, the spectra were similar to those of the porphyrin in TFE. The midpoints of these spectral changes by TFE were almost comparable to the maximum points in the CD spectra (30-40% TFE). The TFE-titration experiments revealed that the chirally twisted orientation of the porphyrins reached to a maximum when the peptide 3D structures were untied to some extent.[2] In higher TFE contents, the porphyrins at side chains are free to move, resulting in complete loss of the chiral orientation. The bundle conformation of Por2α was destroyed by the higher TFE content (40%) than Por1α was (30%), probably due to the covalent bonding of the 2α-peptide.

CD spectra were further measured in the presence of a denaturant, guanidine hydrochloride (GuHCl), at various concentrations in the aqueous solution. Ellipticities at 430 nm of both peptides were decreased steeply with increasing concentration of GuHCl, and disappeared at 3-4 mol dm^{-3}.

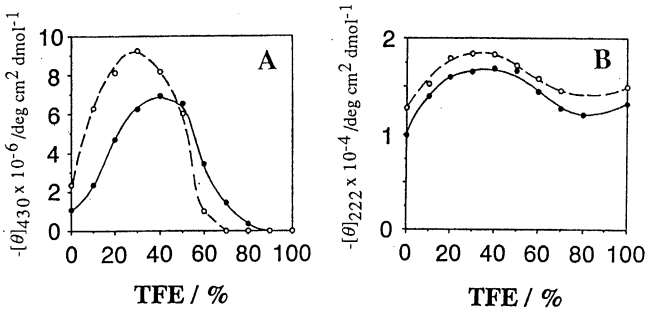

Figure 3. Dependence of the CD spectra at 430 nm (A) and 222 nm (B) on TFE content. (– – – –) Por1α and (———) Por2α. [Por]=2.0 x 10^{-6} mol dm^{-3}, 25 °C.

However, the peptides kept the α-helical structure to some extent (ca. 50% of the ellipticities without GuHCl) even at 7.5 mol dm^{-3}. The secondary structure was very stable, and the chiral twist of the porphyrins was sensitive to the perturbation of the conformation. The porphyrin CD seems to reflect the 3D structure rather than the secondary structure. On the contrary, both the porphyrin orientation and the α-helix structure were rather stable against temperature. Ellipticities at 222 and 430 nm of the peptides at 60 °C were maintained in 90% of those at 25 °C.

The chiral assembling of porphyrins was achieved by the combination of the designed α-helical peptides. The defined spatial orientation of functional groups by polypeptide 3D structures could be applied to elucidation of roles of polypeptide structures on proteinous functions, and obtained information will be useful to design artificial proteins. Metal (Fe and Mn) chelation with the porphyrins was performed and properties of the metal-porphyrin peptides are under investigation.

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References and Notes

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3 The porphyrin was synthesized by the reported procedure by N. Nishino, Kyushu Institute of Technology.

4 Acm, acetamidomethyl; Boc, *t*-butyloxycarbonyl; ClZ, 2-chlorobenzyloxycarbonyl; Fmoc, 9-fluorenylmethyl-oxycarbonyl; Trt, trityl. The synthetic details will be reported elsewhere.

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7 λmax (ε)/buffer 407 nm (43500), 424 (47500), 518 (4000), 555 (1500), 593 (1500), and 645 (1500) for Por1α; 406 (75000), 423 (87000), 517 (5000), 556 (2000), 595 (2000), and 651 (2000) for Por2α. λmax (ε)/TFE 413 nm (279000), 516 (9500), 552 (6000), 589 (4500), and 636 (4000) for Por1α; 413 (547000), 517 (18000), 553 (15000), 589 (14000), 639 (15000) for Por2α; 413 nm (353000), 516 (15200), 553 (10800), 585 (10000), and 637 (10200) for the porphyrin. Fluorescence emissions were at 654 nm (relative intensity 1.0) in buffer and 645 nm (relative intensity 16.0) in TFE for all porphyrins.

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