Enzyme Crystallography

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Insights into the Mechanism of Carbon Monoxide Dehydrogenase at Atomic Resolution**

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The carbon monoxide dehydrogenase (CODH) catalyzes the interconversion between CO and CO_2 in a reaction depicted as $CO + H_2O \rightleftarrows CO_2 + 2H^+ + 2e^-$.[1] This reversible redox reaction is of biological importance in both directions (Figure 1): The oxidation of CO by nickel-containing CODH (Ni-CODH) provides the energy and carbon source for the chemolithoautotrophic growth of anaerobic organisms, such as *Carboxydothermus hydrogenoformans*,[2] whereas the reduction of CO_2 by Ni-CODH is coupled with the formation of acetyl-CoA by an acetyl-CoA synthase (ACS) in acetogenic bacteria, such as *Moorella thermoaceti*ca.[3] Moreover, owing to its unique catalytic capabilities, CODH is an attractive prototype for the development of strategies for CO_2 sequestration (based on CODH-catalyzed CO_2 reduction) and fuel cells (based on CODH-catalyzed CO oxidation), piquing interest in understanding the mechanistic details of this important metalloenzyme.

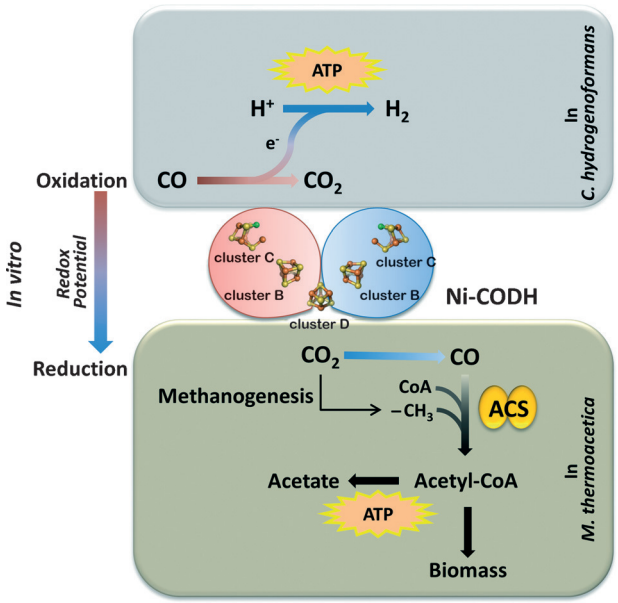

Figure 1. Redox interconversion between CO_2 and CO by Ni-CODH. In *C. hydrogenoformans*, the oxidation of CO by Ni-CODH is tightly coupled with proton reduction by a hydrogenase to generate a proton gradient across the cell membrane for ATP synthesis.[4] In *M. thermoacetica*, the reduction of CO_2 by Ni-CODH is coupled with an acetyl-CoA synthase (ACS) to form acetyl-CoA for cell-mass production or ATP synthesis.[5] Under in vitro conditions, Ni-CODH can catalyze the solution-potential-dependent interconversion between CO_2 and CO.

Discovered in the 1980s, Ni-CODH was initially characterized by biochemical and spectroscopic approaches, which provided important insights into the protein conformation, cluster content, and the physiological roles of this enzyme.[1] Since 2000, a number of crystal structures of Ni-CODH enzymes have been solved by the groups of Drennan, Dobbek, and Chen, which illustrated the unique geometric features of the catalytic cluster C and the role of this cluster in functionalizing the substrates of Ni-CODH.[4] In particular, the CO_2- and CO-bound structures were reported by the groups of Dobbek and Chen, whereas the cyanide (CN^-)-bound structures were described by the groups of Drennan and Dobbek.[5] The seminal work by these groups, along with data derived from biochemical, kinetic, spectroscopic, and theoretical investigations,[6] has led to the proposal of a basic mechanistic model for CO/CO_2 interconversion by Ni-CODH, which features a CO-bound Ni atom with a near-perfect square-planar geometry that is uniquely juxtaposed with a hydroxy-bound Fe atom. Such a conformation suggests a reaction mechanism that involves nucleophilic attack of the Fe-bound hydroxy group at the Ni-bound CO, and this hypothesis is further supported by the observation that CO_2 acts as a bridging ligand between Ni and Fe with μ_2,η^2 coordination.

The mechanism of Ni-CODH inhibition by CN^- and cyanate (NCO^-), on the other hand, is less understood. Structural information regarding the binding of NCO^- to CODH is scarce. In contrast, structural analyses of CN^--bound CODH have led to the proposal that CN^- may adopt two conformations on binding: First, it binds loosely in a bent conformation; then, it slowly rearranges into a linear conformation, which competitively inhibits substrate reduction.[5] Recently, the action of these inhibitors was studied by electrochemical techniques, which has led to a model that depicts the action of inhibitors on C_{red1} and C_{red2}, the two catalytically important oxidation states of cluster C.[7] In this model, CN^- inhibits CO oxidation, but not CO_2 reduction, by

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binding selectively to C_{red1}; whereas NCO^- inhibits CO_2 reduction, but not CO oxidation, by binding selectively to C_{red2} (Figure 2). These results have established basic features

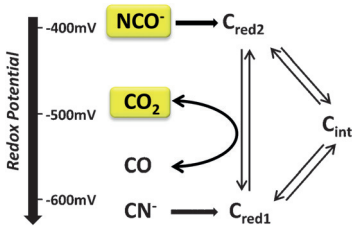

Figure 2. The redox states of cluster C in Ni-CODH and the potentials that permit the binding of substrates and inhibitors to cluster C.[7] The midpoint potential of the CO_2/CO couple is approximately −558 mV, and the potentials for NCO^- and CN^- binding have been estimated to be approximately −400 and −600 mV, respectively. The NCO^-- and CO_2-bound states of Ni-CODH captured by the atomic-resolution structures are highlighted in yellow.[8]

of the redox properties of Ni-CODH in catalysis; however, the lack of information on how substrates/inhibitors interact with Ni-CODH on the molecular level at certain redox potentials prevents an in-depth investigation of the reaction mechanism of this enzyme.

In further studies along this line, the Dobbek group recently solved two crystal structures of the Ni-CODH of *C. hydrogenoformans* (^{Ch}CODH), one featuring the bound substrate (CO_2) and the other one the bound inhibitor (NCO^-), at atomic resolution ($d_{min} < 1.1$ Å).[8] This work describes the molecular details of cluster C at the highest resolution to date, with individual atomic positions unambiguously assigned along with the numbers of electrons associated with these atoms. More importantly, the atomic-resolution structures reveal some previously unobserved features of the substrate/inhibitor bound to Ni-CODH, suggesting that the bound CO_2 moiety is reduced by two electrons and thus more closely resembles a carboxyl group than an anionic $CO_2^{\bullet-}$ radical. NCO^-, however, is bound in a bent arrangement and reduced in a manner similar to that of CO_2, wherein the N atom is reduced by two electrons to form a carbamoyl group. Together, these observations suggest a common two-electron transfer pathway for CO_2 activation and NCO^- inhibition, thereby adding an important piece of the puzzle to the reaction mechanism of Ni-CODH.

The improvement of the resolution to the atomic level played a key role in this work, permitting the analysis of structural data without the inclusion of geometrical restraints and consequently an unbiased determination of important geometric parameters, such as bond lengths and angles. Compared to the previously published high-resolution (1.50 Å) structure of CO_2-bound ^{Ch}CODH,[9] the Ni–C bond is much shorter in the atomic-resolution structure (1.03 Å), although the coordination geometry remains the same (square planar), which implies that the Ni center has an unusual electronic structure (Figure 3). Moreover, the CO_2 ligand is even more bent in the atomic-resolution structure (117°) than in the high-resolution structure (133°), which

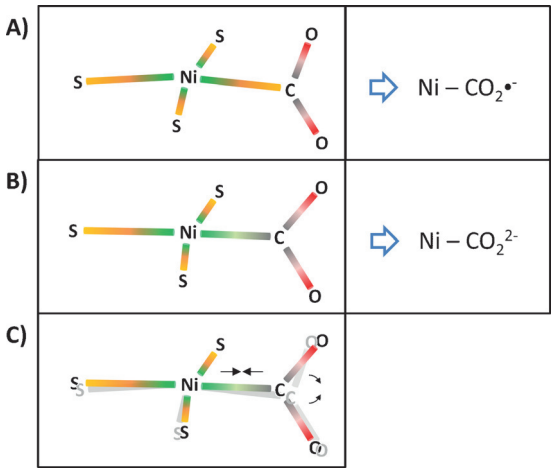

Figure 3. Comparison of the active sites of Ni-CODH in the high-resolution (1.50 Å) structure (PDB: 3B52)[9] and the atomic-resolution (1.03 Å) structure.[8] A) Nickel coordination in the 1.50 Å structure, wherein the bound substrate resembles an anionic $CO_2^{\bullet-}$ radical. B) Nickel coordination in the 1.03 Å structure, wherein the bound substrate resembles formate or Ni-bound carboxylate. C) Overlay of the active sites in A and B, with A shown in gray. The 1.03 Å structure shows a perfect square-planar structure, which is due to a shortened Ni–C bond (indicated by straight arrows) and a more bent O-C-O conformation (indicated by curved arrows), suggesting that the substrate is more reduced in A than in B.

suggests a more reduced state of the CO_2 ligand than the previously proposed one-electron-reduced state (Figure 3). Given the similarity between the geometry of this ligand and that of two-electron-reduced formate or metal-bound carboxyl groups (ca. 120°), this ligand is likely to be reduced by two electrons, apparently stabilized by π-backbonding, and waiting to be protonated as the reaction proceeds.

This work represents the first report of the NCO^--bound structure in CODH research, as previous work had solely focused on the structure of the cyanide- or isocyanate-bound oxidized cluster C.[4,10] Surprisingly, NCO^-, which also undergoes two-electron reduction, is bound with nearly the same geometry as CO_2. Perhaps even more surprisingly, the bond angles and lengths suggest the formation of a carbamoyl (H_2NCO) group upon reduction, which is further supported by the displacement of a His residue that normally forms hydrogen bonds with the N atom of the NCO^- ligand. This observation is unusual, as 1) based on the similarity between the binding geometries of NCO^- and CO_2, a two-electron reduction of NCO^- to form CN^- and H_2O in analogy to the two-electron reduction of CO_2 to CO and H_2O would be anticipated; and 2) given the previous observation of a slow oxidation of *n*-butylisocyanide (CN–Bu) to *n*-butylisocyanate (OCN–Bu),[10] a reduction of NCO^- to cyanide (CN^-) could be speculated in the analogous reverse reaction. The discrepancy between the electron-distribution patterns on the CO_2 and NCO^- ligands underscores the distinction between the substrates and inhibitors of Ni-CODH.

The atomic-resolution structures of ^{Ch}CODH provide compelling evidence for the formation of a two-electron-reduced substrate in the reaction pathway of Ni-CODH. However, the appearance of the one-electron-reduced sub-

strate as a transient intermediate cannot be ruled out and could be confirmed by attempts to generate intermediates that occur during the distinct phases of electron transfer from the metal to the ligand. The observation of the two-electron-reduced NCO^- ligand also raises interesting questions regarding the inhibition mechanism of Ni-CODH. Such a two-electron transfer process was not observed in cyclic voltammetry experiments.[7] Moreover, it implies a dead-end two-electron reduction of the ligand and consequently an unproductive oxidation of the C_{red2} cluster, which seems to contradict the earlier finding that the NCO^- ligand stabilizes the C_{red2} state.[7] Therefore, the electrochemical and spectroscopic data should be re-evaluated and aligned with the results from the atomic-resolution structures to derive a revised model of the inhibition mechanism of Ni-CODH.

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