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Implications of a Carboxylate-Bound C-Cluster Structure of Carbon Monoxide Dehydrogenase**

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Nickel-containing carbon monoxide dehydrogenases (CODHs) catalyze the reversible reduction of CO_2 to CO [Eq. (1)]. They are found in anaerobic

$$CO_2 + 2e^- + 2H^+ \rightarrow CO + H_2O \quad (1)$$

bacteria and archaea that grow chemoautotrophically on simple carbon sources.[1] These organisms play a major role in the global carbon cycle[2] and are among the most evolutionarily primitive forms of life.[3] The enzymes from *Rhodospirillium rubrum* ($CODH_{Rr}$) and *Carboxydothermus hydrogenoformans* ($CODH_{Ch}II$) are monofunctional β_2 homodimers. Acetyl-CoA synthase/carbon monoxide dehydrogenase from *Moorella thermoacetica* ($ACS/CODH_{Mt}$) is a bifunctional $\alpha_2\beta_2$ tetramer that also catalyzes the synthesis of acetyl-CoA. Its β_2 subunits are homologous to the monofunctional CODH β_2 homodimers.

Each β subunit contains the active site responsible for the reaction of Equation (1), known as the C-cluster, as well as an Fe_4S_4 B-cluster. Another Fe_4S_4 cluster (the D-cluster) bridges the two β subunits. B- and D-clusters transfer electrons between the C-cluster and external redox agents. The structures of the C-cluster from $CODH_{Ch}II$ and $ACS/CODH_{Mt}$ have been determined through X-ray crystallography to high resolution (Figure 1a).[4–6] The cluster can be subdivided into an $[Fe_3S_4]$ subsite and a $[Ni\cdots Fe_a]$ subsite, coordinated to each other. Each Fe atom of the $[Fe_3S_4]$ subsite is coordinated by a cysteinate. In the C-cluster of $CODH_{Ch}II$, a square-planar Ni^{2+} ion coordinates two μ^3-sulfide ligands from the $[Fe_3S_4]$ subsite, one cysteinate, and a μ^2-sulfide ion bridged to Fe_a. Fe_a is a high-spin ferrous ion also coordinated by His and Cys residues, and a μ^3-sulfide ion from the $[Fe_3S_4]$ subsite.

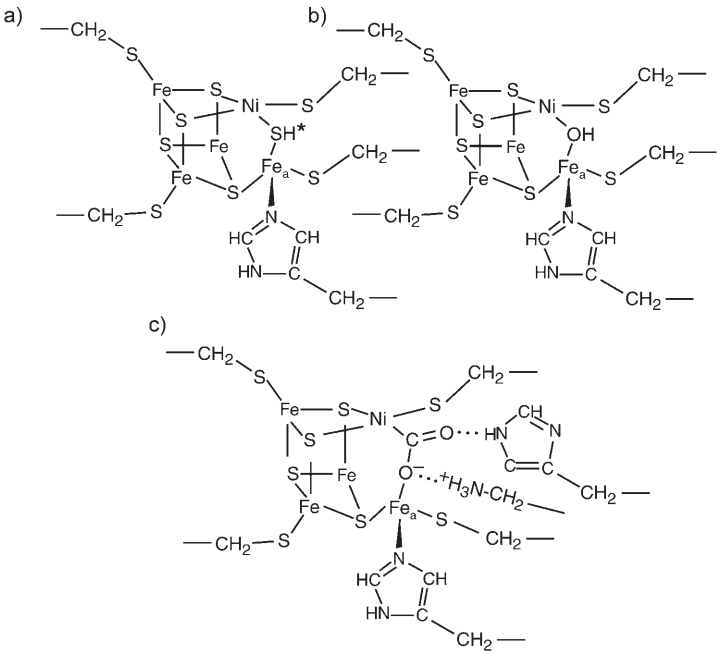

Figure 1. C-cluster structures: a) Structure obtained for $CODH_{Ch}II$ (with the μ^2-sulfide ligand indicated by *)[4,7] and for $ACS/CODH_{Mt}$ (with no μ^2-sulfide ligand).[5,6] b) C_{red1} structure of $CODH_{Ch}II$.[15] c) CO_2-intermediate.[15]

The structure of the C-cluster from $ACS/CODH_{Mt}$ is essentially identical except for the absence of the μ^2-bridging sulfide ligand. This difference has prompted a controversy regarding the mechanistic role of this ion. Dobbek et al. concluded that a sulfide ligand is required for catalytic activity and found that incubation under CO (the conditions used in crystallizing $ACS/CODH_{Mt}$) eliminated the sulfide ion from the C-cluster and inactivated the enzyme.[7] By adding sulfide ion to $CODH_{Rr}$ and $ACS/CODH_{Mt}$, Feng and Lindahl found precisely the opposite, namely that sulfide *inhibited* catalysis and that CODH is *not* inactivated by CO.[8]

EPR studies have established four redox states of the C-cluster, designated C_{ox}, C_{red1}, C_{int}, and C_{red2} (Figure 2).[9,10] C_{ox} has a spin state $S = 0$ and exhibits Mössbauer spectra typical of $[Fe_4S_4]^{2+}$ clusters with no evidence of Fe_a. The one-electron-reduced C_{red1} state exhibits an $S = {}^1/_2$ EPR signal and a Mössbauer doublet for Fe_a.[11] Isomer shift values of the $[Fe_3S_4]$ subsite in the C_{red1} state suggest formal oxidation states of $\{Fe^{2+}\ Fe^{2+}\ Fe^{3+}\}$. The redox and magnetic states of

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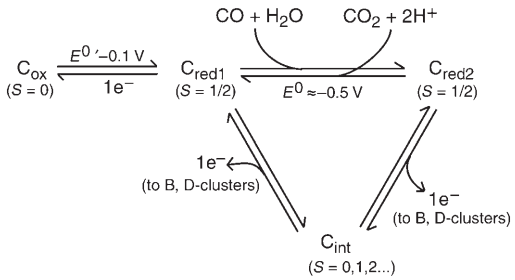

Figure 2. Redox properties of the C-cluster.

related synthetic clusters have been studied by Münck, Holm, et al., who found that such clusters can exist in states ranging from all-ferrous to all-ferric.[12,13] The electronic configuration of C_{red2} is uncertain because of overlap of C_{red2} and reduced B-cluster Mössbauer features, as well as weak magnetic interactions between C_{red2} and the reduced B-cluster. C_{red2} should be 2 electrons more reduced than C_{red1}, but where those electrons localize on the C-cluster is unknown. The magnetic properties of the C-cluster arise predominately from the electronic configuration of the $[Fe_3S_4]$ component. Perhaps Ni^{2+} could accept an electron (forming Ni^{1+}) but placing both electrons on it (forming Ni^0) seems unlikely, in that the negatively charged coordinating cysteinate would destabilize Ni^0.

Sulfide ion is a partial inhibitor of catalysis that binds to the C_{red1} state and is released when the C-cluster is reduced to C_{red2}.[8] Similar effects are observed for the tight-binding inhibitor CN^-, suggesting a common mode of action for both anions. CN^- ions bind Fe_a (perhaps bridged to Ni) in the C_{red1} state.[11] CN^- is slowly released by treating the enzyme with either CO or CO_2 in the presence of a reductant, and does not appear to bind to the C_{red2} state. The proton of a metal-bound hydroxy group can be observed by ENDOR spectroscopy for the C_{red1} state but not for C_{red2}.[14] These data suggest that the substrate H_2O binds in the same mode as SH^- and CN^-—namely to the C_{red1} state, bridging Ni and Fe_a. The binding of CO to Ni may prompt the dissociation of OH^-, facilitating its attack on the C of CO to form the Ni-bound carboxylate.[8]

The recently reported structures of the C-cluster by Jeoung and Dobbek shed abundant new light on the CODH mechanism.[15] They report two important structures, including that from a sample poised at −320 mV vs. NHE, which should correspond to the C_{red1} state, and that from a sample poised at −600 mV and treated with $NaHCO_3$ (Figure 1b and c).[16] The C_{red1} structure includes an OH group bridging Ni and Fe_a. The structure of the reduced sample treated with bicarbonate is remarkable, as it shows CO_2 bound at the C-cluster. The C atom is bound at the Ni center, with one O atom of the carboxylate group hydrogen-bonded to a conserved His residue and the other O atom coordinated to Fe_a and hydrogen-bonded to a conserved Lys group. No sulfide ion bridges Ni and Fe_a in either structure, implying that this ion is not required for catalysis. Given that this bridging position is occupied by an O atom of CO_2 in one structure and by an OH^- ion in the other, it seems likely that sulfide ions are inhibitory.

That the CO_2-bound state of the C-cluster could be isolated and structurally characterized is amazing. The enzyme operates reversibly to interconvert CO and CO_2 with essentially no overpotential,[18] and so it seemed unlikely that the CO_2-bound intermediate would be isolatable. In contrast, the enzyme has been crystallized in the presence of CO, but no unambiguous CO-bound intermediate of the C-cluster has been reported. IR peaks attributed to CO ligands bound to the C-cluster have been reported,[19] but these are unstable, suggesting that such CO ligands are spontaneously oxidized, perhaps by reacting with the bridging OH^- group in the C_{red1} state to form C_{red2} and release CO_2. The CO_2-bound state may be stable because the C-cluster is in the C_{red1} state; indeed, the C_{red1} EPR signal shifts slightly when the enzyme is exposed to CO_2.[9] The shifts are less dramatic than might be expected, but if the Ni center is planar and diamagnetic, binding CO might not affect the magnetic properties of the cluster significantly. CO_2 analogues such as OCN^-, SCN^-, and CS_2 also shift the C_{red1} EPR signal (but not the C_{red2} EPR signal),[9,20] suggesting that these analogues bind to the C_{red1} state. Release of CO_2 probably occurs synchronously with the reduction of C_{red1} to C_{red2}.

The CO_2-bound structure is also remarkable in identifying the roles of the conserved His and Lys residues in the active site. Their prominent location prompted Drennan et al. to suggest that the His residue is involved in proton transfer to and from the C-cluster, and that the Lys residue might stabilize a metal-bound carboxylate intermediate.[21] These roles were supported by mutagenesis studies,[22] but the structures of Jeoung and Dobbek essentially confirm both predictions.[15] The interaction with the positively charged Lys residue suggests that the O atom involved in the interaction is deprotonated. When the mechanism is viewed from the direction of CO formation, protonation of this O atom might promote C–O bond cleavage to form Ni–CO and the bridging OH^- ligand. The CO_2-bound intermediate may be poised to release CO_2 in the next step; what stabilizes this structure is uncertain, but these hydrogen-bonding interactions are undoubtedly important.

Another surprise of the CO_2-bound structure is how similar it is (in terms of distances and geometries) to non-CO_2-bound structures. There is strong spectroscopic evidence indicating that the binding of CO_2 to CODH has a dramatic effect on redox and magnetic properties of the enzyme. CO_2 binding alters the redox potentials of CODH clusters, such that they undergo redox reactions cooperatively.[23] CO_2 alters the saturation properties of the B_{red} cluster, and it increases the rate at which dithionite reduces the C-cluster.[10] The seemingly insignificant structural changes observed upon binding CO_2 must be sufficient to produce these effects, but how they do so remains puzzling.

In summary, the reported C-cluster structures and 20 years of spectroscopic investigations collectively suggest the catalytic mechanism shown in Figure 3. When this mechanism is considered from the direction of CO oxidation, water binds to the C-cluster at the position bridging the Ni and Fe_a centers. This binding promotes the loss of a proton, which is transferred to solvent via a series of His residues and the C_{red1} state forms. CO binds to the C_{red1} state, prompting

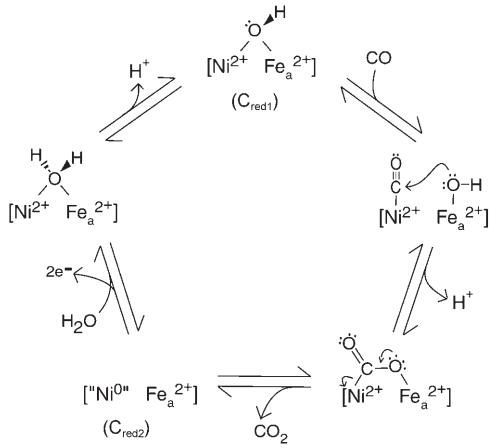

Figure 3. Catalytic mechanism of CODH. "Ni^0" reflects electron bookkeeping rather than a proposed oxidation state.

the release of the bridging OH^- ligand, which attacks the carbonyl carbon atom to form the Ni–CO(OH) intermediate. Loss of the proton from the carboxylate group is stabilized by an interaction with Lys. CO_2 forms as 2 electrons are delivered to the C-cluster, reducing it from C_{red1} to C_{red2}. Two electrons are transferred, one at a time, from the C-cluster to the B-cluster, then to the D-cluster, and ultimately to external electron acceptors. This returns the C-cluster to the C_{red1} state, ready for another catalytic cycle.

Where should future efforts be directed in probing this mechanism further? If CO_2 (and its mimics) indeed bind to the $S = {}^1/_2$ C_{red1} state, it should be possible to probe that binding further by searching for ^{13}C hyperfine interactions by using ENDOR or ESEEM spectroscopy. The electronic structure of C_{red2} remains poorly understood, and future efforts should be placed at preparing site-directed mutants in which the B-cluster is either absent or not reducible. Mössbauer studies of such mutants would allow the C_{red2} electronic structure to be examined in detail. Finally, construction of a mutant in which the C_{red2} state cannot be stabilized might allow a stable CO-bound intermediate to be prepared. The gifted abilities of Dobbek and co-workers in obtaining structures of important CODH reaction intermediates gives one hope that they could use such a mutant to structurally characterize an unambiguous CO-bound intermediate.

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