Copper-Driven Assembly of a Helical-Peptide-Strapped Zinc Porphyrin

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Numerous strapped and capped metalloporphyrins have been prepared for the investigation of ligand binding and oxygenative catalysis as related to heme proteins and enzymes. In these systems, the strap or cap, which is covalently attached to the porphyrin's periphery, generates a substrate-binding cavity at the porphyrin metal center.[1,2] Several laboratories have also investigated peptide−porphyrin assemblies that are designed to be closer analogs to the native heme systems.[1c,3] To the best of our knowledge, no peptide−porphyrin assembly that is analogous to the strapped systems has been described in the literature. Here, we present the preparation of a novel, water-soluble peptide−porphyrin assembly (assembly **I**) in which a conformationally stable peptide is strapped over the face of a metalloporphyrin *without* coordination of a peptide side chain to the porphyrin metal center. A high-yield preparation of the strapped molecule is accomplished by a copper-driven self-assembly process that links the porphyrin to the peptide while simultaneously stabilizing peptide secondary structure.

Assembly **I**, [Zn(Por1)]-Cu_2-Pep1, incorporates a helical peptide strapped over the face of a modified zinc porphyrin, $[Zn(Por1)]^{4-}$ (Figure 1). The α-helical structure in the 22-residue peptide[4] (Pep1) is stabilized by coordination of the copper(II) ions to two His-X_3-His sequences (Figure 1). Several research groups have shown that α-helical structure can be stabilized through the binding of a His-X_3-His sequence to a single metal ion in a bidentate fashion.[5,6] The sequence of Pep1 is designed such that the four histidine side chains can bind two metal ions on the same side of the helix.[7] Molecular modeling indicates that coordination of α-helical Pep1 to two metal ions would place the metal ions 16 Å apart. Thus, $[Zn(Por1)]^{4-}$ was also designed to bind two metal ions at a distance of ∼16 Å. Water-soluble $[Zn(Por1)]^{4-}$ is based upon 5,10,15,20-*meso*-tetraphenylporphyrin, in which the 5 and 15 phenyl groups have iminodiacetate (ida) groups appended at their *meta* positions via methylene bridges. The ida group is ideal since it is well known to form stable metal−ligand complexes via tridentate coordination,[8] thus leaving coordination sites open for metal−histidine coordination.[5c,d,9]

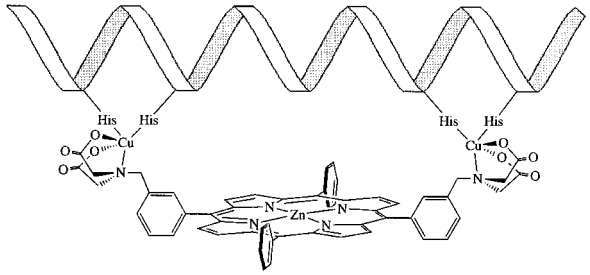

Figure 1. Upper: diagram of assembly **I**. Lower: sequences of peptides Pep1 and Pep2.

The preparation of assembly **I** is accomplished by the addition of 2 equiv of copper(II) to an equimolar solution of Pep1[10] and $[Zn(Por1)]^{4-}$ [11] in 20% (v/v) 2,2,2-trifluoroethanol (TFE)/50 mM phosphate buffer (pH 7). The solution is lyophilized and the peptide−porphyrin assembly is purified by size-exclusion chromatography;[12] isolated yields exceed 90%. A 1:1 peptide: porphyrin stoichiometry was determined from analysis of purified **I**;[13] a spectrophotometric titration also indicates that the binding stoichiometry is 1:1.[14] The monomeric nature (i.e., 1 peptide/1 porphyrin) of the assembly is confirmed by size-exclusion chromatography[15] and electrospray mass spectrometric analysis.[16] EPR spectroscopy indicates that the copper coordination environment in assembly **I** is reasonably assigned as $[Cu(ida)(His)_2]$ as shown in Figure 1.[17] The remarkable yield of **I** demonstrates that the 1:1 peptide−porphyrin complex is the thermodynamically stable product in 20% TFE/buffer. Assembly **I** is a water-soluble molecule that is stable at room temperature in the solid state or in neutral-pH aqueous buffers.[18]

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(7) Lysine glutamate salt bridges are included to provide water-solubility and additional helical stabilization: (a) Lyu, P. C.; Marky, L. A.; Kallenbach, N. R. *J. Am. Chem. Soc.* **1989**, *111*, 2733. (b) Marqusee, S.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8898.

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(10) Crude samples of Pep1 and Pep2 were obtained from PeptidoGenic Research and Co., Inc. (Livermore, CA), purified by HPLC, and characterized by MALDI MS; the purity was judged to be >98% by analytical HPLC.

(11) The zinc porphyrin is isolated as the potassium salt, $K_4[Zn(Por1)]$. Experimental details describing the synthesis of $K_4[Zn(Por1)]$ are available in the Supporting Information.

(12) Purification is accomplished using a Pharmacia Superdex Peptide HR 10/30 size exclusion column (50 mM phosphate buffer, pH 7.

(13) Analysis of **I** entailed flame AA spectroscopy to determine the zinc content and amino-acid analysis (ninhydrin) to determine the peptide content.

(14) A titration of Pep1 into a 20% TFE/50mM phosphate buffer (pH 7) solution containing 1 equiv of $[Zn(Por1)]^{4-}$ and 2 equiv of copper(II) was monitored at 432 nm and indicates that 1 equiv of peptide is bound per porphyrin.

(15) Assembly **I** elutes as a tight band using size-exclusion chromatography, with an apparent MW of 4000 (50 mM phosphate buffer, pH 7; Pharmacia Superdex Peptide HR 10/30 column). MW_{calcd} for $ZnCu_2C_{153}H_{190}N_{40}O_{36}$: 3358.

(16) Negative-ion electrospray MS provides the unit mass of assembly **I**: $MW_{obsd} = 3356$ for $(M - 2H)^{2-}$ (m/z 1678); MW_{calcd} for $ZnCu_2C_{153}H_{190}N_{40}O_{36} = 3358$.

(17) Frozen EPR spectra of assembly **I** ($g_\perp = 2.06$, $g_\parallel = 2.25$, $A_\parallel = 176$ G; 50 mM phosphate buffer, pH 7) resemble those of model complexes with $[Cu(ida)(im)_2]$ (im = imidazole) coordination: Campos, A. C.; Busnot, A.; García, M. E. A.; Zafra, A. G. S.; Pérez, J. M. G.; Gutiérrez, J. N. *Inorg. Chim. Acta* **1994**, *215*, 73.

(18) Assembly **I** is typically stored at 4 °C in 50 mM phosphate buffer (pH 7). When stored under these conditions, no noticeable decomposition is observed over periods of months.

S0002-7863(97)00761-0 CCC: $14.00

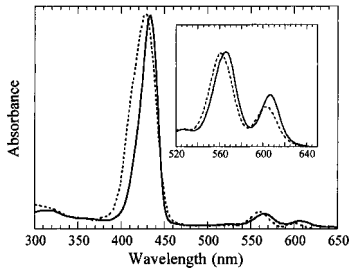

Figure 2. Absorption spectra: (—) assembly **I**; (···) assembly **II**. Spectra were recorded in 50 mM phosphate buffer, pH 7 at 22 °C.

Although TFE is required for the high-yield preparation of **I**, it is not required to stabilize the assembly after addition of copper. The TFE may play a dual role in the preparation by inducing helical structure in the peptide[19] prior to copper binding, while also preventing porphyrin aggregation.

To determine whether the peptide in assembly **I** is strapped across the face of the porphyrin and linked on both sides (closed), we prepared assembly **II** ([Zn(Por1)]-Cu_2-Pep2) using a peptide (Pep2) with only one His-X_3-His sequence (Figure 1). In assembly **II**, the strap is open since the peptide can only be coordinated to copper on one side of the porphyrin. The absorption spectra of assemblies **I** and **II** are shown in Figure 2. In comparison to **II**, the B (Soret) band of **I** is significantly sharper[20] and red-shifted by 4 nm.[21,22] The sharpness of the B band is indicative of a well-defined porphyrin environment in **I**, consistent with a closed strap structure. The broader B band of **II** is attributed to spectral heterogeneity arising from various orientations of the peptide strap relative to the porphyrin. Furthermore, the red-shifted B band of **I** is consistent with the more hydrophobic porphyrin environment expected for the closed strap assembly.[23] To further investigate the assembly process with the two peptides, three spectrophotometric titrations of $[Zn(Por1)]^{4-}$ with copper(II) were carried out: (1) in the absence of peptide, (2) with 1 equiv of Pep2, and (3) with 1 equiv of Pep1.[24] In each case, the spectroscopic data demonstrate that $[Zn(Por1)]^{4-}$ binds to two copper(II) ions,[25] and clean isosbestic behavior is observed. Consistent with the different porphyrin environments expected, the spectral shifts observed in the three titrations vary. In the absence of peptide, the B band of $[Zn(Por1)]^{4-}$ red shifts 2 nm upon addition of 2 equiv of copper(II). With Pep2, the B band red shifts 7 nm, whereas with Pep1, the B band red shifts 11 nm. These titration results provide additional evidence that the closed strap assembly is achieved with Pep1 under the preparative conditions.

The secondary structure of the peptide strap in assembly **I** was investigated using circular dichroism (CD) spectroscopy (Figure 3A).[26] The shape and magnitude of the CD bands clearly indicate a very high degree of α-helicity at room temperature.[27] Interestingly, the CD spectra of **I** are invariant from 0−22 °C (Figure 3A). In contrast, similar alanine-based peptides generally exhibit temperature-dependent CD spectra in this temperature range.[7b,28] In fact, the CD spectra of Pep1 with 2 equiv of $[Cu(bzi)(H_2O)_2]$[29] (bzi = benzyliminodiacetate) are temperature-dependent with an isodichroic point at 204 nm (Figure 3B), consistent with an α-helix−coil equilibrium for Pep1-$[Cu(bzi)]_2$. Estimation of the helical content from the magnitude of the CD bands requires the demonstration of a helix−coil equilibrium. With Pep1-$[Cu(bzi)]_2$, the helical content can thus be calculated (47% at 22 °C and 85% at 0 °C);[30] however, in **I**, the secondary structure is best described as highly helical. It is clear from these CD-temperature studies that the binding of Pep1 to the rigid template [Zn(Por1)]-Cu_2 has a profound effect on the conformations available to the peptide relative to that observed with Pep1-$[Cu(bzi)]_2$.[31] Studies are in progress to further investigate the conformational differences in the peptides of **I** and Pep1-$[Cu(bzi)]_2$.

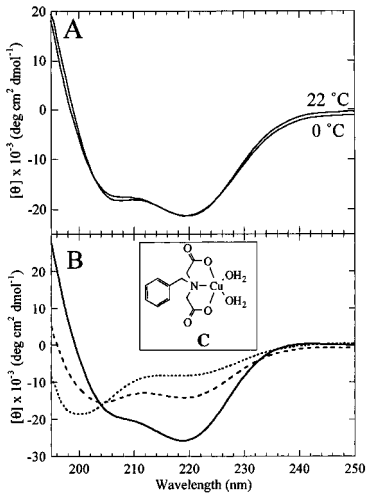

Figure 3. Circular dichroism spectra: (A) Assembly **I** at 0 and 22 °C; (B) (···) Pep1, 22 °C; (- - -) Pep1 plus 2 equiv of $[Cu(bzi)(H_2O)_2]$, 22 °C; (—) Pep1 plus 2 equiv of $[Cu(bzi)(H_2O)_2]$, 0 °C (C); the structure of $[Cu(bzi)(H_2O)_2]$. All spectra were recorded in 50 mM phosphate buffer, pH 7, at 5 μM peptide.

In summary, a high degree of helical structure in the peptide strap of **I** has been demonstrated at room temperature. The conformational stability of the peptide should enable structural control of the pocket above the porphyrin metal center via manipulation of key residues (using natural or unnatural amino acids) in the peptide strap. Extensions of this work to the preparation of double-strapped assemblies and the investigation of assemblies with other metalloporphyrins and with kinetically inert "linker" metal ions are underway in our laboratory.

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Supporting Information Available: Visible CD spectra for assembly **I**, synthetic details for $[Zn(Por1)]^{4-}$, and spectrophotometric titration data (4 pages). See any current masthead page for ordering and Internet access instructions.

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(19) The helix-enhancing effect of TFE is well-known: (a) Cammers-Goodwin, A.; Allen, T. J.; Oslick, S. L.; McClure, K. F.; Lee, J. H.; Kemp, D. S. *J. Am. Chem. Soc.* **1996**, *118*, 3082. (b) Goodman, M.; Rosen, I. G. *Biopolymers* **1964**, *2*, 537.

(20) Spectral width at half the height of λ_{max} (cm^{-1}): **I**, 1240; **II**, 2000.

(21) In 50 mM phosphate buffer (pH 7), λ_{max} (nm): **I**, 434; **II**, 430. The Q-band region in assembly **I** is also red-shifted relative to assembly **II**, λ_{max} (nm): **I**, 566, 606; **II**, 560, 600.

(22) Spectral comparisons of $[Zn(Por1)]^{4-}$ bound to copper(II) in the presence and absence of imidazole demonstrate that the 4 nm B-band shift between assemblies **I** and **II** is not due to histidine−copper coordination.

(23) Sadamoto, R.; Tomioka, N.; Aida, T. *J. Am. Chem. Soc.* **1996**, *118*, 3978.

(24) Spectrophotometric titrations were performed under identical conditions used in the preparation of assembly **I**. Experimental details and spectral data are available in the Supporting Information.

(25) Each binding curve levels off at 2 equiv of copper.

(26) The CD spectra of assembly **I** in the UV are slightly concentration dependent in the range of 2−10 μM. In contrast, the CD spectra of assembly **I** in the visible region are very concentration dependent in the range of 2−10 μM (see the Supporting Information).

(27) (a) Toniolo, C.; Polese, A.; Formaggio, F.; Crisma, M.; Kamphuis, J. *J. Am. Chem. Soc.* **1996**, *118*, 2744. (b) Brahms, S.; Brahms, J. *J. Mol. Biol.* **1980**, *138*, 149.

(28) Ruan, F.; Chen, Y.; Hopkins, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 9403.

(29) Copper(II) *N*-benzyliminodiacetate dihydrate, $[Cu(bzi)(H_2O)_2]$, was prepared according to the synthesis of [Cu(*N*-(4-vinylbenzyl)iminodiacetate)$(H_2O)_2$].[9]

(30) The helical content of Pep1-$[Cu(bzi)]_2$ is calculated using a value of $[\theta]_{220}$(100% helix) = −31 000 deg cm^2 $dmol^{-1}$ for Pep1 (determined in TFE/H_2O solutions at 0 °C).

(31) Temperature-independent helicity from 10−60 °C has been observed in a short alanine-based peptide: Kemp, D. S.; Allen, T. J.; Oslick, S. L.; Boyd, J. G. *J. Am. Chem. Soc.* **1996**, *118*, 4240.