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Biochemistry

Resolution of component proteins in an enzyme complex from *Methanosarcina thermophila* catalyzing the synthesis or cleavage of acetyl-CoA

(methanogenesis/one-carbon metabolism/nickel/corrinoid/iron-sulfur proteins)

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ABSTRACT **An enzyme complex was isolated from acetate-grown *Methanosarcina thermophila* that oxidized CO and catalyzed the synthesis or cleavage of acetyl-CoA. The complex consisted of five subunits ($\alpha_1\beta_1\gamma_1\delta_1\varepsilon_1$) of 89, 71, 60, 58, and 19 kDa. The complex contained nickel, iron, acid-labile sulfide, and cobalt in a corrinoid cofactor. Two components were resolved by anion-exchange chromatography of the complex in the presence of dodecyltrimethylammonium bromide and Triton X-100: a 200-kDa nickel/iron-sulfur protein with the 89- and 19-kDa ($\alpha_2\varepsilon_x$) subunits and a 100-kDa corrinoid/iron-sulfur protein with the 60- and 58-kDa subunits ($\gamma_1\delta_1$). The nickel/iron-sulfur component contained 0.21 Ni, 2.7 Zn, 7.7 Fe, and 13.2 acid-labile sulfide (per $\alpha_1\varepsilon_1$). The corrinoid/iron-sulfur component contained 0.7 Co, 0.7 factor III {Coα-[α-(5-hydroxybenzimidazolyl)]-Coβ-cyanocobamide}, 3.0 Fe, and 2.9 acid-labile sulfide ($\gamma_1\delta_1$). Both components contained iron-sulfur centers. The nickel/iron-sulfur component oxidized CO and reduced methyl viologen or a ferredoxin isolated from *M. thermophila*. The nickel/iron-sulfur component also oxidized CO and transferred electrons to the corrinoid/iron-sulfur component, reducing the iron-sulfur and Co centers. UV–visible spectroscopy indicated that the reduced corrinoid/iron-sulfur component could be methylated with CH_3I. The results suggest that the enzyme complex from *M. thermophila* contained at least two enzyme components, each with a specific function. The properties of the component enzymes support a mechanism proposed for acetyl-CoA synthesis (or cleavage) by the enzyme complex.**

The methanogenic archaebacteria utilize two major energy-yielding pathways. The more extensively studied pathway is the oxidation of H_2 or formate and reduction of CO_2 to CH_4; these studies have revealed unusual coenzymes and new biochemical reactions (1). Less understood is the pathway for conversion of acetate to CH_4 and CO_2, which accounts for approximately two-thirds of the methane produced in most anaerobic environments. This pathway involves cleavage of acetate with transfer of the methyl group to 2-mercaptoethanesulfonic acid (HS-CoM) to form 2-(methylthio)ethanesulfonic acid (CH_3-S-CoM) (2); oxidation of the carbonyl group to CO_2 supplies the electron pair required for reductive demethylation of CH_3-S-CoM to CH_4 (3). The enzyme mechanisms underlying these reactions are unresolved. It has been postulated that CO dehydrogenase (CODH), abundant in acetate-grown methanogens, cleaves the C-C and C-S bonds of activated acetate (acetyl-CoA). Recently, acetyl-CoA synthesis and cleavage activities were reported for an enzyme complex that also contained CODH activity and was isolated from acetate-grown *Methanosarcina thermophila* (4, 5). In addition, an analysis of acetate auxotrophs has recently provided evidence supporting the proposed involvement of CODH in acetyl-CoA synthesis for cell carbon by autotrophic CO_2-reducing methanogens (6). These findings firmly support the proposed functions of CODH from methanogens and make studies on the biochemical mechanism of this enzyme necessary.

CODH also catalyzes the synthesis of acetyl-CoA in the energy-yielding Wood pathway of acetogenic eubacteria (7). The archaebacteria and eubacteria are at the extremes of bacterial evolution (8); thus, it is likely that CODH is an ancient enzyme and that the various present-day CODH enzymes originated from a common ancestor. The comparative biochemistry of this enzyme from widely divergent organisms offers a new direction for studies aimed at understanding the mechanism and evolution of CODH in anaerobes. The enzyme complex from acetate-grown *M. thermophila* was investigated to approach these central questions in the archaebacteria. The five-subunit complex was resolved into two protein components, the properties of which support a proposed mechanism for the enzyme complex during growth of the organism on acetate.

MATERIALS AND METHODS

Protein Purification. All purification steps and manipulation of proteins were done in a N_2-filled anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI) at 23°C.

The five-subunit complex was purified from acetate-grown *M. thermophila* as previously described (9) and stored under N_2 at −20°C. The enzyme was in 50 mM 2-[tris(hydroxymethyl)methyl]amino-1-ethanesulfonic acid (Tes, pH 6.8) that contained 0.4 M KCl, 10% (vol/vol) ethylene glycol, and 10 mM $MgCl_2$. Ferredoxin was purified as described (10).

The nickel/iron-sulfur and corrinoid/iron-sulfur components were isolated as follows. The complex (3.5–5.0 mg/ml of isolation buffer) was incubated with 1% (wt/vol) dodecyltrimethylammonium bromide (DTAB) and 0.3% (vol/vol) Triton X-100 at 26°C for 10 min and then at 4°C for 10 min. The mixture was diluted 3-fold with buffer A [50 mM Tes, pH 6.8/10% (vol/vol) ethylene glycol/10 mM $MgCl_2$, 0.05% (wt/vol) DTAB, 0.1% (wt/vol) Triton X-100]; the complex precipitated in this step when the protein concentration was >5 mg/ml. Samples (50 mg of protein) were applied to a Mono Q HR 10/10 (Pharmacia) ion-exchange column preequilibrated with buffer A. The column was developed with a linear gradient of 0.2–0.65 M KCl in buffer A at 2 ml/min using an FPLC system (Pharmacia). The component proteins were stored under N_2 at −20°C.

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Abbreviations: CODH, CO dehydrogenase; DTAB, dodecyltrimethylammonium bromide.

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Enzyme Assays. The general procedures for anaerobic assays, including the preparation of buffers and reagents, have been described (3, 9). CO-oxidizing activity with methyl viologen as the electron acceptor was assayed as described (9). CO-oxidizing activity with ferredoxin as electron acceptor was measured by following the ferredoxin-dependent reduction of metronidazole at 320 nm (11). Specific activity was defined as μmol of electron acceptor reduced per min per mg of enzyme.

Acetyl-CoA synthesis from CH_3I, CO, and CoA, or exchange of CO with the carbonyl group of acetyl-CoA, was assayed as described (4, 5). The effect of added nickel and iron on activity was determined by addition of 0.23 mM (final concentration) $NiCl_2$ and 0.23 mM $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ to the reaction mixture.

Isolation of the Corrinoid. The corrinoid was isolated as the cyano derivative for the purpose of identification. All procedures were done in the dark. Methanol (4 ml) was added to 4 ml of the enzyme complex (5.0 mg/ml) and the solution was heated at 80°C for 25 min. Protein was removed by centrifugation at 27,000 × *g* for 15 min followed by ultrafiltration of the supernatant solution with a Centriprep-10 (Amicon). The solution containing the corrinoid was concentrated 10-fold under a stream of N_2 at 21°C and desalted with Sephadex G-10 (Pharmacia) by the spun-column technique (12). KCN was added to a final concentration of 0.3 mM and the solution (pH 6.5) was heated to 80°C for 15 min. The isolated corrinoid was identified by HPLC. The corrinoid was quantitated spectrophotometrically after release from the protein and conversion to the dicyano form (9).

Analytical Methods. Protein was determined with protein dye reagent (Bio-Rad) by the method of Bradford (13); the dialyzed and lyophilized enzyme complex was the protein standard.

Protein samples for elemental analysis were dialyzed against four 1-liter changes of 5 mM sodium phosphate buffer (pH 7.2) for 9 hr at 6°C. Elemental analysis was by plasma emission spectroscopy (14). Acid-labile sulfide was determined as described (15).

HPLC of cyanocobamide standards and the corrinoid cofactor isolated from the enzyme complex was done in the dark as previously described (16), with the following modifications. The solvents were 0.1% acetic acid (A) and methanol (B). Samples were applied to a μBondapak RP-18 column (0.39 cm i.d. × 30 cm, Waters) equilibrated with solvent A and eluted using the following solvent system: from 0 to 2 min, isocratic 100% A: from 2 to 3 min, a linear gradient to 75% A/25% B; from 3 to 9 min, isocratic 75% A/25% B; from 9 to 29 min, a linear gradient to 35% A/65% B. Detection was at 254 nm.

Polyacrylamide gel electrophoresis was performed using the Mini-Protean II slab gel apparatus (Bio-Rad). Denaturing electrophoresis was performed with the Laemmli buffer system (17) and 12% polyacrylamide. Nondenaturing electrophoresis was performed using linear gradient gels of 4–30% polyacrylamide (18); the buffer system was according to Laemmli (17) but without sodium dodecyl sulfate (SDS). Molecular mass standards were lactalbumin, 14.2 kDa; ovalbumin, 45 kDa; bovine serum albumin (monomer and dimer), 66 and 132 kDa; β-amylase, 200 kDa; and urease (trimer), 272 kDa. Native molecular masses were also estimated by the method of Hedrick and Smith (19) with 5%, 5.5%, 6%, and 6.5% polyacrylamide gels in the buffer system of Laemmli (17) without SDS. The molecular mass standards were bovine serum albumin (monomer and dimer), 66 and 132 kDa; aldolase, 158 kDa; catalase, 232 kDa; and ferritin, 440 kDa.

Chemicals. The following cyanocobamide standards were a gift from E. Stupperich: factor III {Coα-[α-(5-hydroxybenzimidazolyl)]-Coβ-cyanocobamide}, factor III_m {Coα-[α-(5-methylbenzimidazolyl)]-Coβ-cyanocobamide}, and pseudovitamin B_{12} {Coα-[α-(aden-7-yl)]-Coβ-cyanocobamide}. DTAB, Triton X-100, mersalyl acid {2-[*N*-(3-hydroxymercuri-2-methoxypropyl)carbamoyl]phenoxyacetic acid}, CoA, and CH_3I were of the highest purity available from Sigma.

RESULTS

Resolution of the Enzyme Complex and Composition of Component Proteins. The enzyme complex isolated from *M. thermophila* is composed of five subunits (89, 71, 60, 58, and 19 kDa) and contains nickel, iron, acid-labile sulfide, and cobalt in a corrinoid cofactor (9). Two protein components were resolved by anion-exchange chromatography of the complex in the presence of DTAB and Triton X-100 as described in *Materials and Methods*. The first component (corrinoid/iron-sulfur) was eluted in 0.35 M KCl and contained the 60- and 58-kDa subunits; the second component (nickel/iron-sulfur) was eluted in 0.44 M KCl and contained the 89- and 19-kDa subunits (Fig. 1). These were the only combinations of subunits obtained. The 71-kDa subunit was not recovered. Typically, 20–30% of the complex was unresolved (Fig. 1) and was eluted from the column in 0.55 M KCl.

The corrinoid/iron-sulfur component contained iron, acid-labile sulfide, cobalt, and corrinoid; no nickel was detected (Table 1). This component contained the same molar ratio of corrinoid as did the enzyme complex, which indicated no significant loss of the cofactor during resolution. HPLC of the isolated corrinoid yielded a single peak with a retention time (15.3 min) coincident with that of factor III {Coα-[α-(5-hydroxybenzimidazolyl)]-Coβ-cyanocobamide}. The UV–visible spectrum was comparable to that of factor III (Fig. 2), with absorption maxima at 279, 292, 324, 360, 515, and 545 nm. The spectrum of factor III is significantly different from the UV–visible spectra of pseudovitamin B_{12} {Coα-[α-(aden-7-yl)]-Coβ-cyanocobamide} present in the methanococcales and *Methanoplanus limicola* (20) and of factor III_m {Coα-[α-(5-methylbenzimidazolyl)]-Coβ-cyanocobamide} present in *Methanothrix soehngenii* (21). The results indicate that the corrinoid/iron-sulfur component of the enzyme complex contained only factor III.

The nickel/iron-sulfur component contained nickel, zinc, iron, and acid-labile sulfide; no corrinoid was detected (Table 1). The amount of nickel was low, which could be explained

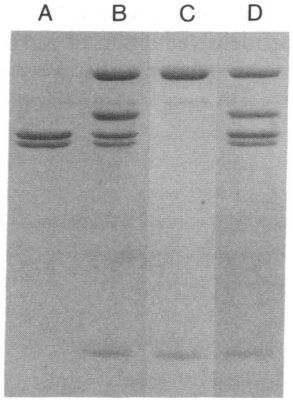

FIG. 1. Denaturing polyacrylamide gel electrophoresis of components obtained by anion-exchange chromatography of the detergent-treated enzyme complex from *M. thermophila*. Lane A, 2 μg of the corrinoid/iron-sulfur component eluted in 0.35 M KCl; lane B, 5 μg of detergent-treated enzyme complex; lane C, 2 μg of the nickel/iron-sulfur component eluted in 0.44 M KCl; lane D, 5 μg of the unresolved complex eluted in 0.55 M KCl. The apparent molecular masses of the subunits in the enzyme complex (lane B) are 89, 71, 60, 58, and 19 kDa (3). The gels were stained with Coomassie blue R-250.

Table 1. Composition of the enzyme complex and component proteins from *M. thermophila*

Protein	Subunit mass, kDa (composition)	Metals and cofactors,* mol/mol of protein					
		Ni	Zn	Co	Factor III	Fe	Acid-labile sulfur
Enzyme complex	89, 71, 60, 58, 19 ($\alpha_1\beta_1\gamma_1\delta_1\varepsilon_1$)	2.3 ± 0.2	1.7 ± 0.5	0.8 ± 0.1	0.8 ± 0.1	21.0 ± 3.0	19.7 ± 0.8
Nickel/iron-sulfur component	89, 19, ($\alpha_1\varepsilon_1$)	0.21 ± 0.02	2.7 ± 0.1	–	–	7.7 ± 1.3	13.2 ± 0.7
Corrinoid/iron-sulfur component	60, 58 ($\gamma_1\delta_1$)	–	–	0.7 ± 0.1	0.7 ± 0.1	3.0 ± 0.6	2.9 ± 0.2

*Minimum of three determinations. All samples were dialyzed as described in *Materials and Methods*. –, None detected.

by loss of the metal during isolation from the complex or losses incurred during dialysis prior to metals analysis.

Scanning densitometry indicated a subunit composition of $\alpha_1\beta_1\gamma_1\delta_1\varepsilon_1$ for the enzyme complex (data not shown), in agreement with previous results (9), which implies that the corrinoid/iron-sulfur and nickel/iron-sulfur components reside in the complex as $\alpha_1\varepsilon_1$ and $\gamma_1\delta_1$ dimers with respective molecular masses of 118 and 108 kDa. Gradient polyacrylamide gel electrophoresis indicated native molecular masses of 102 and 197 kDa for the isolated corrinoid/iron-sulfur and nickel/iron-sulfur components; similar results were obtained by the method of Hedrick and Smith (19). The results are consistent with a subunit composition of $\gamma_1\delta_1$ for the isolated corrinoid/iron-sulfur component. However, the native molecular mass (197 kDa) obtained for the isolated nickel/iron-sulfur component indicated an $\alpha_2\varepsilon_x$ subunit composition. Interestingly, all other methanogen CODHs isolated contain subunits of approximately 90 and 20 kDa and have an $\alpha_2\beta_2$ composition (22–25).

The UV–visible absorption spectra of the nickel/iron-sulfur and corrinoid/iron-sulfur components showed broad absorbance centered at 410 nm, characteristic of oxidized iron-sulfur centers (Figs. 3 and 4). Treatment of either component with mersalyl acid degraded the iron-sulfur centers as revealed by the loss of absorption. The spectrum of the iron-sulfur chromophore of each component was obtained by subtracting the spectrum of the mersalyl acid-treated component from the spectrum of the oxidized component (*Insets*, Figs. 3 and 4).

Enzyme Activities. A ferredoxin from *M. thermophila* has been described (10, 11) that accepts electrons from the enzyme complex. The nickel/iron-sulfur component catalyzed the CO-dependent reduction of ferredoxin or methyl viologen with turnover numbers that were 40% and 73% of the values obtained with the complex (Table 2). The corrinoid/iron-sulfur component was unable to catalyze the reduction of methyl viologen or ferredoxin with CO (data not shown). These results indicate that the nickel/iron-sulfur component is responsible for the CO-oxidizing activity of the enzyme complex.

Addition of dithionite to the nickel/iron-sulfur component decreased absorbance in the 410-nm region, indicating reduction of the iron-sulfur centers (Fig. 3). Identical results were obtained with 101-kPa CO as the reductant (data not shown). The spectrum of the isolated corrinoid/iron-sulfur component (Fig. 4) was characteristic of the Co^{2+} form of the corrinoid/iron-sulfur protein from the acetate-producing eubacterium *Clostridium thermoaceticum* (12). The corrinoid/iron-sulfur component from *M. thermophila* was not reduced by dithionite within 30 min, or with 101-kPa CO. However, addition of catalytic amounts of the nickel/iron-sulfur component (in the presence of CO) resulted in reduction of the iron-sulfur centers of the corrinoid/iron-sulfur component and yielded an absorption band centered at 390 nm (Fig. 4), characteristic of corrinoids in which the cobalt atom is reduced to the Co^{1+} oxidation state (12). The results suggested that the nickel/iron-sulfur component oxidized CO and donated electrons to the corrinoid/iron-sulfur component without the participation of additional electron carriers. The benzimadazolyl base of the corrinoid/iron-sulfur protein from *C. thermoaceticum* is not coordinated to the cobalt atom, thereby stabilizing the strongly nucleophilic Co^{1+} that reacts with CH_3I to form the "base-off" CH_3-Co^{3+} (12). The absorbance of the corrinoid/iron-sulfur component at 390 nm decreased upon addition of CH_3I (Fig. 4), consistent with formation of the methyl-cobalt bond (12); however, EPR studies are necessary before any conclusions can be drawn regarding the coordination state of the cobalt atom.

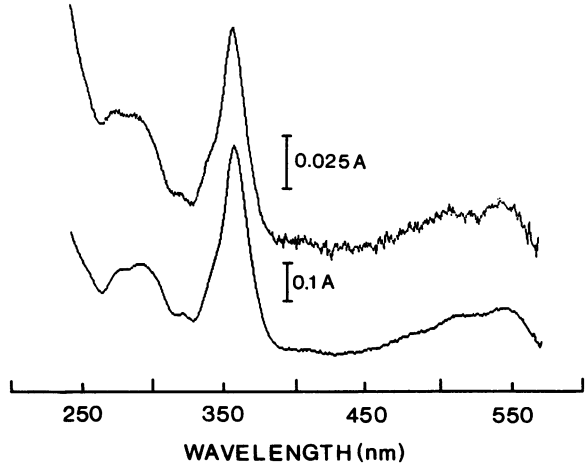

FIG. 2. UV–visible spectroscopy of factor III (33 μg/ml; upper spectrum) and the corrinoid cofactor isolated from the enzyme complex of *M. thermophila* (lower spectrum).

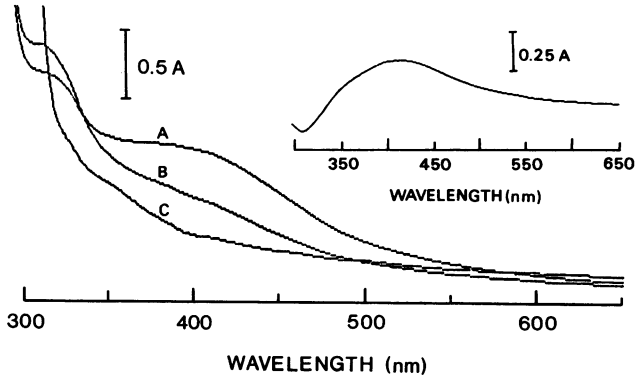

FIG. 3. UV–visible spectroscopy of the nickel/iron-sulfur component of the enzyme complex from *M. thermophila*. Spectra: A, oxidized component (4.3 mg/ml) under 101-kPa N_2; B, reduced with sodium dithionite; C, after addition of two to three crystals of mersalyl acid. (*Inset*) Spectrum of the iron-sulfur centers obtained by subtraction of spectrum C from spectrum A.

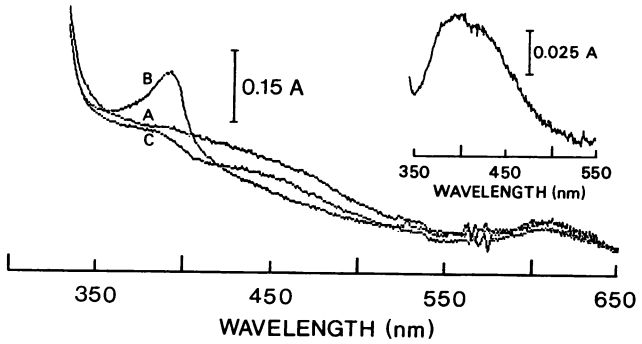

FIG. 4. UV–visible spectroscopy of the corrinoid/iron-sulfur component of the enzyme complex from *M. thermophila*. Spectra: A, the component as isolated from the complex under 101-kPa N_2 (2.1 mg/ml); B, corrinoid/iron-sulfur component (2.1 mg/ml) reduced by the addition of the nickel/iron-sulfur component (0.027 mg/ml) under 101-kPa N_2 and 50-kPa CO for 5 min at 23°C; C, reduced corrinoid/iron-sulfur component treated with 93 μM CH_3I. (*Inset*) Spectrum of the oxidized corrinoid/iron-sulfur component (2.2 mg/ml) after subtraction of the spectrum (not shown) of the mersalyl acid-treated component to reveal the spectrum of the iron-sulfur centers.

The enzyme complex catalyzed the exchange of CO with the carbonyl group of acetyl-CoA (29 nmol per min per mg) and synthesis of acetyl-CoA from CH_3I, CO, and CoA (48 nmol per min per mg). However, the nickel/iron-sulfur and corrinoid/iron-sulfur components were unable to catalyze either activity when assayed separately or combined. Preincubation of the components with Ni^{2+} and Fe^{2+} did not restore activity.

DISCUSSION

The results indicate that the enzyme complex isolated from *M. thermophila* consists of at least two protein components, each with a specific function. The nickel/iron-sulfur component has several properties in common with the CODH (acetyl-CoA synthase) from the acetogenic eubacterium *C. thermoaceticum* (12, 14). Both enzymes are two-subunit nickel-containing iron-sulfur proteins with CO-oxidizing activity that reduce ferredoxin. Each of the enzymes from *M. thermophila* (this report) and *C. thermoaceticum* (12) is associated with a unique two-subunit corrinoid/iron-sulfur protein, both CO-oxidizing enzymes transfer electrons to the respective corrinoid/iron-sulfur proteins in the absence of additional electron carriers. The acetyl-CoA synthase from *C. thermoaceticum* catalyzes the synthesis of acetyl-CoA from CH_3I, CO, and CoA with an absolute requirement for the corrinoid/iron-sulfur protein, which is methylated with

Table 2. CODH activity of the enzyme complex and nickel/iron-sulfur component from *M. thermophila*

Enzyme	Methyl viologen		Ferredoxin	
	SA*	TO†	SA*	TO†
Complex				
Undialyzed	35.8	10,650	1.42	426
Dialyzed	26.3	4,118	ND	ND
Nickel/iron-sulfur component				
Undialyzed	72.3	7,810	1.6	170
Dialyzed	23.7	2,556	ND	ND

Reaction mixtures (1 ml) contained either 12 μg of enzyme complex or 20 μg of nickel/iron-sulfur component. Ferredoxin (100 μg) or methyl viologen (10 mM) was added as electron acceptor as indicated. The assay temperature was 23°C. ND, not determined.
*Specific activity, μmol per min per mg of protein.
†Turnover, min^{-1}, calculated with a molecular mass of 297 kDa (complex) or 108 kDa (nickel/iron-sulfur component).

CH_3I followed by transfer of the methyl group to the synthase (7). The five-subunit enzyme complex from *M. thermophila* also catalyzes the synthesis of acetyl-CoA from CH_3I, CO (or $CO_2 + 2e^-$), and CoA (4); in addition, the complex catalyzes an exchange of CO with the carbonyl group of acetyl-CoA, which demonstrates acetyl-CoA cleavage activity (5). A reduced Ni-Fe center has been proposed as the site of acetyl-group synthesis on the acetyl-CoA synthase of *C. thermoaceticum* (7). The CO-reduced enzyme complex from *M. thermophila* also has a Ni-Fe-C center with an EPR spectrum (26) indistinguishable from the spectrum of the CO-reduced Ni-Fe-C center in the acetyl-CoA synthase from *C. thermoaceticum* (27); the signal from both enzymes is perturbed on addition of acetyl-CoA. Thus, the reduced Ni-Fe center in the enzyme complex from *M. thermophila* is the postulated site of synthesis of acetyl-CoA (26). We propose that the methyl group for acetyl-CoA synthesis is derived from the methylated corrinoid/iron-sulfur component, in analogy to the well-characterized *C. thermoaceticum* system (7, 12, 27, 28). Whole-cell studies with the acetate-utilizing methanogen *Methanosarcina barkeri* suggest that a corrinoid protein is required for synthesis of acetate from CH_3I, CO, and CoA (29). The cobalt atom of the active corrinoid/iron-sulfur protein from *C. thermoaceticum* is in the Co^{1+} state (a strong nucleophile), which reacts with the methyl donor (28). Thus, another proposed function of the nickel/iron-sulfur component from *M. thermophila* is reduction of the corrinoid/iron-sulfur component, which places cobalt in an active redox state. However, EPR studies are necessary to determine the redox state of the cobalt atom and characterize the proposed methylcorrinoid intermediate. We further propose that, during methanogenesis from acetate, the nickel/iron-sulfur component of the *M. thermophila* enzyme complex catalyzes the cleavage of acetyl-CoA followed by oxidation of the carbonyl group to CO_2 and transfer of the methyl group to the corrinoid/iron-sulfur component. Indeed, results with cell extracts of *M. barkeri* show that a corrinoid protein is the methyl acceptor after cleavage of acetyl-CoA (30).

In the absence of the corrinoid/iron-sulfur protein, the acetyl-CoA synthase from *C. thermoaceticum* catalyzes an exchange of CO with the carbonyl of acetyl-CoA, which demonstrates the C-C bond cleavage and synthesis activities of this enzyme (31). Although the nickel/iron-sulfur component of the enzyme complex from *M. thermophila* catalyzed a robust CO oxidation, the component was incompetent in catalysis of the CO/acetyl-CoA exchange reaction. In addition, the nickel/iron-sulfur component was unable to synthesize acetyl-CoA from CH_3I, CO, and CoA when combined with the corrinoid/iron-sulfur component. The results could be explained by (*i*) inactivation of the components during resolution of the enzyme complex, (*ii*) a requirement for the unresolved β (71-kDa) subunit, or (*iii*) incorrect conditions for reconstitution of the components into an active complex.

The results presented here support earlier proposals (9, 29, 30) that CODH and corrinoids are central to the pathway of acetate conversion to methane, and define a starting point for studies on the biochemical mechanism of acetyl-CoA cleavage, methyl transfer, and electron transport in the pathway. In addition, the similarities with the acetyl-CoA synthase system from the acetogenic eubacteria form the basis for comparative studies to facilitate an understanding of the mechanism and evolution of CODHs from anaerobic microorganisms.

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