Resonance Raman spectra of the MMOH-phenol complex (Figure 2) show definitively that the chromophore arises from a phenolate-to-Fe(III) charge-transfer transition derived from an *exogenous* phenol. Two enhanced features appear at 602 and 628 cm^{-1} (brackets in Figure 2B) using 514.5-nm laser excitation that are absent in the spectra of MMOH (150 mg/mL) (Figure 2A), a buffer/phenol solution, and MMOH-phenol with λ_{ex} = 632 nm.[19] As expected, the two Raman bands shift to lower frequency for samples prepared with [^{18}O]-phenol (Figure 2C, 592 and 620 cm^{-1}), [$^{13}C_6$]-phenol (Figure 2D, 582 and 608 cm^{-1}), and [$^{2}H_5$]-phenol (Figure 2E, 580 and 606 cm^{-1}). The ~26-cm^{-1} separation of the two bands in the MMOH-phenol complex is conserved in each of these spectra indicating that the two vibrational features are similar in origin. The observed isotope shifts indicate that these modes have both significant phenol ring deformation and Fe-O stretching character as observed for *p*-cresolate-Fe(III) and phenol-Fe(III) complexes.[20] However these complexes typically exhibit only one mode in this region. Thus the two bands in the MMOH-phenol complex probably arise from two phenolate ligands in distinct environments. At present it is unclear whether these represent two phenols bound to the same diiron cluster or to two slightly different clusters, although the low intensity of the LMCT band might favor the latter interpretation.

The observation of the first visible chromophore of MMOH allows the application of previously inaccessible optical techniques to assess the cluster iron coordination. In general the energy of phenolate-to-Fe(III) LMCT transition reflects the Lewis acidity of the metal center,[20a,21] which in turn is determined by its ligand environment. The visible and CD absorption of the MMOH-phenol complex are remarkably similar to those of Uf[22] which suggests similar cluster ligand environments for the two proteins. The present model[9b,c,10] for the Uf active site consists of a (μ-hydroxo)diiron unit probably supported by a carboxylate bridge with a tyrosine and a histidine on one iron and a histidine and a carboxylate on the other iron. Such a coordination environment, less the tyrosine, is consistent with ENDOR[23] and EXAFS[6] studies of uncomplexed MMOH, but the significant difference in intensity between the LMCT bands of MMOH-phenol and Uf indicates subtle differences in ligand environment. The bearing of these structural differences on unique hydrocarbon oxidation chemistry by MMOH is currently the subject of further spectroscopic and kinetic investigation of the phenolate complexes. The demonstration here that relatively large molecules have access to the diiron cluster may also serve to differentiate MMOH from other proteins that contain related clusters.[24]

(19) The two features are in the metal ligand vibrational region of the Raman spectrum typical of Fe(III)-phenolate complexes.[9a,b,14,20] The phenolate ring vibrational modes at higher frequencies (1100-1600 cm^{-1}), which are characteristic of iron-tyrosine proteins,[14] are not observed above the protein background; these features may be weak as a result of the low intensity of the MMOH-phenol LMCT band and/or the use of the unsubstituted phenol instead of a para-substituted phenol. In an iron porphyrin phenol complex,[20b] only the ~600-cm^{-1} feature is unequivocally observed in the Raman spectrum.

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(24) Ribonucleotide reductase R2-protein has been shown to contain short regions of amino acid sequence homology to MMOH in the cluster binding region suggesting similar cluster ligation.[23] Although exogenous hydrocarbon hydroxylation has not been reported for R2, an endogenous tyrosine placed near the cluster by site specific mutation is hydroxylated. The resulting catechol derivative then appears to bind to the diiron cluster to yield a blue-green chromophore analogous in some ways to the red chromophore described here for MMOH (see: Ormö, M.; deMaré, F.; Regnström, K.; Åberg, A.; Sahlin, M.; Ling, J.; Loehr, T.; Sanders-Loehr, J.; Sjöberg, B.-M. *J. Biol. Chem.* **1992**, *267*, 8711-8714). Thus MMOH-like catalysis may be limited in the case of R2 by site accessibility.

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Characterization of the CO Binding Site of Carbon Monoxide Dehydrogenase from *Clostridium thermoaceticum* by Infrared Spectroscopy†

Manoj Kumar and Stephen W. Ragsdale*

Department of Biochemistry, East Campus
University of Nebraska
Lincoln, Nebraska 68583-0718

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This communication describes the results of an infrared spectroscopic study of the binding site for CO on carbon monoxide dehydrogenase (CODH). The CO molecule was found to be terminally coordinated to one metal in the active site mixed metal cluster containing Ni, 3-4 Fe, and acid-labile sulfide. Since CO is not a bridging ligand, there must be an endogenous bridge between Ni and Fe.

The Wood-Ljungdahl pathway is an autotrophic pathway for growth of *Clostridium themoaceticum* and other anaerobic bacteria in which cell carbon is formed from CO_2, CO, or other organic substrates.[1] CODH catalyzes the final steps in the synthesis of acetyl-CoA from the methylated corrinoid/iron-sulfur protein (CH_3-C/Fe-SP), CO, and coenzyme A ($CoAS^-$) (eq 1). The intermediates are enzyme bound and include methyl-CODH,[2] carbonyl-CODH,[3] and acetyl-CODH[2,4] (see ref 1a for review). After binding of CoA, thiolytic cleavage of the acetyl group produces acetyl-CoA.[5]

$$CH_3\text{-C/Fe-SP} + CO + CoAS^- \rightarrow CH_3\text{-CO-SCoA} + \text{C/Fe-SP} \quad (1)$$

Treatment of CODH with CO reduces the enzyme and elicits an EPR signal with $g_\perp$ = 2.08 and $g_\parallel$ = 2.028.[6] Since this EPR signal is broadened when CODH is enriched with ^{61}Ni or ^{57}Fe and when ^{13}CO is reacted with the enzyme,[7] it has been named the NiFeC signal. The NiFeC species has been shown to be a catalytically competent intermediate in the pathway of acetyl-CoA synthesis.[3] EPR,[7] Mössbauer[8] and ENDOR[7c] spectroscopic studies suggest a working model for the structure of the CO binding site as a [$NiFe_{3-4}S_4$] cluster. Recent work implicates the [$NiFe_{3-4}S_4$] cluster also serves as the site of methylation and acetylation.[3,7c]

* For communications regarding this manuscript, please contact: Dr. Stephen W. Ragsdale, Department of Biochemistry, East Campus, University of Nebraska, Lincoln, Nebraska 68583-0718. Phone: 402-472-2943. Facsimile: 402-472-7842.

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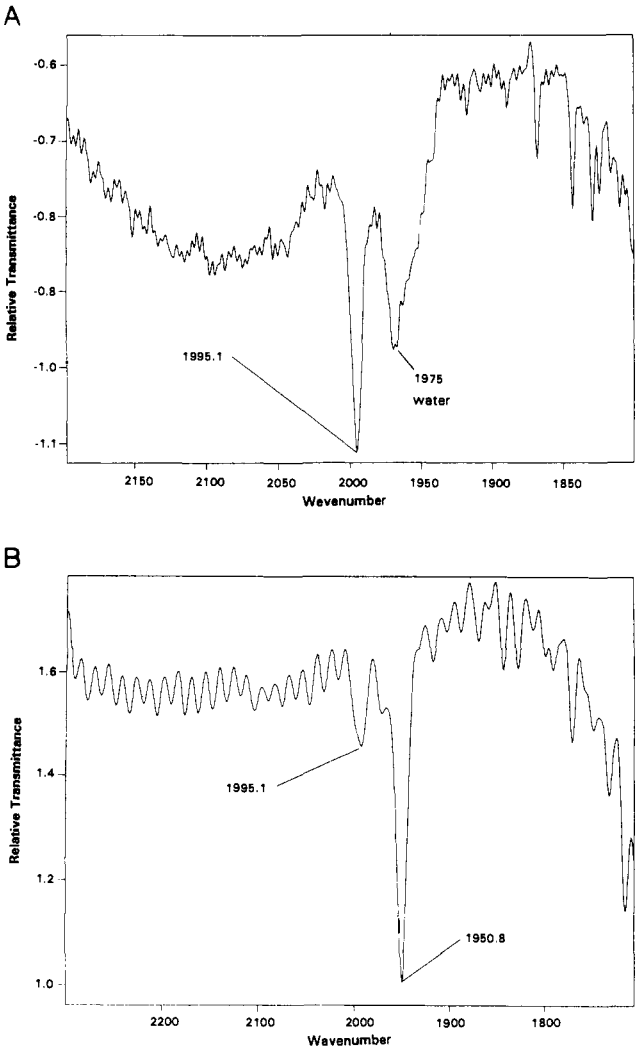

Figure 1. FTIR spectra of the CODH–CO complex. (A) The spectrum of 1.2 mM CODH (8000 scans) was recorded, CODH was removed anaerobically from the Circle cell via a gastight syringe, incubated in ^{12}CO, and reintroduced into the Circle cell, and 8000 spectral scans were recorded. The spectrum of CODH was then digitally subtracted from that of CODH–CO. (B) The spectrum of a 1.37 mM solution of CODH was recorded, and CODH was removed from the cell and incubated under ^{13}CO and 1.37 mM acetyl-CoA. After reintroducing this solution into the Circle cell, spectra were recorded as described above. The resulting spectrum was subtracted from that of the CODH solution. A similar difference spectrum was attained upon incubation of CODH with ^{13}CO in the absence of acetyl-CoA, but lacking the 1995-cm^{-1} peak.

In order to further elucidate the carbonyl binding site of CODH, we have used Fourier transform infrared (FTIR) spectroscopy. Typical of IR spectra of proteins, the primary spectrum of CODH[9] (data not shown) contained two major broad bands with maxima at 1641 and 1107 cm^{-1}. The CODH spectrum obtained after subtraction of that of buffer shows amide I and amide II bands centered at 1546 and 1454 cm^{-1} and a broad, strong band due to water. After incubation of CODH (1.0 mM) with ^{12}CO,[10] the difference spectrum relative to the sample incubated without CO reveals a peak at 1995 cm^{-1} with a band width of 8 cm^{-1} (Figure 1A). There is another broad peak centered at ca. 1975 cm^{-1} which we assign to residual water.[11] The 1995-cm^{-1} band is the major absorption peak in the region of the C–O stretch, and, thus, this IR band is the signature of the major CODH–CO complex formed upon incubation of the enzyme with CO. Based on the signal-to-noise, minor CODH–CO complexes present at less than 5% of the intensity of the major species would not have been detected. Assignment of the 1995-cm^{-1} IR band as the signature of a terminally bound carbonyl, i.e., metal—C≡O, complex is unambiguous since the stretching vibration for CODH–CO is much higher than the values for heterobinuclear or homobinuclear metal–CO–metal complexes. The IR absorption band for a terminally bonded carbonyl is found in the range of 2140–1800 cm^{-1}, whereas that for a bridging carbonyl ranges from 1880 to 1700 cm^{-1}.[12]

When CODH is reacted with ^{13}CO,[10] the IR peak at 1995 cm^{-1} is absent, and a peak appears at 1951 cm^{-1} (described below). Thus, there is an isotopic shift of 44 cm^{-1} which is exactly what is calculated based only on the difference in masses between ^{12}CO and ^{13}CO.[15] The magnitude of the isotopic shift is consistent with the assignment of the 1995-cm^{-1} peak to the stretching mode of a terminally bound metal carbonyl.

A measure of the catalytic activity of CODH in acetyl-CoA synthesis is provided by study of an isotopic exchange reaction between CO and the carbonyl group of acetyl CoA (eq 2).[9a,13]

$$[1\text{-}{}^{*}C]\text{acetyl-CoA} + CO \rightarrow {}^{*}CO + \text{acetyl-CoA} \quad (2)$$

When CODH was reacted with ^{13}CO in the presence of CH_3-^{12}CO-CoA,[14] both CODH-^{13}CO and CODH-^{12}CO stretching vibrations at 1950 and 1995 cm^{-1}, respectively, are observed with peak height ratios of 4/1 (Figure 1B). In the absence of acetyl-CoA, only the 1950-cm^{-1} peak (due to CODH-^{13}CO) was observed. When ^{12}CO and CH_3-^{12}CO-CoA are reacted under similar conditions, only the 1995-cm^{-1} peak was observed. These experiments demonstrate that the metal carbonyl species observed by IR is a catalytically relevant precursor of the carbonyl group of acetyl-CoA. The CO group of the EPR detectable NiFeC complex also was shown to be catalytically competent as the precursor of the carbonyl group of acetyl-CoA.[3] Thus, the 1995-cm^{-1} IR peak and the g = 2.08/2.028 EPR signal are spectroscopic signatures of the same complex and the binding site for CO can be described as [$NiFe_{3-4}S_4$]—C≡O. Since all the components of this complex are part of a single cluster[7,8] and CO is terminally bound, the Ni and Fe components must have an endogenous bridge, X, and the structure of the CO adduct to CODH can be described as [$NiXFe_{3-4}S_4$]—C≡O. Although negative evidence is not convincing, EXAFS studies of the nickel sites of the CODHs from *C. thermoaceticum*[17] and *Rhodospi-*

(9) (a) Purification of CODH: Ragsdale, S. W.; Wood, H. G. *J. Biol. Chem.* **1985**, *260*, 3970–3977. (b) After concentrating CODH to 1.0–1.5 mM in 50 mM Tris-HCl buffer, pH 7.5 using an Amicon macrosolute concentrator, 250 μL of enzyme was introduced into a Circle cell (Spectra Tech). (c) IR spectroscopy was performed on FT-IR Mattson Galaxy 4020 instrument which was purged with N_2 before and during the experiment. The Circle cell contained a Zn–Se crystal and was maintained under anaerobic conditions.

(10) CODH was incubated in either ^{12}CO (Linde) or ^{13}CO (Isotech) for 30 min and then introduced into the Circle cell under a positive pressure of CO. Spectra (8000 scans) were recorded for 33.64 min at 2-cm^{-1} resolution at a forward speed of 6.0 cm/min using an MCT detector. The resulting spectrum was subtracted from that of a CODH solution which had not been incubated with CO. The subtraction factor was from 0.98 to 1.02.

(11) In Figure 1A, there is a broad absorption band centered at ca. 1975 cm^{-1} which is apparently due to moisture in the sample compartment of the IR instrument. It is not present in most samples of enzyme (for example, it is absent in Figure 1B) and does not exhibit isotope substitution with ^{13}CO.

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(14) After incubation of CODH (1.37 mM) for 0.5 h under ^{13}CO, natural abundance acetyl-CoA (1.37 mM) (Sigma Chemicals) in 10 μL buffer was added. After incubation for 20 min, this solution was added to the Circle cell and spectra were recorded as described above. The resulting spectrum was subtracted from that of a solution of CODH which had not been incubated with CO.

(15) The mass effect was calculated using the equation:

$$\nu^{*}/\nu = \frac{(M_A^{*} + M_B^{*})/M_A^{*}M_B^{*}}{(M_A + M_B)/M_AM_B}$$

Many references to this equation are available, for example: Braterman, P. S. *Metal Carbonyl Spectra*: Academic Press, London Ltd.: London, England, 1975; pp 33–36. Thus, $\nu(^{13}C^{16}O)/\nu(^{12}C^{16}O)$ = 2096.071/2143.774 = 0.977 749 and, based on mass effect only, the predicted frequency for the stretching band of the ^{13}CO adduct of CODH is at (1995.12) × (0.977 75) = 1950.76 cm^{-1}, which is identical to the observed value.

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rillum rubrum[18] indicate that Ni is not present within the Fe-S cubane core since there was no evidence for Ni-Fe interactions. Further description of the linkage "X" between Ni and iron and the structure of this complex will require comparison of the properties of biomimetic models with those of CODH and further analyses of the properties of CODH.

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Blocked Photochromism of Diarylethenes

Masahiro Irie,* Osamu Miyatake, and Kingo Uchida

Institute of Advanced Material Study
Kyushu University
Kasuga-Koen 6-1, Kasuga, Fukuoka, Japan
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Photochromic molecules with a clasp, which undergo photoisomerization only when the clasp is freed by a switch molecule, were synthesized. Photochromism has attracted renewed interest because of the recent development of fatigue-resistant compounds. A classical photochromic molecule, 6-nitrospirobenzopyran, loses its photochromic property after 30-50 coloration/decoloration cycles, while several newly synthesized molecules can maintain performance through more than 10^4 repetitions of the cycle.[1-4] Among the molecules, 1,2-diarylethenes containing heterocyclic rings have the potential ability for many applications owing to an additional characteristic, namely, the thermal stability of both isomers.[5] Besides fatigue resistance and thermal irreversibility, a property that is strongly desired but still lacking in existing photochromic molecules is gated photochemical reactivity.[6] Gated reactivity is the property that irradiation with any wavelength causes no molecular change, while a photoreaction occurs when another external stimulation, such as an electric field or chemicals, is present.[7] We designed and synthesized chemical-gated molecules by introducing substituents that have hydrogen-bonding ability into the 1,2-diarylethenes.

1,2-Bis(2-methylbenzo[*b*]thiophen-3-yl)perfluorocyclopentene derivatives with carboxyalkyl groups at the 6 and 6′ positions, **1** and **2**, were synthesized.[8] The perfluorocyclopentene moiety is

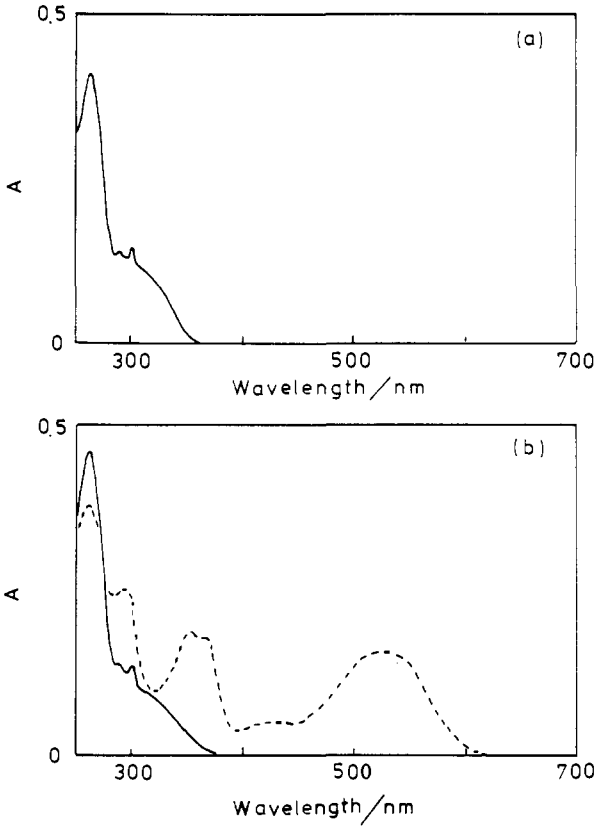
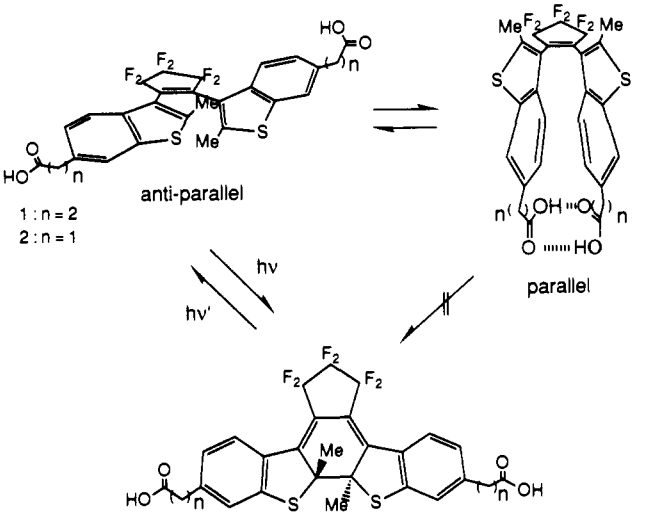


Figure 1. Absorption spectra of **1** (2.5×10^{-5} mol/L) (—) upon irradiation with 313-nm light in (a) cyclohexane and (b) ethanol; the absorption band around 525 nm (– – –) is due to the closed-ring form.

Scheme I



effective in increasing the durability of the molecules.[1a] The photochromic reaction of the 1,2-diarylethenes belongs to a 1,3,5-hexatriene to cyclohexadiene type reaction. According to the Woodward-Hoffmann rule based on π-orbital symmetries, a conrotatory cyclization is brought about by light.[9] When the aryl groups are heterocyclic five-membered rings, the molecule has two conformations, with the two rings in mirror and C_2 symmetries,[3,7] and the conrotatory cyclization can proceed only from the conformation with the rings in C_2 symmetry. This means that the photocyclization is prohibited if the heterocyclic rings are fixed to the mirror symmetry, or parallel orientation, while

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(8) **1**: mp 105-106 °C; ^{1}H-NMR (270 MHz, C_2D_5OD) δ 2.16 (3 H, s), 2.47 (3 H, s), 2.55 (2 H, t), 2.60 (2 H, t), 2.92 (2 H, t), 3.01 (2 H, t), 7.07 (1 H, d, J = 8.06 Hz), 7.29 (1 H, d, J = 8.06 Hz), 7.44 (1 H, d, J = 8.06 Hz), 7.56 (1 H, d, J = 8.06 Hz), 7.50 (1 H, s), 7.60 (1 H, s). Anal. Calcd for $C_{29}H_{22}O_4S_2F_6$: C, 56.85; H, 3.62. Found: C, 56.78; H, 3.72. **2**: mp 219.5-220.5 °C; ^{1}H-NMR (270 MHz, C_2D_5OD) δ 2.21 (3 H, s), 2.49 (3 H, s), 3.62 (2 H, s), 3.71 (2 H, s), 7.14 (1 H, d, J = 8.18 Hz), 7.34 (1 H, d, J = 8.18), 7.50 (1 H, d, J = 8.18 Hz), 7.61 (1 H, d, J = 8.18 Hz), 7.59 (1 H, s), 7.69 (1 H, s). Anal. Calcd for $C_{27}H_{18}O_4S_2F_6$: C, 55.48; H, 3.10. Found: C, 55.56; H, 3.46.

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