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Self-Assembling Porphyrin-Modified Peptides

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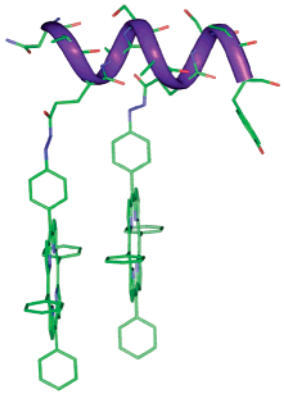
ABSTRACT



We report the synthesis and characterization of a novel supramolecular assembly that features long-range electronic coupling between porphyrins covalently attached to a designed peptide scaffold. The resulting construct self-assembles to form extended organized aggregates in which the porphyrins engage in exciton coupling.

This report constitutes the first part of a more comprehensive study of the use of peptides as scaffolds for the formation of organized porphyrin arrays. One of our goals is to control the structure and electronic properties of the assembly by manipulation of the spacing between the porphyrins along a peptide chain. To this end, we report the synthesis and characterization of a novel supramolecular assembly that features long-range electronic coupling between porphyrins covalently attached to a designed peptide scaffold. Our system shows potential as an important building block for the construction of photonic nanodevices that mimic photosynthetic light-harvesting complexes from plants and bacteria.[1]

Our approach to the synthesis of light-harvesting materials is inspired by biological systems and is guided further by the desire to exert control over the extent of electronic coupling between the pigments. In naturally occurring photosynthetic light-harvesting complexes, biopolymer scaffolds hold pigments at intermolecular distances that optimize electronic coupling, photon capture, and energy transfer.[2] Porphyrins, similar in structure to the naturally occurring (bacterio)chlorophyll pigments but more amenable to synthetic manipulations, have been used extensively in the design of artificial photosynthetic systems.[3] Examples consist of (metallo)porphyrins complexed to a variety of biopolymers, including homopolypeptides,[4] helix bundles,[5] den-

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(1) (a) Fox, M. A. *Acc. Chem. Res.* **1999**, *32*, 201−207. (b) Holten, D.; Bocian, D. F.; Lindsey, J. S. *Acc. Chem. Res.* **2002**, *35*, 57−69.

(2) (a) Hu, X.; Ritz, T.; Damjanovic, A.; Autenrieth, F.; Shulten, K. *Quart. Rev. Biophys.* **2002**, *35*, 1−62. (b) Barber, J. *Curr. Opin. Struct. Biol.* **2002**, *12*, 523−530. (c) Saenger, W.; Jordan, P.; Krauss, N. *Curr. Opin. Struct. Biol.* **2002**, *12*, 244−254.

(3) Konovalova, N. V.; Evsigneeva, R. P.; Luzgina, V. N. *Russ. Chem. Rev.* **2001**, *70* (11), 939−969.

(4) (a) Aubert, N.; Troiani, V.; Gross, M.; Solladiè, N. *Tetrahedron Lett.* **2002**, *43*, 8405−8408 and references therein.

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drimers,[6] and DNA.[7] In a study by Arai et al.[8] a tetraphenylporphyrin moiety was attached via a cysteine residue to a penta- or a heptapeptide sequence to furnish monoporphyrin-linked assemblies. These constructs were shown to aggregate but gave rise to β-sheets that displayed relatively complex spectral properties indicative of heterogeneity. Systems consisting of porphyrins covalently attached to nonpeptidic scaffolds have also been used to examine the distance and angle dependence of energy and electron transfer.[9]

This study reports the first example of a short diporphyrin-linked peptide sequence that self-assembles in a "Lego-like" fashion to yield extended porphyrin arrays with properties that can be controlled by concentration, temperature, and pH.[10] By adjusting the temperature, the growth of the aggregate can be monitored over time.

In our design, 5-(*p*-aminophenyl)-10,15,20-triphenylporphine, is covalently attached via an amide coupling reaction to each of the two Glu side-chains of the decapeptide CH_3CONH-(Asn-Ala-Glu-Ala-Ser-Ala-Glu-Ser-Ala-Tyr)-$CONH_2$ to furnish a diporphyrin-derivatized peptide, **1**. The sequence consists predominantly of helix-promoting residues, Ala and Glu. Ser was incorporated for solubility and a C-terminal Tyr for concentration determination purposes. The Glu residues are separated by three residues in the sequence, i, $i + 4$, thus positioning the porphyrins on the same face. Figure 1 shows CD spectra of **1** dissolved in trifluoroethanol,

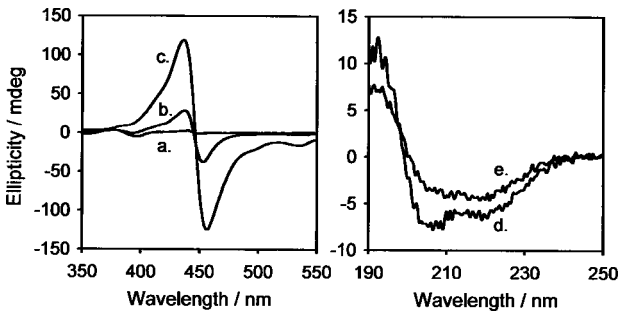

Figure 1. CD spectra of a 15 μM solution of **1** in TFE after storage at 4 °C and pH 7 for (a, d) 4 days, (b) 27 days, and (c, e) 101 days.

TFE, and incubated at 4 °C. Over time, a conservative, negative couplet develops in the 350−500 nm region, the Soret region, of the porphyrin electronic spectrum, with a positive peak at 436 nm and a negative peak at 455 nm.

The simplicity of the spectrum indicates the presence of one major species, and the signal's strength and band shape are indicative of extensive electronic communication between a large number of porphyrin moieties.[11] The free porphyrin, **P**, does not yield a signal in the Soret region of the CD spectrum (data not shown). The formation of the extended porphyrin array is temperature dependent: an increase in incubation temperature increases the rate of aggregation. A red precipitate, triggered by bringing a TFE solution of **1** from pH 4 to 7, immediately forms at 25 °C (Supporting Information). We also investigated the UV−visible absorption and fluorescence properties of **P** and **1**. The free porphyrin, **P**, forms dimers in TFE at pH 6.6, with the monomer and dimer showing Soret bands at 416 and 411 nm, respectively.[12] The blue-shift of the Soret band upon dimerization suggests a cofacial arrangement of the chromophores in the dimer.[13] The Soret-excited fluorescence spectrum of monomeric **P** in TFE at pH 6.6 has bands at 600, 648, and 702 nm. The fluorescence excitation spectra indicate that the 600 nm emission band is associated with the monomeric form of P, whereas the 648/702 nm emission bands are associated with the dimeric form of **P** (Supporting Information).

Figure 2 shows the absorption and fluorescence spectra of **1** after incubation in TFE for 43 days at 4 °C over a range

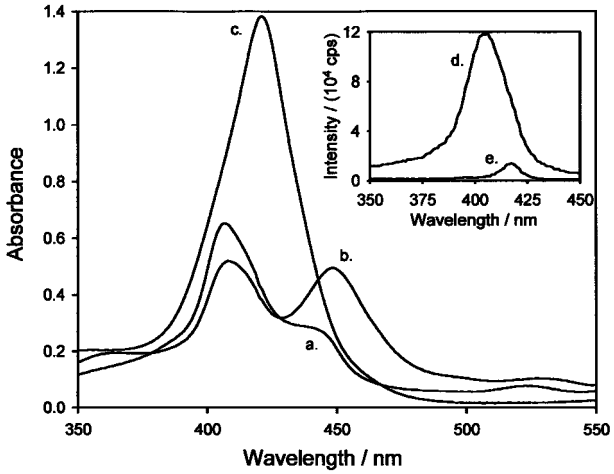

Figure 2. UV−visible absorption spectrum of a 15 μM solution of **1** in TFE after 43 days of incubation at 4 °C at (Soret band positions are noted in parentheses) (a) pH 8.01 (408 and 440 nm), (b) pH 6.37 (408 and 449 nm), and (c) pH 2.01 (420 nm). The inset shows the fluorescence excitation spectra of the solution from spectrum b monitored at (excitation band maxima are noted in parentheses) (d) 670 nm (404 nm) and (e) 600 nm (418 nm).

of pH values. At pH 8.01, the absorption spectrum (Figure 2a) shows a split Soret band with a strong peak at 408 nm and a weaker peak at 440 nm. At pH 6.37 (Figure 2b), the split Soret region consists of two strong components at 408 and 449 nm. The same sample shows fluorescence emission bands at 600 and 670 nm.[14] The fluorescence excitation

(5) (a) DeGrado, W. F.; Summa, C. M.; Pavone, V.; Nastri, F.; Lombardi, A. *Annu. Rev. Biochem.* **1999**, *68*, 779−819. (b) Tommos, C.; Skalicky, J. J.; Pilloud, D. L.; Wand, A. J.; Dutton, P. L. *Biochemistry* **1999**, *38*, 9495−9507.

(6) (a) Zimmerman, S. C.; Wendland, M. S.; Rakow, N. A.; Zharov, I.; Suslick, K. S. *Nature* **2002**, *418*, 399−403. (b) Aida, T.; Jiang, D.-L. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Academic Press: San Diego, CA, 2000; Vol. 3, pp 369−384.

(7) Pasternack, R. F.; Goldsmith, J. I.; Szép, S.; Gibbs, E. J. *Biophys. J.* **1998**, *75*, 1024−1031.

(8) Arai, T.; Inudo, M.; Ishimatsu, T. Akamatsu, C.; Tokusaki, Y.; Sasaki, T.; Nishino, N. *J. Org. Chem.* **2003**, *68*, 5540−5549.

(9) (a) Huang, X.; Nakanishi, K.; Berova, N. *Chirality* **2000**, *12*, 237−255. (b) Clement, T.; Nurco, D.; Smith, K. M. *Inorg. Chem.* **1998**, *37*, 1150−1160.

spectra are shown in the inset of Figure 2. The emission features at 600 (Figure 2d) and 670 nm (Figure 2e) are associated with the absorption bands at 418 and 404 nm, respectively. As an excitation feature is not observed at 449 nm, the data also indicate that the species giving rise to the 449 nm absorption band is not fluorescent.

At pH 2.01, the Soret band is broad with a maximum at 420 nm (Figure 2c). The fluorescence emission spectrum shows a band at 700 nm, which is associated with a peak in the fluorescence excitation spectrum at 437 nm. Samples incubated at this pH did not show CD features in the Soret region.

When considered together, the CD, UV−vis absorption, and fluorescence data of **1** in TFE at 4 °C indicate that samples incubated at pH 2.01 do not form extended porphyrin aggregates. At higher pH values, a band at 440−449 nm can be assigned to a nonfluorescent species, which likely also gives rise to the strong conservative CD feature observed in Figure 1c. Because large chromophore aggregates tend to be nonfluorescent,[15] we assign this absorption to an organized extended aggregate formed by close interactions between porphyrins on different **1** subunits. The red-shifted Soret band (440−449 nm), relative to that of monomeric **P** (416 nm), suggests a J-aggregate, in which the porphyrins are organized edge-to-edge.[14] When compared with the spectral data for **P**, the signals present at 404 and 409 nm in the UV−vis spectrum of **1** may represent a dimeric and a monomeric form of **1**, respectively.

The peptide scaffold is essential for **1** aggregation to occur, as the free porphyrin, **P**, only forms dimers in TFE solution. Interestingly, the peptide region (190−250 nm) of the CD spectrum of **1** incubated at 4 °C (Figure 1d), displays features characteristic of significant secondary structure. After 4 days of incubation, the positive band at 192 nm and the two negative bands at 209 and 222 nm are consistent with an α-helical peptide structure. Thus, the interactions between the porphyrin moieties appear to organize the peptide into a helix, consistent with the model shown in the graphic in the Abstract. Changes in the peptide region occur over time, and Figure 1e shows that incubation of **1** at 4 °C over more than 100 days leads to an overall reduction in intensity of all three peaks and a loss in intensity of the 209 nm band with respect to the 222 nm band. The signal band shape is typical of that seen for moderately aggregated helical spectra in which the spectral changes are consistent with light scattering effects brought about by aggregation.[16] CD spectra taken at higher temperatures, in which aggregation is faster, show less light scattering effects, and the two minima at 209 and 222 nm, characteristic of α-helical peptides, are more pronounced (data not shown).

We have demonstrated that a short diporphyrin−decapeptide complex forms organized extended chromophore arrays. The process is optimal at pH 6−8 in which the rate of aggregation accelerates with increasing temperature. In all, our approach should be useful for the construction of novel biomaterials, including conducting nanowires.

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Supporting Information Available: Experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) First reported: Sandstrom, C.; Young, E.; Baker, P.; Dunetz, J.; Fairman, R.; Johnson, K.; de Paula, J.; Åkerfeldt, K. *Abstracts of Papers*, 223rd National Meeting of the American Chemical Society, Orlando, FL, April 7−11, 2002; American Chemical Society: Washington, DC, 2002.

(11) Pasternack, R. F.; Giannetto, Pagano, P.; Gibbs, E. J. *J. Am. Chem. Soc.* **1991**, *113*, 7799−7800.

(12) Equilibrium constant for dimerization is 4.16 × 106 at 4 °C and was determined by quantitation of the dimer absorption band at 411 nm according to: Tipping, E.; Ketterer, B.; Koskelo, P. *Biochem. J.* **1978**, *169*, 509−516.

(13) (a) Ohno, O.; Kaisu, Y.; Kobayashi, H. *J. Chem. Phys.* **1993**, *99*, 4128−4139. (b) Parkash, J.; Robblee, J. H.; Agnew, J.; Gibbs, E.; Collings, P.; Pasternack, R. F.; de Paula, J. C. *Biophys. J.* **1998**, *74*, 2089−2099.

(14) A third emission band at 700 nm was assigned to residual porphyrin diacid on the basis of the similarity between the fluorescence excitation spectrum of the emission feature at 700 nm and the absorption spectrum of the diacid form of **P**.

(15) (a) Pasternack, R. F.; Schaefer, K. F.; Hambright, P. *Inorg. Chem.* **1994**, *33*, 2062−2065. (b) de Paula, J. C.; Robblee, J. H.; Pasternack, R. F. *Biophys. J.* **1995**, *68*, 335−341.

(16) Arutyunyan, A. M.; Rafikova, E. R.; Drachev, V. A.; Dob, E. N. *Biochemistry (Moscow)* **2001**, *66*, 1378−1380.