Construction of a Multiple Porphyrin System Using a *de novo* Designed Peptide Porphyrin and Hemin

Masato Ushiyama, Fumio Arisaka,† and Takeshi Yamamura*
Department of Chemistry, Faculty of Science, Science University of Tokyo, 1-3 Kagurazaka, Shinjuku-ku, Tokyo 162-8601
†Department of Life Science, Faculty of Bioscience and Bioengineering, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8501

(Received November 10, 1998; CL-980853)

Multiple porphyrin systems were constructed using a Zn(II) porphyrin, **A**, which is a *de novo* designed peptide porphyrin, and Fe(III) protoporphyrin IX chloride, **B**. **A** forms a dimer in buffer solution containing 2,2,2-trifluoroethanol. However, **A** forms a trimer by incorporating three equivalents of **B** per **A**. The results suggest that the **A/B** solution achieves a 12-porphyrin system consisting of different kinds of metalloporphyrins.

Chlorophylls and hemes in photosynthetic reaction centers form multiple porphyrin (derivative) systems, where the chromophores are arranged in close contact with each other, or, packed closely in narrow molecular spaces, and operate as the center of primary charge separation and electron transfer. Chlorophylls in light harvesting proteins are also arranged in a similar manner to achieve effective energy transfer. From this point of view, Rabanal et al.'s hexaporphyrin system[1] was the one which achieved the highest porphyrin multiplicity among the *de novo* designed artificial proteins.[2]

Here, we report on a novel artificial protein, α_4-$(PepA_{18})_4$-ZnTAPP, **A**, where α_4-ZnTAPP=Zn(II) α_4-*meso*-tetra(*o*-amino phenyl)porphyrin[3] and $PepA_{18}$=Glu-Glu-Ala-Leu-Glu-Lys-His-Glu-Lys-Ala-Leu-Glu-Lys-His-Glu-Lys-Ala-Gly. **A** achieved new performance in terms of porphyrin multiplicity, that is, the formation of a 12-porphyrin system in the presence of Fe(III) protoporphyrin IX chloride, **B**.

A was designed according to the template-assembled synthetic proteins (TASP) strategy.[4] $PepA_{18}$ contains two His residues as coordination sites; therefore, **A** makes it possible to construct multiple porphyrin systems by anchoring **B** at its 8 His sites (Figure 1).

A was synthesized in the liquid phase by the Boc strategy using OPac (as the C-terminal protecting group) according to the route shown in Scheme 1.[5,6] In order to increase flexibility at the coupling point, thereby increasing the coupling yield, we

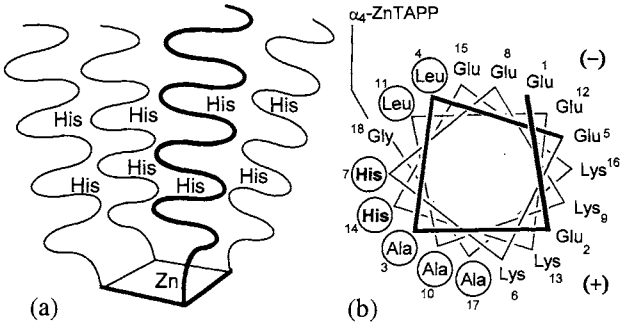
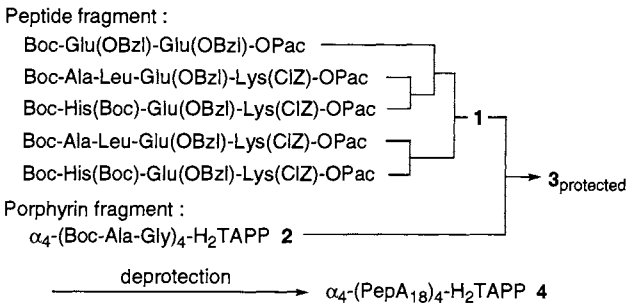


Figure 1. (a) Schematic representation of α_4-$(PepA_{18})_4$-ZnTAPP, **A**. (b) Helical wheel of $PepA_{18}$.



Scheme 1.

divided the amino acid sequence of $PepA_{18}$ into two parts consisting of 16 and 2 residues, and introduced the dipeptide, Ala-Gly, to the amino groups of α_4-TAPP prior to coupling.[7] The most difficult step we faced was the final one in which **1** was coupled to **2** (Scheme 1). It is well known that porphyrins are easily destabilized by neighboring imidazole groups, thus, in the field of peptide synthesis in the liquid phase, there have been only a few examples which combined the peptides containing multiple His residues with porphyrins.[8] After a detailed search, we found that the combination of PyBOP/HOAt/DIEA/DMF afforded a sufficient yield (>40%) when reactions were conducted below −45 °C at least at the initial stage of the coupling reaction.[9] **4** was purified on a reversed-phase HPLC column. **4** and **A** were confirmed by electrospray ionization (ESI) mass spectrometry.[10]

The aggregation behavior of **A** and **B** was studied in buffer solution adjusted to pH 7.0 considering that the pKa value of the His imidazole group is 6.0. A preliminary search for the effect of 2,2,2-trifluoroethanol (TFE) on the helicity of $PepA_{18}$ was

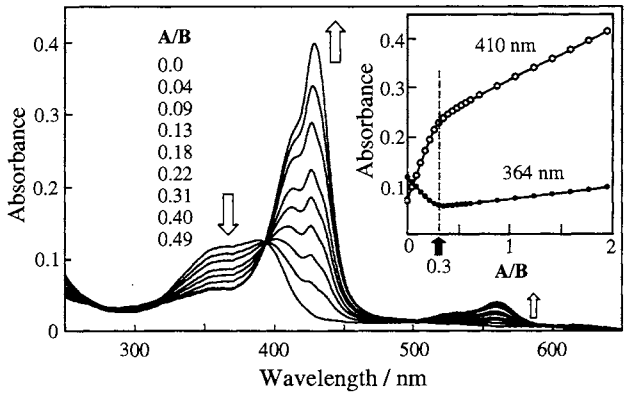


Figure 2. UV-visible spectral changes: 21 μM **B** in 20 mM phosphate buffer, pH 7.0, containing 15% TFE was titrated at 25 °C with 75 μM **A** at shown ratios. Arrows show the trends of changes on titration. Inset: titration curves monitored at 364 and 410 nm.

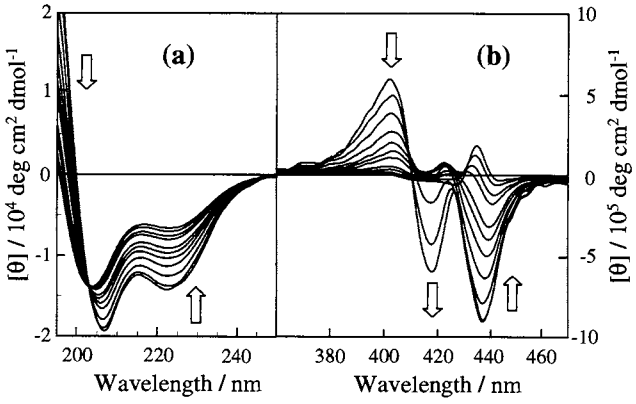


Figure 3. CD spectral changes: (a) UV region and (b) Soret band region of porphyrins. 21 μM **B** was titrated with 75 μM **A** at **A/B** ratios of 0.10, 0.20, 0.30, 0.40, 0.51, 0.61, 0.71, 1.0, 1.2, 1.6, and 2.0. Arrows show the trends of changes on titration. [θ] was evaluated based on the concentration of **A**.

carried out by circular dichroism (CD) spectroscopy. This search revealed that **A** increases its helicity from 7 to 38% ($[\theta]_{222}$) when the content of TFE is increased from 0 to 50%.

UV-vis. and CD titrations were then performed in the presence of 15% TFE by adding **A** into **B** according to the method in the literature.[2b,2c,11] The results of the vis. absorption change observed in the Soret band region are shown in Figure 2. The broad bands at 364 and 389 nm, observed in the solution without **A**, are attributable to aggregated **B**. The successive additions of **A** to this solution cause a gradual decrease in the intensity of these peaks while giving rise to new peaks at 410 and 428 nm. Titration curves observed at appropriate wavelengths show their turning points at about **A/B**=1/3 (see inset), indicating that up to three equivalents of **B** molecules are incorporated into **A**. The CD titration curves (Figure 3b) also show the turning points at about **A/B**=1/3. Here, it should be noted that the CD spectral changes are complicated and accompanied by several isodichroic points, as well as changes in peak positions, linewidths, and peak intensities. Although it may be difficult to grasp from the figures, these complicated changes in CD spectra correspond to those in the UV-vis. absorptions in Figure 2. These changes are to be attributed to those originating from exciton couplings. They suggest that **A** and **B** are located close to each other in molecular space and change their mutual configuration when the **B/A** ratio is varied.

On the other hand, the CD spectral changes in the UV region (Figure 3a) indicate that the helicity of $PepA_{18}$ is increased from 16 to 45% ($[\theta]_{222}$) in the presence of **B** (**A/B**≤0.3). The observed low helicities are explained by the existence of the His residues which are free from the coordination to **B** in $PepA_{18}$, because His residue is well known to destabilize helix formation in general. Here, it should be emphasized that **A** was designed to contain a number of His residues, sacrificing helicity in order to incorporate multiple porphyrins.

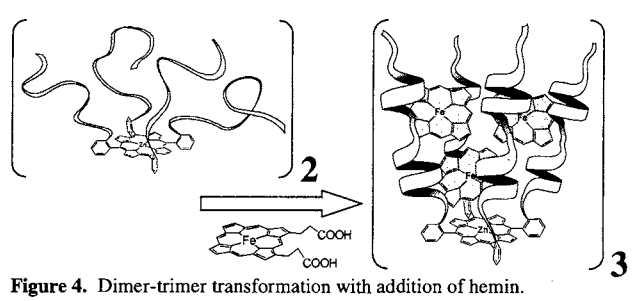


Figure 4. Dimer-trimer transformation with addition of hemin.

Sedimentation-equilibrium ultracentrifugation experiments were performed for **A** only and **A/B**=1/1, 1/2, and 1/3 solutions. Obtained molecular weights for the solutions, 17942, 28070, 31606, and 32952 were close to the values, 17942, 28763, 30612, and 32463, calculated for $\mathbf{A}_2$, $\mathbf{A}_3\mathbf{B}_3$, $\mathbf{A}_3\mathbf{B}_6$, and $\mathbf{A}_3\mathbf{B}_9$, respectively.[12,13] This result indicates that **A** exists as a dimer in the solution, and transformation to a trimer occurs when **B** is incorporated (Figure 4).

Consequently, α_4-($PepA_{18}$)$_4$-ZnTAPP, **A**, achieves a 12-porphyrin system with Fe(III) protoporphyrin IX, **B**. The total aspect of the aggregation can be described by the following equation.

$$3\mathbf{A}_2 \xrightarrow{18\mathbf{B}} 2\mathbf{A}_3\mathbf{B}_9 \qquad (1)$$

We thank Professor Y. Shida of Tokyo University of Pharmacy and Life Science for ESI MS measurement. This work was partly supported by Grants-in-Aid for Scientific Research (Nos. 07640763 and 08454213) from the Ministry of Education, Science, Sports and Culture.

References and Notes

1 F. Rabanal, W. F. DeGrado, and P. L. Dutton, *J. Am. Chem. Soc.*, **118**, 473(1996).
2 (a) H. Mihara, Y. Haruta, S. Sakamoto, N. Nishino, and H. Aoyagi, *Chem. Lett.*, **1996**, 1; H. Mihara, N. Nishino, R. Hasegawa, and T. Fujimoto, *Chem. Lett.*, **1992**, 1805; T. Sasaki and E. T. Kaiser, *J. Am. Chem. Soc.*, **111**, 380(1989); P. A. Arnold, W. R. Shelton, and D. R. Benson, *J. Am. Chem. Soc.*, **119**, 3181(1997); C. T. Choma, K. Kaestle, K. S. Åkerfeldt, R. M. Kim, J. T. Groves, and W. F. DeGrado, *Tetrahedron Lett.*, **35**, 6191(1994). (b) C. T. Choma, J. D. Lear, M. J. Nelson, P. L. Dutton, D. E. Robertson, and W. F. DeGrado, *J. Am. Chem. Soc.*, **116**, 856(1994). (c) S. Sakamoto, S. Sakurai, A. Ueno, and H. Mihara, *Chem. Commun.*, **1997**, 1221.
3 J. P. Collman, R. R. Gagne, C. A. Reed, T. R. Halbert, G. Lang, and W. T. Robinson, *J. Am. Chem. Soc.*, **97**, 1427(1975).
4 M. Mutter and S. Vuilleumier, *Angew. Chem. Int. Ed. Engl.*, **28**, 535(1989).
5 Abbreviations: DIEA=diisopropylethylamine; Boc=*t*-butoxycarbonyl; ClZ=2-chlorobenzyloxycarbonyl; OBzl=benzyl ester; OPac=phenacyl ester; HOAt=1-hydroxy-7-azabenzotriazole; PyBOP=benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate.
6 All the intermediates in Scheme 1 except $\mathbf{3}_{protected}$ were confirmed by ^{1}H NMR, HPLC, FAB mass spectrometry, and elemental analysis (data not shown here).
7 ^{1}H NMR of α_4-$(AG)_4$-H_2TAPP showed single β-pyrrole hydrogens identifying an α,α,α,α-atropisomer.
8 M. Ushiyama, Y. Katayama, and T. Yamamura, *Chem. Lett.*, **1995**, 395; J. P. Collman, J. I. Brauman, K. M. Doxsee, T. R. Halbert, E. Bunnenberg, R. E. Linder, G. N. LaMar, J. D. Gaudio, G. Lang, and K. Spartalian, *J. Am. Chem. Soc.*, **102**, 4182(1980).
9 Details will be published elsewhere.
10 **4**: M_{found}=8909 and M_{calcd}=8908. **A**: M_{found}=8971 and M_{calcd}=8971.
11 I. Hamachi, K. Nakamura, A. Fujita, and T. Kunitake, *J. Am. Chem. Soc.*, **115**, 4966(1993).
12 The linearity of the ln(absorbance) vs radius^2 plots indicates molecular weight homogeneity (data not shown here). The partial specific volume of the peptide moiety was calculated as 0.736 from the amino acid composition of $PepA_{18}$ using those of individual residues. However, we did not adopt this value, since it did not take into account ZnTAPP and hemin. Instead, we adopted 0.761 in order to reproduce the theoretical molecular weight of $\mathbf{A}_2$ within the rational range of the partial specific volume. The effect of porphyrins and TFE on the partial specific volume would be small according to references 2b and 13.
13 C. E. MacPhee, M. A. Perugini, W. H. Sawyer, and G. J. Howlett, *FEBS Letters*, **416**, 265(1997).