Iron Octaethylisobacteriochlorin, a Model System for the Siroheme Prosthetic Group of Nitrite and Sulphite Reductases

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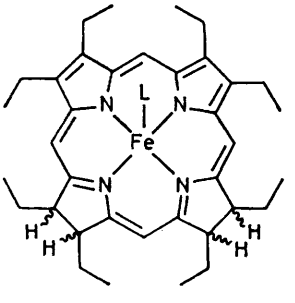
Summary The preparation, spectroscopic, and electrochemical characterization, and the investigation of ligation with biologically relevant ligands are reported here for iron(III) octaethylisobacteriochlorin chloride which is compared with siroheme, the prosthetic group of nitrite and sulphite reductases.

SIROHEME, the first known example of an iron isobacteriochlorin, has been identified as the site of substrate binding in the enzymes nitrite and sulphite reductase, which catalyse the six-electron reductions NO_2^-–NH_3 and SO_3^{2-}–H_2S, respectively.[1] Anaerobic metallation of free base octaethylisobacteriochlorin, H_2(OEiBC),[2] with anhydrous iron(II) acetate in the presence of sodium chloride in glacial acetic acid results in the formation of Fe^{III} octaethylisobacteriochlorin chloride, Fe(OEiBC)Cl (**1**, L = Cl). The



(1)

absorption spectrum of (**1**) in benzene, Figure 1, bears a marked resemblance in the number, general appearance, and relative intensities of the bands to the spectrum of siroheme extracted with acetone–HCl from sulphite and nitrite reductase,[3] (siroheme λ_{max}, 376, 547, and 594 nm; A_{376}/A_{594} 3·5).[4] The diffuse band at 754 nm cannot be correlated to any feature in the spectrum of siroheme, which was only examined below 700 nm. However, analogous bands have been reported for the native enzymes.[4]

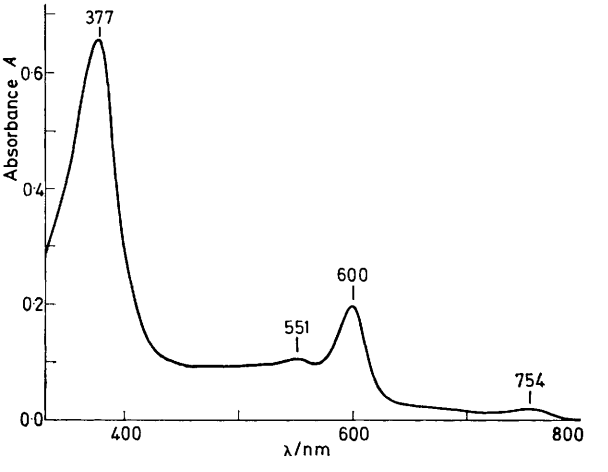


FIGURE 1. Absorption spectrum of *ca.* 10^{-4} M Fe(OEiBC)Cl in benzene.

Cyclic voltammetric experiments with the series of compounds, Fe(OEiBC)Cl, Fe^{III} octaethylchlorin chloride [Fe(OEC)Cl] (**2**),[5] and Fe^{III} octaethylporphyrin chloride [Fe(OEP)Cl], in dichloromethane have afforded a number of important observations. Each complex has two chemically reversible ($i_{p,c}$ *ca.* = $i_{p,a}$) oxidations whose potentials, Table, are *ca.* 90 mV more positive than those of the two corresponding features of the free bases.[2] Oxidation to the dications of (**1**) and (**2**) leads to some dehydrogenation, as shown by the appearance during repetitive scans of waves at the potentials for the next most oxidized compound of the

series. This reaction is typical of reduced metal-free porphyrins[2] but is prevented in siroheme by the presence of methyl groups at the reduced β-pyrrolic positions. The cyclic voltammogram of (**1**) shows $< 1\%$ contamination by (**2**). The potentials of the irreversible Fe^{III}–Fe^{II} couples do not appear to be strongly correlated with the reduction level of the ligand. This contrasts with the structure-sensitive potentials for the first reductions of the free bases,[2] implying that the isobacteriochlorin ring system imparts no exceptional reducing power to Fe^{II}.

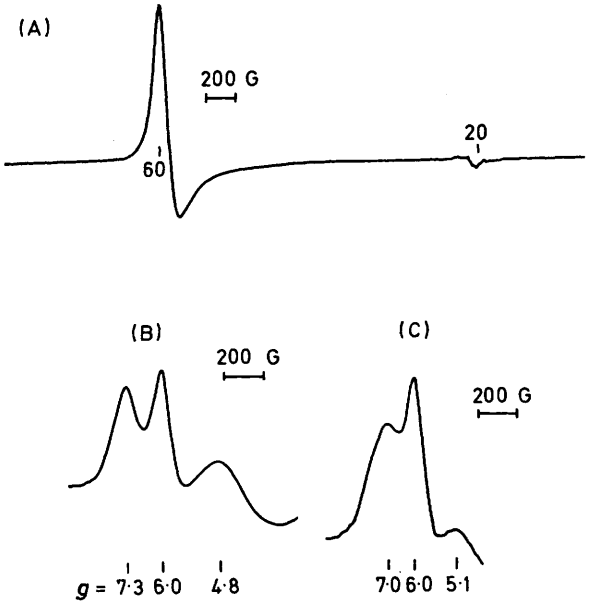


FIGURE 2. *X*-Band e.s.r. spectra at 85 K in toluene. (A); Fe(OEiBC)Cl (B), (C); (A) + 1 equiv. each of RSH and 2,2,6,6-tetramethylpiperidine and excess of RSSR: (B) R = Ph; (C) R = p-$O_2NC_6H_4$. Low field region. The $g = 6$ signal for (B) and (C) is due to residual Fe(OEiBC)Cl. Instrumental parameters differ for each experiment.

The e.s.r. spectra of (**1**), Figure 2(A), and (**2**) are typical of axially symmetric high spin iron(III) complexes. However, the spectra of the oxidized forms of both sulphite[1,6] and nitrite[7] reductase exhibit marked rhombic splittings. Similar observations for cytochrome P-450 have led to the demonstration of axial co-ordination by cysteinate in the protein.[8] Reaction of (**1**) with equivalent amounts of arylthiols and 2,2,6,6-tetramethylpiperidine results in the formation of species with L = SAr which exhibit rhombic high-spin Fe^{III} spectra, Figure 2(B,C). Similar experiments with p-nitrophenol and 2-phenylimidazole result in changes

TABLE. Potentials[a] of Fe(OEP)Cl, Fe(OEC)Cl, and Fe(OEiBC)Cl

Compound	$E_{\frac{1}{2}}(\mathbf{2})^{ox\ b,c}$/V	$E_{\frac{1}{2}}(\mathbf{1})^{ox\ b,c}$/V	$E_{p,c}$/V
Fe(OEiBC)Cl	1·00	0·43	−0·45
Fe(OEC)Cl	1·24	0·72	−0·44
Fe(OEP)Cl	1·39	1·01	−0·52

[a] Cyclic voltammetric measurements at a Pt inlay electrode in dichloromethane (0·05 M tetrabutylammonium perchlorate); scan rate 0·1 V s^{-1}. [b] $E_{\frac{1}{2}} = \frac{1}{2}(E_{p,a} + E_{p,c})$, *vs.* aqueous standard calomel electrode. [c] Separation of $E_{p,a}$ and $E_{p,c}$ was 80—200 mV but the ratios of the peak heights approximated to unity for all cases.

in the optical spectra but show no departure from a high-spin axial e.s.r. spectrum. Thus, by e.s.r. criteria[8] axial co-ordination of the siroheme group by a sulphur ligand is quite likely in both enzymes. This contention is supported by the inhibition of reduced sulphite reductase in the absence of substrate by p-chloromercuriobenzoate.[9] Finally, changes in the thiolate ligand cause an appreciable variation in the rhombic splitting. Reductases from different sources exhibit a range of splittings of the $g = 6$ signal (Δg *ca.* 0·5—1·6) which has been interpreted as arising from substantially different environments.[6] Our results suggest that the different splittings could in fact be due to small changes in co-ordination geometry enforced by the protein structure without change in the axial ligand.

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