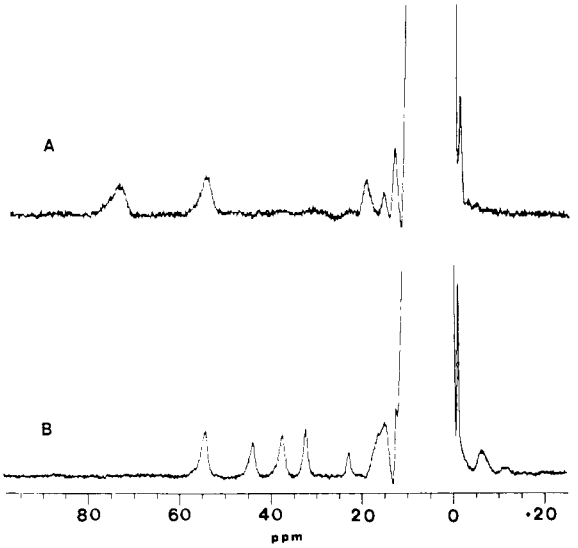

Figure 2. ^{1}H NMR spectra, 300 MHz, of semimethemerythrins. (A) 2.5 mM semimetHrN_3 in 50 mM phosphate buffer, pH 7.5, at 35 °C. (B) 4 mM semimetHrS in 50 mM phosphate buffer, pH 7.5, containing 50 mM $NaClO_4$ at 40 °C.

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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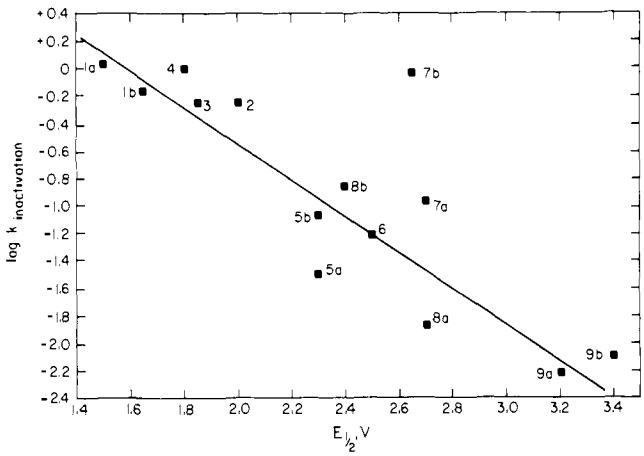

Figure 1. Rates of suidical inactivation of cytochrome P-450 by various cyclopropyl heteroatoms as a function of $E_{1/2}$ for oxidation of the substrates. $E_{1/2}$ values (vs. Ag/AgCl) were estimated by cyclic voltammetry in dry CH_3CN with 0.1 M $(n\text{-}Bu)_4NClO_4$. Inactivation assays were carried out at 37 °C as described elsewhere, and $k_{inactivation}$ is the extrapolated rate at infinite substrate concentration.[2a] The line was drawn from least-squares analysis of the points, excluding **7b**. Substrates included cyclopropylbenzylamine (**1a**), (1-methylcyclopropyl)benzylamine (**1b**), 1-methylcyclopropanol (**2**), cyclopropanone hydrate (**3**), *O*-ethyl cyclopropanone hydrate (**4**), cyclopropyl benzyl ether (**5a**), 1-methylcyclopropyl benzyl ether (**5b**), *N*-(1-methylcyclopropyl)benzamide (**6**), cyclopropyl bromide (**7a**), 1-methylcyclopropyl bromide (**7b**), cyclopropyl iodide (**8a**), 1-methylcyclopropyl iodide (**8b**), cyclopropyl chloride (**9a**), and 1-methylcyclopropyl chloride (**9b**).

Table I. Formation of Benzyl Compounds from **1a**, **1b**, **5a**, and **5b**[a]

substrate	product	nmol product formed/min/nmol cytochrome P-450	inactivation rate, min^{-1}	partition ratio (product formation/inactivation)
1a	benzylamine	trace (gc/ms)	0.10	89
	benzaldehyde	8.9 ± 2.3		
1b	benzaldehyde	14.9 ± 0.6	0.25	60
5a	benzyl alcohol	1.0 ± 0.2	0.009	522
	benzaldehyde	3.7 ± 1.1	0.009	522
5b	benzyl alcohol	1.0 ± 0.1	0.013	485
	benzaldehyde	5.3 ± 1.5		

[a] Assays were carried out with 1 mM substrate under the general conditions described[4] (in triplicate) by capillary GC (OV-17, flame ionization) using acetophenone as an internal standard. Products were confirmed by GC/mass spectrometry (MS) using the same system. Values for product formation are corrected for inactivation rates.

least two steps—single electron transfer from substrate to the enzyme and ring opening of the radical cation—are required to inactivate the enzyme (vide infra); correlation of $k_{inactivation}$ with $E_{1/2}$ requires that ring opening occurs considerably faster than radical formation. Such a view is consistent with enzymatic and nonenzymatic studies of cyclopropyl α-based radicals involving cyclopropyl compounds containing nitrogen,[2,5] oxygen,[6] and bromine.[7]

Table I indicates that heteroatom release from the cyclopropyl compounds occurred with **1a**, **1b**, **5a**, and **5b**. The formation of benzyl alcohol from **5b** can be rationalized by hydrolysis of a ring-opened metabolite but not classic hydrogen atom abstraction and oxygen rebound.[1,8] The unexpectedly high activity of the bromide **7b** for suicide inactivation of cytochrome P-450 cannot be explained at this point; the other halogenated compounds showed more consistent behavior. Since several of the cyclopropyl compounds are blocked by methyl groups at the position α to the heteroatom on the cyclopropane ring, hydroxylation at this position is not involved in inactivation.

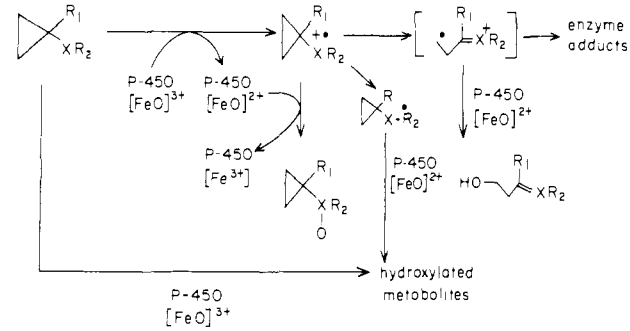

Figure 2. Mechanisms postulated in the oxidation of heteroatomic substrates by cytochrome P-450.

4 was converted to ethyl 3-hydroxypropionate[9] in the presence of the reconstituted cytochrome P-450 system. This is one of the few cases where oxygen rebound to a site generated by rearrangement has been observed.[10] Further, reaction of **4** with Mn^{V}=O(TPP)Cl in dry CH_2Cl_2 under N_2 yielded ethyl 3-hydroxypropionate in 3% yield.

The results support the following mechanism (Figure 2). The formal perferryl cytochrome P-450 complex $(Fe^{V}=O)^{3+}$ initiates the reaction by abstracting an electron from the heteroatom. The cyclopropyl-substituted radical species subsequently undergoes rapid ring opening to form a methylene-localized radical species, which can attack the heme in the enzyme site[11] or form (after oxygen rebound) an aldehyde or ester. When the oxidation potential is relatively large for the heteroatom, other metabolic processes (e.g., aromatic ring hydroxylation, carbon hydroxylation) may also compete; if the heteroatom-centered radical cation is stable (e.g., sulfur), oxygen rebound *to the heteroatomic center* will transpire. Other evidence for this one-electron oxidation mechanism for cytochrome P-450 and heteroatoms comes from correlation of sulfoxide formation with $E_{1/2}$,[12] radical formation by dihydrocollidines,[13] and kinetic deuterium isotope effects in amine dealkylation.[14] Thus, if the activated oxoiron complex of cytochrome P-450 has steric access to heteroatoms, the heteroatoms may undergo one-electron oxidation and yield a variety of products as a consequence.

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