Analysis of an Organometallic Iron Site Model for the Heterodimetallic Unit of [NiFe]Hydrogenase

Donald J. Darensbourg,* Joseph H. Reibenspies, Chia-Huei Lai, Way-Zen Lee, and Marcetta Y. Darensbourg*

Department of Chemistry, Texas A&M University College Station, Texas 77843

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The distinctive electrochemical and spectroscopic signals assigned to the nickel center in [NiFe]hydrogenases ([NiFe]H_2-ase) have inspired the synthesis of many small mononuclear nickel complexes which mimic selected aspects of enzyme structure (a Ni(*S*-cysteine)$_4$ site) and function (catalysis of $H_2 = 2H^+ + 2\ e^-$).[1] Fewer models exist for comparison to the heterobimetallic character of the active site of *Desulfovibrio gigas* [NiFe]hydrogenase,[2] and none thus far have utilized the actual $Fe(CN)_2(CO)$ unit which has been shown by a protein crystal structure to be incorporated into the protein only by two cysteine sulfurs bridging into the Ni site and, in the oxidized form, a μ-oxo or μ-hydroxo.[3] The assignment of this unit, deemed extraordinary in that the "abiological" ligands CN and CO are typically toxic to cells, benefited greatly from infrared spectroscopic studies of the natural and N-15 and C-13 isotopically enriched [NiFe]H_2ase enzyme isolated from *Chromatium vinosum* which showed shifts consistent with two cyanides and one carbonyl ligand.[4] Herein, we describe a small molecule model of the $Fe(CN)_2(CO)$ fragment in the form of the potassium salt of $(\eta^5\text{-}C_5H_5)Fe(CN)_2(CO)^-$ (hereafter, Cp = $\eta^5\text{-}C_5H_5$).[5] Its structural and spectroscopic characterization conforms with the Happe et al.[4] and Volbeda et al.[3] assignment of enzymic $Fe(CN)_2(CO)$ and permits exploration of solvent and counterion effects on the IR spectra of the iron-bound carbonyl and cyanide diatomics.

Golden brown, highly hygroscopic crystals of $K[CpFe(CN)_2(CO)]$ were obtained from EtOH and crystallized in the space group $P2_1/c$ with four molecular weight units per cell; a full X-ray crystal structure report is available in the Supporting Information.[6,7] The Cp and CO moieties in the observed structure are disordered and related by a pseudo mirror plane which contains the iron and cyanides. Figure 1 shows a portion of the structure with selected metric parameters given in the caption. The anion is a typical three-legged piano stool with substantially linear Fe-CN and Fe-CO groups, oriented at ca. 90° angles to each other. The average $Fe-C_{Cp}$ distance is 2.11 Å, the two $Fe-C_{CN}$ distances average to 1.910(8) Å, and the $Fe-C_{CO}$ is 1.73(2) Å. The C−N and C−O distances are typical at 1.15(1) (av) and 1.10(2) Å, respectively. The K^+ interacts with six cyanides in distorted octahedral geometry. Two cyanides are from one $CpFe(CN)_2(CO)^-$ unit, where in fact K^+-C as well as K^+-N interactions are apparent. The remaining $N\cdots K^+$ interactions involve N atoms from individual $(\eta^5\text{-}C_5H_5)Fe$ units, with a ∠C−N−K range of 113−151° and average $N\cdots K^+$ distances of 3.0 Å. Each of the N atoms is in turn bound to two adjacent potassium ions producing an extended structure layered with ionic interactions on one side and hydrophobic regions on the other. The CO is directed into the hydrophobic region of the structure.

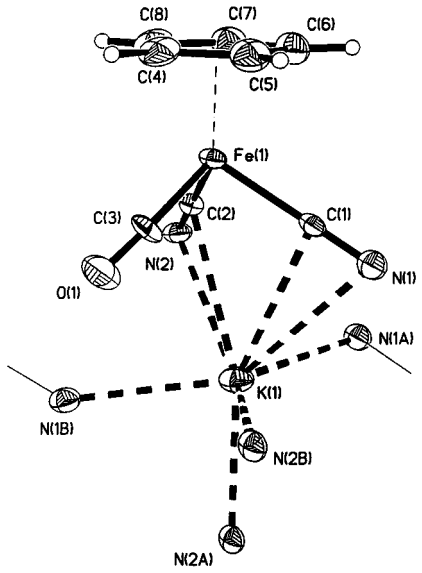

Figure 1. Molecular structure of $K[(\eta^5\text{-}C_5H_5)Fe(CN)_2(CO)]$ (one orientation of the disordered pair) with numbering scheme and a partial view of the interionic interactions of K^+ with cyanides of adjacent units. Selected bond lengths (Å): Fe(1)−C(1), 1.913 (9); Fe(1)−C(2), 1.899 (9); Fe(1)−C(3), 1.73 (2); C(1)−N(1), 1.151 (12); C(2)−N(2), 1.158 (11); C(3)−O(1), 1.10 (2); K(1)−C(1), 3.595(9); K(1)−N(1), 2.934-(8). Selected bond angles (deg): C(1)−Fe(1)−C(2), 90.1 (4); C(1)−Fe(1)−C(3), 95.1 (8); C(2)−Fe(1)−C(3), 94.1 (8); Fe(1)−C(1)−N(1), 179.5(8); Fe(1)−C(2)−N(2), 176.8 (8); Fe(1)−C(3)−O(1), 177 (2).

The $CpFe(CN)_2(CO)^-$ structure was least-squares fit to the current model for the structure of the heterobimetallic site of the oxidized form of [NiFe]H_2ase, extracted from the current full structure.[3,8] Two of the diatomic ligands in the protein structure were assigned as CN groups based upon their close proximity to proposed hydrogen-bonding partners; the CO is in a hydrophobic region of the active site pocket.[8] The modified active site was then fit, with atoms weighted by their electron count, to the $Fe(CN)_2CO$ moiety, and the results are displayed in Figure 2. A reasonable fit is seen with a weighted root-mean-square deviation of 0.205 Å. Of the seven fitted atoms, O(1) and C(3) of the CO group show the largest deviation: 0.29 and 0.24 Å, respectively. The planes of the trigonal faces opposite to the superimposed FeL_3 (L = diatomic ligand) units, the $(\eta^5\text{-}C_5H_5)$ ring for the organometallic, and the $(\mu\text{-SCys})_2(\mu\text{-O})$ bridge between Ni and Fe atoms of the protein are substantially parallel and separated by ca. 0.5 Å (Figure 2). Further details are given in the Supporting Information.

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(6) Crystals examined were small needles (widths ∼0.02 mm). X-ray diffraction data were collected on a Rigaku AFC5 rotating anode diffractometer (oriented graphite monochrometer; Cu Kα radiation) at 193(2) K. The structure was solved by direct methods (exact procedures are given in Supporting Information).[7] Crystallographic data for $C_8H_5N_2OFeK$: fw = 480.18; monoclinic, space group $P2_1/c$, (a = 13.442(3), b = 7.852(2), and c = 8.731(2) Å; β = 99.605°, V = 908.5(3) Å^3, and Z = 4, d_{calcd} = 1.755 g cm^{-3}, μ(Cu Kα) = 17.07 mm^{-1}, 2θ range from 6 < 2θ < 120. Using 1460 reflections total and 866 observed, $R(F)$ = 0.0656 and wR(F^2) = 0.1617 [$I > 2\sigma(I)$].

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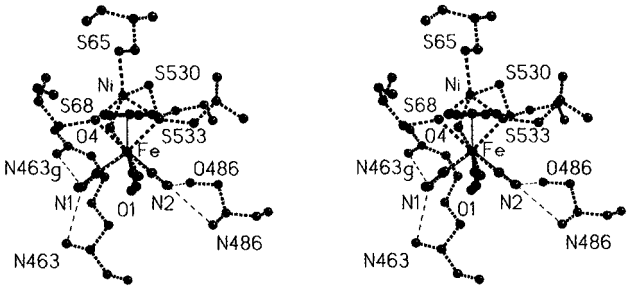

Figure 2. Stereoview of the superposition of $[(\eta^5\text{-}C_5H_5)Fe(CN)_2(CO)]^-$ structure (in blue solid line) with the Ni−Fe heterobimetallic site (in red, dashed lines) of hydrogenase from the crystal structure coordinates reported.[3,8]

Table 1. Infrared Spectral Data of Organometallic Model Complex and $[NiFe]H_2$ase[a] Active Site

	ν(CO), cm^{-1} (s)	ν(CN), cm^{-1} (m)
	$K[(\eta^5\text{-}C_5H_5)Fe(CN)_2(CO)]$	
CH_3CN	1949	2088, 2094
KBr	1954, 1973	2085, 2095
H_2O	1979	2068, 2083
EtOH	1973	2082, 2093
^{13}CN-substitution (CH_3CN)	1951	2044, 2050
$C^{15}N$-substitution (CH_3CN)	1951	2059, 2065
^{13}CO-substitution (CH_3CN)	1907	2088, 2094
^{13}C-labelled (CH_3CN)[b]	1905	2043, 2048
	C. vinosum [4]	
natural abundance	1945	2083, 2093
^{15}N-labeled	1944	2050, 2062
^{13}C-labeled[b]	1900	2036, 2047
	D. gigas[3]	
Ni-A,[c] natural abundance	1947	2083, 2093

[a] IR spectra of *D. gigas* hydrogenase in 100 mM Tris/KCl buffer, pH 8.1.[3] [b] All three ligands are ^{13}C-labeled. In the model system, we have the option of labeling the two types of ligands independently. [c] The redox state Ni-A is the oxidized form.

The infrared spectrum of $CpFe(CN)_2(CO)^-$ in the 2100−1900 cm^{-1} region is predicted to consist of three bands: the symmetric and asymmetric stretching vibrations of the two cyanide ligands and the carbonyl stretching mode. In the aprotic solvent CH_3CN, these are observed at 2094(m), 2088(m), and 1949(s) cm^{-1}, respectively. The spectrum in the solid state (KBr) is similar,[9,10] indicating similar ligand environments, i.e., the $K^+\cdots NC^-$ interactions exist in both media. Isotopic substitutions, $K[CpFe(^{13}CN)_2(CO)]$, $K[CpFe(C^{15}N)_2(CO)]$, $K[CpFe(CN)_2(^{13}CO)]$, and $K[CpFe(^{13}CN)_2(^{13}CO)]$,[11] produce the expected mass difference shifts in the ν_{CN} and ν_{CO} vibrational modes (Table 1). For all selectively isotopically-substituted molecules in both the model complex and the enzymes,[3,4] the unmodified ligands (i.e., $^{12}C^{16}O$ or $^{12}C^{14}N$) maintain their vibrational position. From this we conclude that there is very little vibrational coupling between the ν_{CN} and ν_{CO} modes.

Comparative infrared data for natural and isotopically enriched *C. vinosum* $[NiFe]H_2$ase is listed in Table 1,[4] as well as data measured on *Desulfvibrio gigas*.[3] The relative intensities, the band positions, and the band shifts with isotopic substitution of the enzyme's diatomics match those of $K[CpFe(CN)_2(CO)]$ in CH_3CN solution remarkably well. From the intensity ratio of $\nu_{CN,sym}$ to $\nu_{CN,asym}$ of the organometallic complex in CH_3CN solution, the ∠NC−Fe−CN is calculated to be 92°;[12] the analogous calculation for the enzyme yields 89°.[3] Both values are consistent with the 90° angle determined from the respective crystal structures.

The $K[CpFe(CN)_2(CO)]$ salt is highly soluble in water, and the aqueous solution infrared spectrum is distinct from that recorded in CH_3CN. Notably, in water the ν_{CN} modes are displaced to lower wavenumbers with a greater band separation (2083 and 2068 cm^{-1}), and the CO band is shifted to higher energy (1979 cm^{-1}). These results are attributed to solvation of K^+ with water removing it from the cation/anion interaction at cyanide, with concomitant solvation of the organometallic anion by water. The fact that the IR spectra of the $K[CpFe(CN)_2(CO)]$ complex in the aprotic solvent CH_3CN are so similar to those of the enzyme strongly suggests a similar electronic environment for the $Fe(CN)_2(CO)$ fragment in the two cases. That is, the enzyme reduces electronic charge on the anionic CN ligands by extensive hydrogen-bonding interactions with peptide NH and OH groups and the CO is surrounded by hydrophobic residues, whereas in the organometallic model complex the CN ligands are implicated in ion-pairing interactions with K^+ and the CO ligand is in a hydrophobic environment.[13]

The similarities of structure and IR spectral data discussed above would also suggest that the 6-electron, anionic $(\eta^5\text{-}C_5H_5)$ ligand is similar in donating ability to the $Ni(\mu\text{-}SCys)_2(\mu\text{-}O)$ or $Ni(\mu\text{-}SCys)_2(\mu\text{-}OH)$ donor face in the enzyme bimetallic. In this regard, the cyclic voltammogram of $K[(\eta^5\text{-}C_5H_5)Fe(CN)_2(CO)]$ in acetonitrile finds a quasi-reversible electrochemical event, presumably the $Fe^{III/II}$ couple, at + 0.66 V. This value is rather far removed from redox couples of $[NiFe]H_2$ases (ranging from −0.1 to −0.45 V),[1] which have been attributed to nickel-based or bimetallic electrochemistry.[2c]

The origin of CO and CN in such biological systems is intriguing. The possibility of CO as a useful byproduct in methanogenic bacteria which are functioning to reduce CO_2 is reasonable.

While the presence of Co−CN in coenzyme B_{12} is accepted to be an artifact of isolation,[14] its involvement in $[NiFe]H_2$ase appears to be intrinsic for the role of $Fe(CN)_2CO$. Earlier, we argued for the necessity of exogeneous metal as a thiolate charge-neutralizing moiety in providing easier accessibility of the putative catalytically active Ni^I.[2c]

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Supporting Information Available: A figure of IR spectra, tables of crystallographic data collection parameters, atomic coordinates, and equivalent isotropic displacement parameters, complete listings of bond lengths and bond angles, anisotropic displacement parameters, and packing diagram for $K[(\eta^5\text{-}C_5H_5)Fe(CN)_2(CO)]$ (22 pages). See any current masthead page for ordering and Internet access instructions.

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(11) The preparation of pure samples enriched to greater than 98% in ^{13}CN or $C^{15}N$ was achieved by carrying out the syntheses as described,[5] utilizing the correspondingly isotopically labeled KCN salts, purchased from Cambridge Isotopes, Inc. The sample enriched in ^{13}CO (>90% labeled) was obtained from the photolysis of an ethanol solution of $K[(\eta^5\text{-}C_5H_5)Fe(CN)_2(CO)]$ in the presence of ^{13}CO.

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