Analogues of Bridged Biological Active-Site Assemblies: The Fe_4S_4–Sulfide–Heme Unit

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The active sites of certain metalloenzymes consist of adjacent metal centers connected by one or more covalent bridges. These bridged assemblies, such as occur in the binuclear site of as-isolated cytochrome *c* oxidase (CcO),[1] the cofactor of nitrogenase,[2] and assimilatory sulfite reductase (SiR),[3,4] provide the newest generation of active-site structures whose properties are potentially susceptible to elucidation using synthetic analogues; as one example, a potential analogue of CcO containing the bridge [Fe^{III}–O–Cu^{II}] has recently been prepared.[5] The X-ray structure of *Escherichia coli* SiR reveals an Fe_4S_4 cluster and a siroheme connected by a putative cysteinyl sulfur atom,[3b] an arrangement consistent with ENDOR spectra;[3c] the cluster and siroheme are exchange-coupled in all accessible paramagnetic states.[3a,d,e] SiR from *Desulfovibrio vulgaris* contains the same exchange-coupled components,[4] but chemical analysis suggests that the bridge may be sulfide[4a] instead of cysteinate. We report the synthesis and certain properties of an Fe_4S_4–S–heme bridged assembly as the initial step in analogue development, utilizing subsite-differentiated clusters[6] as key reactants. Selected reactions are depicted in Figure 1; advantage is taken of the extreme sensitivity of isotropically shifted LS_3 ligand resonances to the identity of the ligand at the unique Fe subsite.[6,7]

The key precursor, hydrosulfide cluster **2**, is readily obtained by reaction of $(Bu_4N)_2$[**1**][6d] in CH_2Cl_2 solution with 2.9 equiv of H_2S and was isolated in 84% yield and >95% purity.[7,8] This species is in equilibrium with H_2S and μ-sulfido double cubane **3**; removal of H_2S permits isolation of pure **3**.[7] The initial

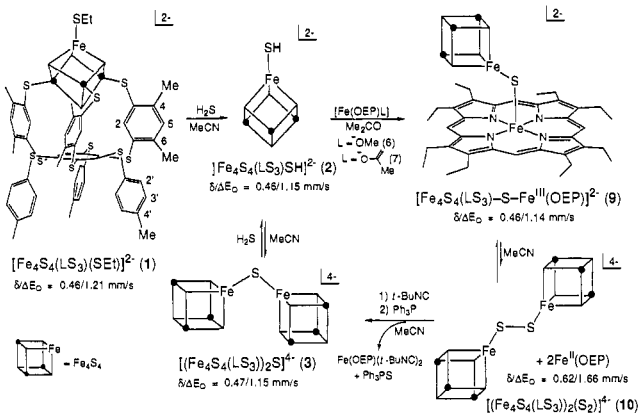

Figure 1. Depiction of a reaction system affording the μ-sulfido-bridged assembly **9** by acid–base coupling reactions of **2**, conversion of **9** to double cubane **3** via **10**, and the LS_3 ligand numbering scheme (**1**). Isomer shifts (δ) and quadrupole splittings (ΔE_Q) at 4.2 K are indicated.

indication of an unsupported Fe_4S_4–S–Fe bridge[9] followed from reaction 1, in which **2** was treated with excess $[Fe(salen)_2]O$[10] (5 equiv) in the presence of 1 equiv of $NaSH/(Et_3NH)ClO_4$ (H_2S generator) in MeCN:C_6H_6:MeOH (1:2:2 v/v). Removal of solvent, extraction of the residue with acetone, and addition of ether to the extract afforded the product **4**, formulated as $[Fe_4S_4(LS_3)$-S-Fe(salen)$]^{2-}$, whose ^1H NMR spectrum[7] is markedly shifted relative to those of the reactants (Figure 2).[10a]

To incorporate a physiologically more relevant heme group, the following coupling reactions of **2** with the indicated Fe(III) porphyrins were developed:[8] (2) $[Fe(OEP)]_2O$[11a] in MeCN/benzene (1:1 v/v, 4 d); (3) $Fe(OEP)(OClO_3)$[11b] (**5**) + $5Et_3N$ + $NaSH/(Et_3NH)ClO_4$ in MeCN (2 h, <0 °C); (4) Fe(OEP)-(OMe)[12] (**6**) in MeCN/benzene (1:1 v/v, 5 h, 0 °C); (5) Fe-(OEP)(OC(Me)=CH_2)[13a] (**7**, from **5** and *t*-BuNP$(NMe_2)_3$[13b] in acetone) + $NaSH/(Et_3NH)ClO_4$ in acetone (0 °C, 2 h); also, (6) $[Fe_4S_4(LS_3)(SSiEt_3)]^{2-}$ [**8**, from **1** + $(CF_3SO_3SiEt_3$ + excess NaSH)] + Fe(OEP)F[14] in MeCN (1–2 days). Reactions 2–5 are directed acid–base processes, while reaction 6 is driven in part by the stability of the Si–F bond. When reaction 2 was conducted on a preparative scale (100 mg of **2**), the product was isolated in 57% yield.

The identity of the reaction products has been established by ^1H NMR. The well-resolved spectrum of **2**, shown in Figure 2, is typical of $[Fe_4S_4(LS_3)L']^{2-}$ clusters.[6] Reactions 2–6 afford an identical product **9**, formulated as $[Fe_4S_4(LS_3)$-S-Fe(OEP)$]^{2-}$. Note that their indistinguishable ^{57}Fe isomer shifts (Figure 1) require that product **9** and clusters **1–3** contain isoelectronic $[Fe_4S_4]^{2+}$ cores; as with oxidized *E. coli* SiR,[3a] heme and cluster Fe atoms in **9** could not be resolved. The spectra of coupling reactant **6** and product **9** have similar meso-H and diastereotopic methylene chemical shifts (Figure 2), demonstrating the presence of the high-spin $[Fe^{III}(OEP)]^+$ fragment in **9**. LS_3 ligand shifts occur at 17 to −11 ppm, with the isotropic shifts +18.0 (2-H), −11.4 (4-Me), −10.7 (5-H), and −13.8 (6-Me) ppm. The pattern

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(7) Chemical shifts (ppm, CD_3CN, 298 K): **1**, 13.20 (CH_2), 8.15 (5-H), 5.1 (2-H), 3.82 (6-Me), 3.69 (4-Me); **3**, 8.66 (5-H), 4.53 (6-Me), 4.25 (2-H), 4.09 (4-Me); **4**, 17.1 (5-H), 16.0 (6-Me), 13.8 (4-Me), −9.00 (2-H); salen, 61.8 (4-H), 35.8 (6-H), −41.6 (5-H), −50.5 (3-H); **8**, 8.17 (5-H), 5.07 (2-H), 3.84 (6-Me), 3.71 (4-Me); **10**, 8.24 (5-H), 5.1 (2-H), 3.87 (6-,4-Me); **11** (acetone), 66.1 (meso-H), 31.7 (CH_2), 11.2 (Me); **12**, 9.64 (meso-H), 3.95 (CH_2), 1.82 (Me), −0.69 (*t*-Bu). See also Figure 2.

(8) Experimental procedures: all reactions were performed under a pure dinitrogen atmosphere at room temperature and utilized equimolar reactant quantities unless indicated otherwise. Clusters were isolated in good yields as Bu_4N^+ salts by standard workup and in ≥90% purity based on integration of the 5-H reactant:product signals; **3** is a minority coproduct of nearly all reactions. Yields were not quantitated for small-scale reactions.

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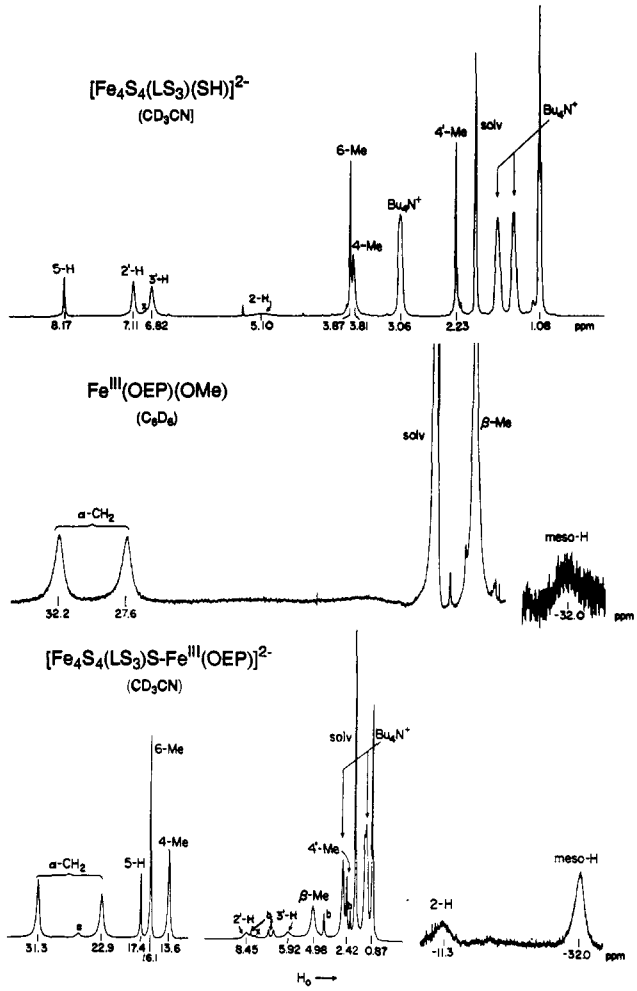

Figure 2. ^{1}H NMR spectra (298 K) of **2**, **6**, and bridged assembly **9** (prepared by reaction 4); signal assignments are indicated. In the bottom spectrum, resonances a and b arise from slight amounts of Fe(OEP) and **10**, respectively, in equilibrium with **9**.

of signs is the same as that for **2** (+1.57 (2-H), −1.59 (4-Me, 6-Me), and −1.44 (5-H) ppm), and the signals correspond to dominant contact interactions,[15] but the shifts are 7–11 times *larger*. Isotropic shifts of **9** increase with decreasing temperature in a linear $1/T$ dependence (240–300 K, CD_3CN) typical of an ordinary paramagnet, whereas those of $[Fe_4S_4(LS_3)L']^{2-}$ increase with increasing temperature[16a] owing to an $S = 0$ ground state and thermal population of paramagnetic states.[16b] This behavior indicates that spin density has been delocalized from the heme to the cluster and dominates the spin distribution in the latter. From the signs of the shifts, we infer antiparallel spin transfer between Fe(III) and μ-S, which is propagated such as to place in π-type orbitals positive spin at S and C (2,4,6) and negative spin at C(5) of LS_3 in the manner of an alternate hydrocarbon. The situation with **4** is the same. The ring-H shifts of precursor $[Fe(salen)]_2O$, restricted to the 1–13-ppm range by the antiferromagnetically coupled $S = 0$ ground state,[10] now occur over 62 to −51 ppm; by comparison with Fe(salen)(OAc),[17] these shifts, also contact in origin, correspond to high-spin Fe(III). The isotropic shifts +15.7 (2-H), −11.7 (4-Me), −10.4 (5-H), and −13.8 (6-Me) ppm are consistent with those of **9**. We conclude that **4** and **9** are sulfide-bridged assemblies in which five-coordinate high-spin Fe(III) and the cluster are electronically coupled, the manifestation of which in NMR is conspicuously increased unpaired spin density in the Fe_4S_4 component. In Mössbauer spectroscopy, this effect will lead to magnetic hyperfine interactions in the cluster, as has been observed for oxidized SiR.[3a]

The ^{1}H NMR spectrum of assembly **9** obtained by all reactions shows very weak additional signals (a,b in Figure 2). We have shown that these arise from μ-persulfido double cubane **10**[7] (b) and Fe^{II}(OEP) (**11**,[7] a) in equilibrium with **9**. Treatment of **9** in acetonitrile with 2 equiv of *t*-BuNC shifts the equilibrium completely to **10** and diamagnetic Fe^{II}(OEP)(*t*-BuNC)$_2$ (**12**,[7] Figure 1). Cluster **10**, which has been independently synthesized from $[Fe_4S_4(LS_3)Cl]^{2-}$ [6a] and Na_2S_2 in MeCN/MeOH (1:1 v/v), reacts cleanly with Fe^{II}(OEP)[18] to afford **9** in high yield. This reaction, together with the preparation of **9** by five different methods, constitutes additional support for the bridged assembly formulation.

The electronic features of **4** and **9**, as expressed in EPR, Mössbauer, and MCD spectroscopies, should serve as distinguishing characteristics for any natural molecule containing the (siro)heme–S–Fe_4S_4 active site in a corresponding oxidation state. Full descriptions of these properties and of the syntheses and reactions of these and other bridged assemblies will be the subjects of future reports.

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