other than fragmentation to alkyl radicals and halide ions should be kept in mind.[17]

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Supplementary Material Available: Derivation of the equation relating cis/trans ratios of 1,2-dimethylcyclopentanes to yields of 2-heptenes and additional description of analytical procedures (2 pages). Ordering information is given on any current masthead page.

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Paramagnetic ^{1}H NMR Spectra of Hemerythrin from *Phascolopsis gouldii*

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Hemerythrin (Hr), a respiratory protein isolated from a number of marine invertebrates, has active sites consisting of binuclear iron centers.[1] Such centers may be prototypical of an emerging class of binuclear iron proteins which include porcine uteroferrin,[2] beef spleen purple acid phosphatase,[2,3] and ribonucleotide reductase from *E. coli*.[4] The binuclear cluster in hemerythrin is known to exist in three formal oxidation states: [Fe(II),Fe(II)] (deoxy); [Fe(III),Fe(II)] (semimet); [Fe(III),Fe(III)] (oxy and met).[1] From a number of physical studies including X-ray crystallography,[1,5] a detailed description of the active site of met- and oxyhemerythrin has been developed. The high-spin ferric centers are coordinated in a confacial bioctahedron by five terminal histidines (two to one iron atom and three to the other) and by bridging oxo and carboxylate groups. The coordination site remaining on the iron atom bound by two histidine ligands is vacant in metHr and occupied by peroxide in oxyHr and by small anions in various synthetic met forms. The two iron atoms are strongly coupled antiferromagnetically ($J \sim -100$ cm^{-1})[6]—a property associated with the ferric oxidation state and the oxo bridge. Considerably less structural information is available for deoxyhemerythrin[7,8] and the mixed-valent forms of the protein. We report here the results of a structural investigation of hemerythrin from *Phascolopsis gouldii* in the met and semimet states using ^{1}H NMR spectroscopy as a probe of the ligand environment and the magnetic properties of the binuclear iron active site.

The ^{1}H NMR spectra of metHr and metHrN$_3$ (Figure 1A,B) feature paramagnetically shifted resonances in the 12–25 ppm region associated with solvent-exchangeable protons and a resonance at 11 ppm associated with nonexchangeable protons. A previous NMR study of this protein failed to observe such features,[9] due to their relative broadness and the use of 75% D_2O as solvent. The exchangeable resonances are assigned to the NH protons of the imidazole groups on histidine ligands. These shifts are small compared to those observed for mononuclear high-spin ferric complexes, where the imidazole NH resonances are observed at ~100 ppm.[10] Since the proton contact shift is dependent on the magnetic susceptibility of the center to which it is attached,[11] the decreased shift observed for these protons in the metHr complexes is consistent with a high degree of antiferromagnetic coupling between the iron atoms. The resonance at 11 ppm is assigned to the β- and γ-CH_2 protons of the bridging aspartate and glutamate, respectively. This assignment is consistent with the methyl resonance for bridging acetate ligands observed at 10.5 ppm in $[Fe_2(HBpz_3)_2(OAc)_2O]$.[12,13] A spectrum obtained for oxyHr closely resembles that of metHrN$_3$.

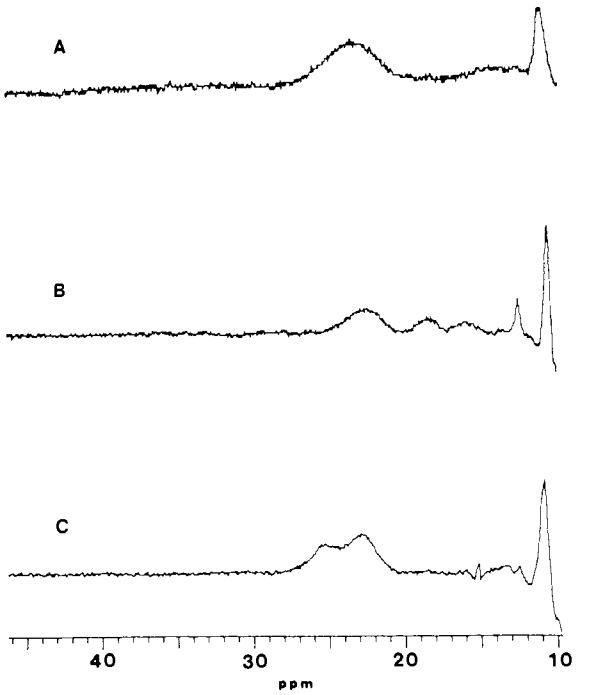

Figure 1. ^{1}H NMR spectra of methemerythrins in 50 mM phosphate buffer, pH 7.5, at 30 °C. (A) 4 mM metHr with added 50 mM $NaClO_4$ (300 MHz). (B) 4 mM metHrN$_3$ (300 MHz). (C) 4 mM metHrS (360 MHz). All spectra were obtained on Nicolet NT-300 and NT-360 spectrometers under conditions described in ref 10.

The spectrum obtained for metHrS (Figure 1C), where the oxo bridge is believed to have been replaced by a sulfido bridge,[14] exhibits two broad peaks associated with exchangeable protons at 23 and 25 ppm and one at 11 ppm that is nonexchangeable. The similarity of this spectrum to those of the other met forms demonstrates that the coupling between the iron atoms in the metsulfido complex is also large and indicates that the sulfido bridge is nearly as effective as the oxo group in mediating the antiferromagnetic interaction. The slightly larger shifts observed for the metsulfido complex are consistent with the somewhat weaker coupling observed for $[Fe(salen)]_2S$ ($J = -75$ cm^{-1})[15] when compared to that for $[Fe(salen)]_2O$ ($J = -95$ cm^{-1}).[16]

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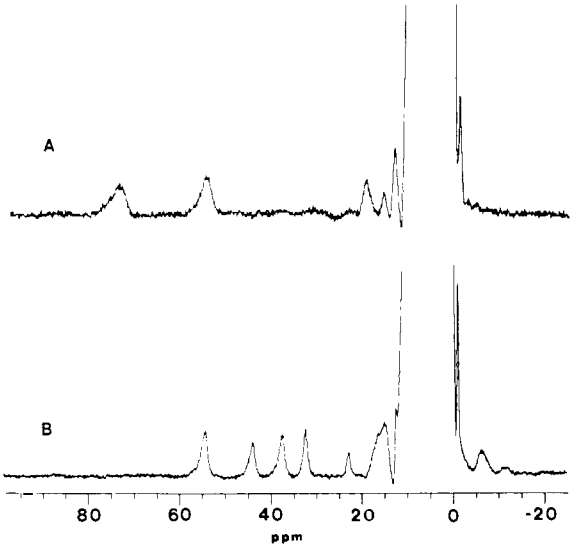

Figure 2. ^{1}H NMR spectra, 300 MHz, of semimethemerythrins. (A) 2.5 mM semimetHrN_3 in 50 mM phosphate buffer, pH 7.5, at 35 °C. (B) 4 mM semimetHrS in 50 mM phosphate buffer, pH 7.5, containing 50 mM $NaClO_4$ at 40 °C.

Semimetherythrins can be prepared by one-electron reduction of metHr[17,18] or by one-electron oxidation of deoxyHr.[17,19] The iron atoms in the mixed-valent clusters are antiferromagnetically coupled, giving rise to EPR signals expected for $S = {}^1/_2$ ground states.[1a,17,18] The ^{1}H NMR spectrum obtained for semimetHrN_3 (Figure 2A), prepared by the method of Nocek et al.,[19] features resonances assigned to the exchangeable protons of coordinated histidines at 73 and 54 ppm at 30 °C. Comparison of these shifts to those expected for the NH protons of imidazole ligands coordinated to mononuclear high-spin ferric and ferrous centers (100 and 65 ppm, respectively)[10] results in the assignment of the peak at 73 ppm to histidines bound to Fe(III) and the peak at 54 ppm to histidines bound to Fe(II). The ratio of the areas of the 54 ppm peak to the 73 ppm peak is 3:2, demonstrating that the iron atom bound to three histidines is the ferrous center. This is consistent with the persistence of the azide-to-Fe(III) charge-transfer band in semimetHrN_3.[1a] The two resonances exhibit Curie temperature dependence in the range 0–50 °C consistent with a $J = -10$ cm^{-1}. This compares well with the temperature-dependent behavior of the resonances observed for porcine uteroferrin ($J = -10$ cm^{-1})[10] and suggests that the two active sites may have similar bridging groups.

In stark contrast to semimetHrN_3, semimetHrS exhibits five peaks in the 20–50 ppm region assigned to the NH protons of histidine ligands (Figure 2B). With the present data, it is not possible to distinguish between resonances associated with histidines bound to Fe(III) and Fe(II). It is clear that there is considerably more magnetic anisotropy and a stronger antiferromagnetic interaction associated with this complex. The temperature dependence of these features is reminiscent of those observed in the spectra of reduced two-iron ferredoxins[20] and is currently under study.

Both semimetHrN_3 and semimetHrS display several nonexchangeable resonances in their ^{1}H NMR spectra in the 10–20 and −10 to 0 ppm regions. Current efforts are aimed at assigning these features as well as structurally characterizing other forms of hemerythrin.

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Mechanism-Based Inactivation of Cytochrome P-450 by Heteroatom-Substituted Cyclopropanes and Formation of Ring-Opened Products

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Cytochromes P-450 catalyze several oxidative transformations of saturated heteroatom-containing substrates, including carbon hydroxylation, heteroatom release (resulting from collapse of α-hydroxy heteroatomic intermediates), and heteroatom oxygenation.[1] Cyclopropylamines and cyclopropyl ethers were among the first *saturated* suicide inhibitors of cytochrome P-450.[2,3] Inactivation was postulated to involve initial heteroatom oxidation to a radical cation and subsequent ring opening to form a highly reactive carbon-centered radical which binds to the proximate heme moiety and destroys enzyme activity.[2a,b,3] We demonstrate here that the rate of inactivation of cytochrome P-450$_{PB-B}$[4] by a series of heteroatom-substituted cyclopropanes is highly correlated with the single-electron oxidation potential ($E_{1/2}$) of the substrate Figure 1) and suggest that such a mechanism for the oxidation of heteroatom-containing cyclopropyl substrates by cytochrome P-450 is general.

The cytochrome P-450 (measured as the ferrous–CO complex) enzyme system had a $t_{1/2} > 200$ min with NADPH in the absence of substrate when scavengers of partially reduced oxygen species (catalase, superoxide dismutase) were present. No inactivation occurred with these substrates when NADPH was omitted. Cyclohexane (at 10 mM), a nonsuicidal substrate, inhibited the rate of inactivation due to compound **7b** by 76%. None of the compounds caused the metabolic conversion of cytochrome P-450 to cytochrome P-420. The decrease in heme levels and catalytic activity paralleled the loss of spectrally determined cytochrome P-450 in the cases of **1a**, **1b**, **2**, **5a**, and **5b**.[2a,3] Substrates **1a**, **1b**, **5a**, and **5b** also appear to be effective in crude microsomal preparations as well as with purified cytochrome P-450$_{PB-B}$.[2,3]

A plot of log $k_{inactivation}$ (maximal rate constant) vs. $E_{1/2}$ is shown in Figure 1. Excluding the bromides **7a** and **7b**, the correlation coefficient for the remaining nine sets of values is 0.946. The values vary over 1.5 V and a 100-fold range of $k_{inactivation}$ and encompass a considerable diversity in substrate structure, functional group disposition, and overall size. We postulate that at

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