Metalloporphyrin - Oligopeptide Complexes

David L. Huffman Final Seminar October 15, 1993

The study of synthetic analogs of heme proteins has been thorough and successful [1-5]. In spite of many accomplishments, however, there remains a fundamental difference between the heme proteins themselves and the small molecules that mimic them: the analogs are generally not made from peptides, and the interactions between the heme and the protein remain largely unexplored. The few exceptions are some digestion products of cytochrome *c*, in which the heme is covalently linked to a short oligopeptide [6], and some synthetic complexes of short oligopeptides covalently attached to the periphery of a metalloporphyrin [7-10]. It is direct metal ligation to polypeptides, however, that holds together most heme proteins. This work provides the first example of direct ligation of polypeptides to a metalloporphyrin to create synthetic heme-peptide complexes.

Little is known about the factors that influence oligopeptide binding to metalloporphyrins in heme proteins. The preparation and characterization of metalloporphyrin-oligopeptide complexes as models of heme proteins address these factors. We are interested both in the influence that the metalloporphyrin has on the properties of the oligopeptide (*e.g.*, structural perturbations) and in the effect that the oligopeptide has on properties of the metalloporphyrin (*e.g.*, ligand binding, redox potentials).

To this end, we have begun the design of synthetic heme proteins by examining the coordination of simple, 15-mer oligopeptides to a synthetic metalloporphyrin, iron(III) coproporphyrinato(I) (Figure 1a). The design of the peptide sequences utilized computer molecular modeling, CPK models, and helix wheel models. In a helix wheel, residues are positioned every 100° around a circle, since one turn of an α-helix is 3.6 residues long.

Ligation from the oligopeptide was provided by the imidazole of histidine, a common ligating residue in heme proteins. The remaining residues of the sequences were chosen for their helix-forming propensity or for their side chain hydrophobicity. The following residues were utilized for their strong intrinsic helix forming ability [11-13]: Ala, Aib, Leu, Lys, Nva. Residues which were used to probe the hydrophobic interaction were: Ala, Leu, Nva, Phe. Solubility of the oligopeptides was provided by positioning lysine residues at every third or fourth position in the sequence. The sequence termini were capped to optimize the helix dipole [14-17]. A linear sequence (Figure 1b) and the corresponding helical wheel (Figure 1c) show the relative residue positions in a putative α-helix when ligated to metalloporphyrin.

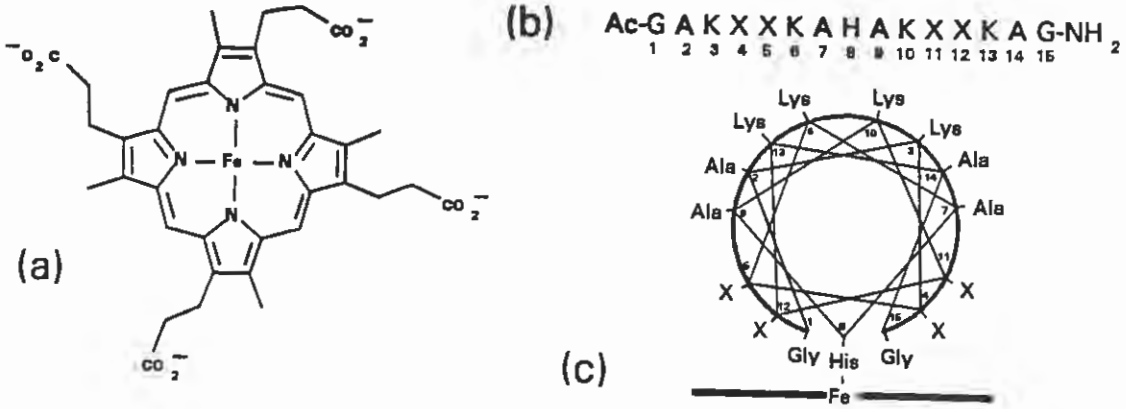

Figure 1. (a) Structure of Fe(Copro-I); (b) sequence of 15-*mer* peptide; X, hydrophobic residue; A, Ala; G, Gly; K, Lys; H, His; (c) helical wheel display of sequence in (b), only one axial ligand shown for simplicity.

Spectrophotometric titrations of the 15-*mer* peptides with Fe(III)(Copro-I)Cl in aqueous, buffered solution (500 mM MOPS, pH 7) were performed and equilibrium constants were determined according to methods described by Suslick [18]. The observed spectra (Figure 2a) and titration data analyses are consistent with the formation of 2:1 oligopeptide-metalloporphyrin complexes with ligation via the imidazole of the histidine. Control titrations indicate that identical spectral changes are seen when histidine alone is bound to Fe(III)(Copro-I).

The equilibrium constants for binding the oligopeptides to Fe(III)(Copro-I) increase as a function of side chain hydrophobicity. In fact, binding constant enhancements of greater than 5000-fold are observed relative to the binding of histidine alone. We attribute the enhanced binding to hydrophobic interactions between the hydrophobic side chains of the amphiphilic peptide and the metalloporphyrin face. Hydrophobic interactions are thought to play a major role in stabilizing heme proteins [19].

The hydrophobic effect is described as the gain in free energy upon transfer of non-polar residues from an aqueous environment to a non-polar environment [20]. The magnitude of the hydrophobic effect has been estimated to be 2.4 kcal/nm^2 [21]. In our system, the surface area of the metalloporphyrin is approximately 1.0 nm^2 per face; thus, the decrease in free energy upon interaction with an oligopeptide should be 2.4 kcal per face of the metalloporphyrin, to give a total of 4.8 kcal. Utilizing the relationship, $\Delta G = -RT\ln K$, and normalizing to the binding constant for histidine, we predict that $K / K_{His} \sim 3100$ for complete overlap of two hydrophobic oligopeptides with the metalloporphyrin faces. We observe that K / K_{His} ranges from 28 (X = Ala, Figure 1b) to 5800 (X = Phe, Figure 1b). Therefore, hydrophobic interactions play a dominant role in the stabilization of the metalloporphyrin-oligopeptide complexes.

In addition, structural changes associated with binding of the oligopeptides to Fe(III)-(Copro-I) were investigated by circular dichroism spectroscopy (CD). Optimum concentrations of oligopeptide and Fe(III)(Copro-I) for CD were determined by solving the equilibrium expression. Experimental constraints limit the amount of helix-induction which can be observed, and the spectrum obtained (Figure 2b) represents the helical content of both bound and unbound peptides. Nonetheless, a definite increase in helical content is observed for all of the peptides in the presence of Fe(III)(Copro-I), based upon the mean residue ellipticity at 222 nm [22]. In all cases the induced helicity is more than doubled.

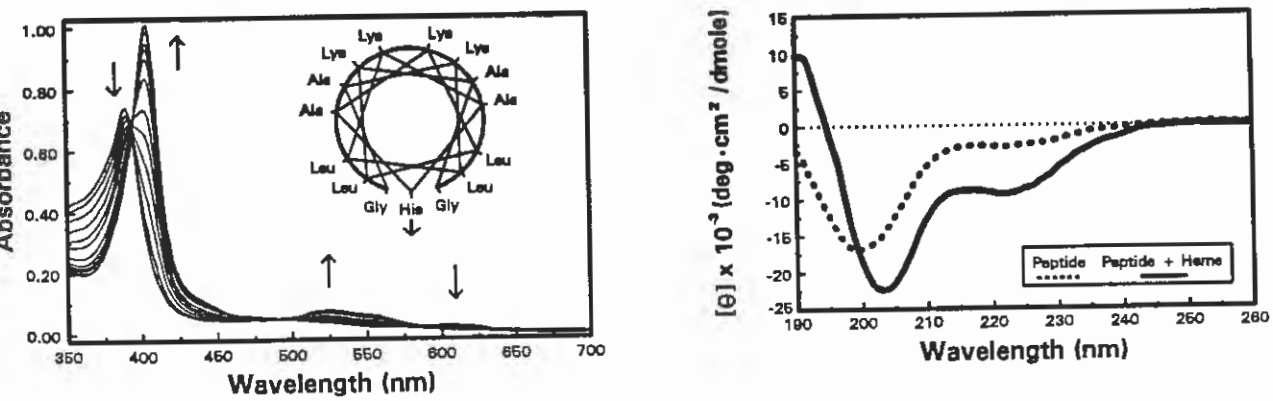

Figure 2. (a, left) Spectrophotometric titration of Fe(Copro-I) with inset sequence, where X (Figure 2a) is Leu; (b, right) heme-induced helix formation for same peptide.

Cyclic voltammetry (CV) was performed to determine the reduction potentials for the metalloporphyrin-oligopeptide complexes. The reduction potentials measured were much

more negative than that reported for cytochrome b_5 by 200-300 mV [23]. The reduction potentials of heme proteins are thought to vary as a function of heme solvent-accessibility [24,25]. In our complexes the heme is very solvent accessible, as determined from a Connelly surface of an idealized complex [26]. The measured reduction potentials for other water-soluble iron porphyrin complexes corroborate our results [9,27,28]. There is an interesting trend in reduction potentials of our metalloporphyrin-oligopeptide complexes: the reduction potentials decrease as the binding constant increases. Thus, the tightest binding oligopeptides favor the ferric state.

References

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