Model Hemoprotein Reduction Potentials: The Effects of Histidine-to-Iron Coordination Equilibrium

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Equilibrium reduction midpoint potentials of natural hemoproteins are influenced by a variety of factors, including the identity,[2] alignment,[3] and basicity[4] of the axial ligands, nonplanar distortions of the heme,[5] local charges,[6] and proton coupling.[7] Heme solvent exposure makes a particularly large contribution.[8] Specifically, it has been shown that decreasing solvent exposure correlates with increasing reduction potential (greater ease of reduction) due to the energetic penalty of burying formally charged ferric heme in a low dielectric environment.[8a] In this report, we provide evidence that the effects of heme Fe(III) and Fe(II) coordination equilibria effectively compete with the observed solvent exposure trend in hemoprotein models having highly solvent-exposed heme.

Histidine (His) to Fe(III) coordination in $\mathbf{1}^+$ is accompanied by modest peptide helix induction (to ca. 36% from 0%) at 25 °C in aqueous solution as determined by circular dichroism spectroscopy.[9] In $\mathbf{2}^+$, alanine-4 in each peptide chain has been replaced by tryptophan (Trp).[10] NMR experiments on the diamagnetic Co(III) analogue of $\mathbf{2}^+$ have shown that the Trp side chains T-stack with the porphyrin,[10] thereby shielding a substantial portion of the porphyrin from contact with solvent. One result of these interactions is a markedly higher peptide helix content in $\mathbf{2}^+$ (ca. 74%) at 25 °C.[9] The amino acid side chains in $\mathbf{1}^+$ have a smaller hydrophobic surface area than those in $\mathbf{2}^+$ and, hence, the porphyrin environment in $\mathbf{1}^+$ is expected to be more polar.

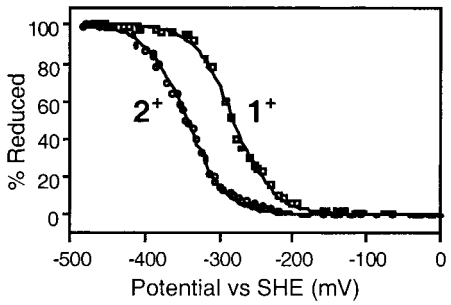


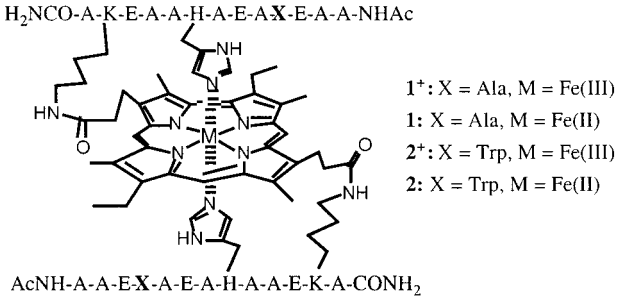
Figure 1. Redox potentiometry of $\mathbf{1}^+$ and $\mathbf{2}^+$ monitored by UV/vis. Conditions: pH 8.0 and 25 °C in 100 mM NaCl, 50 mM Tris/HCl buffer. The curves were fitted to a strict $n = 1$ Nernst equation (solid lines).

Table 1. Potentiometric and Coordination Equilibrium Data

	E_{m8} (mV)	10^5K_{app} (M)	10^7K_a (M)	$10^{-4}\beta_2^{III}$	K_2^{II}
$\mathbf{1}^+$	-281 ± 2	5.01 ± 0.11	3.16 ± 0.16	2.51 ± 0.28	3.0[a]
$\mathbf{2}^+$	-337 ± 6	43.7 ± 1.0	1.74 ± 0.08	631 ± 64	~9[a]

[a] Reference 9.

Based purely on heme solvent exposure precedents in the literature,[8] we therefore expected that the reduction potential of $\mathbf{2}^+$ would be shifted positive relative to $\mathbf{1}^+$.



Data from redox potentiometry[11] studies of $\mathbf{1}^+$ and $\mathbf{2}^+$ in aqueous solution (pH 8.0) at 25 °C are shown in Figure 1, and the results are presented in Table 1. Both reduction potentials are very negative, as is that of bis-imidazole ligated iron mesoporphyrin IX (MPIX; −285 mV at pH 8.5),[12] consistent with the designed bis-His coordination motif in $\mathbf{1}^+$ and $\mathbf{2}^+$ and a high degree of solvent exposure in all three compounds. However, the reduction potentials of $\mathbf{1}^+$ and $\mathbf{2}^+$ do not strictly adhere to the solvent exposure arguments. First, the reduction potentials of $\mathbf{1}^+$ and of the bis-imidazole complex of iron MPIX are essentially identical, despite the presence of hydrophobic peptide−porphyrin packing interactions in $\mathbf{1}^+$[10] which are absent in the MPIX complex. Second, the reduction potential of $\mathbf{2}^+$ is shifted 56 mV negative relative to $\mathbf{1}^+$. This latter result would suggest increased solvent exposure in $\mathbf{2}^+$ relative to $\mathbf{1}^+$, a conclusion at odds with the results of NMR structural studies on the Co(III) analogues of $\mathbf{1}^+$ and $\mathbf{2}^+$.[10] As discussed below, differences in affinity of His for Fe(III) vs Fe(II) in $\mathbf{1}^+/\mathbf{1}$ and $\mathbf{2}^+/\mathbf{2}$ adequately predict the unexpected shift in reduction potential.

The effect of His-to-iron coordination strength on the difference in reduction midpoint potentials of $\mathbf{1}^+$ and $\mathbf{2}^+$ can be determined using eq 1 (derived in Appendix S1, Supporting Information). In eq 1, β_2^{III} is the product of the individual equilibrium constants (K_1^{III} and K_2^{III}) for coordination of the two His ligands to Fe(III) in $\mathbf{1}^+$ and $\mathbf{2}^+$. K_1^{II} and K_2^{II} represent the equilibrium constants

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for binding of the first and second His ligands, respectively, to Fe(II) in **1** and **2**.

$$E_m(\mathbf{1}^+) - E_m(\mathbf{2}^+) = \frac{RT}{nF}\left[\ln\frac{\beta_2^{III}(\mathbf{2}^+)}{\beta_2^{III}(\mathbf{1}^+)} - \ln\frac{K_1^{II}[1+K_2^{II}](\mathbf{2})}{K_1^{II}[1+K_2^{II}](\mathbf{1})}\right] \quad (1)$$

In both $\mathbf{1}^+$ and $\mathbf{2}^+$, the ferric iron center is coordinatively saturated.[10] The absence of high-spin Fe(III) in neutral aqueous solution is demonstrated in both cryogenic EPR and room temperature resonance Raman spectra (Figures S1 and S2), and further supported by the absence of pH-dependent changes in room temperature UV/vis spectra above about pH 5.5. Furthermore, the rR spectra of the two compounds are nearly identical, indicating that perturbation of the Fe(III) porphyrin conformation via nonbonded interactions between the indole side chains of Trp and the porphyrin π system in $\mathbf{2}^+$ is not responsible for the observed reduction potential difference.

We have previously reported results of qualitative pH titrations of $\mathbf{1}^+$ and $\mathbf{2}^+$, which demonstrated that Fe(III)−His coordination is stronger in the latter.[10] Those experiments were performed in 3:1 (v/v) H_2O/CH_3OH, as aggregation was observed to occur below about pH 4 in aqueous solution. For purposes of the present work, we have performed pH titrations of $\mathbf{1}^+$ and $\mathbf{2}^+$ in solutions containing decreasing volume percentage of CH_3OH in H_2O. Fitting of data from each pH titration (see Figure S3) to an equation relating all species that may be present at equilibrium (Appendix S2) reveals that $K_2^{III} > K_1^{III} \gg 1$, as commonly observed for Fe(III) porphyrin model compounds in isotropic solution and consistent with the clean isosbestic behavior of the titrations (Figure S4). Hence the use of β_2^{III} ($=K_1^{III}\cdot K_2^{III}$) in eq 1.

The proton concentration at the midpoint of each pH titration curve (K_{app}) represents the square root of β_2^{III} multiplied by the dissociation constant for protonated, unligated His (K_a) [$K_{app} = K_a(\beta_2^{III})^{1/2}$; Appendix S2]. Because values of K_{app} for $\mathbf{1}^+$ and $\mathbf{2}^+$ in water could not be obtained directly, they were determined from plots of K_{app} versus volume percentage of CH_3OH extrapolated to 0% CH_3OH (Figure S4; Table 1). The K_a values reported in Table 1 were determined by ^{1}H NMR, from pH-dependent changes in the chemical shift of His side chain C−H protons of the peptides used for synthesis of $\mathbf{1}^+$ and $\mathbf{2}^+$ (Figure S5). We propose that these values are relevant to the systems under study because the peptides occupy random coil conformations in the NMR studies as they do in $\mathbf{1}^+$ and $\mathbf{2}^+$ when the His ligands are protonated and uncoordinated. Table 1 lists the values of β_2^{III} for $\mathbf{1}^+$ and $\mathbf{2}^+$ in water, calculated using the relevant values of K_{app} and K_a.

We have recently reported that the reduced compounds **1** and **2** exhibit mono-His/bis-His coordination equilibrium, with the bis-His form slightly favored in aqueous solution at pH 8.0.[9] Values of K_2^{II} for **1** and **2** determined from UV/vis data[9] are listed in Table 1. Because $K_1^{II} \gg K_2^{II} > 1$, we cannot use the product of the individual equilibrium constants in eq 1 as was done for the ferric forms. Experimental difficulties have precluded direct determination of K_1^{II} for **1** and **2**. However, it is reasonable to expect that Trp will exert a similar stabilizing effect on both K_1^{II} and K_2^{II} [e.g. $K_1^{II}(\mathbf{2})/K_1^{II}(\mathbf{1})$ in eq 1 is $\approx K_2^{II}(\mathbf{2})/K_2^{II}(\mathbf{1}) \approx 3$].

The data in Table 1 reveal that Trp side chain−porphyrin interactions stabilize Fe(III)−His coordination to a greater extent than Fe(II)−His coordination. Consequently, eq 1 predicts a 90 mV (2.1 kcal/mol) difference in reduction potentials between $\mathbf{1}^+$ and $\mathbf{2}^+$, with the latter being more negative. The calculated reduction potential difference, based solely on changes in ligand-to-metal coordination equilibria, is larger than the experimentally measured difference (56 mV; 1.3 kcal/mol). Hence, it is less energetically unfavorable to reduce $\mathbf{2}^+$ relative to $\mathbf{1}^+$ than would be the case if ligand-to-metal coordination were the only contributing factor. We propose that the 34 mV (∼0.8 kcal/mol) differential between observed and calculated reduction potentials is primarily due to the smaller extent of porphyrin solvent exposure in $\mathbf{2}^+/\mathbf{2}$ vs $\mathbf{1}^+/\mathbf{1}$, consistent with the established effect of heme solvent exposure on hemoprotein reduction potentials.[8] However, we note that effects due to differences in strength of Fe−His coordination exert the larger influence on reduction potential in this system.

In support of our conclusions, Huffman et al. have recently reported that reduction potentials of intermolecular, bis-His coordinated complexes between peptides and Fe(III) coproporphyrin I are lower than the potential for the bis-His complex of the same porphyrin.[13] The potentials become more negative as His−Fe(III) binding affinity (β_2^{III}) increases. Increasing binding affinity was further correlated with increasing hydrophobic interactions between the ferric porphyrin and amino acid side chains in the untethered peptides. As with $\mathbf{1}^+$ and $\mathbf{2}^+$, porphyrin solvent exposure in these intermolecular complexes is greater than typically occurs in natural hemoproteins.[13]

In the present work, we have provided a quantitative measure of the contribution of His-to-iron coordination to the difference in reduction potentials between two hemoprotein models. The magnitude of this effect, ≈90 mV, is comparable to effects due to local electrostatic interactions (50−80 mV)[8c] and proton coupling (>100 mV)[7] in other systems, but pales in comparison to heme solvent exposure effects (∼500 mV)[8a] and ligand changes (>150 mV).[2c,8c] Thus, the combined results of our studies and those of Hufman et al.[13] suggest two limiting situations. At one extreme, illustrated by the hemoprotein models discussed herein, reduction potential differences are dominated by the relative affinities of the ligands for Fe(II) and Fe(III). Associated changes in porphyrin solvent exposure diminish, but do not fully counter, this effect. At the other limit, exemplified by the electron-transfer hemoproteins,[8] structural changes which decrease heme solvent exposure result in positively shifted reduction potentials. Thus, in hemoprotein mutants with slightly shifted reduction potentials (± 50 mV), changes in strength of metal−ligand coordination may be the operative mechanism.[14] Experiments aimed at further elucidating the delicate balance between heme coordination and solvent exposure will be the focus of future studies in this group.

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Supporting Information Available: Derivation of equations, EPR and rR spectra, and pH titration data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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