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The Design, Synthesis and Characterization of a Porphyrin-Peptide Conjugate

G. Richard Geier III and Tomikazu Sasaki*

Department of Chemistry, University of Washington, Seattle, WA 98195 USA

Abstract: The design and synthesis of a novel peptide strapped porphyrin is described. CD spectroscopy and ^{1}H NMR are supportive of the design. © 1997 Elsevier Science Ltd.

The design and synthesis of artificial proteins has become an increasingly valuable approach for studying protein folding, for understanding the behavior of native proteins, and for developing novel catalysts. Attempts have been made to mimic protein structure using a variety of template molecules to organize short peptide sequences into defined secondary and tertiary structures.[1] Such efforts have lead to a host of elegant helix bundle and β-sheet structures. In addition to mimicking the peptide portion of proteins, chemical models have been developed for the metalloporphyrin prosthetic group found in many proteins. A number of these metalloporphyrins mimic the oxidative chemistry of monooxygenases.[2] Yet, despite the advances made in peptide and porphyrin systems, there are very few reports of designed systems containing both components of native heme proteins.[3]

In this communication, we report the design and synthesis of a porphyrin-peptide conjugate (**5** in Scheme 1). The porphyrin ring functions both as a template for the organization of an α-helical peptide chain and as a potential catalytic site for oxidation of organic substrates. The α-helical peptide portion provides a chiral, hydrophobic substrate-binding site whose dimensions and properties may be readily varied by changing the peptide sequence. This system was designed as a model for the cytochrome P450 monooxygenase family. Crystal structures of native P450 show a protoporphyrin IX group lying in a hydrophobic pocket defined by helical regions of peptide.[4]

The peptide sequence for the conjugate is shown in Figure 1a. The two cysteine residues used to attach the peptide to the porphyrin ring are positioned *i* and *i*+11 residues apart. This placement corresponds to three turns of an α-helix. Figure 1b shows a helical wheel view of the peptide illustrating its amphiphilic nature. The residues toward the porphyrin ring provide a hydrophobic pocket. The residues projecting away from the porphyrin ring are hydrophilic in order to increase the water solubility of the conjugate. The specific residues were selected for their helical propensity[5] and ease of coupling. Finally, the helical net view of the peptide in Figure 1c shows two salt bridges between lysine and glutamic acid side chains to further enhance helicity.[6]

The porphyrin template **4** was chosen for a number of reasons. First, the bromoacetamido groups provide electrophilic sites for attachment to the peptide. Second, the distance between the two bromoacetamido linker arms is consistent with the propagation distance of three turns of an α-helix. Third, tetraphenylporphyrins are resistant to aggregation and auto-oxidation. Fourth, synthetic schemes for the preparation of modified tetraphenylporphyrins are well-developed.[7] Prior to synthesis, design feasibility was confirmed by molecular modeling using *Insight II* by Biosym.

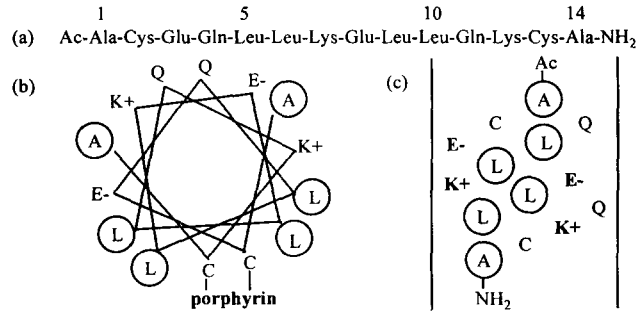
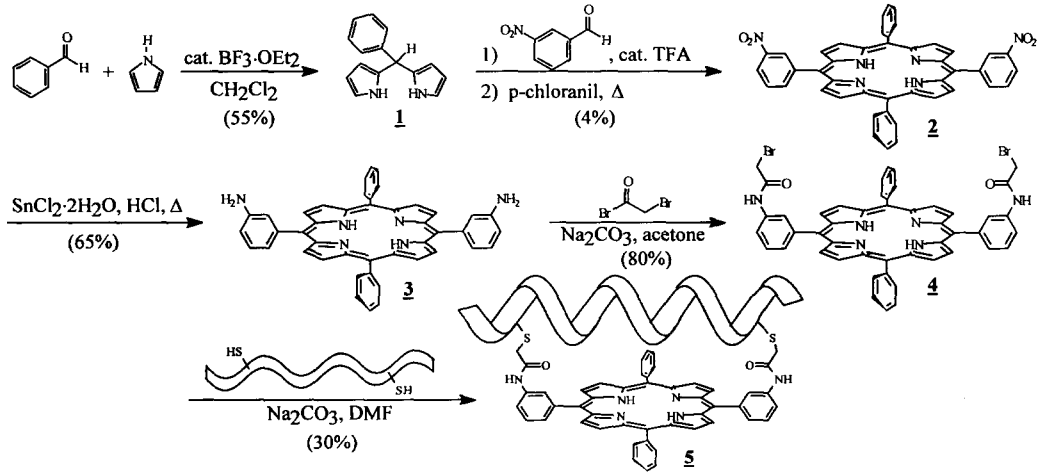


Figure 1: (a) Primary sequence, (b) helical wheel view, and (c) helical net view of the peptide. Hydrophobic residues are circled.

The peptide was synthesized by solid phase methodology on Rink resin using N-terminal Fmoc protection.[8] The porphyrin synthesis and the porphyrin-peptide coupling step are outlined in Scheme 1.[9] By synthesizing **2** via the dipyrrylmethane **1**, **2** was the only porphyrin product obtained.[10] Although the yield of porphyrin was only 4%, no separation of porphyrin isomers was required.[11] The coupling step[12] was monitored by RP-HPLC.[13] Integration of the peak for the conjugate product at both 220 nm and 415 nm suggested a yield of about 60%. The isolated yield after RP-HPLC purification was about 30%. The ESI-MS spectrum of the purified conjugate showed two peaks at m/z, 786.5 and 1178.3, which correspond to $(M+3H)^{+3}$ and $(M+2H)^{+2}$ ions. The product maintained the characteristic porphyrin UV/vis spectrum,[14] yet the material was soluble in water with as little as 10% (v/v) acetonitrile (MeCN) or trifluoroethanol (TFE) added. Finally, amino acid analysis gave ratios consistent with the desired product,[14] and a peak corresponding to the expected modified cysteine residues was observed.



Scheme 1

Circular dichroism spectroscopy was used to examine the peptide secondary structure content of the conjugate (Figure 2). The conjugate displays the classic double minima at 208 nm and 222 nm indicative of an α-helical structure. The conjugate helicity, as judged by the molar ellipticity per residue at 222 nm, increased between 10% and 15% TFE and then remained fairly constant at about 70% helical. A large induced band in the Soret region is the other prominent feature observed in the CD spectra. This band eventually disappeared as the percentage of TFE was increased. Similar behavior was observed in buffer/MeCN and buffer/DMF

mixtures. Also, at lower TFE percentages the Soret band in the UV/vis spectrum was broadened and shifted.[15] This behavior suggests face-to-face aggregation of porphyrin rings in low percentages of organic solvent.[16] This observation is consistent with the amphiphilic nature of the conjugate.

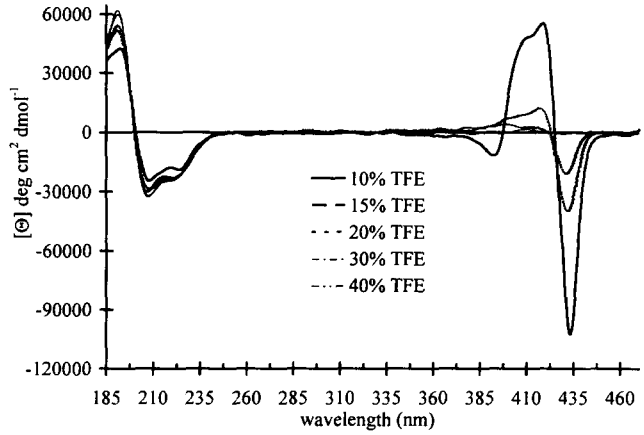


Figure 2: CD spectra of the porphyrin-peptide conjugate in 10 mM phosphate buffer (pH 7) with variable percentage TFE. The conjugate concentration in all samples was 7.0 x 10^{-6} M.

Finally, 500 MHz ^{1}H NMR spectra were recorded in 50% (v/v) TFE-d_3 in D_2O for both the conjugate and the peptide (Figure 3). As the peptide should be positioned close to the porphyrin ring, the chemical shifts of the central amino acid residues should be shifted upfield due to the porphyrin ring current. As expected, the eight leucine methyl groups, which should lie over the porphyrin ring, were particularly affected. Protons near the ends of the peptide, like the methyl groups of the alanines and N-terminal acetyl group, were not perturbed.

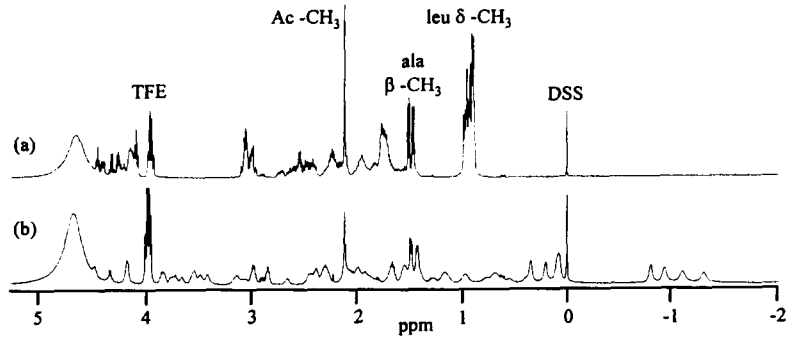


Figure 3: ^{1}H NMR spectra of the (a) peptide and (b) porphyrin-peptide conjugate. Both spectra were recorded in 1:1 TFE-d_3 / D_2O with DSS as an internal standard.

In conclusion, a novel porphyrin-peptide conjugate has been designed and prepared. Preliminary characterization is supportive of that design. Experiments examining the catalytic behavior of the metallated conjugate are currently in progress.

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8. The RP-HPLC purified peptide gave an appropriate ESI-MS, $(M+2H)^{+2}$ = 816.3, and amino acid analysis, Q,E(4) = 3.8, A(2) = 2.0, C(2) = 2.0, L(4) = 3.9, K(2) = 2.0.
9. The product from each step gave satisfactory NMR spectra, mass spectra, and UV/vis spectra. The NMR spectra of **2** and **3** were identical to published spectra.[11]
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12. Porphyrin (10 μmol) and peptide (10 μmol) were dissolved separately in 45 ml of dry, deoxygenated DMF. The two solutions were added over 3 hours to 100 ml of dry, deoxygenated DMF containing 50 mg of sodium carbonate. The reaction was under argon and shielded from light at all times.
13. The HPLC conditions were as follows: 20 minute linear gradient of 40% $MeCN_{(aq)}$ 0.1% TFA to 80% MeCN. A Microsorb-MV, C4 analytical column was used at a 1 ml/min. flow rate. The detector was set at 415 nm. The conjugate elutes at about 75% MeCN and **4** elutes at about 70% MeCN.
14. An extinction coefficient for the Soret band was estimated from amino acid analysis using a valine internal standard. In 50% (v/v) MeCN / water, λ_{max} = 414 nm and $\varepsilon = 4.2(5) \times 10^5\ M^{-1}\ cm^{-1}$. Amino acid analysis ratios were: Q,E(4) = 3.5, A(2) = 2.0, L(4) = 3.8, K(2) = 2.0.
15. At 10% TFE / buffer λ_{max} = 430 nm, 15% TFE / buffer λ_{max} = 418 nm, 20% TFE / buffer λ_{max} = 415, >30% TFE / buffer λ_{max} = 414 nm.
16. In order to probe the nature of the aggregate, sedimentation equilibrium molecular weight determination was performed.[17] In 50% DMF / buffer, an apparent molecular weight (2300) in accord with monomeric conjugate was obtained. At 20% DMF, the conjugate simply pelleted during centrifugation. While that result indicated that aggregation is occurring, the aggregate may be more complicated than a simple face-to-face dimer. All experiments were done at conjugate concentrations equal to those used in CD studies.
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