# Distinguishing Hydroxy from Aqua Bridges in Paramagnetic Diiron(II) and Dicobalt(II) Models of Reduced Dinuclear Non-Heme Iron Sites in Proteins by $^1$H, $^2$H, and $^{19}$F NMR of H/D Isotopomers

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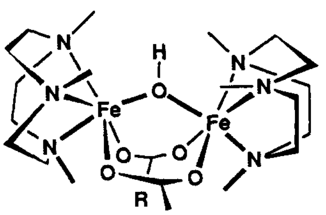
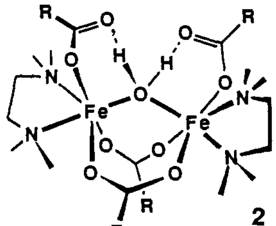
Various physicochemical measurements on the oxy and deoxy forms of the non-heme oxygen transport protein hemerythrin (Hr) suggest that the two iron atoms are bridged by two carboxylates and a hydroxide in the reduced form (deoxyHr) and an oxo (hydrogen-bonded to the bound hydroperoxide) in the oxidized form (oxyHr).[1,2] The extent to which the bridging O is protonated is deduced in part by the magnetic interactions between Fe(II) atoms. The weak antiferromagnetic exchange coupling in deoxyHr ($-12 < J < -38$ cm$^{-1}$) is modulated on binding exogenous ligands, resulting in a net ferromagnetic exchange ($J > 0$) in deoxyHrN$_3$.[3] It has been suggested from these indirect measurements that the hydroxide bridge has been protonated to a water bridge. Model compounds seem to bear this out; a hydroxide-bridged complex ([Fe$_2$(OH)(O$_2$CCH$_3$)$_2$(Me$_3$TACN)$_2$]$^+$, **1**[4,5] has a higher antiferromagnetic coupling ($J = -13$ cm$^{-1}$) than a water-bridged complex ([Fe$_2$(H$_2$O)(O$_2$CR)$_4$(tmen)$_2$], **2**, for R = CH$_3$ and Ph[6]). We now report NMR analyses of new Fe(II) and Co(II) analogs of **1** and **2** which confirm that the solid-state structures are maintained in solution and provide a direct spectroscopic distinction between a bridging hydroxide and a bridging water.



**1**



**2**

The OH$^-$-bridged complexes **1**-Fe and **1**-Co (R = CF$_3$) were isolated under anaerobic conditions from acetonitrile solutions containing a 2:2:2:3:1 ratio of Me$_3$TACN, M(CF$_3$SO$_3$)$_2$·2CH$_3$CN, CF$_3$COOH, Et$_3$N, and H$_2$O in >90% yield.[7] The H$_2$O-bridged complexes **2**-FeF and **2**-CoF (R = CF$_3$) were similarly prepared from acetonitrile solutions containing a 2:2:4:4:1 ratio of tmen, M(CF$_3$SO$_3$)$_2$·2CH$_3$CN, CF$_3$COOH, Et$_3$N, and H$_2$O. Substitution of pivalic acid for trifluoroacetic acid afforded **2**-FeH and **2**-CoH (R = CMe$_3$). The four fluorinated complexes were characterized by crystallography[8] and are similar to the corresponding acetate complexes with the notable exception that the triflate counterion in **1**-Fe and **1**-Co is more strongly hydrogen-bonded to the bridging OH$^-$ (O···OSO$_2$CF$_3$, 2.90 Å) than the disordered perchlorate in **1** (O···OClO$_3^-$, 3.01 Å). There is also a marked increase in the Fe–O–Fe angle of 119° in **1**-Fe (113° in **1**, Co–O–Co 122.0° in **1**-Co), and an 0.08-Å increase in the Fe···Fe distance (3.412 Å in **1**-Fe and 3.33 Å in **1**, Co···Co 3.443 Å in **1**-Co).

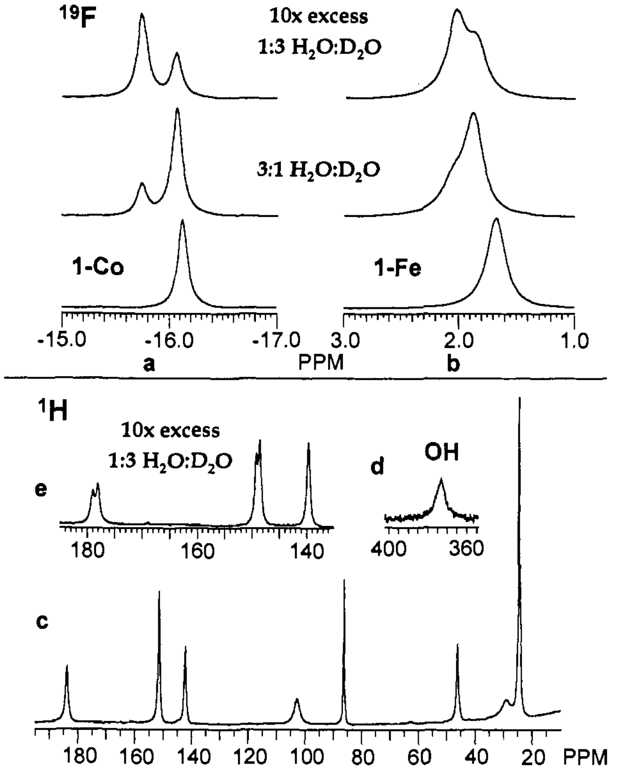


**Figure 1.** 470-MHz $^{19}$F NMR spectra of (a) **1**-Co (vs CFCl$_3$ in CDCl$_3$) with 10-fold excess of 3:1 and 1:3 H$_2$O/D$_2$O; (b) same for **1**-Fe; (c) 500-MHz $^1$H spectrum of **1**-Co with CH$_2$ resonances at 29, 46, 83, 102, 140, and 179 ppm; NCH$_3$ at 23 (*cis* to OH) and 149 ppm (*trans* to OH) vs TMS. Inset d shows OH resonance at 380 ppm, and inset e shows ligand CH and CH$_3$ splittings, at 179 and 149 ppm, in presence of H$_2$O/D$_2$O mixture.

The $^{19}$F NMR spectra of **1**-Fe and **1**-Co (Figure 1) each only show resonances of the bridging CF$_3$CO$_2^-$ ligand and CF$_3$SO$_3^-$ counterion. The $^1$H NMR spectra of **1**-Co indicates $C_{2v}$ symmetry with the eight resonances of the Me$_3$TACN ligand having

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[1] (a) Klotz, I. M.; Kurtz, D. M., Jr. *Acc. Chem. Res.* **1984**, *17*, 16–22. (b) Wilkins, P. C.; Wilkins, R. G. *Coord. Chem. Rev.* **1987**, *79*, 195–214. (c) Wilkins, R. G.; Harrington, P. C. *Adv. Inorg. Biochem.* **1983**, *5*, 51–86. (d) Lippard, S. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 344–361. (e) Solomon, E. I.; Zhang, Y. *Acc. Chem. Res.* **1992**, *25*, 343–352. (f) Wilkins, R. G. *Chem. Soc. Rev.* **1992**, 171–178.

[2] Shiemke, R. E.; Loehr, T. M.; Sanders-Loehr, J. *J. Am. Chem. Soc.* **1986**, *108*, 2437.

[3] (a) Reem, R. C.; Solomon, E. I. *J. Am. Chem. Soc.* **1984**, *106*, 8323–832. (b) Reem, R. C.; Solomon, E. I. *J. Am. Chem. Soc.* **1987**, *109*, 1216–1226.

[4] (a) Wieghardt, K.; Pohl, K.; Gebert, W. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 727. (b) Chaudhuri, P.; Wieghardt, K.; Nuber, B.; Weiss, J. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 778–779. (c) Wieghardt, K.; Pohl, K.; Ventur, D. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 392–393. (d) Hartman, J.-A. R.; Rardin, R. L.; Chaudhuri, P.; Pohl, K.; Wieghardt, K.; Nuber, B.; Weiss, J.; Papaefthymiou, G. C.; Frankel, R. B.; Lippard, S. J. *J. Am. Chem. Soc.* **1987**, *109*, 7387–7396.

[5] Abbreviations: tmen, *N,N,N',N'*-tetramethyl-1,2-diaminoethane; Me$_3$TACN, 1,4,7-trimethyl-1,4,7-triazacyclononane.

[6] Hagen, K. S.; Lachicotte, R. J. *J. Am. Chem. Soc.* **1992**, *114*, 8741–8742.

[7] Anal. Calcd for **1**-Fe, C$_{23}$F$_9$Fe$_2$H$_{43}$N$_6$O$_8$S: C, 32.64; H, 5.12; N, 9.93. Found: C, 32.68; H, 5.11; N, 10.06. Calcd for **1**-Co, C$_{23}$Co$_2$F$_9$H$_{43}$N$_6$O$_8$S: C, 32.40; H, 5.08, N, 9.86. Found: C, 32,47; H, 5.06; N, 10.03. Calcd for **2**-FeF, C$_{20}$F$_{12}$Fe$_2$H$_{34}$N$_4$O$_9$: C, 29.50; H, 4.21; N, 6.89. Found: C, 29.74; H, 4.22; N, 6.89. Calcd for **2**-CoF, C$_{20}$Co$_2$F$_{12}$H$_{34}$N$_4$O$_9$: C, 29.28; H, 4.18; N, 6.83. Found: C, 29.32; H, 4.19; N, 6.84. Calcd for **2**-FeH, C$_{32}$Fe$_2$H$_{70}$N$_4$O$_9$: C, 50.14; H, 9.20; N, 7.31. Found: C, 49.58; H, 8.77; N, 7.41. Calcd for **2**-CoH, C$_{32}$Co$_2$H$_{70}$N$_4$O$_9$: C, 49.74; H, 9.13; N, 7.25. Found: C, 49.64; H, 9.31; N, 7.39.

[8] X-ray analysis: **1**-Fe (**1**-Co in brackets), tetragonal, $P4_32_12$, with $a$ = 11.393(2) Å [$a$ = 11.344(2) Å], $c$ = 26.564(5) Å, [$c$ = 26.512(9) Å,] $V$ = 3448 (2) Å$^3$, [$V$ = 3412(2) Å$^3$], $\rho_{calcd}$ = 1.648 g cm$^{-3}$, [1.660 g cm$^{-3}$], $Z$ = 4. With use of 2674 [2552] unique reflections out to $2\theta$ = 50° Mo K$\alpha$, collected at −100 °C on a single-crystal X-ray diffractometer, the structure was solved by Patterson methods and refined with anisotropic thermal parameters to $R$ = 3.28%, $R_w$ = 3.35% [3.45, 3.38].

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intensities consistent with a mirror plane bisecting the ligand (6 CH, intensity 1; 1 $CH_3$, intensity 3; and 1 $CH_3$, intensity 1.5) and the exchangeable OH resonance at +380 ppm. Additions of 10-fold excesses of $H_2O/D_2O$ mixtures to the $CDCl_3$ solutions of **1**-Co afford the $OH^-$- and $OD^-$-bridged isotopomers which are manifest in resolvable splittings of the $CF_3CO_2^-$ resonance, the resonance of the methyl on the nitrogen *trans* to the OH/OD, and one of the methylene CH resonances. Better resolved spectra are obtained from anhydrous samples isolated from $CDCl_3$ solutions treated with an excess of $CH_3OD$. The broader resonances in **1**-Fe result in a decrease in resolution of the analogous $CF_3CO_2^-$ resonances. The $\mu$-OD resonance was detected (using $^2H$ NMR) at 663 ppm, indicating a large contact shift through strong covalent Fe–OH bonds.[9] Relaxation measurements provide evidence that an ion pair through a hydrogen bond exists in $CDCl_3$ solution: the ratio $T_1(CF_3SO_3^-)/T_1(CF_3CO_2^-) = 4.8$ ($^{19}F$) is in good agreement with the distance ratio $(r(Fe\cdots F_3CSO_3^-)/r(Fe\cdots F_3CCO_2^-))^6 = 5.35$, obtained from the averaged crystallographic Fe···F distances.[10]

The $^{19}F$ NMR spectra of the $\mu$-aqua complexes **2**-FeF and **2**-CoF (Figure 2) each show two $CF_3CO_2^-$ resonances corresponding to bridging ($F_b$) and terminal ($F_t$) ligands ($C_2$ point group). Partial deuteration results in splitting each trifluoroacetate resonance into four signals expected for isotopomers H–O–H, D–O–D ($C_2$ symmetry), and H–O–D ($C_1$ symmetry).[11] The $^1H$ NMR spectrum of **2**-CoF shows the eight resonances of the $Me_2NCH_2CH_2NMe_2$ ligand and the $\mu$-$OH_2$ resonance at −214 ppm, almost 600 ppm upfield from the $\mu$-OH resonance of **1**-Co, which reflects a weaker covalent interaction, as the downfield contact shifts are eclipsed by predominantly upfield dipolar shifts. There is no evidence for isotopomers in the $^1H$ spectrum, possibly because of broadening resulting from rapid conversion between $\delta\delta$, $\lambda\lambda$, and $\delta\lambda$ isomers of the ligand.[12] The $\beta$-CH resonances of pivalate are sharper, and splittings are observed in one of the two pivalate resonances upon partial H/D exchange in **2**-FeH and in both resonances in **2**-CoH (not shown). The broader $\alpha$-CF resonances in **2**-FeF prevent detection of isotopomers by $^{19}F$ or $^1H$ NMR; however, the bridging $D_2O$ resonances are observed at 98 ppm (HOD at 96 ppm, $^2H$ NMR) for **2**-FeH and at −11 ppm for **2**-FeF.

The observed isotopomeric splitting[13] can be attributed to the influence of H-bonds to bridging $OH^-$ or $H_2O$ on the stereochemistry of the isotopomers and/or on modulation of antiferromagnetic coupling between ions. The former is more consistent with the observation that the single HOD isotopomer has two resonances and smaller effects with Fe(II) than Co(II), whose shifts are known to have greater dipolar contribution than Fe(II). The ability to obtain this structural information by multinuclear NMR techniques should prove to be very useful with other synthetic paramagnetic complexes.

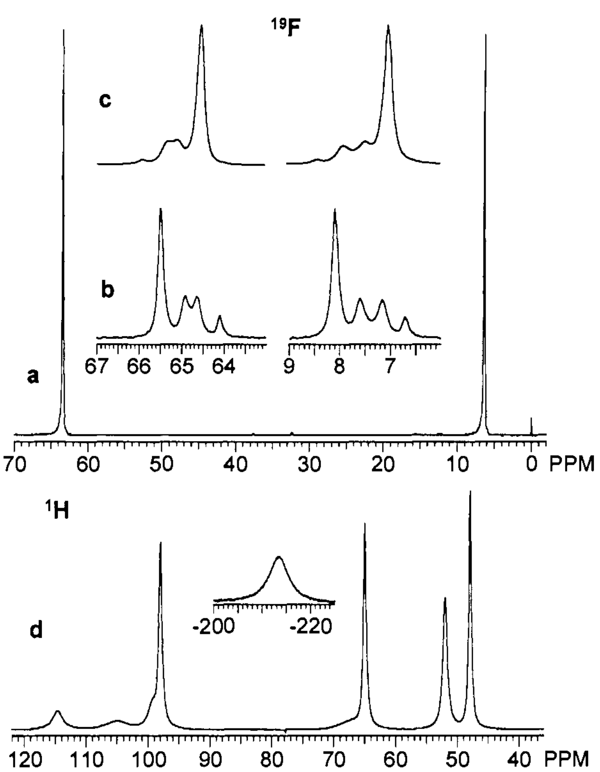

**Figure 2.** 470-MHz $^{19}F$ NMR spectra of (a) **2**-CoF in $CDCl_3$ with (b) excess $CH_3OD$ added, evaporated to dryness, dissolved in $CDCl_3$; (c) ca. 10-fold excess 2:1 $H_2O/D_2O$ 3:1. (d) 500-MHz $^1H$ NMR spectrum of **2**-CoF with $H_2O$ resonance inset at −214 ppm, ligand $CH_3$ resonances at 48, 52, 65, and 98 ppm, and ligand $CH_2$ resonances at 67, 99, 105, and 114 ppm vs TMS.

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**Supplementary Material Available:** ORTEP drawings, crystallographic data, and tables of atomic positional and thermal parameters and bond lengths and angles of **1**-Fe and **1**-Co (16 pages); observed and calculated structure factors (8 pages). Ordering information is given on any current masthead page.

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[9] In $Fe(CH_2Ph)_2$tmen, the $CH_2$ resonance occurs at 947 ppm. Hill, D. H.; Sen, A. *J. Am. Chem. Soc.* **1988**, *110*, 1650–1652.

[10] Swift, T. J. In *NMR of Paramagnetic Molecules*; LaMar, G. N., Horrocks, W. D., Jr., Holm, R. H., Eds.; Academic Press: New York, 1973; Chapter 2, pp 53–83.

[11] $F_t$ and $F_b$ assignments are based on relaxation measurements and spectra of mixed FeCo and FeNi complexes (Lachicotte, R. J.; Kitaygorodskiy, A.; Hagen, K. S., to be submitted for publication). Exchange with excess $H_2O/D_2O$ broadens the $F_b$ resonances of the two H–O–D isotopomers so that they are not resolved although those of $F_t$ are resolved.

[12] This process is slowed considerably at low temperature, where broadening of the tmen resonances is seen, while the carboxylate resonances remain narrow.

[13] The 0.32-ppm splitting in **1**-Co is 0.5% of the 60-ppm isotropic shift and the 1.4-ppm splittings of **2**-CoF are 1.0 and 1.7% of the isotropic shifts of bridging and terminal ligands.