Peptide Helix Induction in a Self-Assembling Hemoprotein Model

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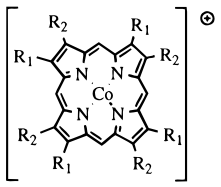
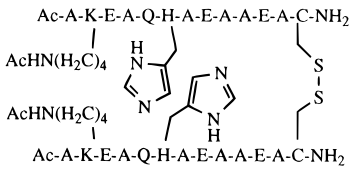
Delineating the forces which impart stabilization to protein structures is a subject of great current interest.[2] Designed peptides which fold into predicted three-dimensional structures provide confirmation that these forces are being properly interpreted and applied.[3] Work from several laboratories has demonstrated that simple conformational constraints can induce short monomeric peptides to adopt helical conformations.[4] We recently employed this stratagem to generate hemoprotein models in which histidine (His) to iron coordination in peptides covalently linked to iron(III) mesoporphyrin IX leads to substantial helix induction.[5] Incorporation of helical peptides into hemoprotein models[6] is desirable due to the ubiquitous occurrence of this conformational motif in heme binding pockets of natural hemoproteins. Herein we introduce hemoprotein models in which helix induction is observed upon simple complexation of cobalt(III) coproporphyrin I (**1**) or Co(III) octaethylporphyrin (**2**) by cystine-dimerized peptide **3**.[7] This



1: R_1 = Me; R_2 = $(CH_2)_2CO_2H$
2: $R_1 = R_2$ = Et



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self-assembly[10] process is analogous to reconstitution reactions of apomyoglobin[11] and apocytochrome b_5[12] with heme, in which a variety of noncovalent interactions between protein and heme lead to enhanced protein conformational stability. A predicted three-dimensional structure of the complex formed from **2** and **3** is shown in Figure 1.

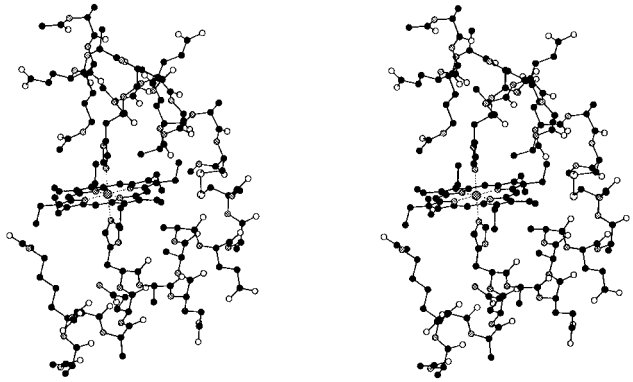

Figure 1. Energy-minimized structure of the proposed complex between **2** and cystine-bridged peptide **3** (**5**), created using the Sybyl suite of molecular modeling programs.[8] Atom colors: carbon (black); nitrogen (gray); oxygen (white); sulfur (speckled). Hydrogen atoms have been omitted for clarity.

The side-chain torsional angles adopted by helix-residing His residues which are ligated to a porphyrin metal determine the orientation of the peptide relative to the porphyrin.[13] With the energetically favorable side-chain torsional angle combination $\chi_1 \approx 180°/\chi_2 \approx -90°$,[13] the angle between the helix axis and the porphyrin plane is ca. 30° (Figure 1), and the C-termini of the peptides make van der Waals contact with the heme. If two such helical peptides are coordinated to opposite sides of a metalloporphyrin, amino acids at position $i + 7$ relative to the coordinating His residues are in close spatial proximity beyond the porphyrin periphery. Molecular modeling[14] suggested the intriguing possibility that a disulfide linkage between two cysteine (Cys) residues in these positions might serve as a conformational trap which could help stabilize helical conformations in the peptides upon complexation of a metalloporphyrin. In contrast to our previously reported peptide-sandwiched mesoheme (PSM),[5] this conformational change would occur in the absence of amide linkages between the peptides and the porphyrin. Because Fe−His bonds are exchange-labile,[15] we explored the use of Co(III) porphyrins, which form six-coordinate, diamagnetic, exchange-inert complexes with imidazoles.[16] Two porphyrins were utilized: **1** (which is water soluble in its tetracarboxylate form)[17] and water insoluble **2**, which can be prepared by oxidation of the commercially available Co(II) form (CoOEP).[18]

Formation of bis-imidazole complexes of Co(III) porphyrins can be followed readily by UV/vis spectroscopy, as the Soret band shifts from 409 nm (high-spin Co) to 417 nm (low-spin

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(7) The monomeric peptide was prepared using Fmoc chemistry[8] on Rink resin, followed by cleavage and deprotection with Reagent K.[9] This and subsequent peptides were purified by HPLC on a Vydac C18 peptide/protein column, using a gradient of acetonitrile in aqueous 0.1% trifluoroacetic acid. Brief treatment with iodine in 50% aqueous acetic acid cleanly yielded the cystine linkage. Acetylation of the Lys Nϵ-amines to give **1** was achieved with acetic anhydride in 100 mM pH 10 borate buffer. Each product is pure as determined by analytical HPLC and gives the correct mass by EIMS.

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(17) Compound **1** was prepared by refluxing coproporphyrin I[18] with excess $Co(OAc)_2$ in acetic acid, followed by elution through Sephadex CM-50 to remove excess metal.

(18) Aldrich Chemical Company, Milwaukee, WI.

S0002-7863(96)03747-X CCC: $14.00

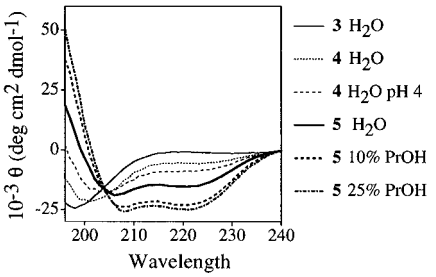

Figure 2. Circular dichroism spectra of **3**−**5**. All samples were buffered to pH 7 with 5 mM potassium phosphate buffer, except as indicated.

Co).[19] Using these spectroscopic changes, a titration of **1** (4 μM) with **3** in 10 mM pH 7 phosphate buffer (Figures S1 and S2, Supporting Information) showed that saturation binding is achieved with 1 equiv of **3**. CD spectroscopy of the 1:1 complex **4** revealed that complexation is accompanied by a modest alteration of the peptide conformation (Figure 2). The increased ellipticity at 220 nm (θ_{220}), and the shift of the band at 195 nm to 202 nm in **4** suggested that the new conformation contained ca. 15% α-helix.[20] Using θ_{220} as an indicator of complexation, a titration of **3** (8 μM) with **1** again indicated that complex **4** has 1:1 stoichiometry (Figure S3, Supporting Information).

At neutral pH, complex **4** has an overall charge of −9. This suggested that electrostatic repulsion between glutamate side chains in the peptides and the propionate groups of the porphyrin may adversely affect peptide helix induction. Reducing the pH from 7 to 4 increased helix content to 30% (Figure 2) without disturbing the Co−His bonds (demonstrated by UV/vis; not shown), strongly supporting this hypothesis. The conformation of uncomplexed peptide **3** was unaffected by the pH change (not shown).

Complexation of **2** by **3** (to give complex **5**) cannot be achieved in aqueous solution due to the low solubility of **2** in water. One useful method of preparing **5** in high purity is to stir a DMSO solution of **3** in the presence of solid CoOEP for several days, with concomitant air oxidation of the metal to Co(III).[19] A more rapid method is to add aliquots of CoOEP dissolved in 2:1 hexane/dichloromethane to a solution of **3** in DMSO, followed by evaporation of the more volatile solvents to prevent peptide precipitation. Dilution of the DMSO solution from either reaction with 10 mM pH 7 phosphate buffer, followed by chromatography on Sephadex CM-50 resin (to remove DMSO and unreacted CoOEP), leads to a wine-red solution. The red color in the aqueous solution suggested that a complex had indeed formed. UV/vis spectroscopy demonstrated that the complex contained exclusively low-spin Co(III) porphyrin.[19] Analytical HPLC (C4) indicated one predominant product with only minor impurities, which was subsequently purified to homogeneity by preparative HPLC (Figure S4, Supporting Information) and analyzed by electrospray ionization (ESI) mass spectrometry operating in the positive ionization mode (Figure 3; Figure S5, Supporting Information). A 1:1 stoichiometry for the complex can be demonstrated by the isotope spacing of the doubly charged peaks at $m/z = 1706.8$ $(M + H)^{2+}$ (Figure 3) and 1725.7 $(M + K)^{2+}$ (Figure S5, Supporting Information). Because HPLC indicates that the sample of **5** is pure, the free peptide observed in the spectrum in Figure S5 (Supporting Information) presumably arises via decomplexation in the mass spectrometer.[21]

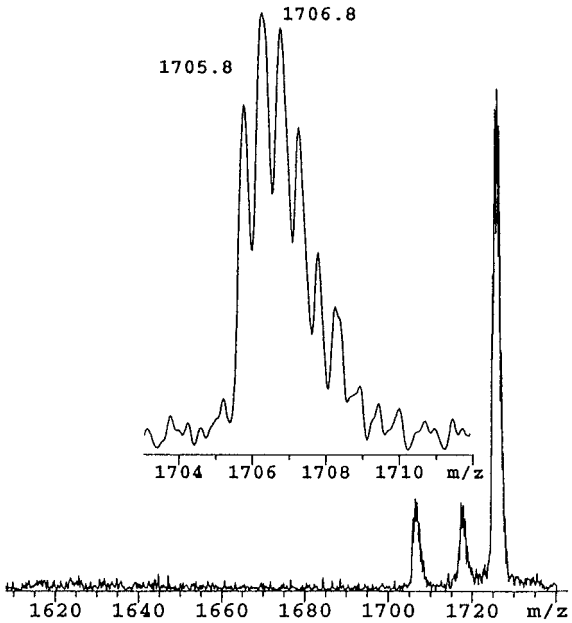

Figure 3. ESI mass spectrum of **5**. The following clusters arise from the complex: $m/z = 1706.3$ $(\mathbf{5} + H)^{2+}$, 1717.7 $(\mathbf{5} + Na)^{2+}$, and 1725.7 $(\mathbf{5} + K)^{2+}$. Corresponding triply charged clusters are also observed for **5**. See also Figure S5, Supporting Information.

CD spectroscopy demonstrates that formation of complex **5** is accompanied by markedly higher helix induction (ca. 55%) than was observed with **4** in neutral aqueous solution (Figure 2). This is similar to the helix induction observed in the PSM, wherein the peptides are attached to the porphyrin via amide linkages.[5] Two factors contributing to the increased helicity in **5** vs **4** are the smaller overall charge on the former (−5) and different alkyl groups on the porphyrin β-positions. Helix content in **5** can be increased (to >90%) by addition of organic cosolvents such as 1-propanol (Figure 2).

In conclusion, we have developed self-assembling hemoprotein models in which peptide helix induction arises solely as a result of metal−ligand bond formation. The simple and rapid method of assembly should allow us to readily prepare many variants with which to probe the influence of peptide sequence and of porphyrin structure on peptide conformational stability.

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Supporting Information Available: Figures S1−S5 (3 pages). See any current masthead page for ordering and Internet access instructions.

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(20) Ellipticity was determined as reported in ref 5. Concentration of the low-spin Co(III) complexes was determined by UV/vis using an extinction coefficient of 2.0×10^5, measured using a DMF solution of the bis-ImH complex of $[CoOEP]^+$.

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