Hydrophobic Interactions in Metalloporphyrin−Peptide Complexes

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Received April 13, 1999

The study of synthetic analogues of heme proteins has been thorough and successful.[1] Despite many accomplishments, however, there remains a fundamental difference between the heme proteins themselves and the small molecules that mimic them; the analogues are generally not made from peptides, and the interactions between the heme and the protein remain largely unexplored.[2] The few exceptions are small covalently linked heme−oligopeptides digestion products derived from cytochrome *c*,[3] short oligopeptides covalently linked to the periphery of porphyrins or coordinated to exchange-inert metalloporphyrins,[4] the elegant helical bundle peptide−heme complexes of DeGrado, Dutton, and co-workers,[2,5] and our recent work on amphiphilic peptide−metalloporphyrin complexes.[6] Studies of this "*meso-molecular*" regime (i.e., from roughly 1000 to 10 000 amu) represent an emerging field that has only recently become synthetically and analytically accessible. In this paper, the direct ligation of polypeptides in synthetic heme−peptide complexes has been characterized. To this end, we have examined the influence of peptide length, sequence, and properties on their complexation to a water-soluble Fe(III) porphyrin.

Recent success in the design of peptides[2] has provided insight into the design of synthetic proteins. Synthetic heme proteins require additionally the integration of a ligating residue into the peptide sequence and the establishment of favorable nonbonding interactions between the metalloporphyrin and the peptide. In addition to metal ligation of amino acid residues, secondary nonbonding interactions may also be important because the heme is sandwiched tightly between two or more helices.[7]

Binding of series of peptides[8] by the Fe^{III} complex of coproporphyrinate-I (3,8,13,18-tetramethyl-2,7,12,18-tetrapropionateporphyrinate, copro) was determined by spectrophotometric titration.[9] Fe^{III}(copro) was chosen because it has a symmetrical distribution of four carboxylates, which increases its solubility and substantially decreases aggregation. Ligand binding studies indicate in all cases that only 2:1 peptide−metalloporphyrin complexes are formed with bisimidazole coordination. The data are shown in Table 1 and Figure 1.

To probe the importance of hydrophobic interactions between the peptides and the porphyrin face, the effects of multiple alanine residues (a strong α-helix former)[10] were examined. Histidine was utilized as the ligating residue in our peptides, analogous to the globins, peroxidases, and *b* cytochromes. Solubility of the peptides was provided by Glu or Ser residues and by *not* end-capping the peptides, thereby having charged amino and carboxy termini. Despite the possibility of helix−turn−helix motifs[11] in our longest peptides,[12] no clear examples of 1:1 binding were observed. In retrospect, this is perhaps not surprising because conformational demands strongly disfavor formation of 1:1 complexes.

Relative binding constants increase by a factor of 1.6×10^4 as the peptide length increases. The cause of this dramatic increase in relative binding cannot be explained by the imidazole ligation to the metal center, which remains constant. Other factors that might contribute to the stability of the complexes include (1) electrostatic interactions between the porphyrin and the peptide, (2) hydrogen bonding between the porphyrin and the peptide, and (3) hydrophobic interactions between the porphyrin and the peptide. Electrostatic interactions are not likely to be favorable in these metalloporphyrin−peptide complexes, since the overall charge is negative for both the metalloporphyrin and the ligand for peptides **5**−**7**. Hydrogen bonding will not explain the systematic trend observed in Table 1 and Figure 1. For the short sequences, hydrogen bonding is structurally untenable to the heme's propionate side chains. For the longer sequences, if the peptides are helical when bound, again hydrogen bonding is precluded. In peptides **5**−**7**, the asparagine or cysteine side chains may possibly hydrogen-bond to the propionate side chains of the metalloporphyrin, but this would not explain the overall trend.

Both these peptides and the π-face of the metalloporphyrin are highly hydrophobic. We therefore suggest that hydrophobic interactions influence peptide binding to heme and are the primary factor responsible for the 1.6×10^4 increase in binding that we observe. As shown in Figure 1, as the number of hydrophobic residues increases, the binding constants increase substantially.

The hydrophobic effect (i.e., gain in free energy on the transfer of nonpolar residues from an aqueous environment to a nonpolar environment) has provided a major unifying concept in under-

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(12) Peptides **4**−**7** were originally designed in hopes of creating 1:1 complexes with a helix−turn−helix motif, with potential coordination of two histidines.

10.1021/ic990405o CCC: $19.00
Published on Web 11/04/2000

Table 1. Peptide Sequences and Equilibrium Constants for 2:1 Complex Formation with Coproporphyrin-I-atoiron(III)

compd	ligand[a]	K (mM^{-2})[b]	K/K_{His}	no. of hydrophobic residues[c]
	His	0.025	1.0	0
	1-MeIm[d]	0.18	7.1	
1	SS**H**A	0.12	4.7	1
2	SSA**H**ASS	0.32	12.5	2
3	SS**H**AAA	1.8	71	3
4	SS**H**AAAAFGPGGFAAAAHSS	55	2200	10
5	EAA**H**AAAAAFNGPGGNFAAAAAHAAE	200	7800	16[c]
6	EAA**H**AAAAAFCNGPGGNCFAAAAAHAAE	430	16 900	16[c]
7	EAA**H**AAAAAAFNGPGGNFAAAAAAHAAE	400[e]	16 000	18

[a] Abbreviations: 1-MeIm, 1-methylimidazole; A, alanine; C, cysteine; E, glutamic acid; F, phenylalanine; G, glycine; **H**, histidine; N, asparagine; P, proline; S, serine. [b] Spectrophotometric titrations were performed at 298.0 ± 0.2 K in 10 mM aqueous phosphate buffer at pH 7.0 with Fe^{III}(copro) at ~60 μM. In a typical titration, aliquots of concentrated buffered peptide were added to a cuvette containing the buffered Fe^{III}(copro). Equilibrium constants were determined from at least three separate titrations from data collected from at least four separate wavelengths (chosen for the largest changes in absorbance). Care was taken to permit thermal and ligation equilibration, and good isosbestic behavior was observed. Formation of μ-oxo dimers was not observed with coproporphyrin-I-atoiron(III) under these conditions. Peptide aggregation did not compete with heme ligation under the conditions used for data analysis. Errors are estimated to be less than ±10%. [c] The number of hydrophobic residues is simply taken as the sum of ala and phe residues; other residues (e.g., cys) can also make minor contributions to hydrophobicity. [d] 1-MeIm is neutral, whereas His is zwitterionic at pH 7. The hydrophobic effect is therefore larger for 1-MeIm than for His, accounting for the difference in K. [e] Errors are estimated to be ±30% for this binding constant because of limited peptide solubility.

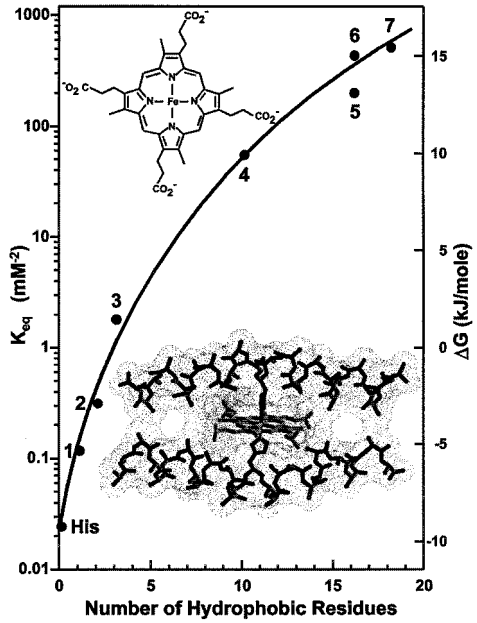

Figure 1. Equilibrium constants for 1:2 complex formation between coproporphyrin-I-atoiron(III) and various peptides as a function of their hydrophobicity, in aqueous solutions at pH 7.0. The number of hydrophobic residues was taken as the sum of alanine and phenylalanine, the strongly hydrophobic residues present in these peptides. The upper insert shows the chemical structure of coproporphyrin-I-atoiron(III). The lower insert shows the molecular mechanics model of the hydrophobic contact between the nonpolar porphyrin face and a putative α-helix conformation of a bound peptide. The dotted surface represents the water-accessible surfaces.

standing the structure and function of biological systems.[13] Estimates of the amino acid hydrophobicity of ~9.9 kJ/nm^2 of water-excluded surface are generally accepted as the magnitude of the hydrophobic effect. We can calculate the amount of buried surface area to account for the largest change in relative binding constants for this system. The surface area of the metalloporphyrin is approximately 1.0 nm^2 per side; when the matching surface of the peptide is counted, a maximum of about 4 nm^2 of hydrophobic surface area could be hidden because of heme−peptide contacts during the formation of a 2:1 complex. The largest change in relative binding constants that we have observed is for peptides **6** and **7**; this change in K_{eq} of 16 000 relative to His is equivalent to a change in ΔG of 24 kJ/mol. This corresponds to approximately 2.4 nm^2 of buried hydrophobic contact, or about 60% of the total excluded surface possible from heme−peptide contacts.

If the only hydrophobic contacts were between the peptide and the porphyrin, we would expect that the increase in the equilibrium constant would sharply plateau as the peptide becomes larger than the size of the porphyrin face (i.e., at roughly eight hydrophobic residues, assuming α-helical structure).[14] With our longest peptides, however, an additional component of hydrophobic contact may occur: interpeptide contacts beyond the porphyrin periphery, as illustrated in the lower inset of Figure 1. Such interpeptide contacts would be templated by coordination to the metalloporphyrin and increase the apparent ligand affinity. We attribute the continued increase in the equilibrium binding peptides **4**−**7** to this effect. The shape of the curve and the change in slope in Figure 1 are consistent with this hypothesis, since the nature of the hydrophobic interactions changes from heme−peptide to peptide−peptide at about eight hydrophobic residues.

Despite our detailed understanding of the structure and function of both heme proteins and their small molecule analogues, the factors that influence peptide binding to metalloporphyrins in heme proteins remain largely unknown. In this examination of metalloporphyrin binding of hydrophobic, alanine-rich peptides, we find that peptide interactions with the hydrophobic face of the porphyrin play an important role in the stabilization of heme−peptide complexes, consistent with our prior studies of a series of 15-mer amphiphilic peptides.[6] In both systems, the presence of noncoordinating, hydrophobic residues flanking the coordinated histidine dramatically increases peptide binding to the heme by >20 kJ/mol. Thus, hydrophobic interactions between the porphyrin face and hydrophobic amino acid residues can be a major component in the formation and stability of heme−peptide complexes and heme proteins.

Acknowledgment. We thank the support of the National Institutes of Health (HL-25934), Dr. M. D. Ball for assistance in early portions of this work, and Dr. M. M. Rosenblatt for helpful discussions.

IC990405O

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