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Supplementary Material Available: ^1H NMR spectra and analytical data for silyl ketones and hydroxy ketones (1 page). Ordering information is given on any current masthead page.

Nickel and Iron EXAFS of Carbon Monoxide Dehydrogenase from *Clostridium thermoaceticum* Strain DSM

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Carbon monoxide dehydrogenase (CODH) from the acetogenic bacterium, *Clostridium thermoaceticum*, has been purified to apparent homogeneity.[1] The native enzyme which has an $\alpha_3\beta_3$ subunit stoichiometry was shown to contain 2Ni, 1-3Zn, 12Fe, and 14 acid labile sulfide per $\alpha\beta$ pair.[1-3] This enzyme catalyzes the reversible reduction of CO_2 to CO[4,5] and in addition catalyzes two isotope exchange reactions: (a) an exchange of labeled CoA with the CoA portion in unlabeled acetyl CoA[3] and (b) the exchange of labeled CO with the unlabeled acetyl CoA carbonyl group.[6] The enzyme is thought to catalyze the C-C bond formation step in the biosynthesis of acetate from C_1 precursors. When CO exchanges with the acetyl CoA carbonyl group in the presence of CODH, the chirality of the methyl group is retained.[20]

The EPR of carbon monoxide dehydrogenase in the presence of CO is due to a nickel-iron-carbon complex, according to hyperfine broadening when ^{61}Ni, ^{57}Fe, or ^{13}CO are present.[3,7] We here characterize the average ligand environments of nickel and iron in the Ni-EPR silent, CO-free form of this biological carbonylation catalyst.

EXAFS data have been collected on both the nickel and iron sites of CODH from *Clostridium thermoaceticum* strain DSM 521.[8,9] The CO dehydrogenase was purified under argon, in a state containing EPR silent nickel as previously described.[10,11]

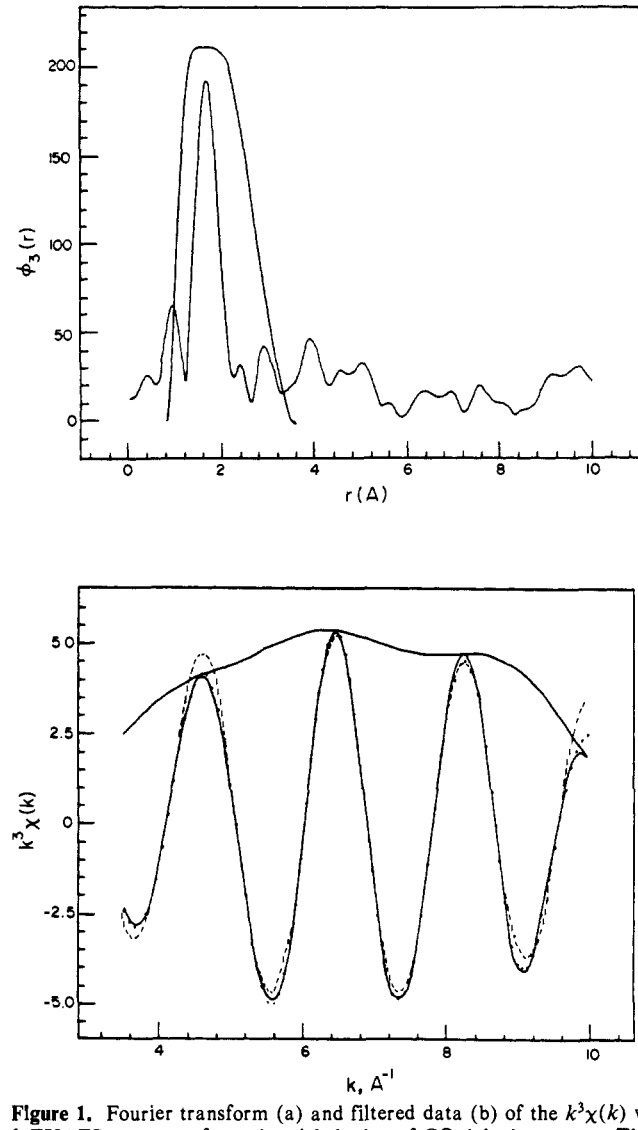

Figure 1. Fourier transform (a) and filtered data (b) of the $k^3\chi(k)$ vs k EXAFS spectrum from the nickel edge of CO dehydrogenase. The upper curve in (a) is the window function used in Fourier filtering. The dashed line in (b) is the one term sulfur fit to the Fourier filtered data (solid line) while the dotted line is the fit of one sulfur plus iron. The amplitude function for the filtered data is also shown.

Table I. Summary of CODH EXAFS Results

compound	bond	bond distance, Å	coord no.	Debye-Waller factor, Å
$Ni_2(TTH)_2$	Ni-S	2.16 ± 0.03	4	0.034 ± 0.005
CO dehydrogenase	Ni-S	2.16 ± 0.03	3.8	0.063 ± 0.004
$Ni_2(TTH)_2$	Ni-Ni	2.85 ± 0.04	1	0.0004 ± 0.003
CO dehydrogenase	Ni-Fe	3.25 ± 0.05	0.42	0.0005 ± 0.005
$(NEt_4)_3Fe_4S_4(SPh)_4$	Fe-S	2.27 ± 0.03	4	0.049 ± 0.004
CO dehydrogenase	Fe-S	2.27 ± 0.03	3.7	0.020 ± 0.003
$(NEt_4)_2Fe_4S_4(SPH)_4$	Fe-Fe	2.74 ± 0.03	3	0.059 ± 0.002
CO dehydrogenase	Fe-Fe	2.75 ± 0.03	2.6	0.030 ± 0.005

Table II. Summary of χ^2 for Different Fitting Models in the Analysis of Ni EXAFS of CODH

model	χ^2	model	χ^2
one term Ni-S	9.96	one term Ni-S plus Fe	2.82
two term Ni-S	6.63	two term Ni-S plus Fe	1.86

Metal content was determined by atomic absorption analysis by using a Perkin Elmer Model 2380 atomic absorption spectrophotometer with a programmable HGA-400 graphite furnace. The purified CODH (0.16 mM) containing 1 mM Ni and 5 mM Fe had a specific activity of 310 (μmol CO oxidized/min)/mg enzyme. Data analysis was performed according to the method

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of Teo[12-14] on the average of 12 nickel and eight iron EXAFS spectra, truncated at 10 and 12 Å^{-1}, respectively.[15,18] Single scans of the nickel[16] and iron[17] models were used.

The first and second peaks of the Fourier transform of the iron EXAFS (data not shown) fit to sulfur and iron, respectively (Table I). Analyses of iron EXAFS could not distinguish iron–nickel interactions among the average three iron–iron interactions per scatterer observed. These data are consistent with but do not rigorously prove the presence of Fe_4S_4 clusters.

The Fourier transform of the nickel EXAFS is shown in Figure 1. The Fourier filtered data was best fit to sulfur (Figure 1b, dashed line) at 2.16 Å. A reasonable fit to oxygen/nitrogen could only be obtained by setting the ΔE_0 to 40 and the Debye–Waller term to 5×10^{-4} Å. A reasonable Ni–S fit was also obtained by assuming two Ni–S bond lengths at 2.22 and 2.11 Å, respectively. Addition of a Ni–M (M = Fe, Ni, Zn) term at 3.25 Å also improved the fit (Figure 1b, dotted line) although a high ΔE_0 (19 eV) was required to obtain a reasonable fit. Similar behavior was observed with the nickel model compound where a Ni–Ni distance could be best fit by setting the ΔE_0 to 21 eV. The calculated χ^2 for various models tested are given in Table II. The nickel in the model compound is a square-planar,[16] and the environment of nickel in CODH may be square planar was well. This would leave open axial coordination sites for CO, methyl, or acetyl CoA.[20]

In summary, the iron EXAFS indicates the presence of iron–sulfur centers, likely Fe_4S_4 clusters. The dominant scatters in the nickel EXAFS are approximately four sulfurs at 2.16 Å. The finding of ^{61}Ni and ^{57}Fe hyperfine splitting in the EPR of the CO complex suggest that both nickel and iron are present in the paramagnetic species produced by CO binding in the enzyme. A nickel–iron interaction at 3.25 Å is plausibly fit to the data but cannot be unequivocally assigned in the present analysis. We emphasize that the present EXAFS results refer to the CO-form of the enzyme. If a Ni–S–Fe complex is present in this form of the enzyme, one would expect, given Fe–S and Ni–S bond lengths of about 2.2 Å, that when the angle of the Fe–S–Ni complex varies between 75° and 180°, the Fe–Ni interaction would vary between 2.7 and 4.4 Å. For thiol bridged Fe–S clusters the angle is found[19] to be about 75°. If the nickel is part of a mixed-metal, cubane-type cluster, one would expect to see an iron atom at about 2.7 Å in the nickel EXAFS, where none in fact is found in the present analysis. Assuming normal bonding strength in the metal–sulfur bonds we conclude that either the Ni–S–Fe angle is larger than 90° or in fact that no direct linkage exists in the CO-free form of the enzyme. If the CO complex can be produced in quantitative yield with respect to the nickel content of the enzyme, further EXAFS measurements may resolve whether CO addition establishes an iron–nickel linkage, perhaps by forming a CO-bridged complex. We also should emphasize that since the enzyme contains two nickel atoms per $\alpha\beta$ dimer, it is possible that the two nickel sites are different in geometry and ligand environment. However, as a minimal working hypothesis, we suggest that the nickel in the EPR inactive state of this enzyme has four sulfurs in an approximately square-planar geometry with a nearby FeS cluster. Further work aimed at characterizing other states of the enzyme and at determining the nature, if any, of the nickel–iron interaction is in progress.

Registry No. $Ni_2(TTH)_2$, 36488-62-7; $(NEt_4)_2[Fe_4S_4(SPh)_4]$, 55663-41-7; carbon monoxide dehydrogenase, 64972-88-9; nickel, 7440-02-0; iron, 7439-89-6.

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(16) The nickel model, $Ni_2(TTH)_2$ where TTH = 1,4,7-trithiaheptane, was assigned a dimeric structure, in which each of the nickel ions is coordinated by four sulfurs in an approximately square-planar geometry. The average Ni–S bond length is 2.18 Å while the Ni–Ni distance is 2.74 Å and the Ni–S–Ni angle is 75.6°: Baker, D. J.; Goodall, D. C.; Moss, D. S. *Chem. Commun.* **1969**, 325.
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Reversible Photodissociation and Thermal Binding of Carbon Dioxide in Oxo-Bridged Copper Dimers

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We report here the reversible sequence of photodissociation and thermal binding of carbon dioxide to a binuclear oxo-bridged copper(II) complex (Scheme I). The carbon dioxide binds the oxo-bridged complex via carbon to μ-oxo and oxygen to copper bonds.[1] The reversible photoexpulsion and thermal binding of carbon dioxide with this type of coordination have not been reported. In general, reversible binding of carbon dioxide to metal complexes is rare. A relatively small number of examples of reversible side-on[2,3] and metal–ligand insertions[2,4] are known.

The (μ-carbonato)dichlorobis(1,10-phenanthroline)dicopper(II) complex (I) and the (μ-oxo)dichlorobis(1,10-phenanthroline)dicopper(II) complex (II) were prepared by using a modification of the literature procedures for similar complexes.[1] For the carbonate complex, a 5 mmol solution of 1,10-phenanthroline (Malincrodt) in 30 mL of anhydrous methylene chloride was flushed with CO_2 for 20 min. Copper(I) chloride[5] (5 mmol) was then added under CO_2, and the mixture was stirred under a stream of CO_2 until all of the copper dissolved. At this point both CO_2 and O_2 were passed through the solution producing a blue-green solution. After an additional 10 min, the gas streams were stopped, and the methylene chloride was removed in a vacuum rotory evaporator leaving a blue-green solid. The oxo complex was prepared in a similar manner except that the system was flushed with nitrogen until the copper dissolved, and then oxygen was passed through the solution to produce a green-brown solution.

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