J. Am. Chem. Soc. 1982, 104, 4711-4712 4711

^{57}Fe ENDOR of the Nitrogenase MoFe Protein

Brian M. Hoffman,* Ronald A. Venters, and
James E. Roberts

Department of Chemistry, Northwestern University
Evanston, Illinois 60201

Mark Nelson and W. H. Orme-Johnson*

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Received May 5, 1982

The resting-state EPR spectrum of the nitrogenase molybdenum–iron (MoFe)[1] protein is unique among biological systems.[2] It is associated with the molybdenum–iron cofactor (FeMo-co; 6-8 Fe, 4–6 S* per molybdenum) and is characteristic of a metal center with total spin $S = {}^3/_2$.[3] Preliminary ^{95}Mo electron nuclear double resonance (ENDOR) spectra of this protein led us to infer that a single molybdenum is integrated into an individual $S = {}^3/_2$ center of FeMo-co and that the molybdenum is most plausibly viewed as being in an even-electron state, for example, Mo(II) or Mo(VI).[4] The best characterization to date of the iron atoms in FeMo-co comes from an elegant analysis of the ^{57}Fe Mössbauer effect of the MoFe protein;[5,6] the MoFe EPR signal is broadened by ^{57}Fe enrichment, but lack of resolution precludes detailed analysis.[5] However, the Mössbauer effect data for the cofactor are obtained by first subtracting from the raw data the spectra of a more than equal number of Fe atoms belonging to the MoFe iron–sulfur clusters. The subsequent fitting procedure is of necessity insensitive to some of the ^{57}Fe hyperfine parameters and is forced to rely on a number of basic, restrictive assumptions. In order to obtain a more complete characterization of the metal atoms in the EPR-visible center of MoFe, we have now performed an ^{57}Fe ENDOR study. Despite the complexity of this multimetal cluster, the experimental circumstances allow us to obtain spectra from six individual and distinct iron sites and to determine the hyperfine coupling tensor of each.

Natural abundance and iron-enriched samples of nitrogenase from *Azotobacter vinelandii* were prepared as described elsewhere.[5] EPR and ENDOR spectra were taken in a spectrometer described previously.[7] ENDOR is performed by observing changes in the EPR signal intensity at fixed applied field, H_0, caused by nuclear transitions induced by a swept radio-frequency field.[8] For a single crystal having a specified orientation (θ, ϕ) with respect to H_0, the ENDOR pattern of an individual ^{57}Fe nucleus, or a set of equivalent nuclei, consists of a pair of lines centered at half the apparent hyperfine coupling constants associated with that field orientation, $A'(\theta,\phi)/2$, and split by twice the nuclear Larmor frequency which is proportional to H_0 ($\nu_{Fe} = 0.440$ MHz at 3200 G; $\nu_{Fe} = 0.220$ MHz at 1600 G):

$$\nu_{obsd} = A'/2 \pm \nu_{Fe} \qquad (1)$$

Because ν_{Fe} is small, at low fields the splitting can be unresolvable. Alternatively, it can happen that only one line has appreciable intensity.[8,7a] When the **g** and hyperfine tensors are coaxial, the hyperfine interaction constants for the lowest Kramers doublet treated as a $S' = {}^1/_2$ system, A_i' (i = 1–3), measured with the field directed along axis i, are related to the principle values of the hyperfine Hamiltonian in the $S = {}^3/_2$ representation A_i (i = 1–3) by the relationship[5]

$$A_i' = A_i g_i / g_e \qquad (2)$$

where g_e is the free-electron **g** value and g_i the ith principal value of the **g** tensor. The superb resolution in the ENDOR spectra reported here is substantially founded in the large electronic magnetic moment of the $S = {}^3/_2$ state, as reflected in the ratios g_3/g_e, $g_2/g_e > 1$ ($g_3 = 4.32$, $g_2 = 3.65$, $g_1 = 2.0$).[5] The spectrum from a cluster comprised of a number of different types of iron atoms is a simple summation of the spectra from the individual atoms.

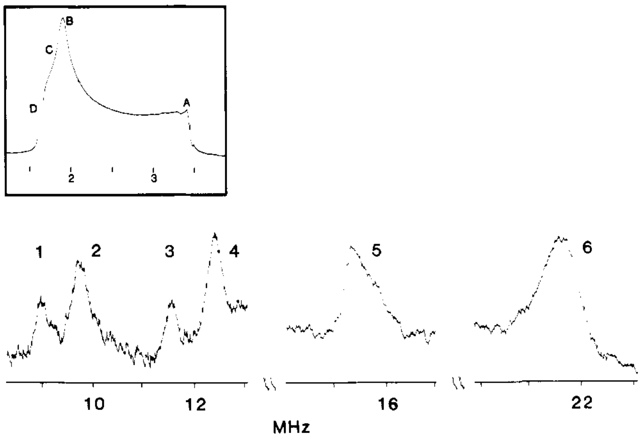

Figure 1. ^{57}Fe ENDOR of *Azotobacter vinelandii* MoFe protein at $g = g_3$. Conditions: $H_0 = 1600$ G (position D of inset); $T = 2$ K; microwave power, 0.63 mW; 100-kHz field modulation, 4 G; RF power, 10 W; RF scan rate, 6.9 MHz/sec. The ENDOR spectrum was taken in segments, as indicated. Each segment represents ca. 5000 scans. Inset: Dispersion derivative EPR spectrum of MoFe (2 K) taken under conditions of rapid adiabatic passage.

The MoFe samples employed in this study are frozen solutions and thus contain centers having a distribution of orientations. The inset to Figure 1 shows the 4.2 K EPR spectrum of such a sample. Field positions at the extreme edges of the EPR spectrum, near either g_1 or g_3 (positions A and D in Figure 1) will give single-crystal-like ENDOR patterns, associated with those molecules having the magnetic field directed along the **g** tensor axes.[9,10] ENDOR signals at field B (Figure 1) will arise from molecules with H_0 along g_2, but to these will be added the signals from all orientations at which the angle-dependent **g** value is equal to g_2. ENDOR at an intermediate field, such as C, similarly involves a range of molecular orientations. ENDOR patterns obtained at fields away from the spectrum edges typically are complex, often are poorly resolved, and can be quite difficult to analyze.[9,10]

Figure 1 presents the single-crystal-like ENDOR spectrum from a ^{57}Fe-enriched sample of MoFe taken with the magnetic field set to the extreme low-field edge of the EPR spectrum and thus corresponding to the g_3 axis of the **g** tensor. These spectra are not observed in unenriched samples. Six clearly articulated ^{57}Fe peaks are observed, indicating the presence of no fewer than six nonequivalent iron sites within the $S = {}^3/_2$ center. This supports the original conclusion that there are in fact six irons per center.[5,6] The sites, labeled 1–6 for convenience, have resonance frequencies that range from ca. 9 to ca. 22 MHz and signal strengths that increase with frequency, as expected.[9] The line width of the two highest frequency resonances is sufficiently broad that the splitting associated with the nuclear Zeeman interaction is not resolved. In addition, these resonances, particularly that of Fe(5), have an asymmetry that indicate a perfect single-crystal spectrum has not

---

(1) Abbreviations: MoFe, molybdenum–iron; FeMo-co, molybdenum–iron cofactor; EPR, electron paramagnetic resonance; ENDOR, electron nuclear double resonance; ESE, electron spin echo.

(2) Orme-Johnson, W. H.; Hamilton, W. D.; Jones, T. L.; Tso, M.-Y. W., Burris, R. H.; Shah, V. K.; Brill, W. J. *Proce. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 3142–3145.

(3) Rawlings, J.; Shah, V. K.; Chisnell, J. R.; Brill, W. J.; Zimmermann, R.; Munck, E.; Orme-Johnson, W. H. *J. Biol. Chem.* **1978**, *253*, 1001–1004.

(4) Hoffman, B. M.; Roberts, J. E., Orme-Johnson, W. H. *J. Am. Chem. Soc.* **1982**, *104*, 860–862.

(5) Munck, E.; Rhodes, H.; Orme-Johnson, W. H., Davis, L. C.; Brill, W. J.; Shah, V. K. *Biochim. Biophys. Acta* **1975**, *400*, 32–53.

(6) Huynh, B. H.; Henzl, M. T.; Christner, J. A.; Zimmerman, R.; Orme-Johnson, W. H.; Munck, E. *Biochim. Biophys. Acta* **1980** *623*, 124–138.

(7) (a) Roberts, J. E.; Hoffman, B. M.; Rutter, R.; Hager, L. P. *J. Biol. Chem.* **1981**, *256*, 2118–2121. (b) Hoffman, B. M.; Roberts, J. E.; Kang, C. H.; Margoliash, E. *J. Biol. Chem.* **1981**, *256*, 6556–6564.

(8) (a) Abragam, A.; Bleaney, B. "Electron Paramagnetic Resonance of Transition Ions"; Clarendon Press: Oxford, 1970. (b) Atherton, N. M. "Electron Spin Resonance"; Wiley: New York, 1973.

(9) Rist, G. H.; Hyde, J. S. *J. Chem. Phys.* **1970**, *52*, 4633–4643.

(10) Roberts, J. E.; Brown, T. G.; Hoffman, B. M.; Peisach, J. *J. Am. Chem. Soc.* **1980**, *102*, 825–829.

0002-7863/82/1504-4711$01.25/0

Table I. ^{57}Fe Hyperfine Interaction Tensors (MHz) for MoFe Protein[a]

α	A_3	A_2	A_1
1	8.3	11.5	9.9
2	9.0	12.6	9.9
3	10.7	11.5	11.8
4	11.5	15.3	18.9
5	14.3	18.2[b]	19.9
6	20.5	17.0	20.6

[a] Calculated from observed hyperfine interactions by assuming coaxial **A** and **g** tensors and $g_3 = 4.32, g_2 = 3.65, g_1 = g_e$. Uncertainties: A_3, A_1, ±0.1 MHz; A_2, ±0.25 MHz (estimated). Assignment of $A_1(\alpha)$ to a particular site (α) made as described in text. [b] The assumption of coaxial tensors is likely to be inadequate for Fe(5),[11] and therefore this entry is likely to be underestimated.

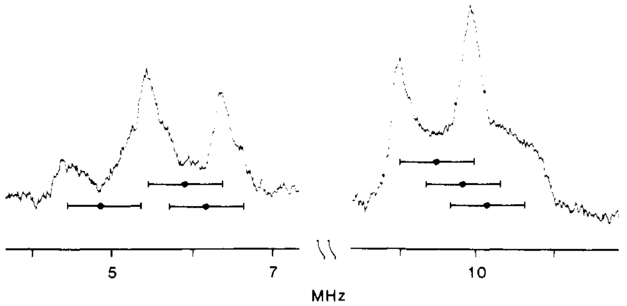

Figure 2. ^{57}Fe ENDOR of *Azotobacter vinelandii* MoFe protein at $g = g_1$. Conditions: H_0 = 3350 G (position A of Figure 1, inset). Other conditions are as in Figure 1.

quite been obtained. The ENDOR responses of irons 1–4 are extremely narrow, and each shows only one of the expected pair of lines, most probably the higher frequency partner. With the provisional assumption the hyperfine and **g** tensor axes are colinear, the frequencies of these resonances may be used in eq 1 to calculate the A_3' principal axis value for six chemically distinct iron sites (Table I).

When the magnetic field is set to the extreme high-field edge of the EPR spectrum, a single-crystal-like ENDOR spectrum is again obtained; this time it arises from those molecules oriented such that the magnetic field lies along the g_1 axis of the **g** tensor (Figure 2). In this case, each of the six iron sites contributes a resolvable doublet (eq 1). The six doublets are grouped into trios having very similar hyperfine parameters, one centered at $A_1'/2 \simeq$ 5–6 MHz and the other at $A_1'/2 \simeq 10$ MHz. A series of spectra have also been taken at fields intermediate between the g_2 and g_3 values. The truly exceptional resolution of the ^{57}Fe endor spectra has allowed us to perform an analysis which gives preliminary values of the A_2' hyperfine constants.[11] Table I presents the ^{57}Fe hyperfine tensor components $A_i(\alpha)$ for the six individual iron atoms (α = 1–6, i = 1–3). The association of an $A_3(\alpha)$ and $A_2(\alpha)$ pair is experimentally determined, but as yet it has not been possible to correlate a particular value of A_1 with its corresponding A_3, A_2 pair. Thus, the table has been completed through aid of the reasonable, but unsupported, device of assigning a measured A_1 to a particular site, α, so that the order of the individual sites in a series having decreasing $A_1(\alpha)$ is the same as that for decreasing $A_2(\alpha)$. This is one of several possible assignments that minimizes the assigned anisotropy of the hyperfine tensors.

Table I shows that the FeMo-co cluster must have an extraordinarily complex structure, for no two iron sites have the same characteristics. Since the site inequivalence must have a structural origin, this result will provide a stringent test of any postulated model for the cluster. Nevertheless, it is possible to see that Fe(1), Fe(2), and Fe(3) are broadly similar and, as concluded from the Mössbauer studies,[6] have roughly isotropic hyperfine tensors, as expected for high-spin ferric ions. The hyperfine constants are much reduced from that of a mononuclear Fe^{3+}, as in rubredoxin,[12] and from that in the two and four iron ferredoxins, as well.[13] This presumably results from the spin coupling that produces the net $S = {}^3/_2$ cluster spin. The other three iron atoms have larger and more anisotropic hyperfine interactions. The anisotropy of Fe(4), Fe(5),[11b] and, to a lesser extent, Fe(6) is suggestive of what might be expected for high-spin ferrous ions engaged in spin coupling.[13] These observations are consistent with the requirement that an Fe$_6$Mo cluster having an even-spin molybdenum[4] must include an odd number of formally ferric (and ferrous) iron atoms in order to achieve the net cluster spin of $S = {}^3/_2$. To the extent that these analogies to iron atoms in well-defined oxidation states correctly reflect the resting state of what must in fact be a complex and highly covalent system, then the ENDOR results give a starting point for discussions of the cluster charge. They also suggest that no more than 3 equiv can be associated with the formally ferric/ferrous iron couple during reduction (or superoxidation) of the molybdenum–iron cofactor of MoFe.

Acknowledgment. This work has been supported by NSF Grant PCM 7681304 and the donors of the Petroleum Research Fund, administered by the American Chemical Society (B.M.H.), and NSF Grant PCM 800087330 (W.H.O.-J.). We thank Drs. Michael Henzl and Joseph Smith for helping prepare isotopically enriched nitrogenase and Dr. Eckard Munck for stimulating discussions.

Registry No. FeMo-*co*, 72994-52-6.

---

(11) (a) To be submitted for publication. (b) Spectra taken at intermediate fields give indications that the provisional treatment in terms of coaxial **g** and hyperfine tensors is inadequate to describe Fe(5). Calculations of the Mössbauer spectrum of this center, based on the values in Table I, give the same indications (E. Munck, private communication).

(12) Rao, K. K.; Evans, M. C. W.; Cammack, R.; Hall, D. O.; Thompson, C. L.; Jackson, P. J.; Johnson, C. E. *Biochem. J.* **1972**, *129*, 1063–1070.

(13) See: Sands, R. H. In "Multiple Electron Resonance Spectroscopy"; Dorio, M. M., Freed, J. H., Eds.; Plenum Press: New York, 1979.

C–C Bond Formation by Reductive Coupling of Two Carbonyl Ligands of Binuclear Transition-Metal Complexes

Donald H. Berry[1] and John E. Bercaw*[2]

Contribution No. 6634 from the Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125

Alan J. Jircitano and Kristin B. Mertes*

Department of Chemistry, University of Kansas Lawrence, Kansas 66044

Received April 15, 1982

Among the most important reactions in organo-transition metal chemistry are those that generate new carbon–carbon bonds. Several different types of processes effect C–C coupling: (i) reductive elimination;[3] (ii) nucleophilic attack of carbanions at carbonyl[4] or olefin[5] ligands; (iii) alkyl migration to carbonyl or carbene ligands;[6] (iv) coupling of alkynes,[7] nitriles,[8] isonitriles,[9]

---

(1) NSF Predoctoral Fellow, 1979–1982.

(2) Camille and Henry Dreyfus Teacher-Scholar, 1977–1982.

(3) Collman, J. P.; Hegedus, L. S. In "Principles and Applications of Organotransition Metal Chemistry"; University Science Books: Mill Valley, CA, 1980; pp 234–245.

(4) Fischer, E. O. *Adv. Organomet. Chem.* **1976**, *14*, 1.

(5) See for example: Holton, R. A. *J. Am. Chem. Soc.* **1977**, *99*, 8083.

(6) (a) Flood, T. C. In "Topics in Inorganic and Organometallic Stereochemistry"; Eliel, E. L., Allinger, N. L., Geoffroy, G., Eds.; Wiley: New York, 1981; Vol. 12. (b) Wojcicki, A. *Adv. Organomet. Chem.* **1973**, *11*, 87. (c) Calderazzo, F. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 299. (d) Threlkel, T. S.; Bercaw, J. E. *J. Am. Chem. Soc.* **1981**, *103*, 2650. (e) Hayes, J. C.; Pearson, G. D. N.; Cooper, N. J. *Ibid.* **1981**, *103*, 4648.

(7) Collman, J. P. *Acc. Chem. Res.* **1968**, *1*, 136. (b) McAllister, D. R.; Bercaw, J. E.; Bergman, R. G. *J. Am. Chem. Soc.* **1977**, *99*, 1666.

(8) Cotton, F. A.; Hall, W. T. *J. Am. Chem. Soc.* **1979**, *101*, 5094. (b) de Boer, E. J. M.; Teuben, J. H. *J. Organomet. Chem.* **1978**, *153*, 53. (c) Wolczanski, P. T. Ph.D. Thesis, California Institute of Technology, Pasadena, CA, 1981.

0002-7863/82/1504-4712$01.25/0