Evidence for Intersubunit Communication during Acetyl-CoA Cleavage by the Multienzyme CO Dehydrogenase/Acetyl-CoA Synthase Complex from Methanosarcina thermophila

EVIDENCE THAT THE β SUBUNIT CATALyzES C–C AND C–S BOND CLEAVAGE*

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The carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS) from Methanosarcina thermophila is part of a five-subunit complex consisting of α, β, γ, δ, and ε subunits. The multienzyme complex catalyzes the reversible oxidation of CO to CO2, transfer of the methyl group of acetyl-CoA to tetrahydromethanopterin (H4MPT), and acetyl-CoA synthesis from CO, CoA, and methyl-H4MPT. The α and ε subunits are required for CO oxidation. The γ and δ subunits constitute a corrinoid iron-sulfur protein that is involved in the transmethylation reaction. This work focuses on the β subunit. The isolated β subunit contains significant amounts of nickel. When proteases truncate the β subunit, causing the CODH/ACS complex to dissociate, the amount of intact β subunit correlates directly with the EPR signal intensity of Cluster A and the activity of the CO/acetyl-CoA exchange reaction. Our results strongly indicate that the β subunit harbors Cluster A, a NiFeS cluster, that is the active site of acetyl-CoA cleavage and assembly. Although the β subunit is necessary, it is not sufficient for acetyl-CoA synthesis; interactions between the CODH and the ACS subunits are required for cleavage or synthesis of the C–C bond of acetyl-CoA. We propose that these interactions include intramolecular electron transfer reactions between the CODH and ACS subunits.

Aceticlastic methanogenic archaea produce methane and carbon dioxide from acetic acid. In aceticlastic methanogenesis, a multifunctional enzyme complex that has been called the carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS)1 complex2 plays key roles (2, 3). This enzyme complex catalyzes the reversible oxidation of CO to CO2 (Reaction 1). It also catalyzes the cleavage (or synthesis) of acetyl-CoA to (or from) a methyl group (methyltetrahydromethanopterin (CH3-H4MPT), carbon monoxide, and Coenzyme A (Reaction 2).

During acetyl-CoA cleavage, a methylcob(III)amide intermediate is formed on a corrinoid iron-sulfur protein (CFeSP), which is a component of the complex. Two related activities that the complex catalyzes are exchange reactions between free CO and the carbonyl group of acetyl-CoA (Reaction 3) and between CoA and the CoA moiety of acetyl-CoA (Reaction 4).

\[
\text{CO + H}_2\text{O} \rightleftharpoons \text{CO}_2 + 2\text{H}^+ + 2\text{e}^- \\
\text{REACTION 1}
\]

\[
\text{CH}_3\text{-CO-SCoA} + \text{H}^+ + \text{H}_4\text{MPT} \rightarrow \text{CH}_2\text{-H}_4\text{MPT} + \text{CO} + \text{HS-CoA} \\
\text{REACTION 2}
\]

\[
\text{CH}_3\text{-CO-SCoA} + \text{CO} \rightleftharpoons \text{CH}_3\text{-CO-SCoA} + \text{CO} \\
\text{REACTION 3}
\]

\[
\text{CH}_3\text{-CO-SCoA} + \text{CoA-SH} \rightleftharpoons \text{CH}_3\text{-CO-SCoA} + \text{CoA-SH} \\
\text{REACTION 4}
\]

The CO/acetyl-CoA exchange reaction is a valuable analytical tool since it requires the disassembly of acetyl-CoA into its three components and its re-synthesis from bound methyl, carbonyl, and CoA moieties. It does not require a corrinoid protein or a methyltransferase, like Reaction 2. Furthermore, this exchange reaction does not involve net redox chemistry, since CO is at the same redox state as the carbonyl group of acetyl-CoA. Reactions 2 and 3 include an intermediate metal-carbon species that is paramagnetic and has been called the NiFeC species (4). Studies of this EPR signal led to the first discovery of a metal cluster in biology containing nickel and iron (4). The acyltransferase reaction between acetyl-CoA and free CoASH (Reaction 4) measures the ability of the enzyme to cleave and re-synthesize only the C–S bond. These reactions have been studied extensively with CODH/ACS from the acetogenic bacterium, Clostridium thermoaceticum (see Refs. 5 and 6 for review). The acetyl-CoA/CoA exchange reaction of the Methanosarcina barkeri (1, 7) and Methanosarcina thermophila (8) CODH/ACS complex also has been studied in great detail. The CO/acetyl-CoA exchange activity of the CODH/ACS complex also has been previously studied (8).

The CODH/ACS complexes from M. thermophila (9, 10), M. barkeri (11), and Archaeoglobus fulgidus (12) (a sulfate reducing archaeon) contain five subunits (α, β, γ, δ, and ε) with molecular masses of 89, 71, 60, 58, and 19 kDa. The α ε dimer contains one [Fe3S4] cluster (Cluster B) and a center called Cluster C, which appears to be a Ni-X-[Fe3S4] cluster (where X is an unknown bridging ligand) and serves as the site of CO oxidation (13). These clusters have very similar properties to those of Clusters B and C in the clostridial CODH subunit (14–17). The γ δ dimer with one corrinoid and one [Fe3S4]

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‡ The abbreviations used are: CODH/ACS, carbon monoxide dehydrogenase/acetyl-CoA synthase; DTAB, dodecyltrimethylammonium bromide; CFeSP, corrinoid iron-sulfur protein; PAGE, polyacrylamide gel electrophoresis; H4MPT, tetrahydromethanopterin; DTT, dithiothreitol.

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2 This complex also has been called the acetyl-CoA decarboxylase/synthase (1).

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cluster is involved in transfer of the methyl group of acetyl-CoA to H4MPT to form methyl-H4MPT (1). The role of the β subunit is controversial.

Evidence suggests that the β subunit contains the molecular machinery to catalyze the first steps in acetyl-CoA utilization, the disassembly of acetyl-CoA to bound methyl, carbonyl, and CoA groups. The M. thermophila β subunit shares 42% sequence identity with the large subunit (acaB) of the C. thermoaceticum enzyme (18), which harbors Cluster A, the site of the acetyl-CoA synthesis (19, 20). However, there is only 16% identity between the β subunit and the small subunit (acaA) of the acetogenic enzyme (18). Furthermore, when the CODH/ACS complex is subjected to limited proteolysis, acetyl-CoA synthesis activity declines in parallel with loss of the intact β subunit; yet the truncated β subunit retains the ability to catalyze the Coa/acetyl-CoA exchange reaction (7). On the other hand, spectroscopic studies of an apparently purified αe dimer indicated that Cluster A is a component of this complex (13).

As reported here, the location of the acetyl-CoA cleavage site was investigated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), electron paramagnetic resonance (EPR) spectroscopy, and kinetic methods. Our results indicate that acetyl-CoA cleavage/synthesis site is located in the β subunit and that interaction between the αe dimer and the β subunit is necessary for breaking and forming the C–C bond of acetyl-CoA.

**EXPERIMENTAL PROCEDURES**

**Materials**—Radioisotopes (63NiCl2 and [1-14C]acetyl-CoA) were purchased from Amersham Pharmacia Biotech. Other chemicals were purchased from Sigma.

**Cell Growth and Enzyme Purification**—M. thermophila TM1 cells were grown, harvested, and lysed as described (21). The CODH/ACS complex was purified essentially as outlined by following the oxidation of CO to CO2 (21). M. thermophila cells were suspended into Buffer A (50 mM Tris/HCl, pH 7.6, 10% glycerol, 0.6% Triton X-100, and 2 mM DTT) and were lysed by sonication. Triton X-100 did not affect the CODH/ACS activities and was needed for purification of heterodisulfide reductase from the same batch of cells. After ultracentrifuging at 32,000 rpm for 2 h, the cell-free extract was loaded on a Q-Sepharose high performance column (2.5 × 40 cm) and washed with Buffer A containing 0.1 M KCl. The CODH/ACS complex was eluted at a 0.1–0.8 M KCl linear gradient. The CODH/ACS complex fractions were pooled, dialyzed, and 3-fold with Buffer B (50 mM Tris/Cl, pH 6.8, 10 mM MgCl2, 10% glycerol, and 2 mM DTT), and applied to a second Q-Sepharose HP column (2.5 × 15 cm). After washing the column with Buffer B containing 0.2 M KCl, the CODH/ACS complex was eluted with the same buffer containing 0.4 M KCl. Fractions containing CODH activity were pooled, concentrated, and loaded on a Superose 6 column (2.5 × 80 cm). The CODH/ACS complex appeared to be homogeneous based on analysis by SDS-PAGE (11.25% polyacrylamide). The purified enzyme exhibited specific activities of CO oxidation and CO/acetyl-CoA exchange of 286 and 0.206 μmol min−1 mg−1, respectively. The CODH/ACS complex was labeled with radioactive nickel by adding 50 μCi of 63Ni (NiCl2) to 5 liters of media containing a total nickel concentration of 2.1 g atm of nickel per mol, assuming an αβδε stoichiometry, in agreement with earlier measurements (2). To determine which subunit(s) contains nickel, the complex was treated with 1% DTAB and 0.3% Triton X-100 (conditions that dissociate the multicomponent complex (2)) and chromatographed on a Q-Sepharose HP column. Two radioactive peaks were observed (Fig. 1). The first peak was highly enriched in the α and ε subunits, whereas the second peak contained a mixture of the β, γ, and δ subunits with traces of the α and ε subunits (Fig. 1, inset). The first peak contained 1.2 g atm of nickel per mol of the αe dimeric unit. Since the ε subunit does not contain metals, the nickel must be associated with the α subunit, as concluded earlier (2). The β subunit was further purified by pooling the fractions in the second peak and treating again with 1% DTAB and 0.3% Triton X-100. After diluting 5-fold, the enzyme solution was loaded on the Q-Sepharose HP column and eluted with a linear gradient of 0.2–0.8 M KCl. The protein and 63Ni radioactivity profiles obtained from SDS-PAGE analysis are shown in Fig. 2. Nickel was clearly associated with the pure β subunit; for example, fraction 16 contains highly purified β subunit with...
Alters the NiFeC EPR Signal by Chymotrypsin Treatment—Limited protease digestion dissociates the components of the M. barkeri CODH/ACS complex and cleaves the \( \beta \) subunit into two fragments (1). The isolated truncated \( \beta \) subunit retains the ability to catalyze an exchange reaction between CoA and acetyl-CoA but is unable to synthesize acetyl-CoA (1). CODH/ACS complex was treated with chymotrypsin at a ratio of 43:1 (CODH/chymotrypsin, w/w) to dissociate the complex and truncate the \( \beta \) subunit. The protease reaction was quenched at different times by adding chymotrypsin inhibitor, which we found to quench rapidly the protease reaction without adversely affecting the NiFeC EPR signal, CO oxidation, or the CO/acytetyl-CoA exchange activity.

The NiFeC EPR signal (Fig. 4), SDS-PAGE-stained protein patterns (Fig. 5), and CO/acytetyl-CoA exchange activity of samples quenched at different times after chymotrypsin treatment were measured (Fig. 6). The CO oxidation activity was not affected by the chymotrypsin treatment over the time course followed. The shape of the NiFeC signal underwent a marked change in morphology. The peak at \( g = 2.08 \) decreased, and the peak at \( g = 2.05 \) increased at the same rate constant of 0.16 min\(^{-1}\) (Figs. 4 and 6). This modified signal was first observed in N-bromosuccinimide-inhibited ACS (33) and has been called the “pseudo NiFeC signal” (34). Essentially all of the NiFeC signal was converted to the “pseudo” form within 1 h of reaction with chymotrypsin, but the total spin concentration did not change significantly, even after 24 h. At approximately the same rate that the EPR signal changed, the CO/acytetyl-CoA exchange activity decayed (0.11 min\(^{-1}\)) and the \( \beta \) subunit underwent degradation (0.08 min\(^{-1}\)) (Fig. 6B).

Western hybridization analysis was performed using an antibody specific to the \( \beta \) subunit. The decrease in band intensity at the position of the \( \beta \) subunit matches the SDS-PAGE results described above. This band disappeared at the same rate as the NiFeC EPR signal intensity and the CO/acytetyl-CoA exchange activity (data not shown). Western analysis allowed us to follow degradation of the \( \beta \) subunit. These results are fully consistent with those reported earlier (1). One of the peptides produced overlapped with the \( \delta \) subunit (58 kDa), which explains why the intensity at the position of the \( \delta \) subunit increases with time of chymotrypsin treatment (see Fig. 5). A smaller fragment (26 kDa) also was observed. The first 10 amino acids of the 26-kDa cleavage product were sequenced and perfectly matched the N-terminal sequence of the \( \beta \) subunit. These results strongly support the concept that the acetyl-CoA cleavage/synthesis site is associated with the \( \beta \) subunit.

Disruption of the NiFeC EPR Signal by Bromelain—We examined the effect of bromelain, another protease, on the integrity of Cluster A. The CODH/ACS enzyme complex was digested with bromelain in the presence of CO, and aliquots of the reaction were freeze-quenched in liquid nitrogen at different times. Unfortunately, we were unable to use bromelain inhibitor to quench the reaction because it alone degraded the \( \beta \) subunit (data not shown). For each time point, the NiFeC EPR signal was measured; SDS-PAGE was performed, and the EPR and SDS-PAGE band patterns were compared (Fig. 7). Whereas the chymotrypsin treatment converted the EPR signal from the NiFeC to the pseudo form, bromelain treatment led to the disappearance of the EPR signal. The degradation of the \( \beta \) subunit and the loss of NiFeC signal intensity followed the same time course, indicating that the EPR signal arose
from the β subunit. Since it is known that the NiFeC signal derives from a CO adduct of Cluster A (34–36), these results bolster the hypothesis that the acetyl-CoA cleavage/synthesis site is associated with the β subunit. In C. thermacetica, Clusters A and C and their related acetyl-CoA synthase and CO oxidation activities are also located on separate subunits (19, 20, 37).

Requirement of CODH Component for Acetyl-CoA Cleavage/Synthesis—As described above, limited proteolysis of the CODH/ACS complex leads to alteration or loss of the EPR signal derived from Cluster A and to loss of acetyl-CoA synthesis activity. To determine if the activity loss results from damage to the β subunit or to separation of the components, the enzyme complex was treated with DTAB in the presence of 0.2 M KCl. At higher salt concentrations, the components of the complex do not precipitate. DTAB treatment has no effect on the CO oxidation activity, demonstrating that Cluster C is unaffected by the DTAB treatment. On the other hand, DTAB treatment results in loss of the CO/acetyl-CoA exchange activity and conversion of the NiFeC EPR signal to the pseudo form. Surprisingly, more than 40% of the CO/acetyl-CoA exchange activity recovers, and the standard NiFeC signal returns after removing the detergent from the solution by extensive ultrafiltration (Fig. 8). Thus, the DTAB treatment is reversible. These results suggest that an association between the β subunit and another component of the complex is required for CO/acetyl-CoA exchange and, therefore, for acetyl-CoA cleavage or synthesis.

DISCUSSION

It has been clear since 1984, when the first methanogenic CODH was isolated from M. barkeri (38), that CO oxidation activity resides in a complex of the α and ϵ subunits. In the acetogenic system, this activity is located in the β subunit (acsA) (19, 39), which has high sequence homology to the methanogenic α subunit (cdhA) (18) and Rhodospirillum rubrum CooS (40). The results described here agree with these conclusions. The isolated α dimer generated after detergent or proteolytic treatment retains high levels of CO oxidation activity.

Which component(s) of the methanogenic CODH/ACS complex is required for formation or cleavage of the C–C and C–S bonds of acetyl-CoA? In the acetogenic enzyme, these activities are located in the α subunit (acsB) (19, 20). Work by Grahame and DeMoll (1) suggests that the β subunit of the methanogenic

FIG. 3. Correlation between the EPR signals and the SDS-PAGE band intensities after DTAB treatment. EPR spectra A and C are from intact CODH/ACS complex (top, SDS-PAGE), and spectra B and D are from the sample that lacks 95% of the β subunit and 60% of the γδ dimer (bottom, SDS-PAGE). Spectra A and B show the NiFeC signal obtained after the samples were treated with CO. Spectrum B was magnified 10-fold. The spin concentrations for A and B are 0.3 and 0.01 spins/mol, respectively. Spectra C and D show the Co(II) cobamide signal from the CFeSP. EPR conditions; 80 K, 40 milliwatts, and 20,000 gain.

FIG. 4. EPR spectra after chymotrypsin treatment. Spectra were collected at the following time points after mixing with chymotrypsin: 0, 1, 5, 10, 30, and 60 min (from the top to the bottom). See “Experimental Procedures” for details. The final CODH/ACS complex concentration was 15 mg/ml. EPR conditions were the same as shown in Fig. 3.

FIG. 5. Effects of chymotrypsin on the CODH/ACS complex. The CODH/ACS complex was treated with chymotrypsin. Lane 1, native CODH/ACS complex; lanes 2–6 show samples quenched 1, 5, 10, 30, and 60 min after chymotrypsin addition, respectively. 7 µg of protein was loaded in each lane.
CODH/ACS complex binds CoA, cleaves (or forms) the C–S bond, and harbors the acetyl-enzyme intermediate. They showed that limited proteolysis of the CODH/ACS complex truncates the β subunit leading to dissociation of the complex and loss of acetyl-CoA synthesis activity (from CO₂, methyl-H₄MPT, and CoA). They also showed that the isolated, truncated β subunit retains the ability to catalyze an exchange reaction between CoA and acetyl-CoA (Reaction 4). Furthermore, the M. thermophila β subunit is homologous to the large ACS subunit of the C. thermoaceticum enzyme, not to the small CODH subunit (18). These results strongly indicate that the β subunit plays an important role in acetyl-CoA synthesis. However, they are in apparent contradiction with two observations. A low level of the NiFeC EPR signal (0.1 spin per mol of homodimer) was observed when the apparently homogeneous ae component from M. thermophila was incubated with CO (13). Furthermore, the two-subunit (ae) Methanothrix soehngenii CODH exhibits a low level of CO/acetyl-CoA exchange activity (35 nmol min⁻¹ mg⁻¹).³ These results suggest that Clus-

³ The CO/acetyl-CoA exchange activity of the M. soehngenii five-subunit complex has not been measured; however, this value is 4–20-fold lower than typical specific activities for this reaction with M. thermophila complex.
acellular CoA synthesis, is associated with the α subunit. How can one explain these two discordant findings? One suggestion is that both α and β subunits contain an ACS-active site (41). One goal of the studies described in this paper was to resolve this conundrum.

We decided to study the simplest acetyl-CoA synthesis reaction that would presumably only involve the ACS-active site. It is difficult to unambiguously define the component(s) required for cleavage or synthesis of the C–C and C–S bonds of acetyl-CoA by studying the total synthesis of acetyl-CoA from CO₂, methyl-H₄MPT, and CoA. This reaction involves CODH (the α component) to reduce CO₂ to CO, the corrinoid iron-sulfur component (the γ component) for two transmethylation reactions, and the ACS component(s) to assemble CO, the methyl group, and CoA to form acetyl-CoA. The exchange reaction between CO₂ and acetyl-CoA also requires CODH as well as the ACS component(s). By studying reactions that, in theory, would only require the component of ACS responsible for C–C and C–S bond cleavage, we reasoned that we might be able to define the ACS component(s) and identify the ambiguous role of the β subunit. We focused on two reactions. The first one is formation of the Cluster A-CO adduct that is the precursor of the carbonyl group of acetyl-CoA.

In the bromelain experiments, the NiFeC EPR signal disappears, whereas in the chymotrypsin experiments, the NiFeC signal is converted into the pseudo NiFeC signal. The pseudo NiFeC signal also is formed when N-bromosuccinimide-inactivated CODH/ACS from C. thermoacetica is treated with CO (33) or when the C. thermoacetica α subunit, isolated after SDS treatment, is reacted with CO (34). The pseudo NiFeC species exhibits ⁶¹Ni, ⁵⁷Fe, and ¹³CO hyperfine interactions (4) and Mössbauer and UV-visible spectroscopic parameters that are very similar to those of the standard NiFeC species (27, 43). Thus, the truncated β subunit contains an altered form of Cluster A that is still able to bind CO. Apparently, the pseudo NiFeC EPR signal reflects the CO adduct of a slightly altered and inactive NiFeS cluster, and the standard NiFeC signal characterizes the active cluster. It is interesting that pseudo NiFeC species is formed by such diverse treatments, including modification of proximal tryptophan residues, SDS, or DTAB treatment, or proteolytic truncation of the β subunit.

The results described above provide significant evidence that the β subunit is an essential component of the CO/acetyl-CoA exchange reaction. Why doesn’t the truncated β subunit alone catalyze the CO/acetyl-CoA exchange reaction since it can still catalyze the CO/acetyl-CoA exchange activity (1), contains Cluster A, and can bind CO? Our results indicate that interactions between the subunits harboring the ACS and CODH components markedly affect the structure of the ACS-active site and are important for cleavage/synthesis of the C–C bond of acetyl-CoA. Since the N terminus of the β subunit remains intact, these intersubunit interactions must involve amino acids near the C terminus. Macromolecular interactions during acetyl-CoA synthesis were further probed by treatment of the CODH/ACS complex with the detergent DTAB, which separates the complex into three components. Dissociation of the complex by DTAB treatment causes a reversible loss of CO/acetyl-CoA exchange activity and alteration of the NiFeC EPR signal. However, DTAB removal causes recovery of the stand-
ard NiFeC EPR signal and of the CO/acetyl-CoA exchange activity. This result indicates that DTAB inhibits by separating the components of the CODH/ACS complex, possibly by disrupting essential interactions between the CODH and ACS subunits.

Why would macromolecular interactions be important for formation of the NiFeC signal or for catalysis of the CO/acetyl-CoA exchange? It is obvious why separation of the CODH and ACS subunits would inhibit acetyl-CoA synthesis from methyl-H4MPT, CO2, and CoA. This reaction involves the CFeSP and the CO2 reduction (CODH) components. Furthermore, both the CODH and ACS subunits would also be required for the CO2/acetyl-CoA exchange reaction. The requirement for the aε component in forming the NiFeC EPR signal also can be rationalized. Cluster A has a low midpoint potential of approximately −2530 mV (44), and reduction of the catalytically active form of Cluster A appears to require CO (45). Therefore, as shown in Fig. 9, generation of the active state of Cluster A could require the Cred2 form of Cluster C (with a similar midpoint potential (36)). However, the CO/acetyl-CoA exchange reaction (see Reaction 3), which is not a net redox reaction, also requires a component(s) of the complex. As shown in Fig. 9, we propose that a covert intramolecular electron transfer reaction occurs between the CO oxidation catalyst (the aε component) and Cluster A (the ACS catalyst) on the β subunit at each catalytic cycle of acetyl-CoA synthesis. This proposal extends the hypothesis offered in 1985 (24), based on the finding that ferredoxin or redox mediators strongly stimulates the CO/acetyl-CoA exchange reaction, that an internal electron transfer reaction occurs during the exchange reaction.

In Fig. 9, the first step of acetyl-CoA synthesis involves CO binding to the reduced state of Cluster A. This step does not require close association between CODH and ACS since the pseudo NiFeC species forms upon incubating the DTAB- or protease-treated complex with CO. This form of Cluster A has been described as the carbonyl adduct of a Ni(I)-X-[4Fe-4S]2+ complex (43). Extensive evidence supports the catalytic competence of the paramagnetic NiFeC species; this intermediate forms and decays at catalytically competent rates and undergoes isotopic exchange with acetyl-CoA (see Refs. 42, 46, and 47 for detailed discussion). In addition, CO binds most tightly to low valent states of transition metals, indicating that CO is bound to the nickel site, as has been proposed earlier (43).

As shown in Fig. 9, the next step is attack by Ni(I) on the methyl group of methylcob(III)amide (bound to the corrinoid iron-sulfur component of the complex) to form methyl-Ni(III) (Reaction 5). Recent results strongly indicate that this methyl transfer is a nucleophilic displacement, not a radical, reaction (46, 48). We propose that it is the next step at which the CODH subunit is required. Methyl-Ni(III) is an extremely strong oxidant. For example, the midpoint for the methyl-Ni(III)/(II) couple of F430 is much more positive than 0 V (49, 50) (Reaction 6). Therefore, capture of one electron from a site on CODH to generate a diamagnetic methyl-Ni(II) species would be extremely favorable.

\[
\text{methyl-Co(III)} + \text{Ni(I)} \rightleftharpoons \text{methyl-Ni(III)} + \text{Co(I)}
\]

REACTION 5

\[
\text{methyl-Ni(III)} + \text{e}^{-} \rightleftharpoons \text{methyl-Ni(II)}
\]

REACTION 6

This proposal can explain why the NiFeC EPR signal disappears when the paramagnetic CO adduct of CODH/ACS is reacted with CH3-H4folate (51) (the acetogenic enzyme) or CH3-H4MPT (52) (the methanogenic complex) in the presence of the methyltransferase and CFeSP. Fig. 9 assumes that the electron originates and is returned from Cluster C of the CODH subunit; however, Cluster B or the “X” or “S” redox centers (14, 53) could also fulfill this role. Another explanation has been offered for the loss of the NiFeC EPR signal upon methylation of the clostridial CODH/ACS (51). It is argued that reaction of a radical with the methyl group donor should generate another
radical. Therefore, it is argued, the "true" carboxylated intermediate on ACS should be diamagnetic, e.g. a Ni(II)-CO species. However, Ni(II) is less nucleophilic than Ni(I), and removal of the methyl group from methyl-Co(III) requires a nucleophile that rivals Co(I).

The next proposed step is formation of the acetyl-enzyme intermediate (see Refs. 6, 7, and 47). Since net redox exchange is not required during the CO-acetyl-CoA exchange reaction, the electron removed from CODH to stabilize the methyl-ACS intermediate must be returned. We speculate that return of the electron to CODH is associated with CoA binding or cleavage of the acetyl-ACS intermediate. Transient kinetic experiments using single turnover conditions are in progress to test this mechanism and, specifically, to identify the one-electron donor.

Given the similarity in structure and function of the methanogenic and acetogenic CODH/ACS, the mechanism described in Fig. 9 is expected to apply to both classes of organisms.

The results described here complement those of Grahame and DeMoll (1); however, they are in apparent contradiction with the occurrence of CO-acetyl-CoA exchange activity and the NiFeC signal, albeit at low levels (described above), in apparently purified samples of ACS. This would explain the occurrence of the NiFeC EPR signal and the ACS subunit of the acetogenic enzyme is insignificant.

In contrast, the cysteine-rich region in the subunit of the methanogenic enzyme only been found tightly bound to CODH. Electron transfer reactions associated with methylation or demethylation of Cluster A during acetyl-CoA synthesis. Perhaps this cryptic electron transfer reaction could help explain why CODH subunits are often found in isolation but active ACS subunits have so far only been found tightly bound to CODH.

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CONCLUSIONS

Our results indicate that the β subunit (cdhC) of the methanogenic CODH/ACS complex contains the NiFeS cluster known as Cluster A, which is responsible for cleavage of the C–C and C–S bonds of acetyl-CoA. Evidence is described that indicates a requirement for intermolecular interactions between the CODH and the ACS subunits. These interactions are considered to include, but are not limited to, electron transfer reactions associated with methylation or demethylation of Cluster A during acetyl-CoA synthesis. Perhaps this cryptic electron transfer reaction could help explain why CODH subunits are often found in isolation but active ACS subunits have so far only been found tightly bound to CODH.
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