Synthesis and Metal Ion Incorporation Reactions of the Cuboidal Fe_3S_4 Cluster

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Received June 15, 1995

The three established types of low-nuclearity biological iron−sulfur clusters contain the core units $Fe_2(\mu_2\text{-}S)_2$, $Fe_3(\mu_3\text{-}S)(\mu_2\text{-}S)_3$, and $Fe_4(\mu_3\text{-}S)_4$. Synthetic analogues of the binuclear and tetranuclear cubane-type clusters are readily synthesized and have proven informative in the elucidation of intrinsic cluster properties, unmodified by protein environment.[1,2] The trinuclear cluster, whose cuboidal structure **1** (Figure 1) has been amply verified by protein crystallography,[3−5] had not yet yielded to synthesis in stable, isolable form.[6] However, Fe_3S_4 has been obtained as a constituent of many heterometal MFe_3S_4 cubane-type cores.[2,7] The finding that unfolding of inactive oxidized aconitase causes isomerization of the $[Fe_3S_4]^+$ cluster core to linear $[Fe_3(\mu_2\text{-}S)_4]^+$,[8] which has otherwise been isolated in the form of the stable clusters $[Fe_3S_4(SR)_4]^{3-}$,[9] implies that the cuboidal geometry may be sustained by protein structure. We report here facile routes to an Fe_3S_4 cluster, unsupported by a protein matrix, that is stable under ambient conditions. This approach utilizes [1:3] site-differentiated clusters, including $[Fe_4S_4(LS_3)(SEt)]^{2-}$ (**2**, Figure 1), whose ^1H NMR shifts are markedly sensitive to the nature of ligands at the unique site[10] and to the cluster spin state.[10,11]

Reaction of $(Bu_4N)_2$[**2**][12] with 1 equiv of $(Et_3NH)(CF_3SO_3)$ in acetonitrile followed by solvent reduction affords pure microcrystalline $(Bu_4N)_2[Fe_4S_4(LS_3)(OSO_2CF_3)]$ ($(Bu_4N)_2$[**3**], Figure 1) in quantitative yield.[13] Its ^1H NMR[14] and Mössbauer[15] spectra are typical of $[Fe_4S_4]^{2+}$ clusters with an $S = 0$ ground state and a low-lying paramagnetic state. The unique iron site in **3** is activated toward terminal ligand substitution; addition of 1 equiv of $(Et_4N)_2$(Meida) (Meida = *N*-methylimidodiacetate) in acetonitrile caused near-quantitative separation of a microcrystalline black solid spectroscopically identified as $(Et_4N)_3[Fe_4S_4(LS_3)(Meida)]$ ($(Et_4N)_3$[**4**]). Cluster **4** retains the $[Fe_4S_4]^{2+}$ core;[14,15] its NMR spectrum (Figure 2A) is consistent with coordination of Meida as a *fac*-tridentate ligand; chelate ring protons H_{ab} are inequivalent (δ 1.65, 3.49), and 5-H exhibits an enhanced downfield shift vs **3** (0.30 ppm[14]) frequently associated with 5- or 6-coordination at the unique Fe site.[10] The same reaction occurs in Me_2SO solution; treatment of **4** in this solvent with 2−3 equiv of $(Et_4N)_2$(Meida) abolishes its spectrum and generates new spectra (Figure 2B). The inset spectrum is that of $[Fe(Meida)_2]^{2-}$ (**5**), so demonstrated by the preparation of an authentic sample of $(Et_4N)_2$[**5**] (from $Fe(CF_3SO_3)_2$ and $(Et_4N)_2$(Meida) in acetonitrile); note the inequivalent methylene protons. The isotropic shifts of the other spectrum are ca. 4× *larger* than those of precursor **4** and are comparable with those of $[Fe_4S_4(LS_3)(RNC)_3]^-$ [11] (**7**) and decidedly similar to those of $[(OC)_3MoFe_3S_4(LS_3)]^{3-}$ [14] (**9**, vide infra), whose $[Fe_3S_4]^0$ cuboidal fragments are in the *spin-isolated* $S = 2$ state because of diamagnetic unique sites.

The sequence **3** → **4** → **6** (Figure 1) proceeds in high yield. $(Et_4N)_3$[**6**] has been isolated in >80% yield from **4** in Me_2SO solution by addition of ether to the reaction mixture followed by washing of the separated solid with THF and acetonitrile. The product contains no impurities observable by ^1H NMR and Mössbauer spectroscopies. The analogous reaction sequence was accomplished with **3** and $(Et_4N)_3$(citrate), including isolation of the intermediate $(Et_4N)_3[Fe_4S_4(LS_3)(citrate)]$, whose chemical shifts in Me_2SO (δ 3.85 (4-Me), 4.47 (6-Me), 8.55 (5-H)) are nearly identical to those of **4**. The product of these reactions is the cuboidal cluster **6**, identified by its solution moment (μ_{eff} = 5.10μ_B, Me_2SO) consistent with $S = 2$ and its Mössbauer spectrum. The latter consists of two partially resolved quadrupole doublets whose parameters[15] are in excellent agreement with those of protein-bound $[Fe_3S_4]^0$ clusters[2,16] such as that in *Desulfovibrio gigas* Fd II.[15]

An alternative approach is based on the findings that $[(OC)_3MoFe_3S_4(SEt)_3]^{3-}$ (**8**)[17] contains a spin-isolated $[Fe_3S_4]$ ($S = 2$) fragment and core Mo−Fe/S bond distances that substantially exceed those in cubane-type $[MoFe_3S_4]^{3+}$ clusters[7a,17] where all metal sites are electronically and structurally tightly integrated into the core. Reaction of this cluster with $L(SH)_3$[19] in acetonitrile solution[13] affords after standard workup $(Et_4N)_3$[**9**] (50%; ν_{CO} 1872, 1763 cm^{-1}, MeCN). The compound $(Et_4N)_3[(OC)_3MoFe_3S_4(Smes)_3]$ (mes = mesityl, 86%; ν_{CO} 1872, 1764 cm^{-1}, MeCN) was prepared similarly; its X-ray structure[20] demonstrates that the long core bonds are not singular to **8** but presumably occur in **9** (Figure 1) as well. A solution of **9** (22 mM) in MeCN/DMF (1.2:1 v/v) containing 5 equiv of $NaPF_6$ was maintained at −60 °C while CO was passed through for 10 min; the solution was allowed to warm to room temperature as the reaction was continued for another 30 min. The initial

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(13) All reactions and measurements were performed under a pure dinitrogen atmosphere. NMR spectra were recorded and magnetic moments determined (by an NMR method) at 299 K.

(14) δ (4-Me, 6-Me, 5-H): **3**, 3.93, 3.96, 8.25 (MeCN); **4**, 3.89 (−1.89), 4.47 (−2.47), 8.55 (−2.08) (Me_2SO); **6**, 8.51 (−6.51), 11.66 (−9.66), 15.48 (−9.01) (Me_2SO); **7**, 10.89 (−8.65), 14.97 (−12.70), 16.55 (−9.91) (MeCN);[11] **9**, 9.04 (−6.88), 12.93 (−10.77), 15.43 (−8.70) (Me_2SO); **10**, 9.07, 11.49, 13.58 (DMF/MeCN, 1.2:1 v/v); **11**, 10.38, 13.89, 15.95 (Me_2SO); **12**, 11.62, 13.76, 15.39 (Me_2SO). Isotropic shifts $(\Delta H/H)_{iso}$ (ppm) = $(\Delta H/H)_{dia} - (\Delta H/H)_{obs}$ of selected compounds are in parentheses.

(15) δ, ΔE_Q (mm/s, 80 K): **3**, 0.45, 1.12; **4**, 0.43, 0.91 (site 1), 0.47, 1.32 (site 2); **6**, 0.46, 1.30 (site 1), 0.32, 0.59 (site 2); **9**, 0.50, 1.34 (site 1), 0.38, 0.64 (site 2); **11**, 0.47, 1.13 (site 1), 0.37, 0.58 (site 2). *D. gigas* Fd II (4.2 K):[16a] 0.46, 1.47 (site 1), 0.30, 0.47 (site 2) (Fd = ferredoxin). Isomer shifts are referenced to Fe metal at room temperature.

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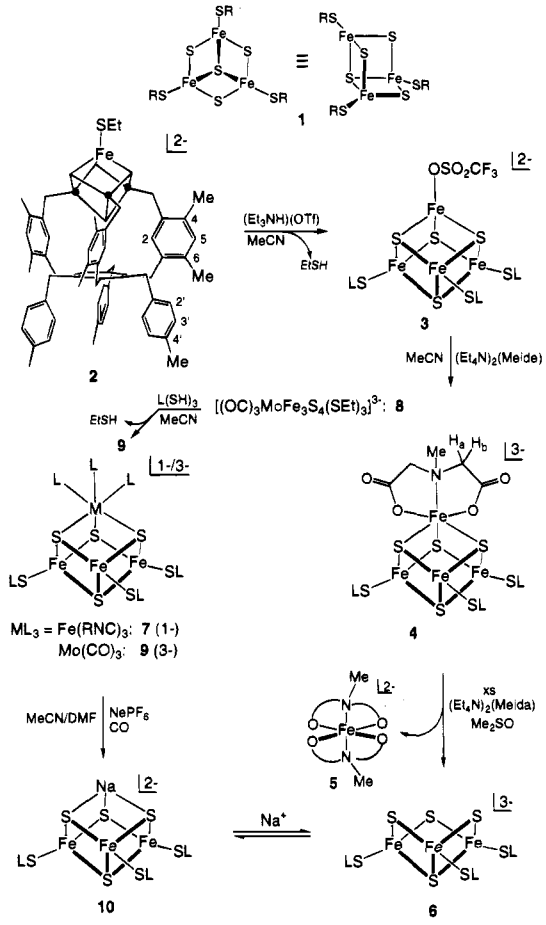

Figure 1. Schematic depiction of the synthesis of $[Fe_3S_4]^0$ cuboidal clusters **6**/**10** using [1:3] site-differentiated clusters stabilized by the LS_3 ligand (shown in **2**); in **3**–**4**, **6**–**7**, **9**, and **10**, 3LS = LS_3.

spectrum of **9** (Figure 2C) was completely replaced by a spectrum whose large isotropic shifts[14] are comparable to those of **6**. This spectrum is assigned to cuboidal cluster **10**, whose small chemical shift differences vs **6** are ascribed to the indicated equilibrium involving free and bound sodium ion. In the absence of CO, the same spectrum appears but the reaction is much slower; for example, 300 equiv of $NaPF_6$ was required for a 75% conversion to product after 1 h.

Under current interpretation,[2,16b] site 1 of protein-bound $[Fe_3S_4]^0$ clusters corresponds to a delocalized pair of iron atoms with mean oxidation state $Fe^{2.5+}$ (as in **3** and **4**) and $S = {}^9/_2$, and site 2 to trapped valence Fe^{3+} with $S = {}^5/_2$. Antiparallel coupling affords the $[Fe_3S_4]^0$ cluster spin $S = 2$. The Mössbauer parameters for **6** are entirely in agreement with this description.[15] The formation of cuboidal **6** and **10** does not require a redox reaction. In a number of proteins,[2] a cubane cluster is oxidized to the $[Fe_4S_4]^{3+}$ state, which loses Fe^{2+} to give $[Fe_3S_4]^+$ ($S = {}^1/_2$); the latter is reducible to $[Fe_3S_4]^0$. In the present scheme, the bound ligand in **4** (and its citrate analogue) must be dissociable in part so as to allow attachment of a second ligand equivalent and eventual removal of Fe^{2+} as **5**, which is formed in situ in an equal amount with **6**. Solutions of **6** under anaerobic ambient conditions are entirely stable for *at least 7 days*. In contrast, when **8** (14 mM) is treated with $10NaPF_6$ and CO (10 min, acetonitrile) and the reaction is monitored by ^1H NMR, $[Fe_3S_4(SEt)_3]^{3-}$ (δ 57.9 {br, CH_2}, 5.09 {Me}) is formed in 25% in situ yield, but after 1 h the only detectable product is $[Fe_4S_4(SEt)_4]^{2-}$ (δ 12.7 {CH_2}, 3.00 {Me}). Clearly, the semirigid cavitand ligand LS_3[19] is conspicuously beneficial to the stability of the cuboidal cluster.

Reaction of **6** with 1 equiv of $Fe(CF_3SO_3)_2$ in Me_2SO solution results in quantitative formation of cubane cluster **3**. Further,

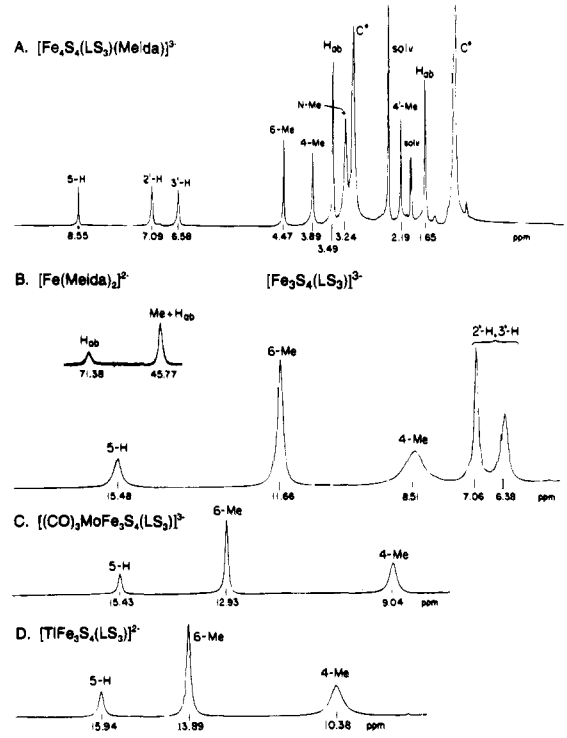

Figure 2. ^1H NMR spectra in Me_3SO:[13] (A) isolated $[Fe_4S_4(LS_3)(Meida)]^{3-}$ (**4**); (B) $[Fe_3S_4(LS_3)]^{3-}$ (**6**) and $[Fe(Meida)_2]^{2-}$ (**5**) generated in situ from the reaction of **4** with excess $(Et_4N)_2(Meida)$; (C) isolated $[(OC)_3MoFe_3S_4(LS_3)]^{3-}$ (**9**); (D) isolated $[TlFe_3S_4(LS_3)]^{2-}$ (**11**). Partial spectra are shown in B–D, emphasizing the isotropically shifted 4-Me, 6-Me, and 5-H resonances; signal assignments are indicated.

when preisolated **6** (61 mM) in DMF was treated with 1 equiv each of $(Et_4N)_2(Meida)$ and $Tl(CF_3SO_3)$ in 6:1 acetonitrile/Me_2SO (v/v) for 50 min followed by addition of ether, pure $(Et_4N)_2[TlFe_3S_4(LS_3)]$ (**11**, 86%) was isolated. The compound exhibits ^1H NMR (Figure 2D) and Mössbauer spectra indicative of a perturbed version of **6**, which must arise from the $[TlFe_3S_4]^+$ core. The compound $(Et_4N)_2[(CF_3SO_3)ZnFe_3S_4(LS_3)]$ (**12**)[14] was obtained similarly. These reactions constitute further structure proof of cuboidal **6**. Protein-bound cuboidal clusters bind certain exogenous metal ions,[2] including Tl^+ [21] and Zn^{2+}.[22] Comparison of properties of proven synthetic cubane MFe_3S_4 clusters[7d,e] and protein/metal ion reaction products[22] demonstrates that the latter have the cubane stereochemistry.

With the attainment of **6** by two independent routes, all structurally defined biological Fe–S clusters have now been synthesized with the exception of the P-cluster of nitrogenase. This work facilitates the investigation of all aspects of the intrinsic chemistry of the cuboidal Fe_3S_4 entity **1**, including redox reactions and structural, electronic, and redox reactions in accessible oxidation levels. Of special interest will be the scope of metal ion incorporation reactions and the properties of the new heterometal MFe_3S_4 clusters. Such studies are in progress.

Acknowledgment. This research was supported by NIH Grant GM 28856.

JA951951T

(20) $(Et_4N)_3[(CO)_3MoFe_3S_4(Smes)_3]\cdot MeCN$ (223 K): monoclinic ($P2_1/n$), a = 13.412(7) Å, b = 19.038(8) Å, c = 26.421(9) Å, β = 97.87-(3)°, 3372 observed data ($F_o^2 > 6\sigma(F_o^2)$), 3° ≤ 2θ ≤ 47°), R (R_w) = 4.93 (6.22%). Data were obtained with Mo Kα radiation, and the structure was solved and refined by standard methods. The mean bond distances Mo–S = 2.62(2) Å and Mo–Fe = 3.22(9) Å emphasize the weak binding of the $Mo(CO)_3$ group to the $[Fe_3S_4]^0$ fragment and its expected lability.

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