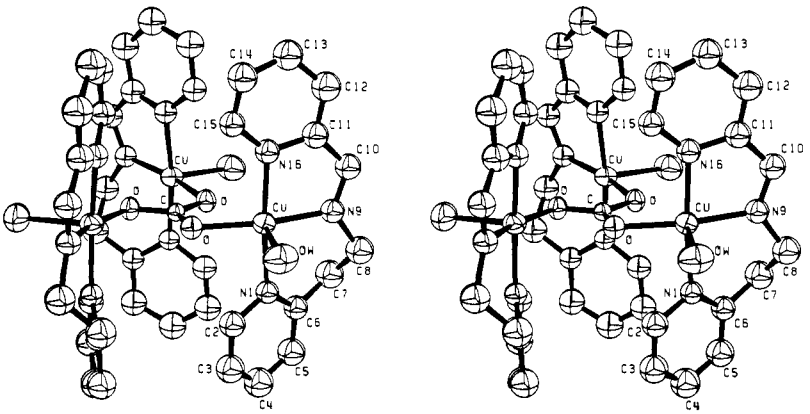

Figure 1. Stereodrawing of the structure of II showing the 35% probability thermal ellipsoids. Hydrogen atoms are omitted. Selected bond distances (in Å; esd ~0.02 Å) are: C–O, 1.294; Cu–O, 1.956; Cu–OW, 2.25; Cu–N1, 2.04; Cu–N9, 1.96; Cu–N16, 2.00. Selected bond angles (in degrees, esd ~0.5°) are: C–O–Cu, 109.2; OW–Cu–O, 97.5; N9–Cu–N1, 92.1; N9–Cu–N16, 81.8; N1–Cu–N16, 172.4. Atoms Cu and O lie −0.06 and 0.42 Å, respectively, out of the best plane fit through N1, N9, and N16. The Cu–Cu distance is 4.63 Å, and each Cu atom has a 2.69-Å contact with another O atom of the CO_3^{2-} group. Only one of the two orientations of the disordered $Cu(pip)(H_2O)^{2+}$ moieties is shown.

The carbonate group[12] lies on a crystallographically required $\bar{6}$ symmetry axis. The $Cu(pip)(H_2O)^{2+}$ moieties (I) are oriented approximately perpendicular to, and are of necessity disordered across, the plane of the carbonate group.[10,13] A water molecule is weakly bound to a fifth, axial, site of each copper atom while an oxygen atom of the carbonate group occupies the space at the other axial position. Hydrogen bonding from the coordinated water molecule to the lattice nitrate groups links units of II into sheets at $z = {}^1/_4, {}^3/_4$, with each unit being associated with six others.

The disposition of Cu atoms about the carbonate group in $[Cu(pip)(H_2O)]_3(CO_3)(NO_3)_4$ is similar to that found for the mineral azurite, $Cu_3(OH)_2(CO_3)_2$.[14] In azurite, the carbonate group is a triply bridging, tridentate ligand with hydroxide ions further linking the $Cu_3CO_3^{4+}$ units into an infinite network.

Magnetic susceptibility data were obtained for both the nitrate and perchlorate salts of II over the range $1.6 \leq T \leq 290$ K by using the Faraday method. Plots of $\chi_M^{cor}T$ vs. T showed ferromagnetic coupling within the $[Cu(pip)(H_2O)]_3CO_3^{4+}$ cations and a very weak intercation antiferromagnetic interaction,[15] perhaps mediated by the hydrogen-bonding linkages described above. Molar susceptibility expressions were derived by assuming isotropic intracluster exchange ($\mathcal{H}' = -2J\sum_{i,j}\hat{S}_i\hat{S}_j$)[16] and allowing for intercluster exchange by the molecular field approximation ($\mathcal{H}'' = -2zJ'\hat{S}^z\langle\hat{S}^z\rangle$).[17] The results are summarized in Table I along with values reported for the polymeric compound $Na_2Cu(CO_3)_2$.[18] The agreement between the magnitude of exchange coupling in the trinuclear and polymeric compounds must be viewed with caution since the bridging carbonate geometries are so different. In the salts of II, the copper atoms interact mainly through the σ system of the carbonate group whereas in the polymer the copper atoms lie 1.64 and −0.38 Å out of the carbonate plane and the superexchange pathway could involve both the σ and π orbitals of the carbonate group.[19]

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(12) Refinement of the bridging ligand as either a carbonate or a nitrate group showed a clear preference for the former as judged by crystallographic significance tests and evaluation of the thermal parameters. The isomorphism and identical magnetic behavior of the nitrate and perchlorate salts of II leave no doubt that carbonate ion is the bridging ligand.

(13) Partial ordering of the $Cu(pip)^{2+}$ orientations about the carbonate group may be responsible for the diffuse reflections of the supercell. Collection of the supercell data is planned, and the details of the ordering will be reported at a later time.

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(15) Zero-field splitting of the cluster quartet state was considered but rejected since it gave a poorer fit to the data and required an unreasonably large value of $|D| = 1.2$ cm^{-1}. No evidence for zero-field splitting occurred in the powder X-band ESR spectrum which consisted of a single symmetric signal at $g = 2.125$ for both complexes.

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(19) **Note Added In Proof**: It has just been reported[20] that in $[Cu\{O(C-H_2CO_2)_2\}]\cdot 0.5H_2O$ the copper atoms, bridged by $-CO_2^-$ groups with geometry similar to that in II, are ferromagnetically coupled with $J = 4.66$ cm^{-1}.

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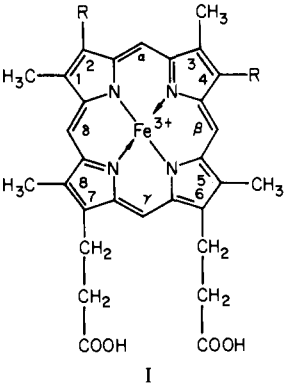
Proton NMR Characterization of Heme Rotational Disorder in Reconstituted Horseradish Peroxidase

Sir:

Elucidation of structure–function relationships in hemoproteins has relied heavily on the comparison of the properties of the native proteins with those of proteins reconstituted with modified porphyrins. The degree that various porphyrins yield different trends in properties in models and in proteins has been interpreted in terms of the nature of the interaction between the 2,4-substituents of I and the apoprotein.[1,2] In particular, deuterohemin (I, R =



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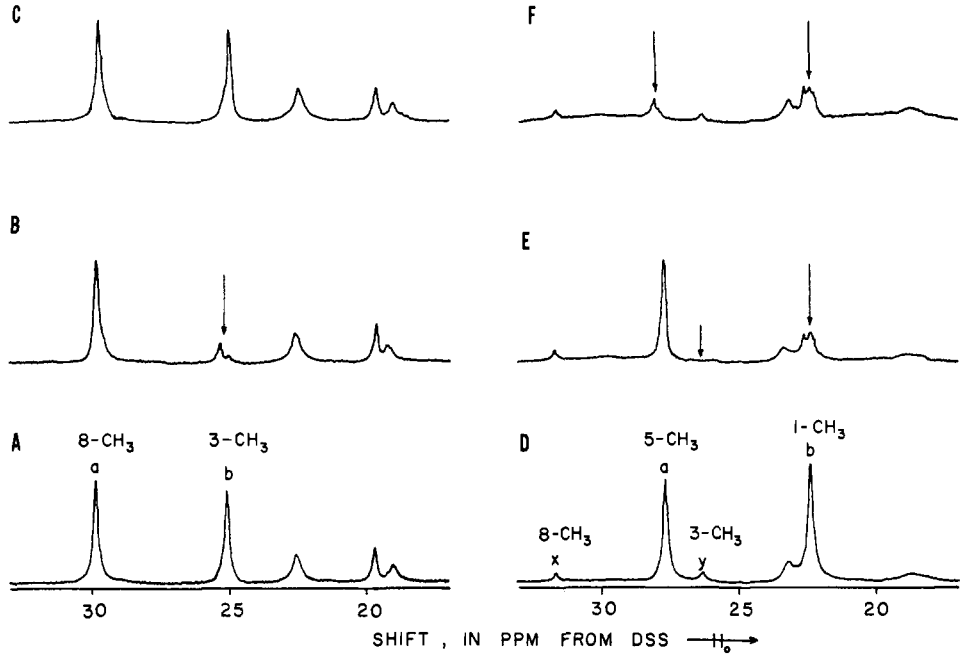

Figure 1. Downfield portions of the 360-MHz proton NMR spectra of native HRP-CN (A), HRP-CN reconstituted with hemin deuterated at methyls-1,3 (B) and methyls-1,5 (C), deuteroHRP-CN (D), and deuteroHRP-CN reconstituted with deuterohemin deuterated at methyls-1,3 (E) and methyls-1,5 (F). Protein concentrations are 0.5–2.5 mm in 0.2 M NaCl–^2H_2O, p^{2}H 7.0, at 35 °C; 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) is the internal calibrant. Reduced methyl intensities due to deuteration are indicated by arrows.

H) has been used extensively to assess the role of the unique 2,4-vinyls in the native protoporphyrins. An implicit assumption in all these comparisons is that the modified and native hemins have the same orientation in the heme pocket. In the case of horseradish peroxidase (HRP), it has been shown not only that the deuterohemin-reconstituted protein (deuteroHRP) exhibits anomalous ligand binding properties[2] when compared to other reconstituted HRP's, but also that the active form compound I, deuteroHRP-I, differs from HRP-I in that the porphyrin cation radical exists in the a_{1u} rather than the a_{2u} orbital ground state.[3,4]

We wish to demonstrate here that a comparison of HRP and deuteroHRP does not reflect simply on the role of the vinyl groups because, although the porphyrin orientation is also essentially unique in deuteroHRP, its orientation differs from that of protohemin in HRP by a 180° rotation about the α–γ meso axis. Our method, outlined in detail elsewhere,[5] relies on the large in-plane asymmetry induced in the unpaired spin distribution in low-spin ferric porphyrins upon incorporation into proteins. This asymmetry results in a large spread in the proton NMR hyperfine shifts for the heme methyls, with the shift reflecting the characteristic environment of a given pyrrole subunit.[5,6] As shown in models and confirmed in deuterohemin reconstituted myoglobin, rotation of the low-symmetry perturbation about the α–γ meso axis results[5] in interchange of environments (i.e., hyperfine shifts) of 1-CH_3 with 3-CH_3 and 5-CH_3 with 8-CH_3.

The downfield portion of the 360-MHz proton NMR spectrum[7] of HRP-CN ($S = {}^1/_2$) (A in Figure 1) is compared with those of HRP reconstituted[8] with protohemin deuterated[9] at methyls-1,3 (B) and methyls-1,5 (C), respectively. This leads to the unambiguous assignment of the two methyl peaks, a and b, to 8-CH_3 and 5-CH_3. The proton NMR trace of deuteroHRP-CN is shown in D of Figure 1, together with those of deuteroHRP-CN reconstituted with deuterohemin deuterated[5] at methyls-1,3 (E) and methyls-1,5 (F), respectively. For deuteroHRP-CN, in addition to the prominent methyl peaks a and b, unambiguously assigned to 5-CH_3 and 1-CH_3, we detect two smaller peaks, x and y, which cannot arise from the major protein form in solution. The observation of two similar satellite peaks near the 2,4-H resonances upfield of DSS with one-third the intensity of x and y (not shown) dictates that x and y are also heme methyls for a minor component ($\sim \leq 5\%$). The deuterium labeling in E and F establishes the origins of x and y as 8-CH_3 and 3-CH_3, respectively. The traces in Figure 1 thus reveal that two forms of the protein exist for deuteroHRP-CN, exhibiting the characteristic interchange[5,10,11] of heme methyl environments indicative of rotational disorder about the α–γ meso axis, and that the *minor* component of deuteroHRP-CN exhibits the same assignment, and hence same heme orientation, as found in native HRP-CN.[12]

We have already shown that several purified native and reconstituted hemoproteins[5,11,13] exist in two forms differing in the rotational position of the porphyrins in the heme pocket. However, in all previous cases the dominant orientation of the porphyrin

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(8) The apoprotein was prepared by the method of M. Yonetani (*J. Biol. Chem.* **1967**, *242*, 5008–5013) and reconstituted according to DiNello and Dolphin.[4] The reconstituted protein was purified by column chromatography on DEAE-cellulose equilibrated in 10 mM Tris-HCl, pH 8.0, to remove excess heme, followed by chromatography on a CM-cellulose column equilibrated in 5 mM acetate, pH 4.4, using 0.05 M NaCl in the equilibrating buffer to elute the protein. The protein was then dialyzed against H_2O and lyophilized prior to redissolving it in ^2H_2O for NMR studies.
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(10) A similar interchange in 1-CH_3 with 3-CH_3 and 5-CH_3 with 8-CH_3 environments is found also in high-spin HRP, deuteroHRP, and HRP-I, deuteroHRP-I.
(11) La Mar, G. N.; Smith, K. M.; Gersonde, K.; Sick, H.; Overkamp, M. *J. Biol. Chem.* **1980**, *255*, 66–70.
(12) The similarity of the methyl shift pattern in HRP-CN and in the monomeric insect HbCN[11] indicates that they have similar heme pocket environments and suggests possibly the same orientations of the proximal histidyl imidazole relative to the porphyrin.[15]
(13) Burns, P. D. Ph.D. Thesis, University of California, Davis, 1980.

was found to be the same as determined by X-ray crystallography,[14-16] although the X-ray data suggested a single orientation. The present deuteroHRP constitutes the first example where the reconstituted protein has essentially the completely reversed heme orientation from that of the native protein.

The different orientations of protohemin and deuterohemin in HRP dictate that a comparison of the two proteins must take into consideration not simply the replacement of vinyl groups by protons, but instead the more complex effects of replacing vinyl by methyl groups and methyl groups by protons in order to rationalize the characteristic differences in physicochemical properties.[2,4] It is now abundantly clear that not only must altered heme orientations be considered in comparing properties of reconstituted hemoproteins but also recognition must be given to the fact that the X-ray crystallographically defined orientation may reflect only one of several orientations adopted in solution.[11] As illustrated here and elsewhere,[5,6,11] proton NMR hyperfine shifts are particularly suited for establishing the presence of variable porphyrin orientations in solution.

The interpretations of the methyl shifts as well as those of other resonances assigned via isotope labeling will be presented elsewhere.

Acknowledgments. We are indebted to the National Institutes of Health (HL-16087, HL-22252) and the UCD NMR Facility for support of this research.

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Structure of Bis(phthalocyaninato)neodymium(III)

Sir:

Since the synthesis of lanthanide(III)-phthalocyanine complexes was established by Kirin and Moskalev,[1] many studies on their compositions and properties have been reported.[2] For instance, intense attention has been directed toward electrochromism of bis(phthalocyaninato)lanthanide(III) complexes, $Pc_2Ln^{III}H$ (Pc and Ln denote a phthalocyanine dianion and a lanthanide cation, respectively). Their electrochromism was first reported by Moskalev and Kirin.[2e] Subsequently, Nicholson and Galiaridi attempted to make electrochromic displays with bis(phthalocyaninato)lutetium(III) and found that a full spectral change of color is generated by adjustment of the applied voltage (from -1.0 to 1.2 V).[3a] They proposed the structure illustrated in Figure 1 for this complex.

With further developments, these electrochromic materials must become useful for full-color imaging and graphic displays, as well as alphanumerics. Recently, Corker et al. investigated the electrochromic behavior of bis(phthalocyaninato)lutetium(III) by using ESR and optical techniques.[4] The structural analysis of bis(phthalocyaninato)lanthanide(III) complexes would provide useful information to investigate their electrochromism and will also be useful to clarify the nature of the complexes. We report here the results of an X-ray diffraction analysis of bis(phthalocyaninato)neodymium(III). The synthesis and purification of the complex were described in a previous paper.[2d] A purple, single crystal of the monosolvated $Pc_2Nd^{III}H$ complex prepared by recrystallization from dichloromethane was used for X-ray analysis. Anal. Calcd for $C_{64}H_{33}N_{16}NdCH_2Cl_2$: Cl, 5.66. Found: Cl, 5.71. Crystal data: orthorhombic; $a = 8.030$ (4), $b = 22.925$ (7), $c = 28.315$ (7) Å; $V = 5212$ (3) Å^3; space group $P2_12_12_1$; ρ(calcd) = 1.60 g cm^{-3}; M_r 1255.2; $F(000) = 2524$ electrons; μ(Mo Kα) = 11.7 cm^{-1}.[5] The neodymium ion occupies a central position between two parallel but staggered (45°) phthalocyanine ligands (Figure 2).

The eight Nd-N bond distances vary from 2.39 to 2.49 Å. While one of the phthalocyanine macrocycles is slightly saucer shaped toward the neodymium atom, the other is planar; angles of tilt range from 2.5 to 7.0°. The pyrrole nitrogen atoms vary from the macrocylic planes by distances ranging from 0.04 to 0.21 Å. The tilted benzimidazole group is an equal distance of 1.47 (1) Å from each of the two least-squares planes containing the four pyrrole nitrogen atoms. An acidic hydrogen in this complex is known to play an important role in the electrochromic properties.[2e] Although an obvious distortion from macrocyclic planarity (13°) is manifest by one benzimidazole moiety, the elusive hydrogen is not directly revealed by the crystallographic results.

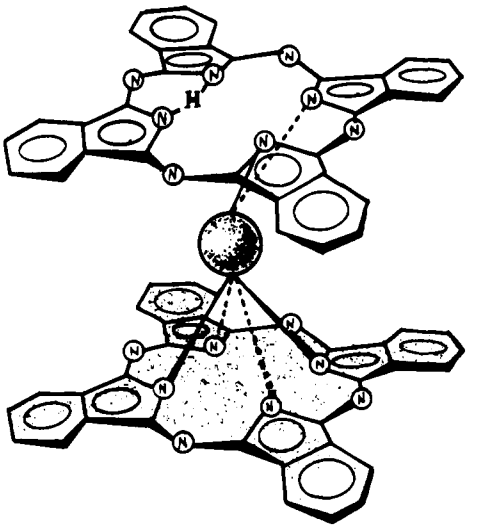

Figure 1. The proposed structure of bis(phthalocyaninato)lutetium(III).[3a]

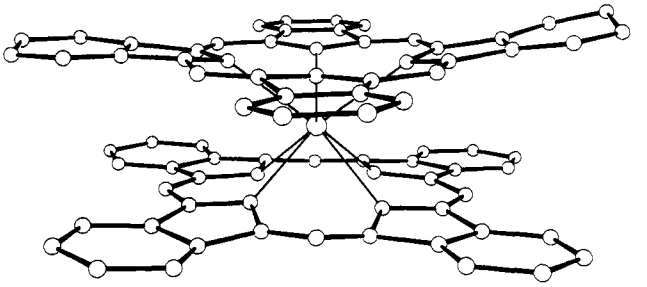

Figure 2. Molecular structure of bis(phthalocyaninato)neodymium(III).

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(5) Intensity data were collected by the Molecular Structure Corporation, College Station, TX 77840. Of the 4736 independent reflections measured, 3505 were classed as being significantly above the background. The structure was solved by the heavy atom method and refined by least-squares calculation to $R = 0.072$.

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