Crystallographic Studies of CO to CO_2 Interconversion in a Ni-Fe-S Cluster-containing Metalloenzyme

While scientists and engineers struggle to develop technologies to remove CO and CO_2 from our environment, anaerobic bacteria such as *Rhodospirillum rubrum* and *Carboxydothermus hydrogenoformans* have the ability to utilize the gaseous pollutant CO as their sole carbon and energy source (1-2). This ability derives from the reversible oxidation of CO to CO_2 catalyzed at a Ni-Fe-S active site metal cluster (C-cluster) of the enzyme carbon monoxide dehydrogenase (CODH). Acetogenic bacteria such as *Moorella thermoacetica* also use CODH in a bifunctional CODH/acetyl-CoA synthase (ACS) enzyme complex to first convert the greenhouse gas CO_2 to a CO intermediate. CO then travels through a tunnel within the CODH/ACS complex to the ACS subunit's active site metallocluster (A-cluster) where it is combined with a methyl group and coenzyme A to form acetyl-CoA. Acetoclastic methanogens also harbor CODH and ACS subunits in the enzyme complex acetyl-CoA decarbonylase/synthase (ACDS); however, in this case, the reaction catalyzed is the degradation of acetyl-CoA to form CO_2 and another greenhouse gas, methane (CH_4). At the heart of these reaction pathways that are vital to the global carbon cycle is the reversible oxidation of CO to CO_2 catalyzed by the active site C-cluster of CODH. In this work, we have used X-ray crystallography performed at SSRL (Beam Line 11-1) and at ALS (Beam Line 5.0.1) to understand the chemistry of this remarkable metallocluster.

The C-cluster is a Ni-Fe-S cluster whose metal content and geometry are unprecedented in biology. Here, Ni is located within a distorted cubane, along with three Fe and four S atoms, with a unique Fe coordinated nearby, liganded by a histidine residue. While several previous crystallographic studies confirmed this distinct arrangement of metals, there was conflicting structural data on the presence (3-4) or absence (5-8) of an additional sulfide ligand bridging the Ni and unique Fe (Figure 1A-B). As the sulfide bridge occupies putative substrate binding sites,

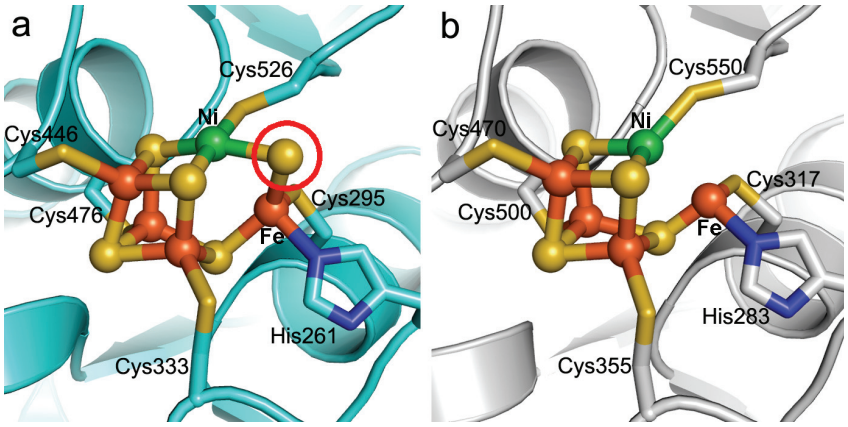

Figure 1. Representative structures of CODH C-clusters **(a)** with and **(b)** without the bridging sulfide. The C-cluster shown in (a) is from the structure of *C. hydrogenoformans* CODH (*Ch*CODH) (PDB ID: 1SU8) and is shown in cyan cartoon with residue numbering following the *Ch*CODH sequence. The bridging sulfide is circled in red. The C-cluster in (b) is from the structure of *M. thermoacetica* CODH/ACS (*Mt*CODH/ACS) solved previously (PDB ID: 1MJG) and is shown in gray cartoon with residue numbering following the *Mt*CODH/ACS sequence. C-clusters are depicted in ball-and-stick, while ligands are shown as sticks: Ni in green, Fe in orange, S in yellow, and N in blue. Ni and the unique Fe of the C-cluster are labeled as Ni and Fe, respectively.

its presence or absence is of considerable catalytic significance. Because these CODH structures did not contain any substrates or analogs bound to the C-cluster, the role of the bridging sulfide and the mechanism of the cluster had been unclear and hotly debated.

In this study, we have solved two crystal structures of the *M. thermoacetica* CODH/ACS complex, with the C-cluster bound by substrate and inhibitor molecules. The first is a native structure, illustrating a substrate water molecule bound to the unique Fe of the C-cluster (Figure 2a). After soaking native crystals in a solution containing potassium cyanide, we obtained a second crystal structure where the substrate water molecule is still seen bound to the unique Fe, but cyanide, an inhibitor which mimics CO binding, is bound to Ni, adjacent the substrate water molecule (Figure 2B). Importantly, neither structure contains the sulfide bridge. With the substrate binding sites now identified, we have contributed in both determining of the catalytic relevance of the sulfide bridge and in uncovering the mechanism of the C-cluster. From the results of this and other recent crystallographic studies (9-10), we have reached a unified catalytic mechanism of the C-cluster that excludes the bridging sulfide. Given the central role of CODH in the global carbon cycle, our detailed mechanistic understanding of this important enzyme may have broader applications in biotechnology and environmental health.

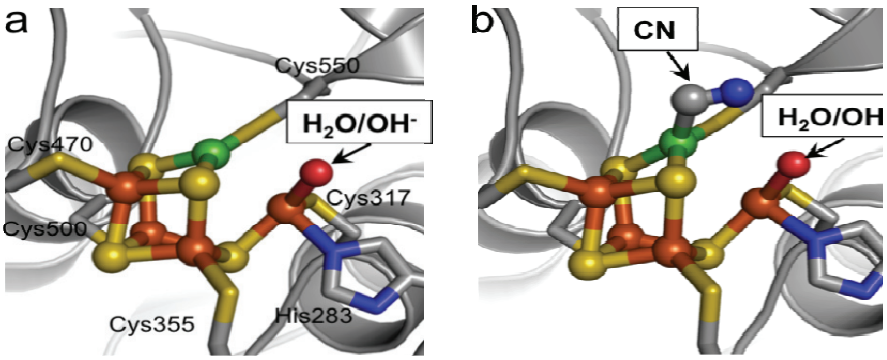

Figure 2. The **(a)** native and **(b)** cyanide-bound C-clusters from *Mt*CODH/ACS, using the same representation and coloring as in Figure 1b.

Primary Citation

Kung, Y., Doukov, T.I., Seravalli, J., Ragsdale, S.W., Drennan, C.L. (2009) Crystallographic snapshots of cyanide- and water-bound C-clusters from bifunctional carbon monoxide dehydrogenase/acetyl-CoA synthase. *Biochemistry*. **48**, 7432-7440.

References

1. Uffen, R.L. (1976) Anaerobic growth of a *Rhodopseudomonas* species in the dark with carbon monoxide as sole carbon and energy substrate. *Proc. Natl. Acad. Sci. U.S.A*. **73**, 3298–3302.
2. Svetlichny, V.A., Sokolova, T.G., Gerhardt, M., Ringpfeil, M., Kostrikina, N.A., and Zavarzin, G.A. (1991) *Carboxydothermus hydrogenoformans* gen. nov., sp. nov., a CO-

utilizing thermophilic anaerobic bacterium from hydrothermal environments of Kunashir Island. *Syst. Appl. Microbiol*. **14**, 254–260.
3. Dobbek, H., Svetlitchnyi, V., Gremer, L., Huber, R., and Meyer, O. (2001) Crystal structure of a carbon monoxide dehydrogenase reveals a [Ni-4Fe-5S] cluster. *Science.* **293**, 1281–1285.
4. Dobbek, H., Svetlitchnyi, V., Liss, J., and Meyer, O. (2004) Carbon monoxide induced decomposition of the active site [Ni-4Fe-5S] cluster of CO dehydrogenase. *J. Am. Chem. Soc*. **126**, 5382–5387.
5. Drennan, C.L., Heo, J., Sintchak, M.D., Schreiter, E., and Ludden, P.W. (2001) Life on carbon monoxide: X-ray structure of *Rhodospirillum rubrum* Ni-Fe-S carbon monoxide dehydrogenase. *Proc. Natl. Acad. Sci. U.S.A*. **98**, 11973–11978.
6. Doukov, T.I., Iverson, T.M., Seravalli, J., Ragsdale, S.W., and Drennan, C.L. (2002) A Ni-Fe-Cu center in a bifunctional carbon monoxide dehydrogenase/acetyl-CoA synthase. *Science.* **298**, 567–572.
7. Darnault, C., Volbeda, A., Kim, E.J., Legrand, P., Vernede, X., Lindahl, P.A., and Fontecilla-Camps, J.C. (2003) Ni-Zn-[Fe_4-S_4] and Ni-Ni-[Fe_4-S_4] clusters in closed and open subunits of acetyl-CoA synthase/carbon monoxide dehydrogenase. *Nat. Struct. Biol*. **10**, 271–279.
8. Doukov, T.I., Blasiak, L.C., Seravalli, J., Ragsdale, S.W., and Drennan, C.L. (2008) Xenon in and at the end of the tunnel of bifunctional carbon monoxide dehydrogenase/acetyl-CoA synthase. *Biochemistry.* **47**, 3474–3483.
9. Gong, W., Hao, B., Wei, Z., Ferguson, D.J.J., Tallant, T., Krzycki, J.A., and Chan, M.K. (2008) Structure of the $\alpha_2\varepsilon_2$ Ni-dependent CO dehydrogenase component of the *Methanosarcina barkeri* acetyl-CoA decarbonylase/synthase complex. *Proc. Natl. Acad. Sci. U.S.A*. **105**, 9558–9563.
10. Jeoung, J.-H., and Dobbek, H. (2007) Carbon dioxide activation at the Ni,Fe-cluster of anaerobic carbon monoxide dehydrogenase. *Science.* **318**, 1461–1464.

SSRL is primarily supported by the DOE Offices of Basic Energy Sciences and Biological and Environmental Research, with additional support from the National Institutes of Health, National Center for Research Resources, Biomedical Technology Program, and the National Institute of General Medical Sciences.