The Structure and Reactions of Heme *A* of Cytochrome *c* Oxidase

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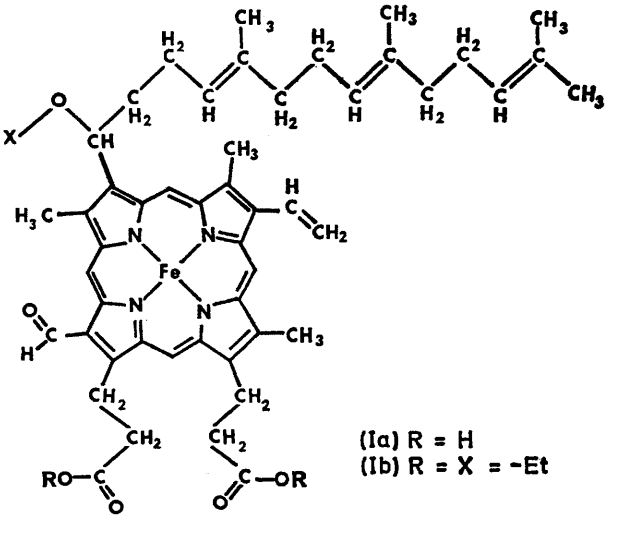
Summary The structure of heme *A* from bovine heart has been established through n.m.r. spectra and other properties except for an acid-labile substituent at C-1 of the 2-(*trans,trans*-farnesyl)ethyl group—a group which may participate in mitochondrial electron-transfer and phosphorylation processes.

DEMONSTRATION of a critical role for cytochrome *c* oxidase in bioenergetics has resulted in wide interest in structure–function relationships in this mitochondrial hemeprotein.[1] However, the structure of the heme moiety, heme *A* has not been fully elucidated.[1b,2,3] Here we report studies of heme *A* derivatives which significantly clarify the structure of native heme *A*.

Heme *A*, isolated from bovine heart as described earlier,[3,4] was subjected to an additional chromatography: a chloroform solution was added to a celite column, elution with chloroform removed any small amounts of residual lipid impurities, and chloroform–pyridine (4 : 1, v/v) elution gave lipid-free heme *A* (Ia). Treatment of (Ia) with ethanol–sulphuric acid (24 : 1, v/v) for 16 hr. at 20° gave a diethyl ester, monoethyl ether (Ib). Similar treatment of (Ia) with methanol–sulphuric acid (24 : 1, v/v) yielded a dimethyl



ester, dimethyl ether (II). Compound (Ib) was shown by ^{1}H n.m.r., i.r., visible, and mass spectra to be an iron complex of 6,7-bis-(2''-ethoxycarbonylethyl)-2-(1'-ethoxy-5',9',13'-trimethyl-4',8',12'-*trans,trans*-tetradecatrienyl)-8-formyl-1,3,5-trimethyl-4-vinylporphin. The n.m.r. spectrum of (Ib) as a diamagnetic iron(II) complex in C_5D_5N is shown in Figure 1. *All* protons have been assigned, this

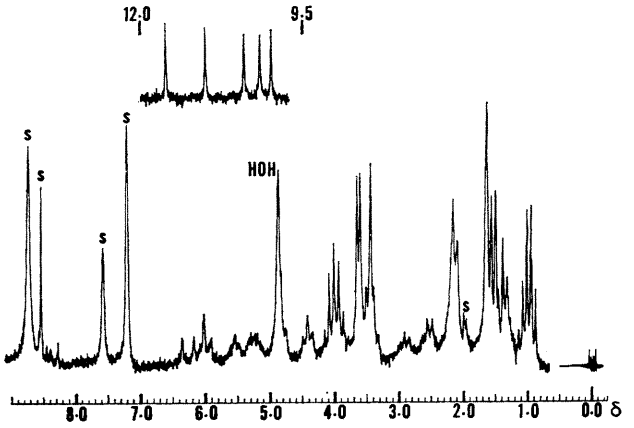
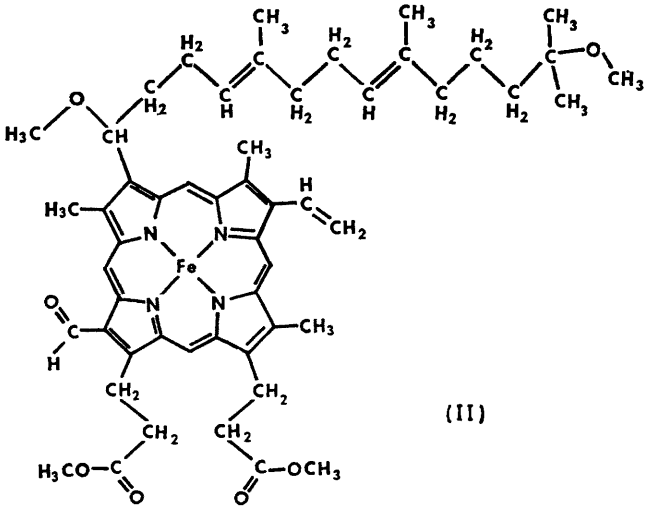


FIGURE 1. 100 *MHz* ^{1}H *n.m.r. spectrum of* 1'*-ethoxy-heme diethyl ester in* C_5D_5N *at* 35°. *Peaks due to solvent are represented by* 'S'.

aided by comparison with spectra of known porphyrins and farnesol derivatives; δ: 11·60(1H, CHO); 11·0(1H, δ-methine); 10·39(1H, α-methine); 10·15(1H, γ-methine); 9·98(1H, β-methine); 8·4(1H, ·C*H*:CH$_2$); 6·2(2H, ·CH:C*H*$_2$); 6·0(1H, proton on C-1'); 5·55(1H, proton on C-4'); 5·25 (2H, protons on C-8' and C-12'); 4·42(2H, ·C*H*$_2$·CH$_2$·CO$_2$Et at position 6); 3·98(6H, ·OC*H*$_2$Me); 3·65 (3H, ring C*H*$_3$); 3·61(3H, ring C*H*$_3$); 3·44(5H, ring C*H*$_3$ and ·CH$_2$·C*H*$_2$·CO$_2$Et at position 7); 2·90(2H, ·CH$_2$·C*H*$_2$CO$_2$Et at position 6);



(II)

2·53(2H, C*H*$_2$ at C-2'); 2·13(10H, C*H*$_2$ at C-3', -6', -7' -10', and -11'); 1·63 (6H, :C·C*H*$_3$); 1·56(3H, :C·C*H*$_3$); 1·49(3H, :3·C*H*$_3$); 1·38(3H, ·OCH$_2$·C*H*$_3$ at C-1'); 0·95, 1·01(6H, ·CO$_2$CH$_2$·C*H*$_3$). A shoulder appearing on the water peak indicates that the α-methylene of the propionic ester group at position 7 occurs at *ca.* δ 4·7. A *trans,trans*-stereochemistry for the double bonds at C-4' and -8' was supported by chemical shifts of adjacent protons.[5]

Compound (II) was formulated as an iron complex of 6,7-bis-(2''-methoxycarbonylethyl)-8-formyl-2-(1',13'-dimethoxy-5',9',13'-trimethyl-4',8'-*trans,trans*-tetradecadienyl-1,3,5-trimethyl-4-vinylporphin with an n.m.r. spectrum that, apart from ester alkoxyls, differed significantly from that of Figure 1 only in the long side-chain resonances, δ (C_5D_5N): 5·9(1H, proton on C-1'); 5·55(1H, proton on C-4'); 5·27(1H, proton on C-8'); 3·56(3H, OC*H*$_3$ at C-1'); 3·13(3H, OC*H*$_3$ at C-13'); 2·53(2H, C*H*$_2$ at C-2'); 2·12(8H, C*H*$_2$ at C-3', -6', -7', and -10'); 1·50, 1·49(6H, C*H*$_3$ on C-5' and -9'); 1·29(4H, C*H*$_2$ at C-11' and -12'); 1·12(6H, C*H*$_3$ at C-13').

The n.m.r. spectrum of (Ia) as an iron(II) species in C_5D_5N (Figure 2) shows all the protons found for (Ib)

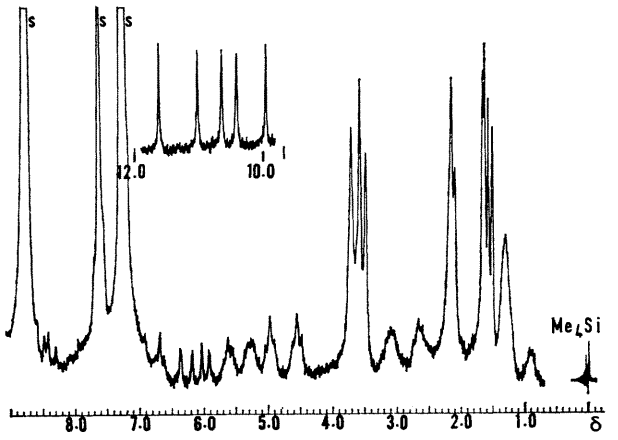


FIGURE 2. 100 *MHz* ^{1}H *n.m.r. spectrum of bovine heart muscle heme* A *in* C_5D_5N *at* 35°. *Peaks due to solvent are represented by* 'S'.

(Figure 1) except for the three ethyl groups. Thus the C_{17} group appears the same in (Ia) and (Ib) except for an as yet undetermined group (X)[3] in (Ia) which is replaced by ethyl upon the formation of (Ib). In the native heme *A*, X appears bound to C-1' *via* oxygen because the chemical shift for the C-1' proton is in the same region as that found for (Ib), (II), and hematoporphyrin.[6] In addition to the evidence from i.r., elemental analysis, and molecular-weight data, [2,3] the presence of several additional protons attributable to X provide evidence against X being H. Those protons found earlier[2] at saturated CH_2 and CH_3 regions (*e.g.* δ 0·9 and 1·2, Figure 2) which made the n.m.r. spectrum inconsistent with a 5',9',13'-trimethyl-4',8',12'-tetradecatrienyl group as the sole source of such protons in heme *A*[2] are removed upon displacement of X by an ethyl or methyl group as are several protons found at low field (*i.e.* δ6·5—8·7). Studies to assign the protons of X (either heme-bound or free) and to distinguish X from very small amounts of possible impurities are in progress.

These data thus extend and confirm structural interpretations reported earlier for native heme *A*[2,3] and demonstrate the presence of a 2-(*trans, trans*-farnesylethyl) group containing a labile substituent on the 1-position and the nature of the other seven groups on the porphyrin ring. Structures suggested for heme *A* which have a long side-chain with 5- or 6-membered oxygen heterocycles,[7] or with

no double bonds[8] are not compatible with these findings. If there are two different heme *A* structures—one for cytochrome *a* and one for cytochrome a_3, it now appears clear such differences must reside in the X groups.[9]

We suggest that the long side-chain may have an important role in electron transfer and/or coupled phosphorylation processes. Ubiquinone which serves near oxidative phosphorylation sites I and II[10] and heme *A* at site III each possess similarly unsaturated isoprenoid side-chains. Of possible relevance here is the greater reactivity shown by the terminal double-bond (C-12′) compared with the other two (C-4′ and -8′). Models reveal that the terminal double-bond may readily assume a position immediately beside the central iron atom should reaction with iron or with a ligand bound to iron be required. Also, the observation of selective broadening of n.m.r. bands for protons near X in a Cu^{II}–heme *A* complex isolated from beef heart[1f] may signify that the polar OX group serves as a ligand to copper in the oxidase.

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