Unraveling the Structure and Mechanism of Acetyl-Coenzyme A Synthase

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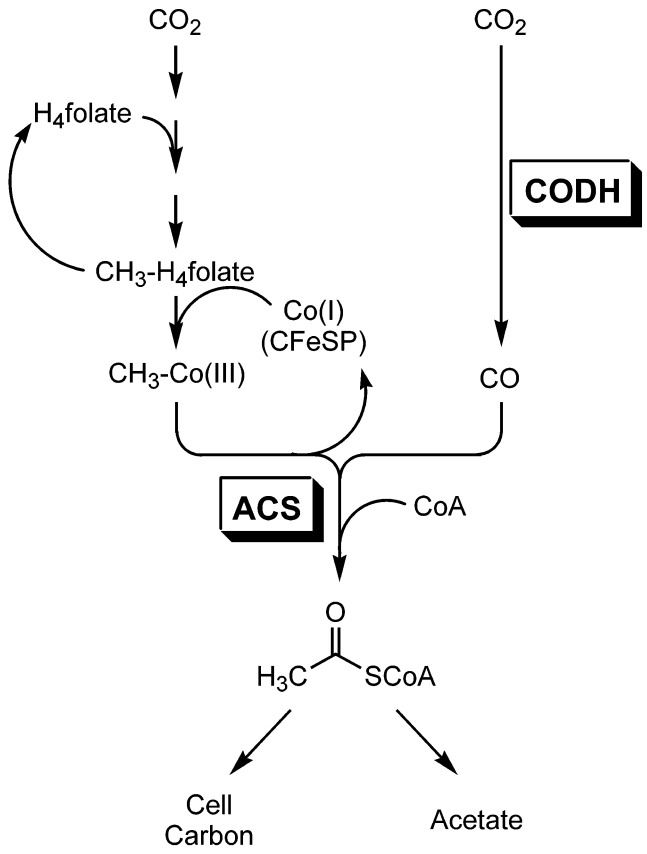
ABSTRACT

The bifunctional enzyme carbon monoxide dehydrogenase/acetyl-coenzyme A (CoA) synthase (CODH/ACS) is a key enzyme in the Wood−Ljungdahl pathway of carbon fixation. Carbon monoxide is combined with a methyl group and ultimately converted to acetyl-CoA at a unique Ni-containing bimetallic site in the A-cluster of this enzyme. Despite years of extensive biochemical and spectroscopic studies and the recent report of three separate crystal structures, the mechanism by which acetyl-CoA is synthesized is still unknown. Over the past two years there have been a number of significant developments regarding ACS. This Account critically examines these recent developments and especially focuses on those areas that are still a matter of debate.

Introduction

Acetyl-coenzyme A (CoA) synthase (ACS), together with carbon monoxide dehydrogenase (CODH), is a key player in the metabolism of anaerobic microorganisms via the Wood−Ljungdahl pathway (Figure 1) and a major component of the global carbon cycle.[1,2] CODH catalyzes the reversible reduction of CO_2 to CO (reaction 1), while ACS catalyzes the formation of acetyl-CoA from CO, coenzyme A, and a methyl group provided by the corrinoid iron−sulfur protein (CFeSP) according to reaction 2.[1] Autotrophic anaerobes utilize CODH/ACS and the Wood−Ljungdahl pathway to form acetyl-CoA from CO_2; the acetyl-CoA is then either converted into more complex organic molecules or respired as acetate depending on the metabolic needs of the cell. Conversely, the CODH/ACS pathway can also be used by acetotrophic anaerobes during energy production.

Because of the importance of CODH/ACS to the global carbon cycle as well as to our understanding of biological C1/C2 chemistry, this bifunctional enzyme has come under intense scrutiny from a number of different labs. The seemingly "simple" reactions catalyzed by CODH/ACS belie the complexity of this enzyme system. Indeed, despite the availability of three high-resolution crystal structures of ACS[3−5] and nearly two decades of thorough spectroscopic and biochemical studies, many questions remain concerning the mechanism. This Account critically examines the conflicting data, the proposed reaction mechanisms of ACS, and the contributions provided by biochemical and modeling studies toward unraveling the details of this fascinating and complex enzyme.



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

$$CO + CH_3\text{-}CFeSP + CoA \rightleftharpoons \text{acetyl-CoA} + CFeSP \quad (2)$$

FIGURE 1. Diagram of the Wood−Ljungdahl pathway used in one-carbon metabolism of anaerobic organisms.[2] Reactions catalyzed by carbon monoxide dehydrogenase (CODH) and acetyl-CoA synthase (ACS) are shown in eqs 1 and 2, respectively.

Structure of ACS: What Is the Identity of M?

The first reported crystal structure of the bifunctional enzyme CODH/ACS[3] from *Moorella thermoacetica* was an exciting development for a couple of different reasons. First, it demonstrated on a molecular level what had been proposed earlier on the basis of biochemical studies[6]—that the CODH and ACS activities are integrally linked with a gas channel connecting the two active sites so that the CO produced by CODH is specifically incorporated into acetyl-CoA. Second, it revealed a number of surprises in the active site of ACS itself. Atomic absorption,[7] UV−vis,[7,8] electron paramagnetic resonance (EPR),[7−10] electron-nuclear double resonance (ENDOR),[10] and Mössbauer[8,9] spectroscopies correctly deduced the presence of a Ni ion linked by some unknown bridge to an $[Fe_4S_4]$ cluster,

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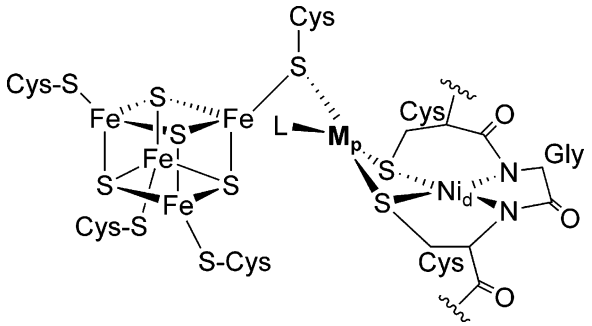


FIGURE 2. Representation of the ACS active site. The site distal to the $[Fe_4S_4]$ cluster is occupied by a Ni(II) ion in a square-planar environment (Ni_d). It is now agreed that Ni is the catalytically relevant ion in the proximal site (M_p),[4,5] although tetrahedral Cu(I)[3] and Zn(II)[4] have also been observed crystallographically in this site. The coordination sphere of M_p is completed by an exogenous ligand L of unknown identity.

and extended X-ray absorption fine structure (EXAFS)[7,8] accurately predicted a Ni ion ligated by two S and two N/O ligands. What was completely unanticipated, however, is that there are actually two metal ions at the active site (Figure 2). There is one square-planar Ni ion distal to the $[Fe_4S_4]$ cluster (Ni_d) that is ligated by an unusual Cys-Gly-Cys (C·G·C) motif consisting of two deprotonated amides and two cysteine thiolates. The second ion, proximal to the $[Fe_4S_4]$ cluster (M_p), is now known to be nickel, although until recently the identity of M_p was a matter of debate.

In the first structure reported by Drennan and co-workers,[3] Cu^+ resided in the M_p site in a distorted tetrahedral environment. In support of this structure, they noted that ACS activity appeared to correlate with copper concentration given the two data points available at that time. A later and more thorough study by Ragsdale and co-workers[11] seemed to corroborate the earlier conclusion that copper content correlated with ACS activity. Had Cu^+ indeed been physiologically relevant, it is not surprising that it would have stayed hidden because the copper content in ACS was rarely measured and the d^{10} electronic configuration of Cu^+ provides few convenient spectroscopic signatures.

Approximately 6 months after Drennan's structure was published, Fontecilla-Camps and co-workers published a competing CODH/ACS structure.[4] Interestingly, two forms of the ACS subunit were crystallized, an "open" form and a "closed" form (discussed in more detail below). Although the overall structure of the two forms generally agreed with Drennan's structure, the "open" form contained a Ni ion at the proximal M_p site, while in the "closed" form a Zn ion occupied the proximal site (Figure 3). Fontecilla-Camps and co-workers proposed that only the $[Fe_4S_4]-Ni_p-Ni_d$ cluster is active and argued against copper being physiologically relevant in ACS. A third high-resolution crystal structure of a monomeric form of ACS from an additional species, *Carboxydothermus hydrogenoformans*, also revealed two Ni ions in the active site.[5] Significantly, ACS activity exhibited a negative correlation with Zn and Cu content, and the presence of Ni in the proximal site was confirmed by X-ray absorption spectroscopy (XAS).

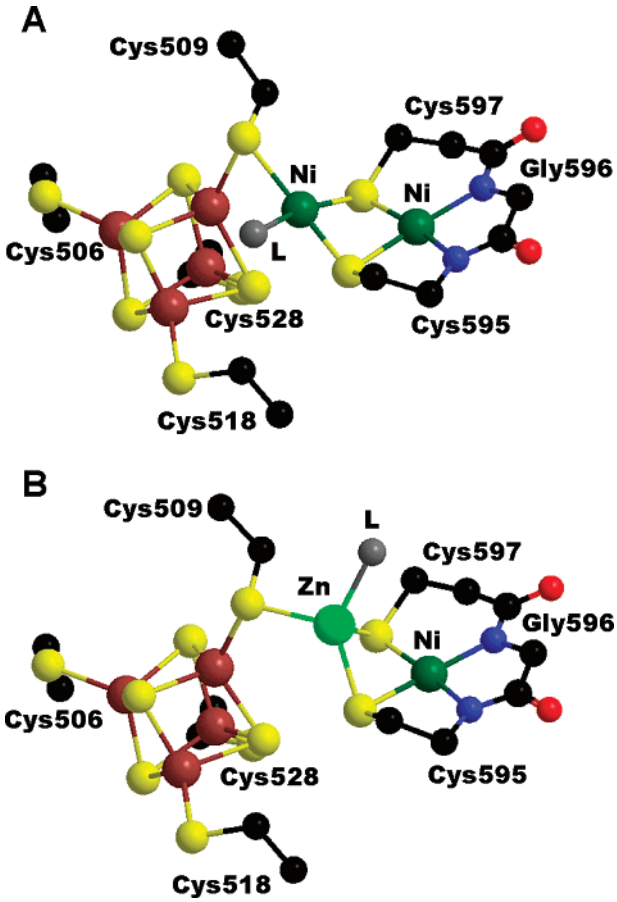


FIGURE 3. Two forms of the ACS active site observed in the crystal structure of Fontecilla-Camps and co-workers.[4] In panel A, the M_p site is occupied by a square-planar Ni(II) ion ("open" conformation), while in panel B, a tetrahedral Zn(II) ion resides in the M_p site ("closed" conformation). The exact identity of the exogenous ligand L is unknown, although in panel A it was refined as acetyl and in panel B, it was refined as SO.

The importance of Ni at the M_p site of ACS was a sentiment echoed by Lindahl. Over 10 years ago, Shin, Anderson, and Lindahl used phenanthroline to remove "labile" Ni from ACS/CODH and completely inhibited ACS activity while leaving CODH activity intact.[12] Addition of $NiCl_2$ to Ni-depleted ACS restored both key spectroscopic features and enzymatic activity. More recently, they reported that the addition of either $CuCl_2$ alone or $CuCl_2$ and an equimolar amount of the reductant titanium(III) citrate to Ni-depleted ACS did not restore activity or key spectroscopic features and that the presence of $CuCl_2$ actually inhibited the ability of Ni to do so as well.[13] One possibility was that the Ni that was removed in their experiments came from the distal site (Ni_d). This scenario seems unlikely, however, given the available biochemical data and the fact that our synthetic models of the Ni_d site are not perturbed by phenanthroline,[14] suggesting that the "labile" Ni ion is indeed at the proximal site.

Support for Ni at the M_p site was also obtained using the monofunctional enzyme acetyl-CoA decarbonylase/synthase (ACDS). Gencic and Grahame reported that ACDS from *Methanosarcina thermophila* could be over-expressed in *Escherichia coli* in the apo form and that

incubation of the inactive enzyme with $NiCl_2$ resulted in the insertion of two Ni^{2+} ions per active site,[15] and additional spectroscopic studies support this finding.[16,17] While other ions such as Cu^{2+} could bind to ACDS with relatively high affinity, only Ni^{2+}-reconstituted ACDS was catalytically active (although mixed-metal systems such as Cu_p-Ni_d were not reported).

Very recently, Ragsdale and co-workers published perhaps the most thorough study to date addressing the issue of copper content and ACS activity. Using a combination of metal-specific chelators and 15 data points over a considerably wider range of Cu/Ni ratios than used previously, they concluded that Cu content did, in fact, correlate negatively with ACS activity,[18] a conclusion that contradicted their earlier findings.[11] In addition, they noted that there was a positive correlation between Ni content and ACS activity and that the highest activity was achieved at a point at which both the proximal and distal sites were presumably occupied by Ni.[18]

Given that Ni is now known to be the physiologically relevant metal at the proximal site, it is interesting to conjecture why both copper[3] and zinc[4] were found crystallographically in the active site. Clearly the proximal site is quite labile as evidenced by the substoichiometric amount of Ni in the "as isolated" state and the ability of Ni-chelators to remove Ni_p. The proximal site also appears to be quite promiscuous, and copper, zinc, cobalt, iron, and manganese (in addition to nickel) have all been shown biochemically or spectroscopically to bind to ACS/ACDS.[5,13,15] Furthermore, Lindahl and co-workers observed that ACS containing Cu at M_p could not be reactivated by the presence of excess $NiCl_2$,[13] although it is less clear whether Cu can replace Ni in the absence of a chelator.[13,18] What is clear, however, is that zinc can replace nickel at the proximal site. Interestingly, when $ZnSO_4$ is added to ACS, the ACS activity initially remains unchanged but then decreases rapidly within minutes.[19] The authors have interpreted these data as evidence that in the absence of chelators, Ni_p is replaced during turnover conditions, presumably when the enzyme is in the "open" conformation, thus providing perhaps the first biochemical evidence that the two ACS conformations observed structurally by Fontecilla-Camps and co-workers[4] are catalytically relevant. The ability of Cu^+, Zn^{2+}, and Ni^{2+} to bind to the same site was recently highlighted by Darensbourg and co-workers using model complexes containing a similar coordination environment to that found in ACS.[20] Thus, finding adventitiously bound copper[3] and zinc[4] in the M_p site in the early structures is completely understandable and, in retrospect, even predictable given our current knowledge of the lability and promiscuity of this site.

Proposed Mechanisms of ACS

Given the complexity of the CODH/ACS system and the difficulty in even determining the metal ions involved, it is not surprising that many questions remain concerning the reaction mechanism of ACS despite nearly two decades of research. The various mechanisms that have been proposed to date[3,4,11,15] have significant differences relating to the role of each of the metal centers and the redox states of the various intermediates. What is generally agreed upon is that the crystal structures of CODH/ACS reveal the presence of a hydrophobic channel connecting the CODH active site (C-cluster) to the ACS active site (A-cluster). This putative gas channel opens at the metal proximal to the $[Fe_4S_4]$ cluster, suggesting that Ni_p is the site of CO binding.[3,4] As isolated, CODH/ACS is in an oxidized and EPR-silent state, consistent with an $[Fe_4S_4]^{2+}$ cluster linked to two $S = 0$ Ni^{2+} ions.[7,10] This species does not exhibit ACS activity and requires the addition of an electron to become catalytically competent, presumably reducing the $[Fe_4S_4]^{2+}$ cluster as deduced by Mössbauer and EPR spectroscopy.[9] The addition of CO to this species results in the formation of the "NiFeC" or "A_{red}-CO" state containing the diagnostic NiFeC EPR signal ($g = 2.08$, 2.07, and 2.03).[9,10] No other species in the ACS pathway has been successfully characterized, and there is little further agreement.

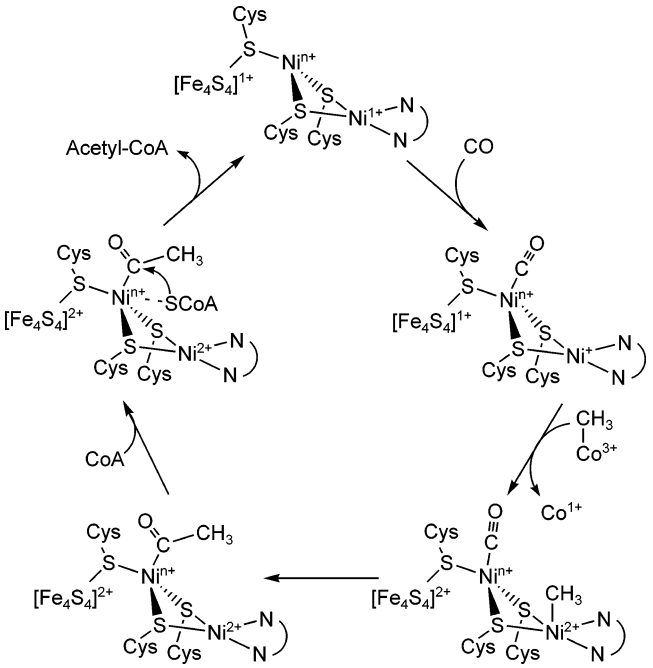


FIGURE 4. Reaction mechanism of ACS adapted from Ragsdale[11] and Drennan.[3] The original mechanism utilized Cu^+ in the proximal site. In the mechanism shown, CO binds first, although the authors note that the order could be reversed.[3] In addition, it is noteworthy that the CH_3^+ group coordinates to the Ni_d site.

A two-metal mechanism was proposed by Drennan[3] and later refined by Ragsdale.[11] Although Drennan and Ragsdale initially utilized Cu^+ in the M_p site for their mechanism, an identical reaction can be envisioned using Ni (Figure 4). In this mechanism, CO binds to Ni_p^{n+} (forming what Ragsdale proposes to be NiFeC), while CH_3^+ is delivered to Ni_d. Internal electron transfer to Ni_d from the reduced $[Fe_4S_4]^+$ cluster concomitant with CH_3^+ binding results in a Ni_d^{II}-CH_3 species. Methyl migration to $Ni_p^{n+}-CO$ leads to the formation of a $Ni^{n+}-$acetyl complex. One potential problem with the proposed mechanism is that it was recently reported that the rate of

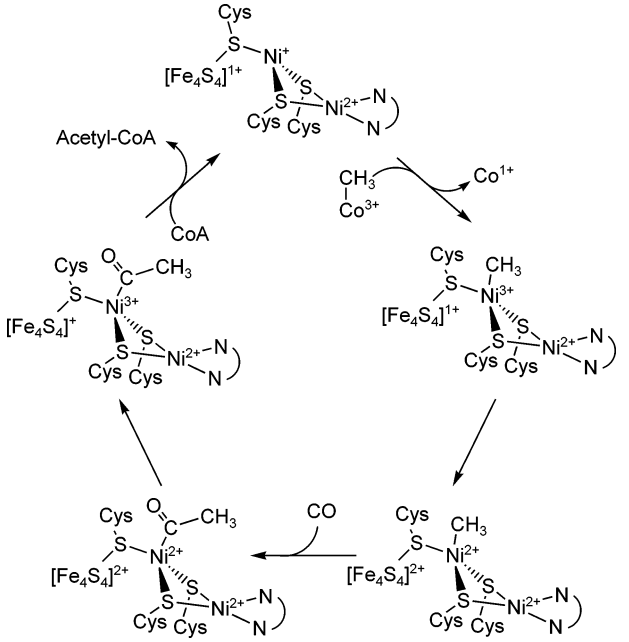


FIGURE 5. A possible reaction mechanism of ACS modified from Grahame and co-workers.[15] In the original mechanism, Ni_p and Ni_d were not distinguished and no specific oxidation states were assigned to the $[Fe_4S_4]$ cluster. Clusters originally listed as oxidized are depicted as $[Fe_4S_4]^{2+}$ in this scheme. In this mechanism, all of the "chemistry" occurs at a single nickel site.

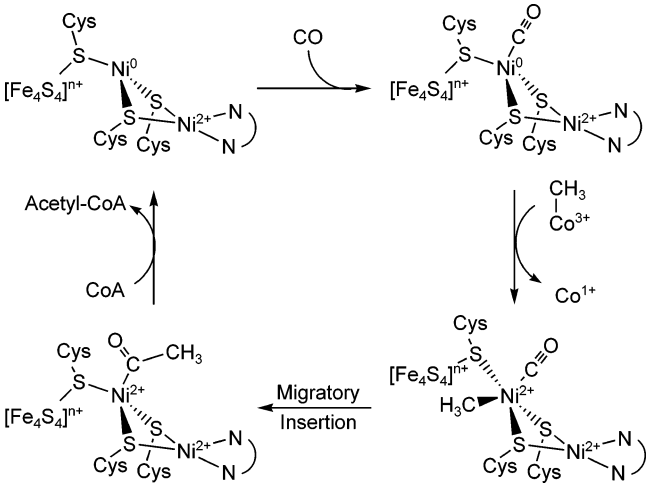


FIGURE 6. A possible reaction mechanism of ACS proposed by Fontecilla-Camps and co-workers.[4] In this mechanism, ACS needs to stabilize a Ni^0 species.

electron transfer to and from the $[Fe_4S_4]^{+/2+}$ cluster is 200-fold slower than the rate of methyl group transfer,[21] making it questionable whether the $[Fe_4S_4]$ cluster is capable of directly participating in the catalytic cycle. In addition, our preliminary results (see below) do not support the idea that the Ni^{2+} ion in a C·G·C motif can be reduced under physiological conditions. Finally, while two-metal catalysis is well established in certain pathways, the use of two metals to effect the *intramolecular* formation of acetyl from Ni_p–CO and Ni_d–CH_3 would be largely unprecedented.

There is, however, abundant chemical precedent supporting acetyl formation from CO and $-CH_3$ at a single nickel center. Stavropoulos et al.[22] used a $[N(SR)_3]$ ligand set to synthesize and crystallographically characterize a Ni^{II}–$C(O)CH_3$ complex generated by reacting the corresponding Ni^{II}–CH_3 complex with CO. The acetyl group could also be transferred to an alkyl or aryl thiolate (forming RS–$C(O)CH_3$), thus mimicking the reaction observed at the active site of ACS. Three other systems in which a thioester is formed following CO "insertion" into a LNi^{II}–alkyl bond have been reported; two of these systems utilize a bipy–thiolate ligand set,[23,24] whereas the third uses a (N–S–SR) coordination environment.[25] In addition, recent theoretical work also supports a mechanism where all of the "chemistry" occurs at a single Ni center.[26] Thus, although there is currently no biochemical data that can distinguish between a two-metal and one-metal mechanism in ACS, chemical and theoretic results favor a mechanism utilizing a single Ni ion.

A model proposed by Gencic and Grahame[15] (Figure 5) utilizes such a "mononuclear" mechanism. For the sake of comparison, the catalytic nickel in the Grahame mechanism is depicted as Ni_p and the oxidation states of the $[Fe_4S_4]$ cluster have been deduced, although the original mechanism did not distinguish between Ni_p and Ni_d or assign specific oxidation states to the iron–sulfur cluster. The catalytic cycle begins with the core in the $[Fe_4S_4]^+$–Ni_p^I–Ni_d^{II} state followed by the addition of Me^+ to generate a CH_3–Ni_p^{III} center. The binding of CO then leads to the formation of a Ni^{III}–$C(O)CH_3$ species, which is utilized to form acetyl CoA and regenerate the Ni^I starting complex. The role of the second Ni ion is not addressed. Once again, a potential problem with this mechanism is that it invokes electron transfer at the $[Fe_4S_4]^{+/2+}$ cluster in the catalytic cycle despite some recent evidence to the contrary.[21] In addition, the redox state of the species that enters the catalytic cycle (presumably $[Fe_4S_4]^+$–Ni_p^I–Ni_d^{II}) appears to contain one more electron than spectroscopy suggests ($[Fe_4S_4]^+$–Ni_p^{II}–Ni_d^{II}).[9] An important feature of this model is that the species that gives rise to the characteristic NiFeC EPR signal represents a CO-inhibited form of the enzyme and is *not* part of the catalytic cycle,[27] a conclusion supported by Lindahl and co-workers[28] but contrasting with the data reported by Ragsdale's lab.[29] In large part on the basis of the conclusion that addition of CO in the absence of the methyl group leads to an inactive state, Grahame concludes that Me^+ must bind first.[15]

Finally, the crystallographically inspired mechanism proposed by Fontecilla-Camps and co-workers[4] also has all of the "chemistry" occurring at the proximal Ni_p site (Figure 6). In this case, however, Ni_p cycles between Ni^0 and Ni^{2+}. While this is an intriguing possibility with literature precedent in chemical systems,[22–25] a stable Ni^0 species would be unprecedented in biology. Furthermore, there is simply no biochemical evidence that Ni_p can be reduced to the zero-valent state[17] (although recent theoretical work leaves this point open to debate[26,30]). Ironically, while this mechanism avoids the potential problem of the catalytic competency of the $[Fe_4S_4]$ cluster by having it remain in a single, undefined oxidation state, it does so by incorporating another potential problem.[31] That is, in

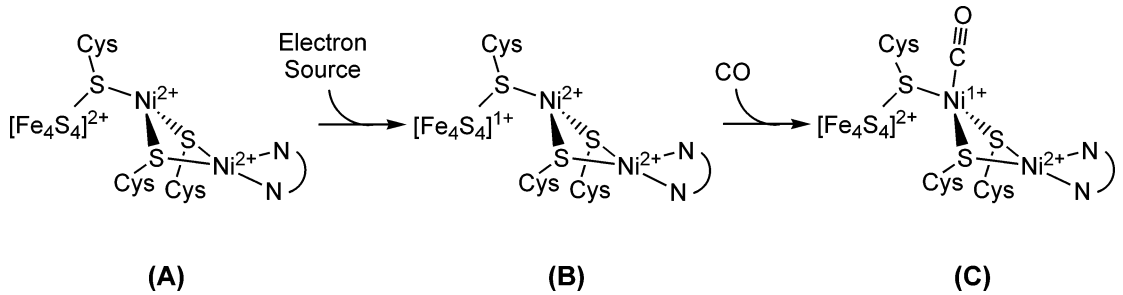


FIGURE 7. Possible pathway for the formation of the NiFeC species. In this scenario, species A would be the oxidized "as isolated" state, while species B is one-electron reduced from species A. Addition of CO to species B followed by internal electron transfer could then lead to this putative NiFeC species C.

the absence of an additional redox active cofactor to donate an electron during catalysis, the enzyme must stabilize Ni over a range of two oxidation states (in this case both Ni^0 and Ni^{2+}).

Based on structural consideration, the Fontecilla-Camps model predicts CO binding before the methyl group in the catalytic cycle. As previously mentioned, the structure reported by Darnault et al. revealed two different conformations for the α-subunits containing the ACS active site.[4] In the "closed" form of the enzyme, similar to the structure reported by Doukov et al.,[3] the hydrophobic channel connecting CODH to ACS opens at Ni_p. In the "open" form, however, this channel is blocked and the active site is much more solvent-accessible. Thus, it was reasonably proposed that following CO binding the enzyme undergoes a conformational change that blocks the CO channel but opens the active site and makes it accessible to the corrinoid iron−sulfur protein (CFeSP) to deliver CH_3^+. However, while the structures appear to suggest an ordered binding mechanism, there is currently little direct evidence to support either CO or CH_3^+ binding first, and in vitro ACS can accept the components in either order.[1,32]

Challenges in Unraveling the ACS Mechanism

Clearly the proposed mechanisms are quite different, and this can be explained largely by the fact that there are three major obstacles researchers must confront when proposing a mechanism of acetyl-CoA biosynthesis. The first major challenge is the uncertainty concerning the identity of NiFeC and whether it is part of the catalytic pathway. Based on EPR,[10] Mössbauer,[9] ENDOR,[9] and IR[33] spectroscopies, as well as theoretical work,[30] one likely description of NiFeC is $[Fe_4S_4]^{2+}-Ni_p^{+}(CO)-Ni_d^{2+}$. This species could easily be formed from ACS that is one-electron-reduced relative to the "as isolated" state by the addition of CO and internal electron transfer (Figure 7). One obvious consequence of this interpretation is that it is natural to propose that NiFeC is part of the catalytic cycle. This is the position taken by Ragsdale and co-workers, and considerable work in his lab measuring the rate of both formation and decay of the NiFeC species supports the notion that NiFeC is on the catalytic pathway.[29] The labs of both Grahame[15,17] and Lindahl[28] disagree with this sentiment, preferring instead to ascribe the NiFeC species to a CO-inhibited form of the enzyme. While it is true that very high concentrations of CO inhibit ACS, it is entirely possible that the CO-inhibited form is *not* the same species as NiFeC. For example, the CO-inhibited form could contain two carbonyls on Ni_p, thus deterring the binding of CH_3^+. (The NiFeC species itself cannot contain two carbonyls based on IR data showing only a single CO stretch at 1996 cm^{-1}.[33]) Grahame and co-workers also point to the fact that in their ACDS preparations an electron is needed to enter the catalytic cycle but not to observe the characteristic NiFeC EPR signal. This result, however, does not agree with the data reported from other labs and can be partially explained if a residual amount of dithionite from their purification buffer remained with the protein. Furthermore, in their interpretation of their XAS data, Grahame and co-workers claim that the Ti^{3+}-reduced form of the enzyme is actually $[Fe_4S_4]^{2+}-Ni_p^{+}-Ni_d^{2+}$ (as opposed to $[Fe_4S_4]^{+}-Ni_p^{2+}-Ni_d^{2+}$) and that addition of CO results in the oxidation of Ni_p^{+} to Ni_p^{2+}(CO). These results currently cannot be fully explained, do not seem to be consistent with previous spectroscopic data on ACS, and are chemically counter-intuitive.

The second challenge is that with the exception of the NiFeC species, none of the other potential intermediates in the catalytic cycle have been characterized and none appear to be EPR active. The inability to trap an EPR active species is curious if the $S = {}^1/_2$ NiFeC species really is part of the catalytic pathway. All of the "chemistry" occurring in acetyl-CoA biosynthesis is formally two-electron chemistry. Thus, each intermediate should be EPR active. Even if the intermediates are unstable and quickly decompose, the resulting products should still have an uneven spin state. This conundrum can be explained, however, by noting that in vitro studies are almost invariably performed in the presence of excess reductant. As a result, any oxidized species that is formed during the reaction cycle following the addition of CH_3^+ could get reduced by one electron and become EPR silent. Nonetheless, this inability to trap either directly or indirectly any intermediates often means that there is very little evidence either for or against a proposed mechanism.

The third challenge researchers face is a conceptual one. In biological systems, nickel typically donates or accepts only a single electron. The differences in redox potentials between Ni(I) and Ni(III) species are simply too large for both states to be accessed in a single biocatalytic

cycle unless either Ni(I) or Ni(III) is accessed only as a transition state.[31] This appears to be true even if multiple thiolate ligands are utilized such that the charge is easily delocalized. One is therefore inevitably left with the question of how enzymes can perform two-electron chemistry at a nickel center. The most obvious explanation in the case of ACS is that there must be an additional redox-active center. Prior to the availability of the structures, Lindahl and co-workers proposed the presence of a "D" site (most likely a disulfide bond) that is able to participate in two-electron chemistry,[32] which would also explain why no EPR-active intermediates are observed. Looking at the crystal structures, however, it is not clear where this so-called "D" site would reside. Svetlitchnyi and co-workers[5] suggested that perhaps the two cysteine residues in the C·G·C motif could form a disulfide bond and be the "D" site, but this scenario seems unlikely on chemical grounds given the fact that both of the sulfur atoms bridge the Ni_d and Ni_p sites. Furthermore, the distance between the two sulfur atoms is ~3.1 Å[4,5] (far longer than a typical disulfide bond), and model complexes suggests that the Ni_d site is far too rigid to allow the cysteine residues significant motion.[14] Another possibility, of course, is that the $[Fe_4S_4]$ cluster could participate in the redox chemistry. As noted previously, Lindahl and co-workers have reported that electron transfer at the $[Fe_4S_4]$ cluster is 200-fold slower than the rate of methyl transfer.[21] If this turns out to be correct, then the $[Fe_4S_4]$ cluster cannot be part of the catalytic cycle and its role would instead be similar to that of a pilot light, shuttling the initial electrons required to get the reaction started, rereducing the Ni_p site upon accidental oxidation, or both. Literature precedent for an $[Fe_4S_4]$ cluster acting as a "pilot light" can be found in the corrinoid iron–sulfur protein (CFeSP).[34]

Should the $[Fe_4S_4]$ cluster be incapable of participating in the catalytic cycle, one other possible redox-active center is the Ni_d site. In this scenario, Ni_d would be an active participant in the catalytic process instead of merely being required to perform a structural role or to "tune" the reactivity of the Ni_p site. While this would be an unusual role for nickel, it is an intriguing possibility. The unusual and highly anionic C·G·C ligand motif (containing two alkyl thiolates and two deprotonated amides) might very well lower the $Ni^{II/III}$ redox couple into the physiologically relevant range. Furthermore, the rigid coordination environment could promote rapid electron transfer by reducing the reorganization energy. A compelling aspect to this possibility is that it would provide an important and chemically reasonable role for the unusual Ni_d(C·G·C) site. Preliminary data from our lab using model complexes (see below), however, seems to call this theory into question.

Insights from Model Complexes

Many uncertainties remain concerning the reaction mechanism of ACS, and model complexes that serve as well-defined structural and functional mimics are well positioned to address some of these questions and to test various mechanistic proposals. By carefully mapping the scope of chemistry available to the models and by defining the limits of reactivity, model complexes can provide important insight into the mechanism of this complicated enzyme system.

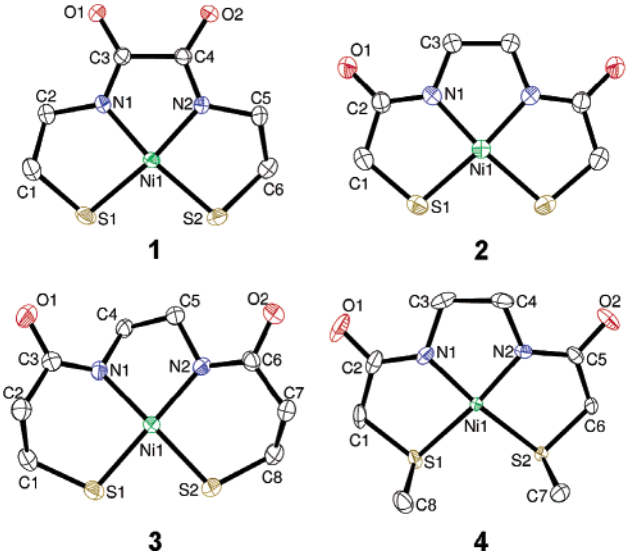


FIGURE 8. Models of the Ni_d site.[14] Complex **2** (first reported by Holm and co-workers)[38] is sitting on a mirror plane. Complexes **1**–**3** are dianionic, while **4** is neutral. Counterions and solvent molecules, which form hydrogen bonds to the amide oxygen atoms in **1**–**3**, have been omitted for clarity.

As previously discussed, there is abundant literature precedent for acetyl formation at a mononuclear Ni site. Since the publication of the ACS crystal structure, however, effort has obviously been directed at modeling both the Ni_p and Ni_d site. Because of the difficulties associated with synthesizing well-characterized metal–peptide complexes, we[14] and others[20,35–37] have initially focused on peptide mimics when designing models of the Ni_d site (Figure 8). Ironically, Holm and co-workers developed some of the earliest models of the Ni_d(C·G·C) site (including complex **2**) when preparing models for the active site of [NiFe]-hydrogenase,[38] well before the active site structure of either enzyme was known.

In ACS, the thiolates of the C·G·C motif bridge two metals, and this is obviously *not* the case in complexes **1**–**3**. Complex **4** addresses this concern, and while **4** is clearly not an exact structural mimic for the ACS active site, there is ample evidence that charge neutralization of thiolates via metalation and alkylation are functionally equivalent.[39] Thus, complex **4** should be a reasonable electronic mimic for the Ni_d center. All of the complexes depicted in Figure 8 maintain certain key features of the C·G·C motif observed in ACS. The Ni(II) ions exhibit a square-planar geometry, each is coordinated to two deprotonated amides and two alkyl thiolates, and the Ni–N and Ni–S bond lengths are similar to those found in ACS as determined by EXAFS spectroscopy.[7,8] However, none of the sulfurs is bridging a second metal ion.

With use of complexes **2** and **3** as synthons, multinuclear complexes have been prepared. When **3** is reacted

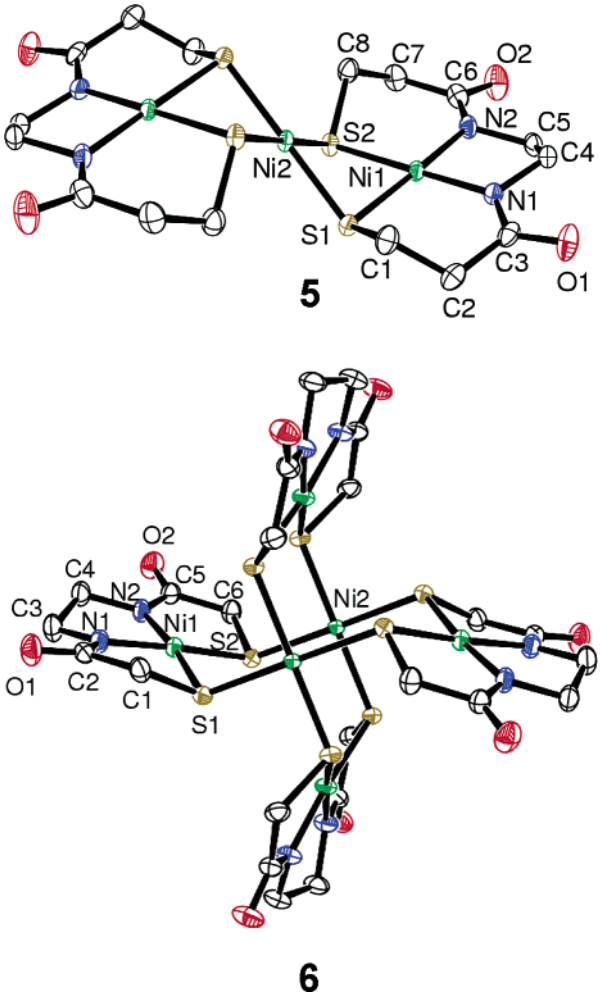
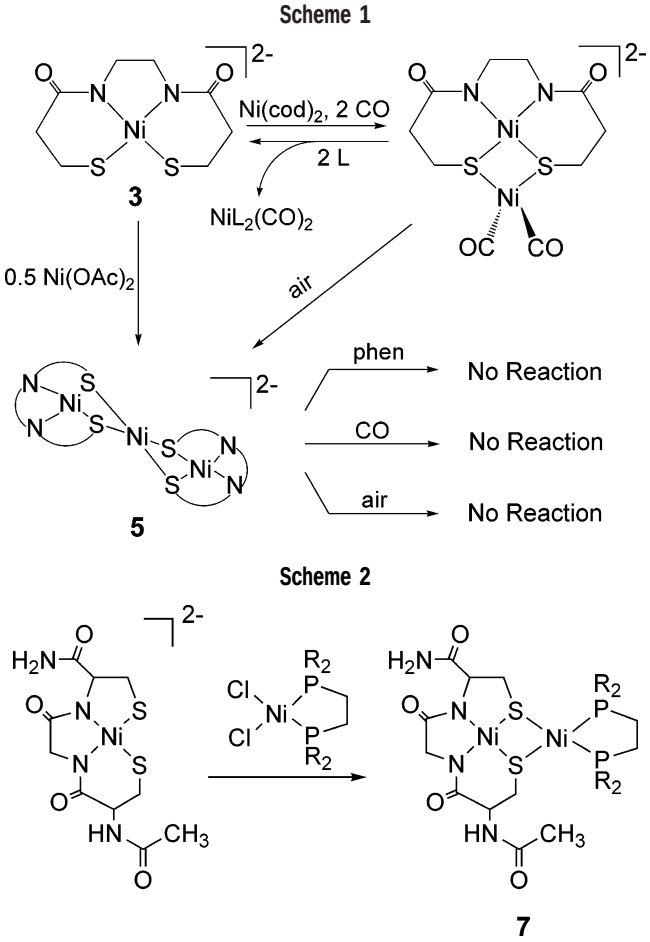


FIGURE 9. Multinuclear complexes generated by the addition of $Ni(OAc)_2$ to Ni_d models.[14] Counterions have been omitted for clarity.



with $Ni(OAc)_2$, a trinuclear complex **5** is generated in which a square-planar Ni(II) ion bridges two molecules of **3** (Figure 9).[14] Complex **5** cannot be decomposed with excess phenanthroline and is not reactive toward a number of small molecules. Rauchfuss and co-workers have also utilized complex **3**, reacting it with $Ni(cod)_2$ in the presence of CO to form a dinuclear complex (Scheme 1).[35] Unlike complex **5**, this dinuclear species is quite reactive at the Ni(0) center, decomposing in the presence of either O_2 or a variety of donor ligands (e.g., phosphines). Contrasting with complex **3**, when **2** is incubated with $Ni(OAc)_2$, a multinuclear paddlewheel complex **6** is obtained (Figure 9). Similar multimeric structures were reported previously by the labs of Darensbourg[20] and Riordan[36] using related ligand systems. In solution, complex **6** slowly decays to the corresponding trinuclear species. Our inability to trap an analogous paddlewheel complex using **3** as a synthon highlights the importance of ligand design and the sensitivity of these systems to relatively minor perturbations.

More recently, Schröder and co-workers reported a dinuclear nickel(II) model in which the "Ni_p" site was capped by a bidentate phosphine ligand.[37] In perhaps the best structural model reported to date, Krishnan and Riordan utilized the tripeptide Cys-Gly-Cys to generate the Ni_d site and reacted this complex with $(R_2PCH_2CH_2PR_2)NiCl_2$ to generate a dinuclear Ni(II) complex (Scheme 2).[40] Complex **7** exhibits two one-electron reductions, potentially modeling the reductive activation of ACS. Interestingly, when electrochemistry was performed in the presence of CO, only a single, irreversible two-electron reduction is observed.

What insights can be drawn from these model complexes? Electrochemical experiments have confirmed that methylation of the thiolates significantly alters the charge density on the Ni(II) center.[14] While **2** has a quasi-reversible $Ni^{II/III}$ couple at −160 mV vs NHE, in complex **4** this same couple is shifted considerably to 1.24 V vs NHE. In fact, the $Ni^{I/II}$ couple is now accessible for **4** (although still outside the physiological range) with a reversible wave centered at −1.26 V vs NHE. The mechanism proposed by Ragsdale and co-workers suggests that Ni^{II}(C·G·C) is reduced to the +1 state prior to methylation by CFeSP;[11] our results are not consistent with this hypothesis. Furthermore, because excess CO is known to inhibit ACS, the lack of reactivity of complex **4** with CO (or with various methyl donors) provides additional evidence that all of the "chemistry" occurs at the Ni_p site.

The stability of complex **5** is also noteworthy. The fact that the central Ni ion cannot be removed with phenanthroline is consistent with the general lack of reactivity

of **5**. Together the data suggest that complex **5** does not properly model the electronics of the Ni_p despite the fact that two separate crystal structures[4,5] show Ni_p as a square-planar nickel coordinated to three "bridging cysteine thiolates and an unidentified exogenous ligand."[4] How might complex **5** be modified to make it a better functional mimic of ACS? One potential problem with **5** is the rigidity of the central Ni ion. Although the crystal structures show Ni_p in a square-planar environment, this site is obviously quite flexible as demonstrated by the presence of the tetrahedral Zn^{2+} and Cu^{+} in the proximal site. Furthermore, according to XAS spectroscopy the Ni_p site is best described as a nonplanar or distorted tetrahedral Ni(II) center in the "as isolated" state.[5,16] This provides additional evidence for conformational flexibility at the Ni_p site and is consistent with this ion playing a key role in the catalytic cycle. Thus, the challenge of synthetic chemists is to design dinuclear "Ni_p−Ni_d(C·G·C)" complexes where the "Ni_p" ion coordinates biologically relevant thiolate ligands, contains two cis-oriented sites available for binding exogenous ligands, participates in reversible redox chemistry, does not polymerize, and still retains conformational flexibility. The fact that no such complexes have yet been reported highlights the difficulty of this challenge.

Summary

Extensive biochemical and spectroscopic studies, the recent report of three separate crystal structures, and the preparation of a number of model complexes has not yet resolved the uncertainties surrounding the reaction mechanism of acetyl-CoA synthase (ACS). The data currently available would suggest that all of the "chemistry" occurs at the Ni_p site, but the relevant oxidation states are still far from clear. ACS performs two-electron chemistry, and thus the enzyme must either stabilize Ni over a range of two oxidation states or provide an electron from either a "D" site, the $[Fe_4S_4]$ cluster, or some other unidentified redox-active cofactor. All of these scenarios contain potential problems, and thus many questions remain to be answered. It is clear that unraveling this mystery will require the synergistic cooperation of biochemists, spectroscopists, crystallographers, and synthetic bioinorganic chemists.

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References

(1) Ragsdale, S. W.; Kumar, M. Nickel-Containing Carbon Monoxide Dehydrogenase/Acetyl-CoA Synthase. *Chem. Rev.* **1996**, *96*, 2515−2539.

(2) Ragsdale, S. W. The Eastern and Western Branches of the Wood/Ljungdahl Pathway: How the East and West were won. *Biofactors* **1997**, *6*, 3−9.

(3) Doukov, T. I.; Iverson, T. M.; Seravalli, J.; Ragsdale, S. W.; Drennan, C. L. A Ni−Fe−Cu Center in a Bifunctional Carbon Monoxide Dehydrogenase/Acetyl-CoA Synthase. *Science* **2002**, *298*, 567−572.

(4) Darnault, C.; Volbeda, A.; Kim, E. J.; Legrand, P.; Vernède, X.; Lindahl, P. A.; Fontecilla-Camps, J. C. Ni-Zn-$[Fe_4$-$S_4]$ and Ni-Ni-$[Fe_4$-$S_4]$ Clusters in Closed and Open α Subunits of acetyl-CoA Synthase/Carbon Monoxide Dehydrogenase. *Nat. Struct. Biol.* **2003**, *10*, 271−279.

(5) Svetlitchnyi, V.; Dobbek, H.; Meyer-Klaucke, W.; Meins, T.; Thiele, B.; Römer, P.; Huber, R.; Meyer, O. A Functional Ni-Ni-[4Fe-4S] Cluster in the Monomeric Acetyl-CoA Synthase from *Carboxydothermus hydrogenoformans*. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 446−451.

(6) Maynard, E. L.; Lindahl, P. A. Evidence of a Molecular Tunnel Connecting the Active Sites for CO_2 Reduction and Acetyl-CoA Synthesis in Acetyl-CoA Synthase from *Clostridium thermoaceticum*. *J. Am. Chem. Soc.* **1999**, *121*, 9221−9222.

(7) Xia, J.; Dong, J.; Wang, S.; Scott, R. A.; Lindahl, P. A. EXAFS, EPR, and Electronic Absorption Spectroscopic Study of the α Metallo Subunit of CO Dehydrogenase from *Clostridium thermoaceticum*. *J. Am. Chem. Soc.* **1995**, *117*, 7065−7070.

(8) Russell, W. K.; Stålhandske, C. M. V.; Xia, J.; Scott, R. A.; Lindahl, P. A. Spectroscopic, Redox, and Structural Characterizations of the Ni−Labile and Nonlabile Forms of the Acetyl-CoA Synthase Active Site of Carbon Monoxide Dehydrogenase. *J. Am. Chem. Soc.* **1998**, *120*, 7502−7510.

(9) Xia, J.; Hu, Z.; Popescu, C. V.; Lindahl, P. A.; Münck, E. Mössbauer and EPR Study of the Ni-Activated α-Subunit of Carbon Monoxide Dehydrogenase from *Clostridium thermoaceticum*. *J. Am. Chem. Soc.* **1997**, *119*, 8301−8312.

(10) Fan, C.; Gorst, C. M.; Ragsdale, S. W.; Hoffman, B. M. Characterization of the Ni-Fe-C Complex Formed by Reaction of Carbon Monoxide with the Carbon Monoxide Dehydrogenase from *Clostridium thermoaceticum* by Q-Band ENDOR. *Biochemistry* **1991**, *30*, 431−435.

(11) Seravalli, J.; Gu, W.; Tam, A.; Strauss, E.; Begley, T. P.; Cramer, S. P.; Ragsdale, S. W. Functional Copper at the Acetyl-CoA Synthase Active Site. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3689−3694.

(12) Shin, W.; Anderson, M. E.; Lindahl, P. A. Heterogeneous Nickel−Iron Environments in Carbon Monoxide Dehydrogenase from *Clostridium thermoaceticum*. *J. Am. Chem. Soc.* **1993**, *115*, 5522−5526.

(13) Bramlett, M. R.; Tan, X.; Lindahl, P. A. Inactivation of Acetyl-CoA Synthase/Carbon Monoxide Dehydrogenase by Copper. *J. Am. Chem. Soc.* **2003**, *125*, 9316−9317.

(14) Hatlevik, Ø.; Blanksma, M. C.; Mathrubootham, V.; Arif, A. M.; Hegg, E. L. Modeling Carbon Monoxide Dehydrogenase/Acetyl-CoA Synthase (CODH/ACS): A Trinuclear Nickel Complex Employing Deprotonated Amides and Bridging Thiolates. *J. Biol. Inorg. Chem.* **2004**, *9*, 238−246.

(15) Gencic, S.; Grahame, D. A. Nickel in Subunit β of the Acetyl-CoA Decarbonylase/Synthase Multienzyme Complex in Methanogens. *J. Biol. Chem.* **2003**, *278*, 6101−6110.

(16) Gu, W.; Gencic, S.; Cramer, S. P.; Grahame, D. A. The A-cluster in Subunit β of the Acetyl-CoA Decarbonylase/synthase Complex from *Methanosarcina thermophila*: Ni and Fe K-edge XANES and EXAFS Analyses. *J. Am. Chem. Soc.* **2003**, *125*, 15343−15351.

(17) Funk, T.; Gu, W.; Friedrich, S.; Wang, H.; Gencic, S.; Grahame, D. A.; Cramer, S. P. Chemically Distinct Ni Sites in the A-Cluster in Subunit β of the Acetyl-CoA Decarbonylase/Synthase Complex from *Methanosarcina thermophila*: Ni L-Edge Absorption and X-ray Magnetic Circular Dichroism Analyses. *J. Am. Chem. Soc.* **2004**, *126*, 88−95.

(18) Seravalli, J.; Xiao, Y.; Gu, W.; Cramer, S. P.; Antholine, W. E.; Krymov, V.; Gerfen, G. J.; Ragsdale, S. W. Evidence That NiNi Acetyl-CoA Synthase Is Active and That the CuNi Enzyme Is Not. *Biochemistry* **2004**, *43*, 3944−3955.

(19) Tan, X.; Bramlett, M. R.; Lindahl, P. A. Effect of Zn on Acetyl Coenzyme A Synthase: Evidence for a Conformational Change in the α Subunit during Catalysis. *J. Am. Chem. Soc.* **2004**, *126*, 5954−5955.

(20) Golden, M. L.; Rampersad, M. V.; Reibenspies, J. H.; Darensbourg, M. Y. Capture of Ni^{II}, Cu^{I} and Zn^{II} by Thiolate Sulfurs of an N_2S_2-Ni Complex: A Role for a Metallothiolate Ligand in the Acetyl-Coenzyme A Synthase Active Site. *Chem. Commun.* **2003**, 1824−1825.

(21) Tan, X.; Sewell, C.; Yang, Q.; Lindahl, P. A. Reduction and Methyl Transfer Kinetics of the α Subunit from Acetyl Coenzyme A Synthase. *J. Am. Chem. Soc.* **2003**, *125*, 318−319.

(22) Stavropoulos, P.; Muetterties, M. C.; Carrié, M.; Holm, R. H. Structural and Reaction Chemistry of Nickel Complexes in Relation to Carbon Monoxide Dehydrogenase: A Reaction System Simulating Acetyl-Coenzyme A Synthase Activity. *J. Am. Chem. Soc.* **1991**, *113*, 8485−8492.
(23) Matsunaga, P. T.; Hillhouse, G. L. Thianickelacycles by Ring Opening of Cyclic Thioethers and Their Subsequent Carbonylation to Thioesters. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1748−1749.
(24) Tucci, G. C.; Holm, R. H. Nickel-Mediated Formation of Thio Esters from Bound Methyl, Thiols, and Carbon Monoxide: A Possible Reaction Pathway of Acetyl-Coenzyme A Synthase Activity in Nickel-Containing Carbon Monoxide Dehydrogenases. *J. Am. Chem. Soc.* **1995**, *117*, 6489−6496.
(25) Sellmann, D.; Häussinger, D.; Knoch, F.; Moll, M. Transition Metal Complexes with Sulfur Ligands. 117. A Reaction Cycle for Nickel Mediated Thioester Formation from Alkyl, CO, and Thiolate Groups Modeling the Acetyl-Coenzyme A Synthase Function of CO Dehydrogenase. *J. Am. Chem. Soc.* **1996**, *118*, 5368−5374.
(26) Webster, C. E.; Darensbourg, M. Y.; Lindahl, P. A.; Hall, M. B. Structures and Energetics of Models for the Active Site of Acetyl-Coenzyme A Synthase: Role of Distal and Proximal Metals in Catalysis. *J. Am. Chem. Soc.* **2004**, *126*, 3410−3411.
(27) Grahame, D. A.; Khangulov, S.; DeMoll, E. Reactivity of a Paramagnetic Enzyme-CO Adduct in Acetyl-CoA Synthesis and Cleavage. *Biochemistry* **1996**, *35*, 593−600.
(28) Tan, X. S.; Sewell, C.; Lindahl, P. A. Stopped-Flow Kinetics of Methyl Group Transfer Between the Corrinoid-Iron-Sulfur Protein and Acetyl-Coenzyme A Synthase from *Clostridium thermoaceticum*. *J. Am. Chem. Soc.* **2002**, *124*, 6277−6284.
(29) Seravalli, J.; Kumar, M.; Ragsdale, S. W. Rapid Kinetic Studies of Acetyl-CoA Synthesis: Evidence Supporting the Catalytic Intermediacy of a Paramagnetic NiFeC Species in the Autotrophic Wood-Ljungdahl Pathway. *Biochemistry* **2002**, *41*, 1807−1819.
(30) Schenker, R. P.; Brunold, T. C. Computational Studies on the A Cluster of Acetyl-Coenzyme A Synthase: Geometric and Electronic Properties of the NiFeC Species and Mechanistic Implications. *J. Am. Chem. Soc.* **2003**, *125*, 13962−13963.
(31) Maroney, M. J.; Pressler, M. A.; Mirza, S. A.; Whitehead, J. P.; Gurbiel, R. J.; Hoffman, B. M. In *Mechanistic Bioinorganic Chemistry*; Thorp, H. H., Pecoraro, V. L., Eds.; Advances in Chemistry Series 246; American Chemical Society: Washington, DC, 1995; pp 21−60.
(32) Barondeau, D. P.; Lindahl, P. A. Methylation of Carbon Monoxide Dehydrogenase from *Clostridium thermoaceticum* and Mechanism of Acetyl Coenzyme A Synthesis. *J. Am. Chem. Soc.* **1997**, *119*, 3959−3970.
(33) Chen, J.; Huang, S.; Seravalli, J.; Gutzman, H., Jr.; Swartz, D. J.; Ragsdale, S. W.; Bagley, K. A. Infrared Studies of Carbon Monoxide Binding to Carbon Monoxide Dehydrogenase/Acetyl-CoA Synthase from *Moorella thermoacetica*. *Biochemistry* **2003**, *42*, 14822−14830.
(34) Menon, S.; Ragsdale, S. W. The Role of an Iron−Sulfur Cluster in an Enzymatic Methylation Reaction. Methylation of CO Dehydrogenase/Acetyl-CoA Synthase by the Methylated Corrinoid Iron−Sulfur Protein. *J. Biol. Chem.* **1999**, *274*, 11513−11518.
(35) Linck, R. C.; Spahn, C. W.; Rauchfuss, T. B.; Wilson, S. R. Structural Analogues of the Bimetallic Reaction Center in Acetyl CoA Synthase: A Ni−Ni Model with Bound CO. *J. Am. Chem. Soc.* **2003**, *125*, 8700−8701.
(36) Krishnan, R.; Voo, J. K.; Riordan, C. G.; Zahkarov, L.; Rheingold, A. L. Thiolate-Bridged Nickel−Copper Complexes: A Binuclear Model for the Catalytic Site of Acetyl Coenzyme A Synthase? *J. Am. Chem. Soc.* **2003**, *125*, 4422−4423.
(37) Wang, Q.; Blake, A. J.; Davies, E. S.; McInnes, E. J. L.; Wilson, C.; Schröder, M. Structure and Electronic Properties of an Asymmetric Thiolate-Bridged binuclear Complex: A Model for the Active Site of Acetyl CoA Synthase. *Chem. Commun.* **2003**, 3012−3013.
(38) Krüger, H. J.; Peng, G.; Holm, R. H. Low-Potential Nickel(III,II) Complexes: New Systems Based on Tetradentate Amidate-Thiolate Ligands and the Influence of Ligand Structure on Potentials in Relation to the Nickel Site in [NiFe]-Hydrogenases. *Inorg. Chem.* **1991**, *30*, 734−742.
(39) Grapperhaus, C. A.; Patra, A. K.; Mashuta, M. S. First $\{Fe-NO\}^6$ Complex with an N_2S_3Fe-NO Core as a Model of NO-Inactivated Iron-Containing Nitrile Hydratase. Are Thiolates and Thioethers Equivalent Donors in Low-Spin Iron Complexes? *Inorg. Chem.* **2002**, *41*, 1039−1041.
(40) Krishnan, R.; Riordan, C. G. Cys-Gly-Cys Tripeptide Complexes of Nickel: Binuclear Analogues for the Catalytic Site in Acetyl Coenzyme A Synthase. *J. Am. Chem. Soc.* **2004**, *126*, 4484−4485.

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