BiC bond lengths suggest multiple-bond character as has been previously found for the E-C bonds of heteroferrocenes **6-9**.

In conclusion, the overall structural data on **10** emphasizes its close relationship to the series of the heteroferrocenes.

Acknowledgment. We are grateful to the Research Corporation, to NATO Scientific Affairs, and to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this work.

Supplementary Material Available: Full experimental details for the procedures described herein, tables of complete crystallographic data, atomic coordinates, bond lengths and angles, anisotropic thermal parameters, hydrogen atom coordinates, and planes of **10**, and an ORTEP plot of **10** (14 pages); tables of observed and calculated structure factors of **10** (13 pages). Ordering information is given on any current masthead page.

^{2}H Mims Pulsed ENDOR of Hydrogen Bonds and Exogenous Ligands to the Metal Clusters of Iron-Sulfur Proteins

Chaoliang Fan,† Mary Claire Kennedy,‡ Helmut Beinert,‡ and Brian M. Hoffman*,†

Department of Chemistry, Northwestern University 2145 Sheridan Road, Evanston, Illinois 60208-3113 Department of Biochemistry and National Biomedical ESR Center Medical College of Wisconsin 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226

Received August 30, 1991

Hydrogen bonding to the [*m*Fe-*n*S] clusters in Fe-S proteins is of interest because this interaction may well modulate cluster function.[1] In addition, multimetal clusters in proteins can act in a catalytic role,[2] and thus it is important to probe their interaction with exogenous ligands, such as those that come from solvent, H_xO (HO^-, H_2O). We report that Mims[3a,4] deuteron pulsed electron-nuclear double resonance (ENDOR) spectroscopy of H/D exchanged Fe-S proteins provides significant new opportunities both for probing H bonding to metal clusters and for examining exogenous ligands to such clusters. We find that frozen solutions of H/D exchanged proteins give extremely well resolved deuteron Mims ENDOR spectra[3a,4] from small samples (~10-20 μL), and that the data permits direct measurements of ^{2}H hyperfine and quadrupole couplings for individual interacting deuterons. Indeed, these unmatched opportunities exist for *any* nucleus that experiences small hyperfine couplings.[4c,e,5e] As the first applications of this approach, we report that the [2Fe-2S]$^+$ cluster in *Anabaena* 7120 ferredoxin (Fd)[6] is involved in at least one direct H bond with significant covalency, and we provide data supporting the suggestion based on multifrequency continuous wave (CW) ENDOR that the [4Fe-4S]$^+$ cluster of aconitase binds OH^- in the absence of substrate, but H_2O in its presence.[5c]

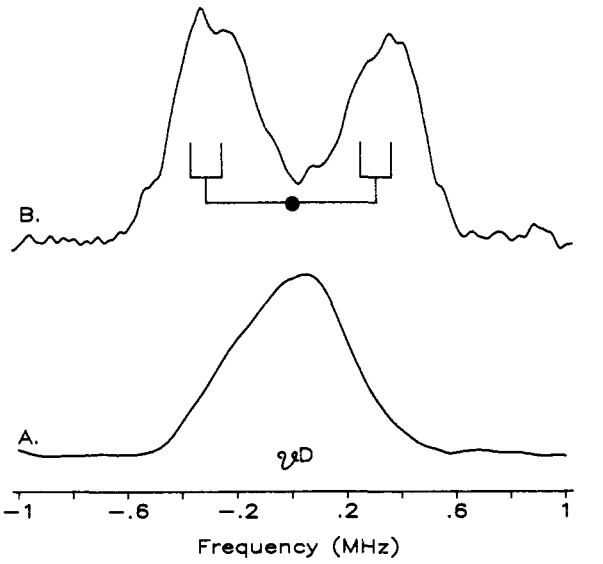

Figure 1. CW ENDOR (A) and Mims ENDOR (B) spectra of the [2Fe-2S]$^+$ cluster of *Anabaena* ferredoxin in D_2O solvent. For comparison, spectra are plotted as $\delta\nu = \nu - \nu_D$. Experimental conditions for spectrum A: ν_e = 34.54 GHz; H_0 = 12670 G; ν_D = 8.3 MHz; scan rate 0.25 MHz/s; 200 scans. For spectrum B: ν_e = 9.15 GHz; H_0 = 3357 G; microwave pulse width, 16 ns; rf pulse width, 40 μs; τ_{12} = 420 ns; ν_D = 2.2 MHz; 64 scans.

The [2Fe-2S]$^+$ cluster of *Anabaena* Fd exhibits a rhombic EPR spectrum with $g_{1,2,3}$ = 2.00, 1.96, 1.92. A ^{2}H Q-band CW ENDOR spectrum[7a] (Figure 1A) of the D_2O-exchanged protein[8] shows only a featureless signal at the deuterium Larmor frequency, ν_D (7.9 MHz at H_0 = 12000 G), and one cannot determine to what extent this signal is due to "distant ENDOR"[9] from deuterons that are not hyperfine-coupled to the cluster. The electron spin echo envelope modulation (ESEEM) technique also typically shows ^{2}H modulation only with a frequency ν_D.[3] In contrast, the Mims pulsed ENDOR technique yields highly resolved local ^{2}H

† Northwestern University.
‡ Medical College of Wisconsin.

(1) See: Backes, G.; Mino, Y.; Loehr, T. M.; Meyer, T. E.; Cusanovich, M. A.; Sweeney, W. V.; Adman, E. T.; Sanders-Loehr, J. *J. Am. Chem. Soc.* **1991**, *113*, 2055-2064.

(2) For example, see the following. (a) Aconitase: Beinert, H.; Kennedy, M. C. *Eur. J. Biochem.* **1989**, *186*, 5-15. (b) Nitrogenase: Burgess, B. K. *Chem. Rev.* **1990**, *90*, 1377-1406. (c) CO dehydrogenase: Ragsdale, S. W.; Wood, H. G.; Antholine, W. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*(10), 6811-6814.

(3) (a) Mims, W. B. In *Electron Paramagnetic Resonance*; Geschwind, S., Ed.; Plenum Press: New York, 1972; p 263. (b) Mims, W. B.; Peisach, J. In *Advanced EPR, Application in Biology and Biochemistry*; Hoff, A. J., Ed.; Elsevier: New York, 1989. (c) Mims, W. B.; Davis, J. L.; Peisach, J. *J. Magn. Reson.* **1990**, *86*(2), 273-292. (d) Orme-Johnson, N. R.; Mims, W. B.; Orme-Johnson, W. H.; Bartsch, R. G.; Cusanovich, M. A.; Peisach, J. *Biochim. Biophys. Acta* **1983**, *748*, 68-72. (e) Morgan, T. V.; McCracken, J.; Orme-Johnson, W. H.; Mims, W. B.; Mortenson, L. E.; Peisach, J. *Biochemistry* **1990**, *29*, 3077-3082.

(4) (a) Mims, W. B. *Proc. R. Soc. London, A* **1965**, *283*, 452-457. (b) This technique employs a pulse sequence that consists of three $\pi/2$ microwave pulses, which produce a stimulated electron spin echo; an rf pulse is inserted between pulses 2 and 3. The ENDOR response consists of a change in the spin echo amplitude when the rf matches a nuclear resonance frequency. (c) The widely perceived disadvantage of Mims ENDOR, "blind spots" for hyperfine couplings where A (MHz) = n/τ (μs), n = 1, 2, ..., is not relevant for small couplings, $A < 1/\tau$. (d) Gemperle, C.; Schweiger, A. *Chem. Rev.* **1991**, *91*, 1481-1505. (e) **Note Added in Proof**: As an example, a ^{14}N Mims ENDOR study has appeared recently. Thomann, H.; Bernardo, M.; Adams, M. W. W. *J. Am. Chem. Soc.* **1991**, *113*, 7044-7046.

(5) (a) Hoffman, B. M.; Gurbiel, R. J.; Werst, M. M.; Sivaraja, M. In *Advanced EPR, Applications in Biology and Biochemistry*; Hoff, A. J., Ed.; Elsevier: New York, 1989; p 541. (b) Hoffman, B. M. *Acc. Chem. Res.* **1991**, *24*, 164-170. (c) Werst, M. M.; Kennedy, M. C.; Beinert, H.; Hoffman, B. M. *Biochemistry* **1990**, *29*, 10526-10532. (d) Houseman, A. L. P.; Oh, B.-H.; Kennedy, M. C.; Fan, C.; Werst, M. M.; Beinert, H.; Markley, J. L.; Hoffman, B. M. *Biochemistry*, in press. (e) Manuscript in preparation.

(6) (a) Rypniewski, W. R.; Breiter, D. R.; Benning, M. W.; Wesenberg, G.; Oh, B.-H.; Markley, J. L.; Raymond, I.; Holden, H. M. *Biochemistry* **1991**, *30*, 4126-4131. (b) Skjeldal, L.; Westler, W. M.; Oh, B.-H.; Krezel, A. M.; Holden, H. M.; Jacobson, B. L.; Rayment, I.; Markley, J. L. *Biochemistry* **1991**, *30*(30), 7363-7368.

(7) Descriptions of apparatus. (a) Q-Band, CW: Werst, M. M.; Davoust, C. E.; Hoffman, B. M. *J. Am. Chem. Soc.* **1991**, *113*, 1533-1538. (b) X-Band, pulsed: Fan, C.; Doan, P. E.; Davoust, C. E.; Hoffman, B. M. *J. Magn. Reson.*, in press.

(8) Deuterium exchange of *Anabaena* Fd followed a procedure (ref 5d) that is a slight modification of that of Backes, et al. (ref 1); that for aconitase is given in ref 5d. The concentrations were *Anabaena* Fd, ~1.5 mM, aconitase, ~1 mM.

(9) (a) Wenckebach, W. Th.; Schneurs, L. A. H.; Hoogstraate, H.; Swanenburg, T. J. B.; Poulis, N. J. *Physica* **1971**, *52*, 455-480. (b) Boroske, E.; Möbius, K. *J. Magn. Reson.* **1977**, *28*, 325-338. (c) Pattison, M. R.; Kim, Y. W. *J. Magn. Reson.* **1981**, *43*, 193-206.

0002-7863/92/1514-374$03.00/0

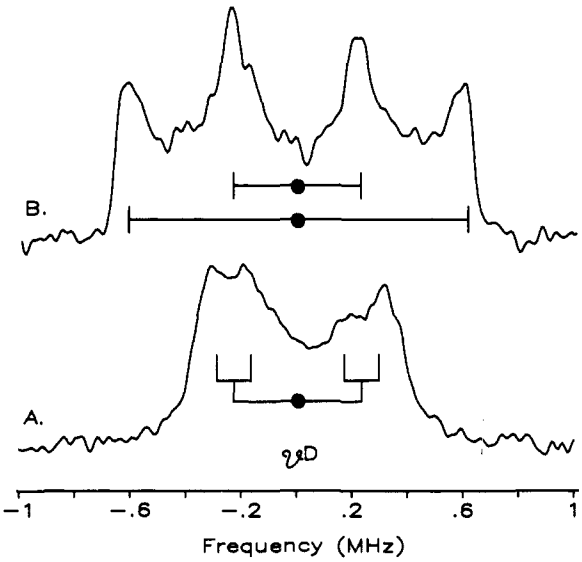

Figure 2. Mims ENDOR spectra of $[4Fe-4S]^+$ cluster of aconitase (A) without substrate and (B) with substrate (citrate). Spectra are plotted as $\delta\nu = \nu - \nu_D$. Experimental conditions: $T = 2$ K; $\nu_e = 9.14$ (A), 9.09 (B) GHz; $H_0 = 3525$ (A), 3645 (B) G; microwave pulse width, 16 ns; rf pulse width, 50 μs; $\tau_{12} = 380$ (A), 440 (B) ns; (A) 168, (B) 880 scans.

ENDOR spectra from which hyperfine and quadrupole splittings can be determined directly. Figure 1B is a single-crystal-like[5a,b] ^{2}H Mims ENDOR spectrum taken[7b] at the high-field (g_3) edge of the *Anabaena* Fd EPR absorption envelope, using the same small sample used at the Q-band.[5d] It shows a pair of peaks centered at ν_D and separated by 0.60 MHz. This splitting, which is far too large to be associated with the quadrupolar interaction, represents a deuteron hyperfine coupling, $A^D \approx 0.60$ MHz.[10] The peaks further show a partially resolved quadrupole splitting of $2P^D \approx 0.1$ MHz. As no exogenous ligands are associated with the cluster, this local deuteron can be assigned to a hydrogen N–H···S bond, presumably one of the two putative strong H bonds seen in the crystal structure of the oxidized protein:[6a] arginine 42 H-bonded to a cysteinyl mercaptide sulfur bound to iron or arginine 258 H-bonded to a bridging cluster S^{2-}.

The ^{2}H splitting varies little in the ENDOR spectra taken at magnetic field values across the EPR envelope (data not shown), which indicates that the coupling is nearly isotropic.[5a,b] This is equivalent to an isotropic proton interaction, $A^H = (g_H/g_D)A^D = 6.51A^D \approx 3.9$ MHz, whose size indicates that this H bond must have significant covalency. Variations in the quadrupole term indicate that $K^D \equiv 3e^2qQ/2h \lesssim 0.2$ MHz, in agreement with the expectation from model compounds.[10]

The explanation for the difference in resolution of the CW and pulsed deuterium signals is likely as follows. In the former, distant ENDOR signals from noninteracting deuterons[9,5d] overwhelm the local ENDOR of H-bonded deuterons. In pulsed ENDOR, an individual pulse sequence ($\lesssim$50 μs) is shorter than the time for the spin diffusion processes that are the basis of the distant CW ENDOR response; thus the distant ENDOR response is "quenched", and local ENDOR signals become visible.

The $[4Fe-4S]^+$ cluster of aconitase has one iron ion (Fe_a) that has been shown by ^{17}O CW ENDOR to be coordinated to a solvent species, H_xO.[2a,5c] The enzyme shows a rhombic EPR signal, with $g_{1,2,3} = 2.06$, 1.93, and 1.86 in the absence of substrate, and $g_{1,2,3} = 2.04$, 1.85, and 1.78 in the presence of substrate (citrate). Figure 2A is a single-crystal-like ^{2}H Mims ENDOR spectrum of the enzyme in D_2O without substrate, taken at the high-field edge ($g_3 = 1.86$) of the EPR absorption envelope. It shows one pair of peaks from D_xO at $\delta\nu_\pm = \nu_\pm - \nu_D \approx \pm 0.25$ MHz, with each showing a further small splitting of $\sim$0.12 MHz. We assign the spectrum to a deuteron with hyperfine interactions, $A^D \approx 0.50$ ($A^H \approx 3.25$) MHz, and quadrupole splitting, $2P^D \approx 0.12$ MHz. The pulsed ENDOR spectrum taken at $g_3 = 1.78$ for the enzyme with substrate (nondeuterated citrate) in D_2O (Figure 2B) shows a more complex pattern, with four deuterium peaks at $\delta\nu_\pm \approx \pm 0.61$ and ± 0.23 MHz. An assignment to a single deuterium with $A^D = 0.84$ MHz and quadrupole splitting $2P^D = 0.38$ MHz can be discounted because the latter is improbably large;[10] instead, the peaks are assigned to two inequivalent nuclei that have $A^D = 1.22$ and 0.46 ($A^H = 7.9$, 3.0) MHz and no observable quadrupole splitting at this field. Comparison with the ^{1}H resonances lost upon H/D exchange[5c] confirms the latter assignment. The ^{2}H Mims ENDOR spectrum of enzyme without substrate has significantly better resolution than the corresponding Q-band CW ENDOR spectrum; that of enzyme with substrate also is improved.[5c] These high-resolution spectra support the earlier suggestion that the H_xO species coordinated to the $[4Fe-4S]^+$ cluster of the enzyme with substrate is a water molecule, whereas it is a hydroxyl ion in the enzyme without substrate.[2a,5c]

The data presented here clearly show that ^{2}H Mims pulsed ENDOR examination of H bonds and H_xO coordinated to metal clusters provides an important complement to multifrequency CW ENDOR[5] and its pulsed analogue, Davies ENDOR,[4d,7b] which typically do best with larger couplings, and to ESEEM, which is well-suited for measuring distances to and numbers of dipole-coupled exchangeable protons in the active-site vicinity.[3c]

Acknowledgment. This work could not have been performed without the expert assistance of Mr. Clark E. Davoust and supportive discussions with Dr. Peter E. Doan. The *Anabaena* 7120 Fd was kindly provided by Professor John L. Markley. This work was supported by the NIH [HL 13531 (B.M.H.), GM 34812 (H.B.)], NSF [DBM-8907559 (B.M.H.)], and USDA [90-37120-5604 (B.M.H.)]. Equipment was purchased with a grant from the NIH (DRR 04936) and received support from the Materials Research Center of Northwestern University (DMR-88 21571).

(10) (a) Recall (refs 5a,b) that ENDOR frequencies for ^{2}H(I=1) have the form $\nu_\pm(m) \approx |\pm A^D/2 + \nu_D + P^D(2m - 1)|$, $m = 0$, 1, for a given field orientation. (b) Studies on model compounds (ref 10c) show that the maximum deuterium quadrupole splitting falls in the range $2|P_{zz}| \equiv K^D \approx$ 0.26–0.30 MHz for R_1R_2ND that is H-bonded, $2|P_{zz}| \approx 0.20$–0.36 MHz for OD^-, and $2|P_{zz}| \approx 0.30$–0.38 MHz for D_2O. (c) Edmonds, D. T. *Phys. Rep.* **1974**, *29*(4), 233.

Stereochemical Analysis of Totally Stereoselective, Competing [1,2]- and [2,3]-Wittig Rearrangements. Inversion at the Lithium-Bearing Carbon Atom

Erik J. Verner and Theodore Cohen*

Department of Chemistry, University of Pittsburgh Pittsburgh, Pennsylvania 15260

Received July 18, 1991

We present the first stereochemical analysis of [1,2]-[1] and [2,3]-[2]Wittig rearrangements which includes the stereochemical change that occurs at the lithium-bearing carbon atom. Counterintuitively, the [2,3]-rearrangement requires a trans disposition of the two substituents in a 2-lithio-6-vinyltetrahydropyran, and both rearrangements occur with *inversion of configuration at the lithium-bearing carbon atom.*[3] This inversion in the case of the

(1) (a) Review: Schöllkopf, U. *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 763. For references to more recent work, see: (b) Schreiber, S. L.; Goulet, M. T. *Tetrahedron Lett.* **1987**, *28*, 1043. Adam, S. *Tetrahedron* **1989**, *45*, 1409. Azzena, U.; Denurra, T.; Melloni, G.; Piroddi, A. M. *J. Org. Chem.* **1990**, *55*, 5532. (c) Schlosser, M.; Strunk, S. *Tetrahedron* **1989**, *45*, 2649.

(2) Reviews: Reference 1a. Hoffmann, R. W. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 563. Nakai, T.; Mikami, K. *Chem. Rev.* **1986**, *86*, 885.

(3) Carbon nearly always displaces lithium at nonplanar carbon with retention. Fleming, I.; Rowley, M. *Tetrahedron* **1986**, *42*, 3181 and citations therein. Chassaing, G.; Lett, R.; Marquet, A. *Tetrahedron Lett.* **1978**, 471 and citations therein.

0002-7863/92/1514-375$03.00/0