A Refined Model for $[Fe_3S_4]^0$ Clusters in Proteins**

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In 1987 Mössbauer spectra of the $[Fe_3S_4]^0$ center in *Desulfovibrio gigas* ferredoxin II (*Dg* Fd II) were interpreted in terms of a pair of $Fe^{2.5+}$ ions and a single Fe^{3+} ion. The two $Fe^{2.5+}$ ions were shown to possess a ground-state subspin $S_{12} = 9/2$, which is antiferromagnetically coupled to the $S_3 = 5/2$ of the Fe^{3+} ion. The overall ground-state spin is thus $S' = 2$.[1] Similar results were obtained for the $[Fe_3S_4]^0$ cluster of *Azotobacter vinelandii* Fd (*Av* Fd) at high and low pH.[2]

Analogous analyses were performed on $[Fe_4S_4]^{3+}$ and $[Fe_4S_4]^{+}$ clusters containing a pair of $Fe^{2.5+}$ ions and two Fe^{3+} ions or two Fe^{2+} ions, respectively.[3, 4] The ^{1}H NMR resonance signals of the cysteine ligands bound to the cluster exhibit hyperfine shifts that were shown to be related to the oxidation state of the iron center to which the cysteine is bound.[5, 6] By exploiting the high-resolution and sensitivity of NMR spectroscopy it was found that the various iron centers are not equivalent. This was attributed to equilibria between forms with different charge locations within the cluster.[7]

Several attempts have been made to study $[Fe_3S_4]^0$ cluster containing proteins by ^{1}H NMR spectroscopy, but no resonance signals from the β-CH_2 protons of the cluster cysteine ligands could be detected.[7–12] The Fe_7S_8 ferredoxin from *Bacillus schlegelii* (*Bs* Fd), containing one $[Fe_3S_4]$ and one $[Fe_4S_4]$ cluster, was labeled with deuterated cysteine groups.[13] The ^{2}H nucleus has a magnetogyric ratio that is 6.5 times smaller than that of the ^{1}H nucleus and the resonance lines should be 42 times narrower. Therefore, if paramagnetism is responsible for proton line broadening beyond detection, then the narrower ^{2}H signals should be detected. This was not the case.[13] Although based on only negative evidence, it was proposed that the line broadening beyond detection might be because of chemical exchange, that is that the exchange of valence between $Fe^{2.5+}$ and Fe^{3+} could occur with a rate constant of the order of the expected hyperfine shift difference (about $10^5 - 10^6\ s^{-1}$).

The $[Fe_3S_4]^0$ clusters are formally derived from the so-called "cubane" $[Fe_4S_4]^{2+}$ cluster by removal of one Fe^{2+} ion. As one vertex of the cubane is deprived of an iron atom, only one of the four sulfide ions (μ_3-S ion) bridges three iron centers, while the other three sulfide ions (μ-S ions) each bridge two iron centers. Realizing that the reduction of the $[Fe_3S_4]^0$ cluster at physiological pH requires a proton,[14–17] and following the idea that the proton could bind to any of the three μ-S ions,[2, 16–19] we propose the hypothesis that the proton could jump among the μ-S ions at the above rate and provide the required exchange mechanism. To demonstrate this model, we planned an NMR experiment at pH > 9, that is, well above the pK_a for the release of H^+ ions. Under these conditions, the mechanism responsible for the proton exchange within the reduced $[Fe_3S_4]$ cluster is deactivated, and the ^{1}H NMR signals should appear. A relatively low magnetic field of 4.7 T (200 MHz proton resonance frequency) was chosen to minimize the adverse effect of Curie line broadening, which is predicted to be sizable for a high-spin ($S' = 2$) metal cluster.[20]

The *Bs* ferredoxin was characterized during this work by electrochemical measurements. The potential for the one-electron reduction and reoxidation of the $[Fe_3S_4]^+$ cluster is -337 mV at pH 7.2. The pH dependence is very similar to that of the homologous *A. vinelandii* ferredoxin I (*Av* Fd I).[15] The slope $dE^{\circ\prime}_{obs}/d(pH)$ in the linear part of the pH dependence curve is -51 mV (pH unit)$^{-1}$ (-53 mV (pH unit)$^{-1}$ for *Av* Fd I), close to the -59 mV (pH unit)$^{-1}$ predicted for the coupling of the one-electron transfer to the transfer of a single proton. Fitting[15, 21] of the pH dependence data gave $E^{\circ\prime}_{alk} = -390$ mV and $pK_a = 8.1$ at 293 K; the corresponding values for native *Av* FdI at 273 K are -430 mV and 7.7, respectively.[15]

The paramagnetic ^{1}D ^{1}H NMR spectrum of concentrated *Bs* Fd (ca. 8 mM), reduced at pH 9.7 with sodium dithionite shows, besides some resonance signals shifted downfield in the $\delta = 10 - 50$ region (Figure 1 A), a broad signal at $\delta = 60$

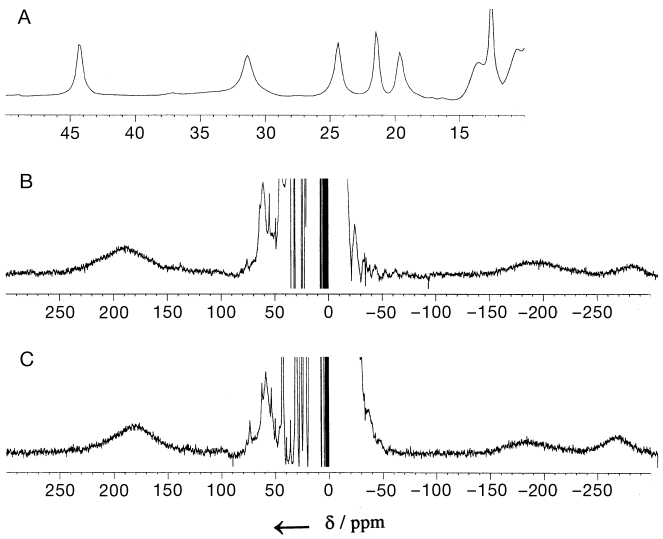

Figure 1. 200 MHz ^{1}H NMR spectra of dithionite-reduced *Bs* Fd at pH 9.7 (200 mM borate/NaOH buffer). A) spectrum at 297.3 K showing the $\delta = 10 - 50$ region containing the resonance signals of the cysteine ligands of the $[Fe_4S_4]^+$ cluster. B) Spectrum at 297.3 K showing the spectral window from $\delta = -310$ to 300 containing resonances from the cysteine ligands of the $[Fe_3S_4]^0$ cluster. C) The same sample and window as in B) but at 306.8 K.

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and three extremely broad signals at $\delta = 190$, -192, and -282 (Figure 1B). A search for further signals shifted beyond $\delta =$ 200 or -300 gave negative results. The shifts of the four hyperfine shifted signals show a Curie-type temperature dependence (Figure 1C). The intensity ratio of the three most shifted signals is approximately 2:1:1. These signals must be attributed to the cysteine residues coordinated to the $[Fe_3S_4]^0$ cluster, which to the best of our knowledge have never been observed before.

From established models based on exchange coupling among the three iron centers,[1, 2] the two upfield signals at $\delta = -192$ and -282 in Figure 1B correspond to the β-CH_2 protons of the cysteine residue coordinated to the Fe^{3+} ion. The signal shifted downfield at $\delta = 190$ has a relative intensity of 2, thus it could be assigned to the two β-CH_2 protons of one of the two cysteine units ligated to the mixed-valence pair of iron centers. The resonance signals of two more β-CH_2 protons are expected to be shifted even further and broadened, and therefore can not be observed.[13] A modest inequivalence of the two mixed-valence iron centers may be present, as suggested by the NMR spectra[8, 11, 22] of the oxidized form,[8, 11, 22] which lacks the cluster-bound proton over the pH range of interest. If the four downfield-shifted signals are pairwise inequivalent, only the less shifted and less broadened pair would be observed. The resonance signal at $\delta = 60$ can be assigned tentatively to a Hα proton of one the cysteine ligands bound to the mixed-valence pair of iron centers, presumably of the cysteine that gives rise to the most shifted resonance signal.

The present experiments are relevant to the biochemical behavior of the $[Fe_3S_4]^0$ cluster, and account for the Mössbauer and NMR spectroscopy data reported. The Mössbauer data indicate the presence of an Fe^{3+} ion and a pair of $Fe^{2.5+}$ ions.[1, 2] The NMR data at neutral pH are consistent with the presence of a chemical exchange process with a rate constant of the order of $10^5 - 10^6\ s^{-1}$[13] which wipes out the NMR signals. Electrochemistry shows that at neutral pH the uptake of the electron is associated with the uptake of a proton. Consistent with results from magnetic circular dichroism (MCD) and circular dichroism (CD) experiments,[15–17, 23] and following suggestions of Armstrong and co-workers that the protonation occurs at one of the three μ-S ions[15] and that "wherever the proton is bound to the cluster, it can be moved easily…",[19] a reasonable model emerges (Figure 2) in which 1) each of the three μ-S ions is protonated for a fraction of time (including the μ-S_1 that has been proposed to be the gateway for proton exchange[18, 19, 24]); 2) the protonated μ-S ion determines the location of the mixed-valence pair as being the two iron centers that are bridged by that sulfide. The exchange of a proton among the three μ-S ions determines the interchange of the valences of each iron; 3) the NMR signals of the cysteine ligands coordinated to each iron jump by hundreds of ppm from upfield to downfield because of this valence interchange, and are exchange-broadened beyond detection if the exchange occurs with a rate constant of $10^5 - 10^6\ s^{-1}$.

At a pH higher than the pK_a value the proton is released, and the ^{1}H NMR signals appear in the far-shifted regions. The signals are still broad and shifted strongly because of the high resulting total spin state $S' = 2$ and large hyperfine coupling constants. The resulting picture at high pH values is that of a cluster with a single valence distribution. This is in contrast to the $[Fe_4S_4]^{3+}$ clusters which show fast exchange equilibria on the NMR time scale.[7]

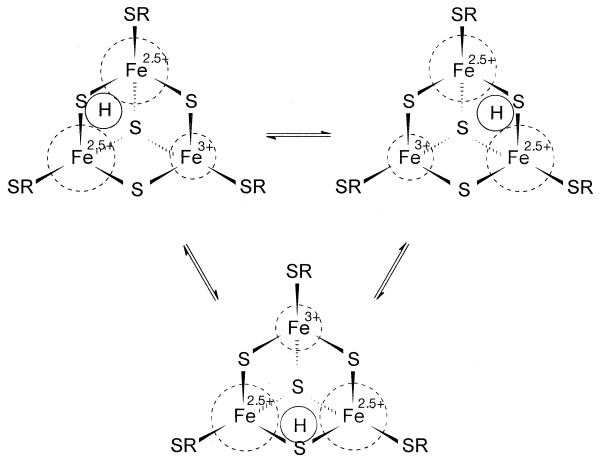

Figure 2. Schematic model of the proton–electron exchange in the low-pH form of the $[Fe_3S_4]^0$ cluster. According to this model, 1) the cluster is protonated at one of the three μ-S ions; 2) the mixed-valence pair is the one bridged by the protonated μ-S ion; 3) each of the three μ-S ions is protonated for a fraction of time; 4) the proton jumps from one μ-S ion to another at a rate of $10^5 - 10^6\ s^{-1}$, thereby causing a "merry-go-round" of iron valences in the cluster.

Experimental Section

The Fe_7S_8 Fd from *Bs*[25] was reduced with a small amount of solid sodium dithionite (Fluka) under an argon atmosphere at pH 9.7 in H_3BO_3/NaOH buffer (200 mM) and 99% D_2O.

The ^{1}D ^{1}H NMR spectra of reduced *Bs* Fd were recorded at 297.3 and 306.8 K on a wide-bore Bruker MSL 200 spectrometer with a superWEFT pulse sequence (180-tau-pulse-acquisition).[26] The maximum spectral window of 125 kHz was applied.

Voltammetric measurements were carried out at 293 K with solutions containing 100 μM of *Bs* Fd and 0.1 M NaCl in buffers of different pH values (5.9–10.1) using a standard three-electrode cell in a procedure already described.[21]

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The Total Synthesis of the Annonaceous Acetogenin, Muricatetrocin C**

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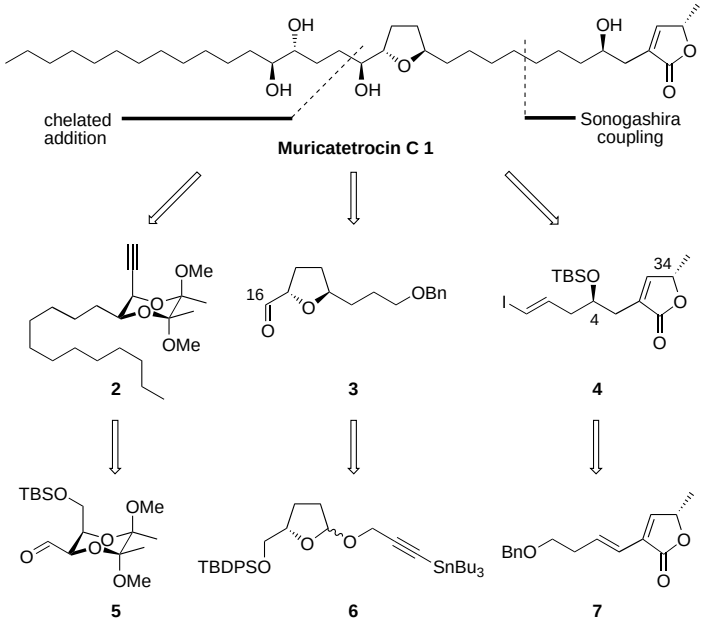
The annonaceous acetogenins comprise a rapidly growing class of natural products exhibiting a broad spectrum of biological properties. These include antibacterial, antimalarial, in vivo antitumor, parasiticidal, and pesticidal effects.[1] Perhaps most exciting is their novel and selective mode of action as inhibitors of oxidative phosphorylation, which offers a unique potential for these compounds as anticancer agents. The acetogenins are known to be powerful inhibitors of complex I (NADH:ubiquinone oxidoreductase) in mammalian and insect mitochondrial electron transport systems and of NADH oxidase found in the plasma membranes of cancer cells.[2, 3] These actions lead to ATP deprivation and subsequent apoptosis.[4] More recently the annonaceous acetogenins have also been shown to overcome resistance in multidrug resistant (MDR) tumors.[5] Thus, for the above reasons and by virtue of their limited availability from natural sources, these compounds have been targeted for total synthesis by a number of research groups.[6]

In 1996 McLaughlin and co-workers reported the isolation of muricatetrocin C (**1**; see Scheme 1) from the leaves of *Rollina mucosa*.[7] The molecule exhibits potent inhibitory action against PC-3 prostatic adenocarcinoma, PACA-2 pancreatic carcinoma, and A-549 lung carcinoma. Herein we report the first total synthesis of **1**, which was achieved by using a highly convergent synthetic strategy.[8]

The synthetic plan to **1** hinged on the enantioselective preparation of fragments **2**, **3**, and **4** and their subsequent coupling reactions (Scheme 1). It was believed that in addition to allowing an efficient synthesis of **1**, these three components would provide an excellent platform for both the evolution of existing group methodology and the potential development of new synthetic tools. The key features of our approach are: the application of the recently reported (*R*′,*R*′,*R*,*S*)-2,3-butanediacetal-protected butane tetrol **8** (see Scheme 2) as a building block for the *anti*-1,2-diol component **2** through selective chemical differentiation of the incongruous hydroxy termini;[9] the use of the recently developed anomeric O – C rearrangement protocol for the stereoselective construction of the 2,5-*trans*-disubstituted THF ring component **3**;[10] and finally the implementation of a new approach to the (*S*)-hydroxy-butenolide terminus **4**, using a hetero-Diels – Alder (HDA) reaction to simultaneously install the 1,5-stereochemical relationship and mask the butenolide double bond.



Scheme 1. Synthetic plan for **1**. Bn = benzyl, TBS = *tert*-butyldimethylsilyl, TBDPS = *tert*-butyldiphenylsilyl.

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