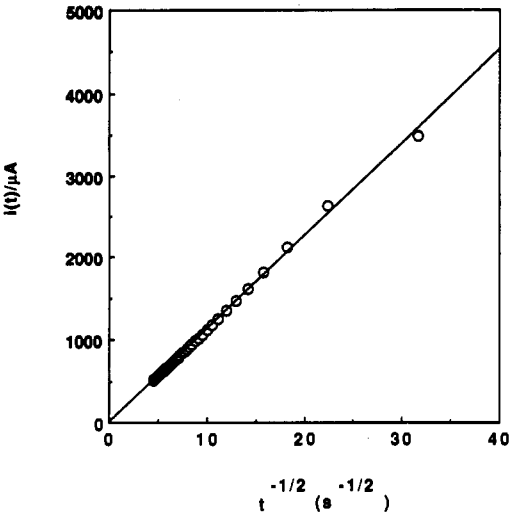

Figure 3. Chronoamperometry of O_2-saturated 0.95:1 melt. Potential step was from 0.0 to -1.2 V vs Al/Al(III) for 50 ms. Other conditions are as in Figure 1.

The fact that O_2 is capable of oxidizing $FeCl_4^{2-}$ in the melt shows that there is a rather large overpotential for its reduction at the GC surface. This is analogous to the behavior observed in aqueous media. Our laboratory has noted previously that O_2 is capable of oxidizing species with $E^{\circ\prime} \leq 1.4$ V vs Al/Al(III) in acidic melts, e.g., $Ru(bpy)_3^{2+}$ (bpy = 2,2'-bipyridine).[13] While we have not determined the exact value of $E^{\circ\prime}$ for the O_2/products couple in the basic melt, it must be more positive than +0.267 V vs Al/Al(III).

Double-potential step chronoamperometry, for steps to potentials negative of the CSV peak of Figure 1 (-1.2 V vs Al/Al(III)) gave linear Cottrell plots ($i(t)$ vs $t^{-1/2}$), for step times between 10 and 250 ms. A typical result is shown in Figure 3. The current axis intercepts for reduction were essentially zero in these cases. No reoxidation current was observed for the reverse potential step (i.e., stepping from -1.2 to 0.0 V) at the pulse times investigated. Potential steps were performed at a potential negative enough that interference from heterogeneous electron-transfer kinetics should be precluded. However, we found that integration of the current-time curve (e.g., to quantify adsorption) gave charge $-t^{1/2}$ plots with significantly negative charge intercepts. This indicates that there may still be some kinetic complication to charge transfer, even at -1.2 V. The diffusion coefficient of O_2 in the melt, D, was determined as 1.5 (±0.2) × 10^{-4} cm^2/s (based on a 4 e^- transfer, e.g., $O_2 + 4e^- + 4HCl \rightleftharpoons 2H_2O + 4Cl^-$) and $[O_2]$ = 0.63 mM). This is an unusually large value for a small, diffusing species in the melt (absolute viscosity of 0.95:1 melt = 20.6 cP). From the Stokes-Einstein relation, using an aqueous diffusion coefficient of 1.0 × 10^{-5} cm^2/s,[14] we have estimated D in the melt as 4.8 × 10^{-7} cm^2/s (25 °C).

The large, apparent value of D suggests that the mechanism of O_2 reduction in the melt probably involves regeneration of O_2, e.g.[15-17]

$$O_2 + e^- \rightleftharpoons O_2^{\bullet-} \quad (2)$$

$$O_2^{\bullet-} + HCl \rightarrow HO_2^{\bullet} + Cl^- \quad (3)$$

$$2HO_2^{\bullet} \rightarrow O_2 + H_2O_2 \quad (4)$$

where O_2 evolved in disproportionation of the perhydroxyl radical can be reduced further at the electrode surface. Such a reaction scheme is typical of the chemical step accompanying O_2 reduction in aprotic solvents containing Brønsted acids.

Attempts were made to conduct voltammetric experiments in the absence of protons, in order to stabilize the $1e^-$ reduction product, $O_2^{\bullet-}$.[18,19] Protons can be removed from basic melts by heating at 75 °C under vacuum.[20] Removal of protons was confirmed by the absence of the characteristic voltammetric wave for proton reduction at ca. -0.2 V using a Pt electrode. However, we found that during the course of saturating the melt with O_2, proton was introduced to the extent of about 1 mM. This was sufficient to preclude stabilization of superoxide. Given the experimental problems encountered with fouling of the electrode (necessitating its removal from the cell for polishing after each voltammetric scan), it is not possible to maintain a rigorously proton-free melt, over the time required to collect data.

Acknowledgment. The work at SUNY was supported in part by SDIO/IST and managed by the Office of Naval Research; the work at the University of Mississippi was supported by the National Science Foundation.

Registry No. ImCl, 65039-09-0; O_2, 7782-44-7; $AlCl_3$, 7446-70-0; H^+, 12408-02-5; $AlCl_4^-$, 17611-22-2; Fe, 7439-89-6; carbon, 7440-44-0.

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Heme Rotational Isomerism Is Not Required for the Production of Q-Band Splitting in the Spectra of Iron-Porphyrin Proteins‡

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Received June 19, 1990

The phenomenon of heme orientational isomerism is currently a topic of some interest and is often functionally significant in heme proteins.[1-11] It has recently been reported[12] that splitting of the α band (the $Q_{0,0}$ band) in the optical absorption spectra of some reconstituted-heme proteins is due to the presence of superimposed spectra arising from rotational isomers of the porphyrin in the protein. These studies were performed exclusively with non-iron porphyrins, and it was concluded that the proximal histidine can adopt "two different coordination modes" depending on the orientation of the porphyrin in the protein, this conclusion being extended to iron heme proteins as well. While the conclusions presented by the authors indeed appear applicable to closed-shell non-iron porphyrins, another interpretation[13,14] must be considered regarding the observed splitting of the α band in certain native-heme (iron porphyrin) protein derivatives. Figure 1 depicts the spectra of the $Fe^{III}NO$ derivatives of two heme proteins: *Glycera dibranchiata* hemoglobin major component (Hb_c),[13-15] a monomeric hemoglobin with the distal (E7) substitution His → Leu,[16] and equine myoglobin (Mb), which possesses the archetypal histidine distal residue.[17]

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‡ Abbreviations: $Hb_c^{III}NO$, nitrosyliron(III) form of *Glycera dibranchiata* monomeric hemoglobin major component; $Mb^{II}CO$, carbonyliron(II) form of equine myoglobin.

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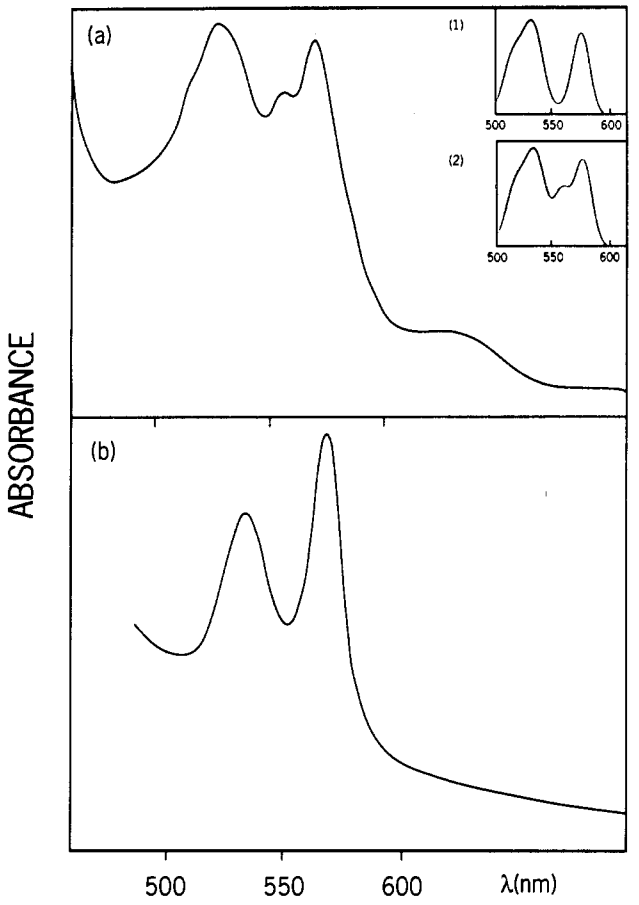

Figure 1. The α and β band region in the electronic absorption spectra of (a) $Mb^{III}NO$ and (b) $Hb_c^{III}NO$, each in 0.1 M KH_2PO_4 buffer, pH = 7, at 20 °C. The absorbance scales for the spectra are similar but not identical. The insets depict the simulated spectrum of $Mb^{III}NO$ assuming similar Gaussian line widths for the two heme isomers' absorption band components and component ratios of (inset 1) 95:5 and (inset 2) 60:40. Similar results are obtained by using Lorentzian line shapes.

The relevant feature regarding the spectra of these proteins is the differing appearances of the β and particularly the α bands for the spectra of the nitrosyliron(III) derivatives. The optical spectrum of $Hb_c^{III}NO$ shows a sharp, unsplit α band of greater intensity than the β band, while $Mb^{III}NO$ displays a weaker, broadened, and split α band. The observed splitting of the α band in the spectrum of $Mb^{III}NO$ has been attributed to nondegeneracy of the $e_g(\pi^*_{x,y})$ orbitals of the porphyrin resulting from d_{xz}, d_{yz} nondegeneracy traced to the stereochemical influence of the distal histidine.[13-15] The absence of splitting in the α band of the $Hb_c^{III}NO$ spectrum is consistent with the absence of a distal histidine in this hemoglobin. A similar, though less pronounced splitting is seen for $Mb^{II}CO$, again in contrast with the $Hb_c^{II}CO$ analogue.[13] In both derivatives, the structural feature of the iron coordination leading to the interaction with the distal histidine is the putative linearity of the Fe–X–O moiety. Moreover, both adducts are considered to entail closed-shell (t_{2g}^6) hemes.[13]

The implication[12] that the observation of split α bands signals the presence of heme rotational isomers needs to be examined in the case of iron–porphyrin proteins in light of the available data concerning heme rotational disorder in such native heme proteins and the previously proposed insensitivity of the optical spectra to heme orientation.[18] Both Hb_c[1-4] and Mb[9,19-24] are reported to have primarily one heme rotational isomer, although the respective orientations of the porphyrins in each protein are opposite: Hb_c has 90–95% of the "inverted" heme rotational isomer,[1-3] while Mb is similarly ca. 95% isomerically pure, as the "normal" isomer.[5,23,25] In spite of this, the spectrum of $Mb^{III}NO$ shows a split α band. With regard to $Mb^{III}NO$, it seems unlikely that a 5% component would lead to such an intense shoulder on the α band. Indeed, simulation of the 500–600-nm region of the spectrum for $Mb^{III}NO$ shows that in order to duplicate the experimentally observed split α spectrum, one must assume either that the line width of the minor component α band is much less than that of the major component or that the percentage of the minor component is of the same order of magnitude as the major component. The former scenario would be difficult to justify, while the latter is in discord with the reported proportions of heme rotational isomers for Mb.[5,23,25] Inset 1 of Figure 1 shows the simulated spectrum of $Mb^{III}NO$, generated by using similar line widths for two heme rotational isomer components' absorption bands with a rotational isomer ratio of 95:5. Inset 2 shows the simulated spectrum for $Mb^{III}NO$ assuming similar line widths for the two heme rotational isomer absorption band components but with an isomer ratio of 60:40. In the simulated spectra, the wavelengths of the component transitions were optimized to best reflect the experimentally observed spectrum of $Mb^{III}NO$. It is apparent from Figure 1, that a heme rotational isomer ratio of 60:40 would need to be employed in order to duplicate the experimental spectrum through a simulation that attributes the splitting to heme rotational isomers; this seems unrealistic in terms of what is currently known for the protein. In addition, the suggestion[12] that the lack of α band splitting in the spectrum of deoxy-Mb may be caused by the presence of one isomer in excess appears to be inconsistent with the marked splitting of the α band observed in the spectrum of the also nearly isomerically monodisperse $Mb^{III}NO$. Contrary to previous indications,[26] recent work[27] has shown that in native-heme Mb (yellowfin tuna) the precise value of the heme orientational equilibrium constant is dependent to a 4-fold factor on the iron's oxidation state and distal ligand. The values for the equilibrium heme orientation isomer compositions of $Mb^{III}NO$ and $Hb_c^{III}NO$ have not been precisely determined experimentally, but their Kuhn anisotropy spectra[13] demonstrate

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unequivocally that the dominant heme orientations are the same as those of their other derivatives, including the aquamet proteins precursory to the iron(III) nitrosyls.[15] In order to account for the different band shapes among the above derivatives, the rate of any ligand-dependent heme reorientation would need to be fast relative to the time required to prepare an adduct and obtain its optical spectrum. This seems unlikely: when horse heart myoglobin is reconstituted[15,28] from apoprotein by using Fe(CO)(PPIX) at pH 7 (μ = 0.2 phosphate, 1 atm CO, 20 °C), the subsequent equilibrative reordering of the heme from its initially randomized orientational distribution may be followed by circular dichroism spectroscopy and is observed to occur with a first-order rate constant of only $(3.9 \pm 0.2) \times 10^{-7}$ s^{-1}. Although the reorientation rates are coordination-dependent[25] (with high-spin Fe(III) presumably having a lower activation barrier than low-spin Fe(II) hemes), all reorientations so far observed are very slow processes, the fastest having half-lives of a few hours.[29,30] Furthermore, when the slow nitrosyl autoredox reaction is inhibited by hexacyanoferrate(III), the optical spectra of $Mb^{III}NO$ and $Hb_c^{III}NO$ formed from NO and the aquamet proteins are stable in the 10^{-2}–10^{6}-s time scale.[13,31] Finally, the spectrum of oxymyoglobin shows no splitting of the α band; perturbations of the α band in myoglobin are intrinsically dependent on the nature of the distal ligand.

Therefore, we conclude that the splitting of the α band in iron heme proteins cannot readily be explained by the presence of different heme rotational components. Clearly, distal effects play an important role in determining the appearance of the α band in iron–porphyrin proteins.

In conclusion, although porphyrin orientational isomerism may lead to splitting of the optical absorption spectra of heme proteins reconstituted with non-iron porphyrins, the reverse logic does not apply for natural (iron) hemes.

Acknowledgment. We thank Drs. Gray and Cowan for helpful discussion.

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Physical Properties of a Manganese Tetramer with All-Oxygen Coordination

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Received July 13, 1990

The role of manganese in photosynthetic water oxidation is under intense study.[1] Since crystallographic data for the active site of photosystem II (PS II) are not available, current strategies include synthesis of model complexes whose properties correlate with existing physical data for the natural system.[2-5] Recent studies of PS II, particularly by EPR and X-ray spectroscopy,[1] have led to a greater understanding of the structure of the manganese water oxidation catalyst and have created a growing need for polynuclear manganese model complexes having suitable coordination geometries and oxidation states. The current data available for the natural system indicate that four manganese atoms are present with two distinct metal–metal separations of 2.7 and 3.3 Å[6-8] and that the coordination environment is primarily O donor in character.[9] Protein sequence data and indirect evidence for the location of the Mn binding site[10] are consistent with the primary protein-derived ligands being carboxylate groups; manganese coordination is completed by water-derived oxo, hydroxo, or aqua ligands.

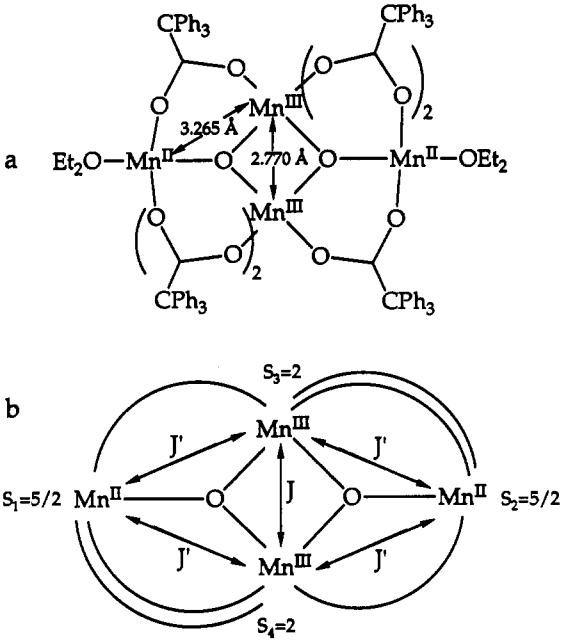

Figure 1. (a) Structure of complex **1**, $Mn_4O_2(O_2CCPh_3)_6(OEt_2)_2$. (b) Vector-coupling scheme used for the analysis of the temperature-dependent magnetic susceptibility of **1**.

Recently, we reported the structure of a tetranuclear manganese complex ($Mn_4(\mu_3\text{-}O)_2(O_2CCPh_3)_6(OEt_2)_2$ (**1**)) consisting of a central planar core of metal atoms (two Mn^{II}, two Mn^{III}) bridged by two μ_3-oxo ligands (Figure 1a).[11] The remaining ligands are carboxylate and Et_2O, leading to an all-oxygen coordination environment for each manganese center. As we have pointed out,[2b]

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