CD Investigation of Porphyrin–Porphyrin Interaction with Links to Acyclic β-Sheet Peptide Self-Assembled in an Aqueous Media

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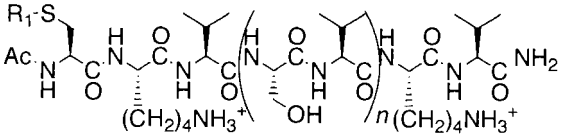
Amphiphilic peptide, Ac-Cys(Por)-Lys-Val-(-Ser-Val-)$_n$-Lys-Val-NH$_2$ (n = 0 or 1, Cys(Por) = side-chain porphyrin-linked Cys), self-assembled to form the β-sheet in buffer solution-2,2,2-trifluoroethanol and showed exciton coupled Cotton effects in the porphyrin region due to the closely oriented porphyrins.

Recently, various natural systems with a number of porphyrins have been found such as antenna-chlorophylls[1] and some redox enzymes.[2] Many studies have focused on the hitherto unknown functions of multi-porphyrins using multi-step synthesized porphyrin oligomers as the models.[3] However, the self-constructing properties of both the porphyrins and the polypeptides form the natural porphyrin–polypeptide conjugates. Therefore, if both porphyrin and polypeptide tend to self-assemble in an artificial porphyrin–polypeptide conjugate, we can easily obtain a variety of multi-porphyrin supramolecules. In the amphiphilic β-sheet polypeptides linking porphyrins in the hydrophobic face, the hydrophobic interaction between porphyrins and the hydrogen bonding between peptides may function coorperatively.[4] A number of porphyrins may be arranged in the hydrophobic face, to which we can expect 1) a multi-porphyrin assembling due to the polymeric nature of β-sheet, 2) facile syntheses of various monomeric porphyrins with links to acyclic peptides, and 3) a soluble β-sheet due to the one-face covered porphyrins. These features of multi-porphyrins on the β-sheet, though rarely studied, may open to a novel functional molecule for photochemistry, electron transfer, device, and catalysis. Thus, we synthesized the porphyrins with acyclic β-sheet peptides.



1: n=0, R_1=H , **2**: n=1, R_1=H, **3**: n=0, R_1=CH_2CO-*p*-Por
4: n=0, R_1=CH_2CO-*o*-Por, **5**: n=1, R_1=CH_2CO-*p*-Por
(*p*- or *o*-)Por: tri(*p*-tolyl)porphyrin-5-yl-C_6H_4-(*p*- or *o*-)NH-

Figure 1. Polypeptide and porphyrin-linked peptide.

The acyclic peptides with alternative hydrophobic/hydrophilic sequences, Ac-Cys-Lys-Val-Lys-Val-NH$_2$ (**1**) and Ac-Cys-Lys-Val-Ser-Val-Lys-Val-NH$_2$ (**2**) were manually synthesized using the Rink-amide resin (Figure 1).[5] These peptide reacted smoothly with (5,10,15-tritolyl-20-(*p*- or *o*-)bromoacetamidophenyl)porphyrin in DMF-diisopropylethylamine,[6,7] affording Ac-Cys(*p*- or *o*-linked-Por)-Lys-Val-Lys-Val-NH$_2$ (**3** or **4**) and Ac-Cys(*p*-linked-Por)-Lys-Val-Ser-Val-Lys-Val-NH$_2$ (**5**). These porphyrin–peptide hybrids and the free peptides were purified by RP-HPLC and characterized by FAB-high MS (for instance, found for **1**, 617.3826; calcd for [**1**+H]$^+$, 617.3808; found for **3**, 1328.6813; calcd for [**3**]$^+$, 1328.6806) and ^{1}H NMR (in DMSO-d_6, 1D and 2D) spectra.

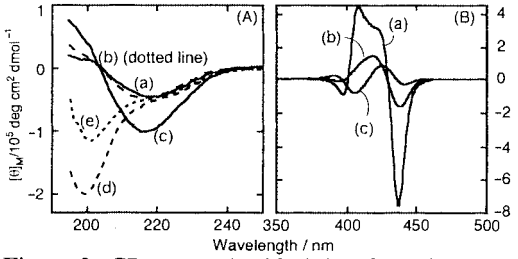


Figure 2. CD spectra (amide (A) and porphyrin (B) region) of (a) **3**, (b) **4**, (c) **5**, (d) **1**, and (e) **2** in pH 9 buffer–20% TFE.

CD spectra suggested that the porphyrin–polypeptide hybrids adopted the β-sheet structure (Figure 2A). In pH 9 buffer (10 mM 1,3-bis{tris(hydroxymethyl)methylamino}propane)-20% 2,2,2-trifluoroethanol (TFE), **3**, **4**, and **5** (40 μM, determined by amino acid analyses) showed the negative Cotton effects characteristic of the β-structure at around 217 nm,[8] with the $[\theta]_M$ (molar ellipticity) of –47000, –49500, and –103000 deg cm^2 dmol^{-1}, respectively. The larger Cotton effect for **5** than those of **3** and **4** suggested the more stable β-structure with the heptapeptide. In the same solvent, the free peptides (**1** and **2**, 40 μM) were random structure showing their Cotton effects at around 200 nm with a shoulder at 222 nm.[8] These observation suggests that the porphyrins linked at Cys induced the β-structure. In the porphyrin region (~420 nm), the conjugates **3**, **4**, and **5** showed strong Cotton effects (Figure 2B) with various $[\theta]_M$ and peak shapes (see below). Such strong exciton coupled Cotton effects indicated that porphyrins were located nearby in the chiral orientation in the self-assembled strructure.[4,6,9] The hydrophobic porphyrins would stack in the aqueous system, which affected the gathering of the peptide chains, then the peptides formed the β-sheet structure. In fact, **3**, **4**, and **5** were eluted in the void volume region (molecular weight > 20000) during the SEC analyses (YMC DIOL-60, pH 8 buffer-20% CH_3CN). The Cotton effect of **3** was the highest at pH 9, which is likely due to the deprotonation of the Lys side chains. The CD spectra of **3**, **4**, and **5** changed little under 10 μM condition.

The Cotton effects of **3** in the porphyrin region (440, 426, 409, and 402 nm, Figure 3A) were strong with the higher TFE content (20, 30 and 40%) in pH 9 buffer. Hydrophobic TFE may stabilize the peptide hydrogen bondings. However, the hydrophobic interaction between the porphyrins was broken in

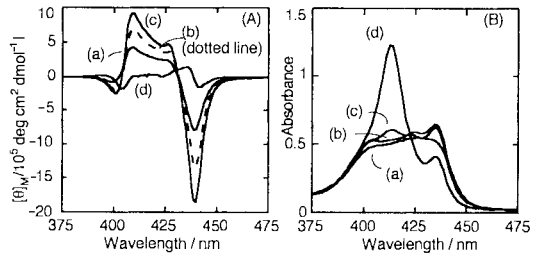


Figure 3. CD (A) and UV (B) spectra of **3** in (a) pH 9 buffer-20% TFE, (b) 30% TFE, (c) 40% TFE, and (d) 50% TFE.

50% TFE which caused the weak Cotton effects. The large $[\theta]_{440}$ (-18×10^5) in 40% TFE is consistent with the exciton coupled CD from the chirally arranged porphyrins located nearby. The origin of these distorted CD spectra is not clear, but these spectra might be the sum of two sets of Cotton effects, for instance 440(–)/426(+) and 409(+)/402(–). The appearance of two sets of Cotton effects implied that more than two porphyrins generated the Cotton effects (see below). The UV–vis spectra also supported the assembled structure of **3** (Figure 3B). In 50% TFE, **3** showed two absorptions at 414 and 436 nm. The absorption at 414 nm arises from the monomeric porphyrin (**3** showed sharp absorption at 414 nm in TFE–1% triethylamine). The peak at 436 nm appears to be from the assembled porphyrins.[10] In 30% TFE, **3** showed multiple absorption at 436, 423, 414, and 402 nm, which may arise from the multiple interactions between porphyrins, as in the case of the CD spectra. If more than three **3** molecules formed β-sheets, at least two porphyrin–porphyrin interactions occur (between the first and the second chains, and between the first and the third chains). The CAChe®-MM2 study (Figure 4, tetrameric structure was a model of β-sheet structure) of the antiparallel **3** suggested that the center-to-center distance of two porphyrins between the first and the second was 2.3 nm, and 0.9 nm between first and the third. Such a multi-porphyrin assembly may arise the distorted CD and the multiple UV absorptions. The parallel and antiparallel orientations might arise the multiple Cotton effects. However, a parallel one is sterically unlikely. The MM2 structure also shows that the two porphyrins of the first and the second are in the edge to edge fashion, which might stabilize their assembly (see below).

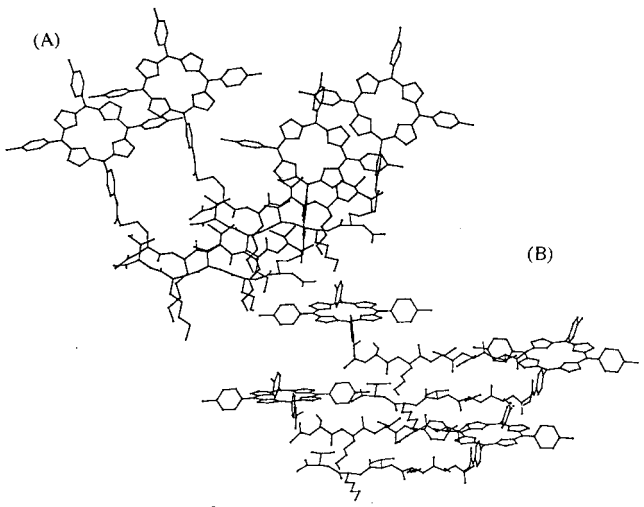


Figure 4. CAChe®-MM2 structure of the tetrameric **3** with multiple exciton couplings ((A) front and (B) upper view).

The pentapeptide linking the porphyrin at the *ortho*-phenyl position, **4**, showed weak Cotton effects (Figure 2B). Such smaller Cotton effects of **4** compared with **3** may be due to the steric repulsion of the porphyrins, preventing the porphyrins from close proximity. Interestingly, the Cotton effect of **4** was the largest in 10% TFE and decreased by adding TFE, which suggested the weaker stacking interaction of **4**. The simple CD pattern of **4** supported the formation of the smaller aggregates.

The heptapeptide–porphyrin **5** also showed weak Cotton effects (Figure 2B). Because the β-sheet structure of **5** should be more stable than those of **3** and **4** (Figure 2A), the weak Cotton effect of **5** would not be due to its weak aggregation. In the antiparallel β-sheet structure of **5**, the porphyrins are probably separated. The CAChe®-MM2 structure of the **5** assembly (not shown) suggested that the heptapeptide was a little too long to bring two porphyrins closely. Without the edge to edge interactions of porphyrins, **5** formed less stable assembly. The CD spectrum of **5** of (+–) sign ($[\theta]_{426}$/ $[\theta]_{407}$) also suggested the separate porphyrin rings. Because the β-sheet peptide chains are left-twisted,[11] the porphyrin rings attached to the β-sheet peptide may be left-twisted, which would show a Cotton effect with (–+) sign,[9] as **3** and **4**. The Cotton effect of the two porphyrins attached to the cyclic β-sheet peptide was also similar.[4] For **5**, the two porphyrins of the neighboring two chains may be separated and the interaction of two porphyrins in the first and third chains might be dominant, which might show the (+–) sign of the Cotton effect.

In conclusion, **3** formed a stable β-sheet assembly, which cannot be broken up to 40% TFE content. The CD and UV spectra of **3** showed several interaction of assembled porphyrins. MM2 model of the antiparallel β-sheet structural **3** suggested that the porphyrin rings in the neighboring peptides located in the edge to edge fashion. The edge to edge interaction of porphyrins seemed to be essential for the stable assembling, by comparing to the analogous **5**. A more detailed investigation including a ^{1}H NMR study is now under way. However, ^{1}H NMR signals of the hydrophobic moiety of **3** were substantially broadened probably due to their restricted motions.[12]

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References and Notes

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