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Iron–Sulfur Clusters

The Complete Characterization of a Reduced Biomimetic [2Fe-2S] Cluster[**]

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Protein-bound iron–sulfur clusters of the ferredoxin and Rieske types, including binuclear [2Fe-2S] sites, are ubiquitous biological cofactors.[1] Their dominant function is electron transfer, where the Fe/S core shuttles between the [2Fe-2S]$^{2+}$ and [2Fe-2S]$^{1+}$ states.[2] Synthetic analogues for thiolato-coordinated [2Fe-2S] cores are well established in bioinorganic chemistry,[3] but well-characterized [2Fe-2S] complexes with other terminal ligands are still relatively rare;[4,5] a model complex for Rieske-type clusters comprising a heteroleptic set of ligands was published only recently.[6] Most reported analogues of [2Fe-2S] sites have been synthesized exclusively in the all-ferric state, while the mixed-valent [2Fe-2S]$^{1+}$ state could, if at all, only be accessed by electrochemical methods.[7] Gibson and Beardwood reported some reduced [2Fe-2S] clusters that were generated by chemical reduction in situ and investigated in solution.[8] Furthermore they were able to isolate and characterize one species in the [2Fe-2S]$^{1+}$ state using a bidentate bis(benzimidazolato) terminal ligand; it was studied by Mössbauer spectroscopy, and an $S = 1/2$ ground state with partially delocalized mixed valence has been proposed for the system.[9] However, neither has a crystal structure of any [2Fe-2S] cluster in the mixed-valent [2Fe-2S]$^{1+}$ state been published nor have magnetic measurements of such reduced clusters been performed; such measurements could confirm the proposals based on Mössbauer data. Herein we report the first crystal structure and SQUID magnetic susceptibility data of a synthetic mixed-valent [2Fe-2S] cluster, together with its complete spectroscopic characterization.

We decided to also use a bidentate bis(benzimidazolato) ligand since these heterocyclic systems have proven to sufficiently stabilize the mixed-valent form. A phenyl group was appended at the ligand backbone to improve solubility and crystallization (Figure 1). The homoleptic all-ferric cluster **1** was synthesized in a standard ligand-exchange reaction by addition of an excess of the deprotonated ligand to $(NEt_4)_2[Fe_2S_2Cl_4]$.[10]

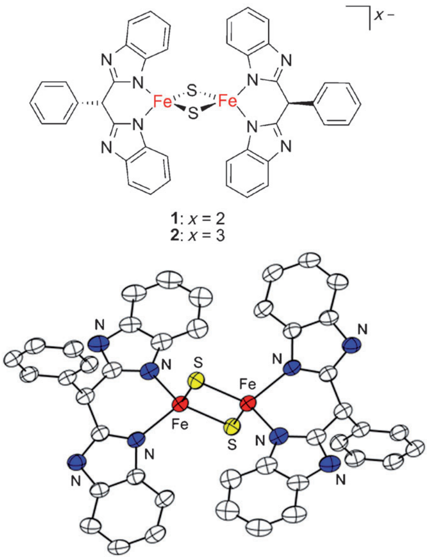

Figure 1. Top: Schematic view of bis(benzimidazolate) coordinated [2Fe-2S] clusters **1** and **2**. Bottom: ORTEP plot of the molecular structure of cluster **2** (thermal ellipsoids set at 50% probability). For clarity all hydrogen atoms and counterions are omitted.

Redox properties of **1** were studied by cyclic voltammetry in MeCN/0.1 M $n\mathrm{Bu_4NPF_6}$ at room temperature (Figure 2, top). Two reversible one-electron reduction processes are assigned to the formation of the mixed-valent species at $E_{1/2} = -1.14$ V and the all-ferrous species at $E_{1/2} = -2.10$ V vs. the Fc/Fc$^+$ couple (Fc = $[(C_5H_5)_2Fe]$). The potentials are shifted to more positive values compared to related thiolato-coordinated complexes and are relatively high for synthetic [2Fe-2S] clusters, a result of the electron withdrawing character of the bis(benzimidazolato) capping ligands. Electrochemical potentials of cysteine-coordinated [2Fe-2S] ferredoxines usually lie in the range −1.05 V to −0.75 V versus the Fc/Fc$^+$ couple.[11] The large separation between the two half-wave potentials revealed significant stabilization of the mixed-valent species **2** ($K_c = 1.7 \times 10^{16}$), a fact that allowed the chemical reduction of **1** (using decamethylcobaltocene) and isolation of **2**, which proved to be stable in the solid state under nitrogen even at room temperature for several hours.

The UV/Vis spectrum (Figure 2, bottom) of **1** shows two prominent bands at 411 nm ($\varepsilon = 7340\,\mathrm{M^{-1}cm^{-1}}$) and 528 nm ($\varepsilon = 7020\,\mathrm{M^{-1}cm^{-1}}$) and a minor band at

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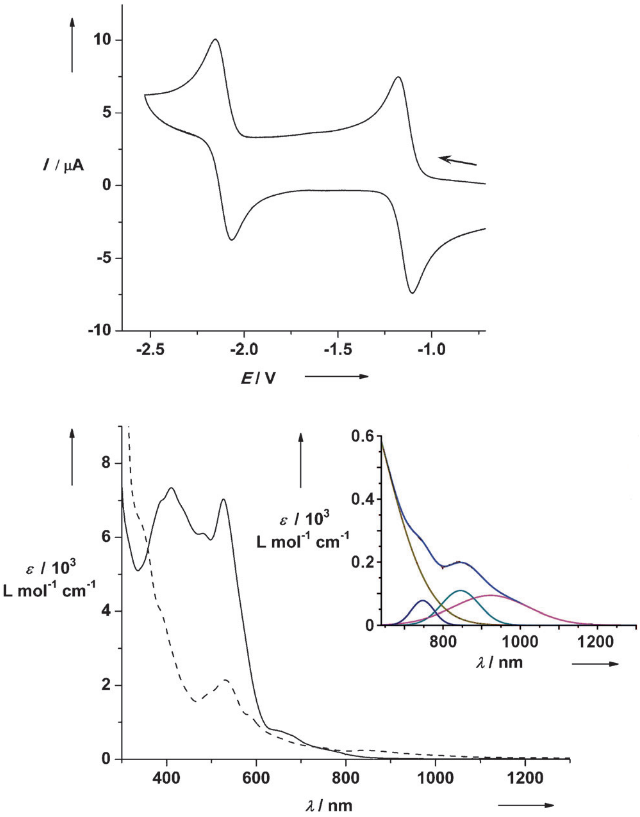

Figure 2. Top: Cyclic voltammogram of **1** in MeCN ($c = 1.0 \cdot 10^{-3}$ M) at a scan rate of 100 mV s^{-1}. Bottom: Potentials are given in volts vs. the Fc/Fc^{+} couple. Electronic absorption spectrum of **1** (——) and **2** (-----) recorded in DMF solution at room temperature. Inset: deconvolution of the low-energy bands.

484 nm ($\varepsilon = 6100\,\mathrm{M^{-1}cm^{-1}}$). In the course of reduction the overall intensity in the visible region drops significantly. In the spectrum of **2** the band at 531 nm ($\varepsilon = 2150\,\mathrm{M^{-1}cm^{-1}}$) remains, a shoulder at 583 nm ($\varepsilon = 1220\,\mathrm{M^{-1}cm^{-1}}$) and two shoulders at 384 nm and 341 nm ($\varepsilon = 4000$ and $6500\,\mathrm{M^{-1}cm^{-1}}$) appear. Furthermore, a broad band at about 842 nm ($\varepsilon = 240\,\mathrm{M^{-1}cm^{-1}}$) develops (Figure 2, inset). After exposing a solution of **2** to oxygen the absorption spectrum appears to revert almost to the spectrum of **1** (Supporting information, Figure S3) with three isosbestic points at 359 nm, approximately 730 nm and approximately 1200 nm. Differences to the spectrum of cluster **1** can be explained by an absorption band of the $[\mathrm{Cp^*_2Co}]^+$ ion at 339 nm ($\mathrm{Cp^*} = \mathrm{C_5Me_5}$).

Crystals of **1** and **2** were obtained by slow diffusion of diethyl ether into DMF solutions of the products, allowing the molecular structures of biomimetic [2Fe-2S] complexes in both relevant oxidation states to be compared by X-ray crystallography for the first time.

Inspection of the core structures of all-ferric **1** (Supporting information, Figure S12) and mixed-valent **2** (Figure 1) shows only minor changes in geometric parameters. Relevant distances and angles are collected in Table 1. Both compounds crystallize in the triclinic space group $P\bar{1}$ with crystallographically imposed inversion symmetry. Interestingly the two iron atoms of the mixed-valent species **2** are not distinguishable, a result of partial delocalization of the unpaired electron. The [2Fe-2S] rhombus remains almost unchanged upon reduction: the Fe···Fe distances of both clusters are similar ($d(\mathbf{1}) = 2.70$ Å vs. $d(\mathbf{2}) = 2.69$ Å) and the bond lengths and angles between the iron and the sulfur atoms differ only by 0.02 Å and 1.5°, respectively. In **2** the bonds between iron and nitrogen atoms are elongated by $d = 0.06$ Å, and therefore the N-Fe-N angles in **2** are smaller by 5.1°. An overlay of both structures is in the Supporting Information (Figure S14)

Table 1: Selected interatomic distances [Å] and angles [°] for **1** and **2**.

	d(Fe···Fe)	d(Fe–N)	d(Fe–S)	∡(N-Fe-N)	∡(S-Fe-S)
1	2.70	1.89/1.99	2.19/2.21	92.84	104.27
2	2.69	2.04/2.05	2.22/2.23	87.73	105.80

Magnetic susceptibility measurements (SQUID; Figure 3) reveal strong antiferromagnetic coupling of the Fe$^{\mathrm{III}}$ ions and an $S = 0$ ground state for **1** ($J = -179\,\mathrm{cm^{-1}}$ in a $-2JS_1 \cdot S_2$ model);[12] such behavior is typical for the $[2\mathrm{Fe}\text{-}2\mathrm{S}]^{2+}$ core. For the mixed-valent cluster **2** the magnetic moment μ_{eff} was also found to decrease upon lowering the temperature from 295 to 75 K owing to antiferromagnetic coupling, but below 75 K it remains constant at 1.82 μ_{B} in accordance with an $S = 1/2$ ground state. Also this behavior can be simulated with a Heisenberg spin-coupling model by adopting $S_1 = 5/2$ and $S_2 = 2$ for the Fe$^{\mathrm{III}}$ and the Fe$^{\mathrm{II}}$ ions in the mixed-valent $[2\mathrm{Fe}\text{-}2\mathrm{S}]^{1+}$ core, and an effective coupling constant, J_{eff}, which comprises the exchange interaction as well as the competing effects of intrinsic electron transfer (double exchange)[13–15] and charge localization[16] arising from the static and vibronic coupling to the environment.[8,17] The effective coupling constant[18] is determined to be $J_{\mathrm{eff}} = -134\,\mathrm{cm^{-1}}$, which is well within the range found for Rieske and Rieske-type proteins ($-65\,\mathrm{cm^{-1}}$ to $-270\,\mathrm{cm^{-1}}$ with a median around $-150\,\mathrm{cm^{-1}}$).[19,20]

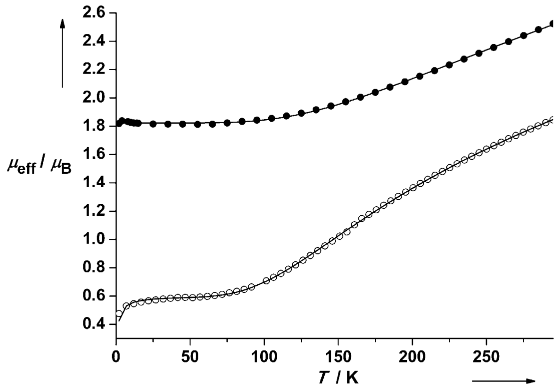

Figure 3. Temperature dependence of μ_{eff} for **1** (○) and **2** (●) at a field of $B = 0.5$ T. The solid lines represent spin-Hamiltonian simulations.

Unfortunately, we cannot probe directly the strength of double exchange in **2**, because no intervalence band can be detected in the investigated range of the UV/Vis/NIR spectrum (200–3000 nm). However, substantial charge delocalization is clear from the Mössbauer and EPR spectra of **2**.

The EPR spectrum in frozen solution (Supporting information, Figure S11) shows pronounced rhombic splitting with $g = (2.016, 1.935, 1.885)$, which is similar to those of the Rieske-type proteins[21–23] and the related model complex.[6] This result is in line with expectation as the g anisotropy is mainly determined by the properties of the Fe^{II} sites of the mixed-valent dimers,[22,23] which all have comparable $\{S_2N_2\}$-coordination. However, the average g value of **2** ($g_{av} = 1.96$) is significantly closer to the free-electron value ($g = 2.0023$) than those of the Rieske centers and the model compound ($g_{av} =$ 1.91 and 1.92, respectively). Mouesca and Orio have pointed out that this behavior indicates significant partial valence delocalization.[23]

The zero-field Mössbauer spectrum recorded at 4.2 K (Figure 4, bottom) shows a superposition of two distinct quadrupole doublets (I and II), as expected for a mixed-valent dimer with fast spin relaxation in the solid state (collapsed paramagnetic hyperfine splitting). The parameters, however, $\delta^{I} = 0.47\ \mathrm{mm\,s^{-1}}$, $\Delta E_Q^{\ I} = 1.41\ \mathrm{mm\,s^{-1}}$, $\delta^{II} = 0.69\ \mathrm{mm\,s^{-1}}$, $\Delta E_Q^{\ II} = 2.90\ \mathrm{mm\,s^{-1}}$, deviate from those expected for valence-trapped Fe^{III} and Fe^{II} ions in quasi tetrahedral $\{S_2N_2\}$-coordination and indicate appreciable mixing of Fe^{II} and Fe^{III} characters for the individual sites. The partial delocalization is particularly clear for the Fe^{III} subspectrum, the isomer shift of which ($\delta^{I} = 0.47\ \mathrm{mm\,s^{-1}}$) clearly exceeds that of the corresponding $\{Fe^{III}\}_2$ compound **1** ($\delta = 0.27\ \mathrm{mm\,s^{-1}}$ at 4.2 K). The empirical correlation[24] $\delta(x) = [1.43–0.40x]\ \mathrm{mm\,s^{-1}}$, found for δ and the oxidation number (x) of $\{FeS_4\}$ units, would predict a difference of 0.4 $\mathrm{mm\,s^{-1}}$ for fully localized Fe^{III} and Fe^{II} sites (I) and (II), whereas for **2** we find only half as much (0.2 $\mathrm{mm\,s^{-1}}$).

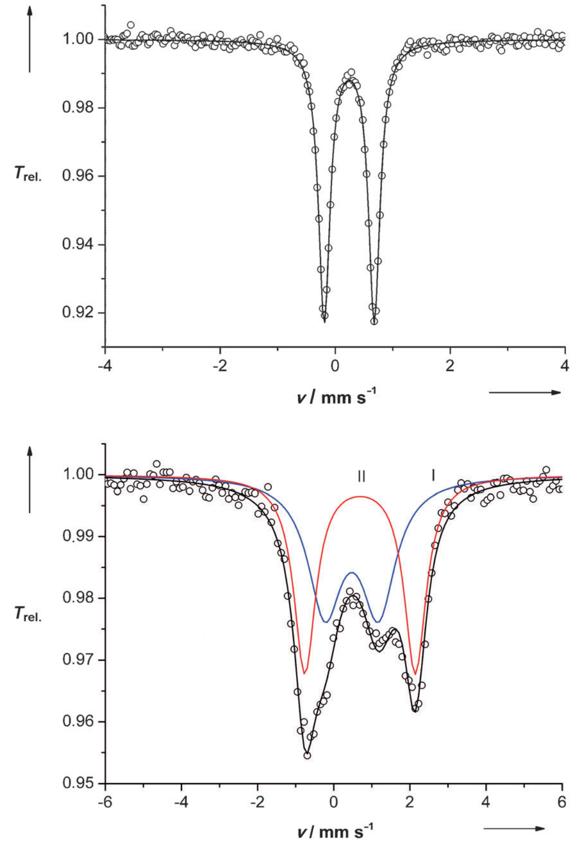

Figure 4. Zero-field Mössbauer spectra of **1** recorded at 80 K (top) and of **2** recorded at 4.2 K (bottom). The solid lines are fits with Lorentzian doublets to the experimental values using the following isomer shifts and quadrupole splittings: $\delta = 0.24\ \mathrm{mm\,s^{-1}}$, $\Delta E_Q = 0.87\ \mathrm{mm\,s^{-1}}$ for **1**, and $\delta = 0.47\ \mathrm{mm\,s^{-1}}$, $\Delta E_Q = 1.41\ \mathrm{mm\,s^{-1}}$ for the "Fe^{III}" contribution and $\delta = 0.69\ \mathrm{mm\,s^{-1}}$, $\Delta E_Q = 2.90\ \mathrm{mm\,s^{-1}}$ for the "Fe^{II}" contributions of **2**, respectively.

The actual mixing of the electronic configurations [Fe^{2+}-Fe^{3+}] ("A") and [Fe^{3+}-Fe^{2+}] ("B") was determined from a comparison of the isomer shifts and magnetic hyperfine data with those of the Rieske cluster and of the closely related trianion $[Fe_2S_2(DMBB)_2]^{3-}$ (**A**) (DMBB = dimethylmethanebisbenzimidazolato).[21,25] Since **A** and **2** show almost identical Mössbauer parameters,[9,12] we also adopt the same 20% valence mixing for the partially delocalized states "A" and "B" of **2** (coefficients $a^2 = 0.8$, $b^2 = 0.2$).[9] However, in contrast to this similarity, the splitting of the spin doublet ground state and the quartet excited state found for **2** deviates considerably from that reported for **A** ($\Delta_S = 402\ \mathrm{cm^{-1}}$ vs. 105 $\mathrm{cm^{-1}}$). We tend to assign the deviation to low accuracy of the measurement of **A** (an intricate analysis of paramagnetic relaxation rates).[9]

The magnetic and spectroscopic properties of **2** can be rationalized by using a phenomenological model that describes the energies of the spin states of a mixed-valent iron dimer in terms of the exchange coupling constant J, a double exchange parameter B accounting for delocalization, and an effective energy difference Δ_{AB} of the configurations "A" and "B" that summarizes the charge-localizing interactions due to static site differences as well as vibronic coupling. The corresponding double-exchange Hamiltonian[9,12,13,15,26] cannot be solved with experimental data for only two variables,[9] J_{eff} and a^2. However, if in addition we adopt $B = 700\ \mathrm{cm^{-1}}$ as determined by DFT calculations,[23] we can fit parameters and obtain $J = -341\ \mathrm{cm^{-1}}$, and $\Delta_{AB} = 1050\ \mathrm{cm^{-1}}$. The exchange coupling constant is in good agreement with $J = -360\ \mathrm{cm^{-1}}$ estimated from an analysis of the covalency in $[2Fe\text{-}2S]^{1+}$ clusters using ligand K edge X-ray absorption spectroscopy.[27] Moreover, this set of parameters is consistent with Mouesca and Orio's combined DFT and EPR analysis for $[2Fe\text{-}2S]^{1+}$ clusters.[23] Our results predict an intervalence band at 1750 $\mathrm{cm^{-1}}$ (5714 nm).[28] Apparently such transitions are difficult to detect, and we didn't find it for **2**, but the result rules out previous tentative assignments of intervalence bands around 540 nm;[29] such large double-exchange splitting of total spin states would not be consistent with the $S = 1/2$ ground state of $[2Fe\text{-}2S]^{1+}$ clusters.

In summary, for the first time we have isolated, crystallized, and thoroughly characterized a synthetic ferredoxin-type [2Fe-2S] cluster in both relevant oxidation states, namely the $[2Fe\text{-}2S]^{2+}$ and mixed-valent $[2Fe\text{-}2S]^{1+}$ forms. Magnetic measurements confirm antiferromagnetic coupling of the Fe^{II} and the Fe^{III} ion in the mixed-valent complex resulting in an $S = 1/2$ ground state with $J_{eff} = -134\ \mathrm{cm^{-1}}$. This value provides some solid experimental basis for predicting the position of the intervalence band in the IR region. The unpaired electron in the $[2Fe\text{-}2S]^{1+}$ species is partially delocalized over the cluster core, and the two iron ions are not distinguishable by X-ray crystallography. The [2Fe-2S] core structure undergoes only minor geometric changes upon reduction, which contributes to the high stability of the mixed-valent species **2** and reflects the low reorganization energies that make these [2Fe-2S] clusters preferred electron-transfer sites in biology. Future work will address whether reduction of the [2Fe-2S] core can be coupled to protonation of the backside N atoms of the benzimidazolates, similar to what has been proposed for the imidazole ligands of the natural Rieske cluster.[30]

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[12] For further details see Supporting Information.

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[18] J_{eff} is defined as one third of the energy splitting Δ_S between $S = 1/2$ and $S = 3/2$ states, which is 402 cm^{-1} for **2**.

[19] Values converted to our notation $\mathbf{H} = -2\,J_{eff} S_1 S_2$.

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