Resonance Raman Evidence for Non-Heme Fe−O Species in the [6Fe−6S]-containing Iron−Sulfur Proteins from Sulfate-Reducing Bacteria

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Spectroscopic (EPR, Mössbauer, and MCD) and analytical data have provided strong evidence for novel iron−sulfur clusters containing six Fe atoms in proteins of unknown function isolated from *Desulfovibrio vulgaris* (Hildenborough)[1] and *Desulfovibrio desulfuricans* (ATCC 27774).[2] These monomeric proteins contain 6−7 non-heme Fe and S^{2-} per 58−60 kDa subunit, and the primary structures have been deduced from the nucleotide sequences.[3] While the nature of the Fe−S cluster in these proteins is still under investigation, the presence of a prismane-type [6Fe−6S] cluster has been proposed on the basis of the analytical data and the similarity in the EPR properties of the dithionite-reduced proteins with those of crystallographically defined synthetic prismane-type clusters with $[6Fe-6S]^{3+}$ cores.[1] Redox titrations indicate that the cluster can be stabilized in four redox states: $[6Fe-6S]^{6+}$ ($S = 0$ ground state), $[6Fe-6S]^{5+}$ (mixed spin with $S = {}^9/_2$ (≥90%) and $S = {}^1/_2$ (≤10%) ground states), $[6Fe-6S]^{4+}$ (mixed spin with $S = 4$ and 0 ground states), and $[6Fe-6S]^{3+}$ ($S = {}^1/_2$ ground state), and the midpoint potentials (vs NHE at pH 7.5) for the transitions are in the range +285 to +370, −25 to +50, and −220 to −113 mV, respectively. Here we report on resonance Raman studies for the *D. vulgaris* and *D. desulfuricans* [6Fe−6S] proteins. The results are consistent with a new structural type of biological Fe−S cluster with μ_3-bridging sulfides and reveal the presence of a non-heme Fe=O or monobridged Fe−O−Fe species in the aerobically purified protein ($[6Fe-6S]^{5+}$ core oxidation state).

Resonance Raman spectra[4] of the recombinant [6Fe−6S] proteins from *D. desulfuricans* (natural abundance Fe sample in $H_2{}^{16}O$ and $H_2{}^{18}O$ buffers) and *D. vulgaris* (^{54}Fe-enriched sample in $H_2{}^{16}O$ and $H_2{}^{18}O$ buffers and ^{57}Fe-enriched sample in $H_2{}^{16}O$ buffer) are shown in Figures 1 and 2, respectively.[5] In the Fe−S stretching region, 200−430 cm^{-1}, the frequencies and pattern of band intensities observed in both proteins are most similar to those observed for structurally characterized Fe−S proteins containing clusters with one or more μ_3-bridging sulfides, i.e., $[3Fe-4S]^{+}$, $[4Fe-4S]^{2+}$, or $[4Fe-4S]^{3+}$ clusters in low-molecular-weight ferredoxins or high-potential iron−sulfur proteins.[6] On the basis of the vibrational assignments for these clusters and the negligible ^{57}Fe/^{54}Fe isotope shift (<0.5 cm^{-1}), the most intense band at 344−345 cm^{-1} is assigned to symmetric stretching primarily involving motion of μ_3-bridging sulfides. The frequency of this mode has been found to correlate approximately with the average Fe oxidation state (333−340 cm^{-1} for $Fe^{+2.5}$ in $[4Fe-4S]^{2+}$ clusters; 341−344 cm^{-1} for $Fe^{+2.75}$ in $[4Fe-4S]^{3+}$ clusters; 347−348 cm^{-1} for Fe^{+3} in $[3Fe-4S]^{+}$)[6] and is consistent with the average $Fe^{+2.83}$ oxidation state of a putative $[6Fe-6S]^{5+}$ cluster. While the spectra of the two [6Fe−6S] proteins are remarkably similar in the Fe−S stretching region, the bands are significantly broader, and the frequencies differ in detail from any of the structurally characterized [3Fe−4S] or [4Fe−4S] clusters. The spectra are, therefore, consistent with a new type of higher nuclearity Fe−S core unit with μ_3-bridging sulfides. A prismane-type [6Fe−

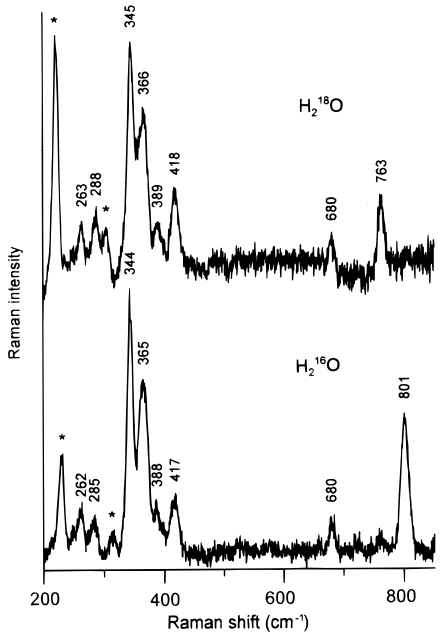

Figure 1. Resonance Raman spectra of *D. desulfuricans* [6Fe−6S] protein as purified (lower spectrum) and after exchange into a $H_2{}^{18}O$ buffer (upper spectrum). The samples (2−3 mM in protein) were in 20 mM Tris-HCl buffer, pH 8.0, with 100 mM NaCl. The spectra were obtained using 457.9 nm excitation by collecting scattered light from the surface of a frozen droplet at 25 K. Each spectrum is the sum of 50 scans with each scan involving photon counting for 1 s every 0.5 cm^{-1} with 6 cm^{-1} spectral resolution. Bands marked with an asterisk are lattice modes of the frozen buffer solution.

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(4) Raman spectra were recorded with an Instruments SA U1000 scanning spectrometer fitted with a cooled RCA 31034 photomultiplier tube, using lines from a Coherent Innova 100 10-W Ar^+ or 200-K2 Kr^+ laser. Scattering was collected at 90° from the surface of a frozen droplet of protein solution on the cold finger of an Air Products Displex model CSA-202E closed cycle refrigerator (~25 K). All absolute frequencies are accurate to ±1 cm^{-1}, and isotope shifts are accurate to ±0.5 cm^{-1}.

(5) Samples were purified aerobically from cells grown on natural abundance Fe, ^{54}Fe-enriched (using 99.85% ^{54}Fe), or ^{57}Fe-enriched (using 95.2% ^{57}Fe) media using the published procedures.[1c,2b] Isotopic enrichment is estimated to be approximately 80% on the basis of previous studies.[1c] All samples were in 20 mM Tris-HCl buffer, pH 8.0, with 100 mM NaCl and were exchanged into the equivalent buffer prepared using $H_2{}^{18}O$ (>95% enrichment) by repeated dilution and concentration using centricon ultrafiltration.

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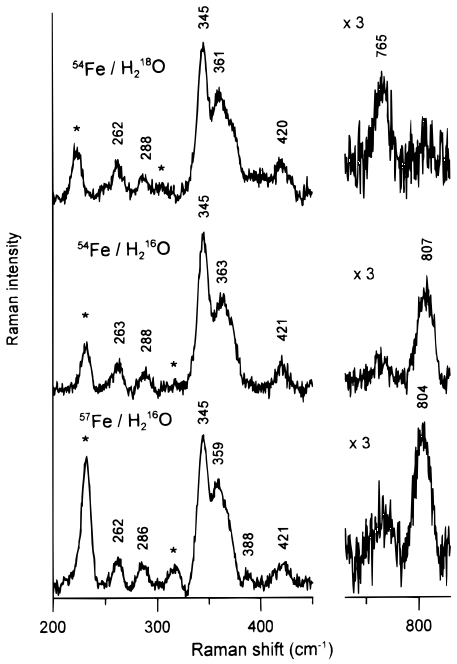

Figure 2. Resonance Raman spectra of ^{54}Fe- and ^{57}Fe-enriched samples of *D. vulgaris* [6Fe−6S] protein as purified: (upper spectrum) ^{54}Fe-enriched sample after exchange into a $H_2{}^{18}O$ buffer; (middle spectrum) ^{54}Fe-enriched sample as purified; (lower spectrum) ^{57}Fe-enriched sample as purified. Buffer solution, sample concentration, and conditions of measurement are as described in the legend of Figure 1.

6S] core is clearly an excellent candidate in light of the available analytical and biophysical data. More detailed vibrational analysis of the Fe−S stretching modes will require ^{34}S/^{32}S isotope shifts, and the appropriate isotopically labeled samples are in preparation.

In the higher frequency region, 430−1600 cm^{-1}, the [6Fe−6S] proteins each exhibit two pronounced bands at 680 and 801 cm^{-1} (804 cm^{-1} in the ^{57}Fe-enriched *D. vulgaris* [6Fe−6S] protein in $H_2{}^{16}O$ buffer). While the former is easily rationalized as the first overtone of the symmetric breathing mode of the [6Fe−6S] cluster (2 × 344 cm^{-1}) and/or the S−C stretching mode of coordinated cysteine residues, an intense band at 801 cm^{-1} has never been observed before for any Fe−S protein and cannot be attributed to an overtone/combination band or an impurity. A preliminary excitation profile (not shown) indicates that this vibration is maximally enhanced with 458 nm excitation and has much less enhancement with 406 and 530 nm excitation. This band is clearly identified as a solvent exchangeable Fe−O stretching mode by the 38−42 cm^{-1} $H_2{}^{16}O/H_2{}^{18}O$ downshift and the 3 cm^{-1} ^{54}Fe/^{57}Fe downshift; see Figures 1 and 2.[7]

Fe−O stretching modes occurring near 800 cm^{-1} can only be attributed to an Fe−O−Fe[8] or Fe═O unit.[9] All biological and synthetic examples of multiply bridged Fe^{III}−O−Fe^{III} centers (Fe−O−Fe angles in the range 119−135°) exhibit asymmetric stretching modes in the range 720−790 cm^{-1} with ^{18}O downshifts between 25 and 45 cm^{-1}.[8] However, for these centers, the symmetric Fe^{III}−O−Fe^{III} stretching mode occurs in the range 450−550 cm^{-1} with ^{18}O downshifts between 9 and 17 cm^{-1} and is invariably more intense than the asymmetric stretch in the Raman spectrum.[8] Hence, a multiply bridged Fe^{III}−O−Fe^{III} unit is not consistent with the observed spectra. In contrast, synthetic compounds with monobridged Fe^{III}−O−Fe^{III} groups (Fe−O−Fe angles in the range 150−180°) exhibit higher frequency asymmetric stretching modes, 795−885 cm^{-1} with ^{18}O downshifts in the range 39−44 cm^{-1}, and lower frequency symmetric stretching modes, 363−458 cm^{-1} with smaller ^{18}O downshifts that can be negligible for angles approaching 180°.[8] Moreover, in structurally asymmetric Fe−O−Fe complexes, the asymmetric stretching mode can be much more intense than the symmetric stretch.[8] Hence, it is possible that one of the bands attributed to Fe−S stretching modes in the 360−420 cm^{-1} region may correspond to the symmetric Fe−O−Fe stretch, or alternatively, this mode may be obscured by the Fe−S modes. Biological examples of Fe^{IV}═O species have been confined to hemoproteins, and the range established for the $\nu(Fe^{IV}{=}O)$ stretching frequency is 745−852 cm^{-1} with ^{18}O downshifts between 31 and 46 cm^{-1}. To our knowledge, Raman data is only available for one synthetic non-porphyrin-based compound, OFe(salen) (salen refers to the *N*,*N*′-ethylenebis(salicylideneaminato) ligand). In this compound the Fe^{IV}═O vibration occurs at 851 cm^{-1} (35 cm^{-1} ^{18}O isotope downshift) and is maximally enhanced with 458 nm excitation via an O → Fe^{IV} charge transfer transition.[9a]

While the resonance Raman results provide strong evidence for a Fe═O or monobridged Fe−O−Fe species in the as-purified samples of both [6Fe−6S] proteins investigated in this work, it remains to be determined if the Fe−O unit is an intrinsic part of the Fe−S cluster or involves at least one additional Fe site. The Fe analyses do not rule out the possibility of an additional Fe site, but the published Mössbauer data have been interpreted in terms of six distinct iron sites with no evidence for a Fe^{IV}═O species.[1c,2a] However, recent Mössbauer studies of the *D. vulgaris* [6Fe−6S] protein in the +5 oxidation state have provided evidence for an additional Fe site with high-spin Fe^{II} character that is coupled to the cluster spin.[10] A model involving a μ_2-O^{2-} as the sole bridging ligand between a localized-valence Fe^{II} site and a cluster Fe would explain the vibrational data and is currently our best working hypothesis. Although the current data does not permit distinction between a Fe−O−Fe and a Fe═O site, we conclude that at least one of the Fe sites of the [6Fe−6S] clusters in these proteins has a unique oxo functionality, and it seems probable that this is related to the yet-to-be-determined biological function.

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(7) For an isolated ^{57}Fe−^{16}O unit with ν_{Fe-O} = 800 cm^{-1}, the predicted ^{18}O and ^{54}Fe isotope shifts are 35 and 5 cm^{-1}, respectively. Coordination of the Fe would have the effect of increasing the effective mass of the Fe, thereby increasing the ^{18}O isotope shift and decreasing the ^{54}Fe isotope shift.

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