Peptide Complexes of Metalloporphyrins

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Considerable efforts using both experimental[1] and theoretical[2] approaches have contributed greatly to the understanding of the factors and forces that stabilize protein structures[3]. One approach that has yielded tremendous insight is that of *de novo* protein design[4]: the design of stable tertiary structures that have little sequence homology to known proteins. Within the last 15 years, both _-helical[5,6] _-sheet[7], and more complex mixed domain topologies[8], have been designed. Initially the designs demonstrated progress in making desired secondary structures[9]. The problem, however, was in producing systems which showed "native-like" properties (i.e. those characteristics seen in natural proteins, exhibiting singular solution conformation). Several reports have recently shown that it is possible to produce designed systems with "native-like" properties [10,11].

Our objective is to understand the role of the porphyrin in contributing to the structure of proteins. To understand this role, we have carried out work directed toward designing small oligo-peptides (15-34 amino acids) which not only bind to metallo-porphyrins, but also form _-helical structure in the presence of the porphyrin. Our prototypical sequence, $AcGAKAAKAHAKAAKAGNH_2$, contains several elements necessary for heme-induced helix formation[12]. The central histidine, H8, was intended to bind to a water-soluble metalloporphyrin, Fe^{III}Coproporphyrin-I, shown in Figure 1. Figure 2 shows a helix wheel[13]

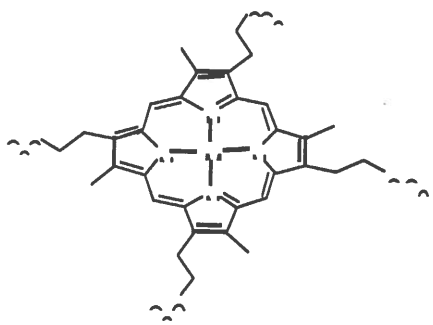

Figure 1

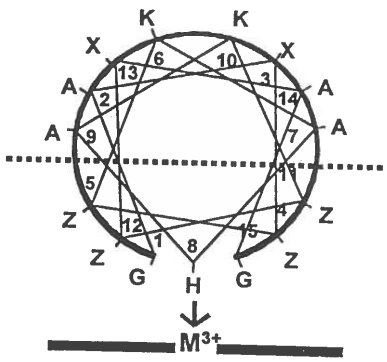

Figure 2

representation of this peptide. In this convention, the reader is looking down the helical axis, about 4-turns. In our design of an amphiphillic helix, one face of the helix is composed of polar residues, like lysine, which promote water solubility. The other face is composed of hydrophobic residues (Z) that are composed of aliphatic and aromatic hydrocarbons, designed to from stable hydrophobic interactions[14] with the porphyrin. Alanine, (or Aib(a-amino isobutyric acid)[15] in position 2,7,9,and 14) was implemented to promote helix formation.

Initially a library was constructed that contained a variety of putative hydrophobic residues (ala, norvaline, leu, and phe). It was found that the Aib containing peptides, having putative phenylalanines bound almost 6000x tighter than histidine. This observation provided

strong evidence for a hydrophobic interaction between the porphyrin and the peptides. Circular dichroism studies indicated that the amount of secondary structure formed was equivalent to 5 residues in a helical conformation (~30%). Measurement of redox potentials revealed that the complexes became increasingly difficult to reduce as their stability is increased. To study the structure of the porphyrin bound peptides, an exchange-inert metalloporphyrin Ru^{II}Coproporphyrin-I (CO) (RuCopro) was employed. Binding affinities for the peptides to the RuCopro revealed that all of the complexes, regardless of putative residues were highly stable (>3Kcal/mole). CD analysis revealed helicities of almost 40%. NMR analysis revealed that the peptide is only structured in the presence of the porphyrin. In addition, inclusion of a salt bridge (between i,i+4 glutamates and lysines) or addition of the helix forming solvent TFE, appears to stabilize more secondary structure by increasing the number of turns in the helix from 2 to 3. Small addition (0.1-equivalents/ addition) of porphyrin stock solutions to the peptide allowed for the analysis of folding stability. The data indicate that the engineered salt bridged system (E3K; E13K) is about 1.4 Kcal/mole more stable than the intial system, consistent with other studies[16]. Studies of a small library indicate that linear and aromatics side-chains interact favorably with the porphyrin, _-branched peptides do not.

To improve the binding affinity of the peptides to the porphyrin, disulfide bridges were added to either the N-terminus (tweezers) or both N and C termini (cyclic) of the 15-mer, to produce disulfide cross-linked dimers[17]. Binding affinities of the cyclic and tweezers system are substantially higher than the 15-mer precursors. CD studies show that the tweezers and cyclic systems are 70 and 90% helical, respectively. NMR studies reveal that the 15-mers form a high spin (S=5/2), mono-peptide heme complex, before saturation. The cyclic systems on the other hand bind the peptides cooperatively, with the complex always being low-spin (S=1/2).

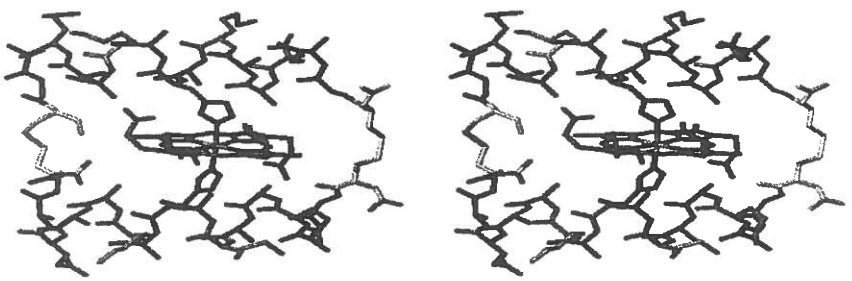

Figure 3

Structural studies, using multidimensional, high-resolution[18] NMR have allowed for the determination of a solution structure (Figure 3.) The structure is a cyclic peptide (Cyclic-3) complexed with Co^{III}Coproporphyrin-I. Cyclic-III has the sequence-(CGAE AAKAHAKAAEAGC)$_2$-. The salt bridges between E3:K6 and K10:K13 have been shown, by binding and CD studies to stabilize the folded complex. The structure reveals that the helices are tilted so that they are maximally separated from each other. Thermal unfolding studies reveal cooperative thermal unfolding, typical of small native proteins. Chemical denaturation studies in the presence of urea indicate the unfolding of the Co^{III} complex of Cyclic-I and Cyclic-III is on the order of 3 Kcal/mole. These numbers are confirmed by folding studies, obtained from analysis of CD titration data.

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