Kinetic Characterization of the Carbon Monoxide–Acetyl-CoA (Carbonyl Group) Exchange Activity of the Acetyl-CoA Synthesizing CO Dehydrogenase from *Clostridium thermoaceticum*†

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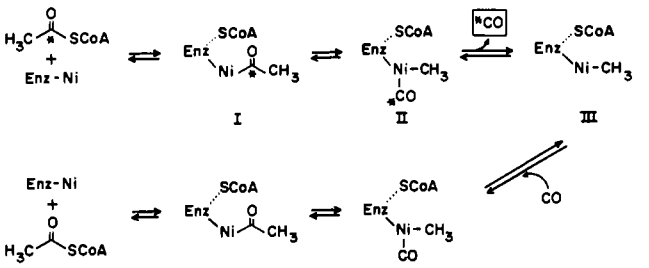
ABSTRACT: CO dehydrogenase from *Clostridium thermoaceticum* is a nickel-containing enzyme that catalyzes both the reversible conversion of CO_2 to CO (for incorporation into the carbonyl group of acetate) and the synthesis of acetyl-CoA from methyl corrinoid, CO, and CoASH. The latter activity is conveniently assayed by monitoring the exchange of [1-^{14}C]acetyl-CoA (carbonyl group) with ^{12}CO. Kinetic parameters for the highly oxygen sensitive exchange activity have been determined: K_m (acetyl-CoA) = 600 μM; V_{max} = 440 min^{-1}. In addition, coenzyme A analogues have been tested as inhibitors of the exchange to probe the active site of the enzyme; each has no effect on the $CO_2 \rightleftharpoons$ CO activity of CO dehydrogenase. Coenzyme A, the substrate for acetate biosynthesis, is a potent competitive inhibitor, K_I = 7 μM. Comparison of this value with that for desulfo-CoA (K_I = 6000 μM) suggests that a key mode of binding is through the sulfur atom, possibly to a metal site on the enzyme. The relatively high affinity of the enzyme for CoASH relative to acetyl-CoA is consistent with its proposed operation in the acetogenic direction. The differential sensitivity to oxygen and storage of the two activities of CO dehydrogenase as well as the contrasting effect of coenzyme A inhibitors suggests that acetate assemblage occurs at a site distinct from that for CO dehydrogenation.

Acetogenic and methanogenic bacteria growing on CO_2 as the sole carbon source generate acetate units by a novel carbon–carbon bond forming process. In an extended series of studies the groups of Wood, Ljungdahl, and others (Wood et al., 1986; Ljungdahl & Wood, 1982; Diekert & Thauer, 1978; Diekert et al., 1979) demonstrated that one CO_2 molecule is successively reduced by six electrons to yield the organocobalt methyl coenzyme B12 species as the proximal source of the acetyl methyl group while the second CO_2 molecule is reduced by two electrons to CO by a nickel–iron enzyme, termed CO dehydrogenase. The carbon–carbon bond formation step is then catalyzed by this same nickel enzyme acting as an acetyl-CoA[1] synthase. This is the first indication of a possible enzyme-mediated organometallic carbonylation in carbon skeleton biogenesis, and as such this unique enzyme is an object of unusual mechanistic interest. The enzyme from the acetogenic *Clostridium thermoaceticum* is best characterized and was shown by Ragsdale et al. (1983) to be an $\alpha_3\beta_3$ hexamer of molecular weight 440000 with 6 nickel, 3 zinc, 33 iron, and 42 acid labile sulfur atoms.

The crucial role of this CO dehydrogenase/acetyl-CoA synthase was recently and conclusively established in a seminal contribution by Ragsdale and Wood (1985), who demonstrated that homogeneous enzyme, without other proteins, catalyzed the isotopic exchange of CO with the carbonyl group of acetyl-CoA. This is a process best explained by reversible fragmentation of acetyl-CoA to a methyl fragment, a CoASH fragment, and a CO fragment stored in the enzyme active site, selective dissociation of bound CO and exchange with CO in solution, and then enzymic reassembly of the acetyl-CoA. A turnover number of 70 min^{-1} was estimated for the carbonyl exchange, a value 2500-fold slower than the CO dehydrogenase activity, assayed by CO-dependent reduction of methylviologen.

Scheme I



Using the carbonyl exchange assay to monitor acetyl-CoA synthase activity, we recently examined the stereochemical outcome at C-2 of acetyl-CoA after enzyme-catalyzed carbon–carbon breakage and reformation (Raybuck et al., 1987). Complete retention of configuration was observed after decarbonylation/recarbonylation of both *R* and *S* chiral methyl (H, D, T) acetyl-CoA. Such a result is easily accounted for by envisioning carbon–carbon bond formation as a metal-based CO insertion into a metal–methyl bond, known to proceed with retention of stereochemistry. Given EPR evidence that both CO and CoASH interact with the nickel site and the organometallic precedents for nickel-based carbonylations (Kohara et al., 1979), we ascribed this unique acetyl-CoA synthase ability to nickel and postulated the biosynthesis of

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[1] Abbreviations: acetyl-CoA or AcCoA, acetyl-coenzyme A; CoA or HSCoA, coenzyme A; CODH, carbon monoxide dehydrogenase; DTT, dithiothreitol; Enz, enzyme; EPR, electron paramagnetic resonance; HPLC, high-performancce liquid chromatography; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

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acetate occurring via methyl–nickel and acetyl–nickel species (see Scheme I). In our continuing efforts to elucidate the carbon–carbon bond forming mechanism of this complex metalloenzyme, we have begun to characterize further the carbonyl exchange of [1-^{14}C]acetyl-CoA as a probe of the acetyl-CoA synthase activity and report our initial efforts in this area.

Materials and Methods

General. [1-^{14}C]Acetyl-CoA (54 mCi/mmol) was purchased from either New England Nuclear Research Products or Amersham Corp. [1-^{14}C]Propionyl-CoA (50 mCi/mmol) and [^{3}H]acetyl-CoA (2.5 Ci/mmol) were purchased from Amersham. Pantetheine 11-pivalate was synthesized by the method of Davis et al. (1987). Coenzyme A and all other coenzyme A derivatives were purchased from Sigma Chemicals and checked for purity by HPLC. HPLC analysis of coenzyme A thioesters and reaction mixtures was conducted on a Waters Associates liquid chromatograph equipped with a 214-nm detector and a Vydak 10-μM C18 reverse-phase analytical column that was eluted isocratically with 15% methanol and 0.1 M potassium phoshate buffer, pH 5.5. Retention times are identical with those reported by Patel and Walt (1987). Prepurified carbon monoxide and argon gases were purchased from Matheson Gas Products and Airco, respectively; oxygen scrubbers were from VWR or MG Scientific. Mixtures of carbon monoxide in argon at room temperature were prepared on a gas manifold system in flasks containing a small quantity of alkaline pyrogallol solution and were allowed to equilibrate over a 24-h period with rapid stirring. EPR spectra were collected at 173 or 77 K on a Varian E-9 EPR spectrometer operating at X-band. Enzyme samples containing 20–50 μM nickel were prepared anaerobically and stored in liquid nitrogen at 77 K.

Protein Purification. C. thermoaceticum (DSM 521) was grown as described (Diekert & Thauer, 1978); the cells were harvested under argon and frozen in liquid nitrogen. All protein purification was carried out by using CO- and argon-saturated buffers inside a helium-filled anaerobic chamber. CO dehydrogenase was prepared from *C. thermoaceticum* by the method of Ragsdale and Wood (1985), using DEAE-Sepharose, phenyl-Sepharose, hydroxylapatite, and agarose-CoA chromatography, in that order. For kinetic studies, the enzyme was used after the phenyl-Sepharose column, at approximately 25% purity as determined by SDS–PAGE.

Assays. Protein was determined by using the method of Bradford (1976). CO dehydrogenase activity was determined at 55 or 25 °C with 5 mM methylviologen under carbon monoxide as previously described (Ragsdale & Wood, 1985). The acetyl-CoA synthase activity was determined by using the carbonyl exchange assay, converting [1-^{14}C]acetyl-CoA to ^{14}CO under a CO atmosphere (Ragsdale & Wood, 1985; Raybuck et al., 1987). The standard assay mixture contained 0.85 mL of KP_i/DTT (150 mM/1 mM), pH 6.0 at 55 °C, 0.2 mM methylviologen, 6.25 nmol of [1-^{14}C]acetyl-CoA (specific activity 54 mCi/mmol), 194 nmol of acetyl-CoA, and 0.5 mg of CO dehydrogenase. All buffers and the reaction vessel containing a magnetic stirbar and fitted with tandem septum seals were rigorously degassed under carbon monoxide (Bienert et al., 1978). The enzymic solution was preincubated at 55 °C for 5 min under a CO atmosphere at 1.2 atm, and the assay was initiated by the addition of acetyl-CoA substrate via gastight syringe. For kinetic studies 100-μL aliquots were taken at $t = 0$ and regular intervals, and the extent of exchange was calculated from the difference in the amount of radioactivity remaining in the aqueous phase. For the determination of K_m values for CO, initial rates were measured from assays conducted by using mixtures of CO in argon prepared as described earlier. The carbonyl exchange assay using [1-^{14}C]propionyl-CoA was performed in the same manner with 2 mM substrate and 2 mg of CO dehydrogenase. No carbonylation was detected after a 2-h period at 55 °C.

Kinetic Analysis. Initial exchange velocities were calculated from time course data by using the equation derived by Segel (1975) for a pair of molecules undergoing isotopic exchange

$$v^* = -\frac{[CO][AcCoA]}{[CO] + [AcCoA]}\frac{1}{t}[\ln (1 - F)] \qquad (1)$$

where F is the fraction of isotopic equilibrium at time t and the substrate concentrations are a measure of the total number of molecules available for isotopic exchange. Under the conditions of the assay (55 °C vigorous stirring) the CO in solution will be in rapid equilibrium with that in the gas phase; using 100% CO, all the radioactivity initially present in [1-^{14}C]acetyl-CoA will be converted to ^{14}CO at isotopic equilibrium as [CO] $\gg$ [AcCoA]. This simplifies eq 1 to

$$v^* = -[AcCoA](1/t)[\ln (1 - F)] \qquad (2)$$

Plots of t vs ln (1 − F) were linear at short reaction times, from which the slope (equal to $-v^*/[AcCoA]$) and subsequently the initial exchange velocity could be determined (Figure 1). Michaelis constants were derived from Lineweaver–Burk analysis of initial velocity data. Lineweaver–Burk plots at three concentrations of inhibitor (including [I] = 0) were used to determine inhibition constants (K_I) of the carbonyl exchange assay for coenzyme A (see Figure 2) and propionyl-CoA (data not shown). The nature of the inhibition in both cases was analyzed by the methods of Cleland (1979). Of the simple inhibition patterns, reasonable kinetic parameters could only be obtained by assuming competitive inhibition; neither set of data could be fit to noncompetitive or uncompetitive inhibition patterns. A small contribution of mixed inhibition cannot, however, be rule out. All other inhibitors were tested under standard assay conditions (varying [I]); inhibition constants (K_I) were determined from Dixon plots.

In determining the K_m for CO, CO was not always in great excess over the concentration of acetyl-CoA, and eq 1 was used in determining initial rates for mixtures containing 3% CO or less. Michaelis constants were derived as above. The concentration of CO in solution was calculated to be 0.58 mM (100% CO, 1.2 atm, 55 °C) by using a Bunsen coefficient of 0.1310, obtained by interpolation of values at 50 and 60 °C (Allamaguy, 1976).

Results and Discussion

CO dehydrogenase/acetyl-CoA synthase from *C. thermoaceticum* requires rigorously anaerobic conditions for purification and assay. We have been able to purify the enzyme to homogeneity on several occasions following modifications of the Diekert and Ritter (1983) or Ragsdale et al. (1983) procedures, including assaying the CO dehydrogenase activity by CO $\rightleftharpoons$ CO_2 conversion with reduction of the artificial electron acceptor methylviologen. However, we repeatedly find that the acetyl-CoA synthase activity, as assayed by the decarbonylation/recarbonylation of [1-^{14}C]acetyl-CoA in the presence of CO, is extremely labile and *sensitive to the slightest traces of oxygen.* Further, some cell extracts, after brief aerobic exposure, yield enzyme with full CO dehydrogenase activity but essentially no acetyl-CoA synthase ability. This differential lability of the two activities was mentioned by Ragsdale and Wood (1985) in their initial report,

noting that some samples lose exchange activity during storage faster than the CO dehydrogenase activity. We find that, during a dozen or more separate enzyme preparations, careful monitoring of both activities indicated the enzyme stored with scrupulously anaerobic conditions at 4 °C under CO is active for carbonyl exchange for only 10–12 days whereas CO-dependent methylviologen reduction activity is undiminished for months.

We suggest two conclusions: First, we note that the reduction of CO_2 to CO may occur on this complex metalloenzyme elsewhere from the site of assembly of the methyl and carbonyl fragments to acetyl groups. Diekert et al. (1984) showed CO formation from CO_2 was inhibited by cyanide in whole cells of *C. thermoaceticum*, although no effect of cyanide on the incorporation of CO into acetate was seen, an observation consistent with the presence of two distinct sites for the two activities of CO dehydrogenase. This concept is also supported by Ragsdale and Wood's (1985) data on selective inhibition of the carbonyl exchange by arginine group reagents and by coenzyme A. We have extended this list with the present study of coenzyme A analogues (vide infra) that serve as inhibitors of the acetyl-CoA synthase activity, none having any effect on the CO $\rightleftharpoons$ CO_2 assay (at a concentration of 2 mM).

Second, we emphatically restate that a positive CO $\rightleftharpoons$ CO_2 assay, the usual convenient measure of CO dehydrogenase activity, by no means ensures the enzyme preparation is active in carbon–carbon bond formation and cleavage. We therefore recommend that *both* assays be used routinely to characterize this enzyme, *the carbonyl exchange activity being the sole present criterion for active acetyl-CoA synthase*, absent the methyl donor system. The differential lability of the two activities also calls into question the interpretability of spectroscopic probes of the metal center(s), particularly in ascertaining the unique role of nickel, when only the CO dehydrogenase (CO $\rightleftharpoons$ CO_2) assay is used to assure the activity of the preparation.

Kinetic Studies. Because of the lability of the [1-^{14}C]-acetyl-CoA $\rightleftharpoons$ CO exchange, we have routinely used enzyme of 20–40% homogeneity, rapidly isolated anaerobically, to acquire an initial kinetic characterization of the decarbonylation activity of this enzyme. We do observe a 2000/1 ratio of CO-linked methylviologen reduction/carbonyl exchange just as reported by Ragsdale and Wood. In their initial paper no time course or other kinetic data for the carbonyl exchange were presented, and the maximal exchange detected, at a single 30-min time point, was at best 52%. Following the Ragsdale and Wood assay conditions and taking aliquots to monitor radioactivity not yet exchanged into the gas phase as ^{14}CO, we again reproduced their observations and validated that the exchange ceased at 40–50% loss of radioactivity from the aqueous phase even at very large excess of CO gas. HPLC analysis of the assay solution at the end of the run revealed only 7% of the initial radioactivity remained in the acetyl-CoA fraction, suggesting a competing reaction of the acetyl group. As less than 5% of the radiolabel was observed in the acetate fraction, enzymic hydrolysis of acetyl-CoA to acetate was ruled out, and attention was focused on the 5 mM dithiothreitol present in the assay mixture with the possibiity that enzyme-mediated acetyl transfer to DTT was a competing process. Indeed, as the DTT concentration was lowered from 5 to 0.5 mM, the extent of decarbonylation rose from 50% to >85%, and the initial rate increased almost 2-fold. One cannot lower the DTT concentration further because the enzyme appears to require reduced thiols for the carbonyl exchange.

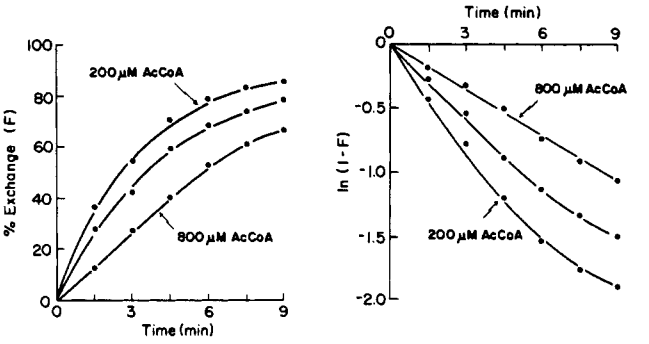

FIGURE 1: (Left) Typical time course of the CO dehydrogenase catalyzed carbonyl exchange: [1-^{14}C]acetyl-CoA + CO $\rightleftharpoons$ ^{14}CO + acetyl-CoA. Acetyl-CoA concentrations are 200, 400, and 800 μM. (Right) Semilog plot of the data at left from which initial rates and kinetic constants are derived. All reactions proceed to 85% exchange at 40 min (data not shown).

At 1 mM DTT, by use of a mixture of [1-^{14}C]acetyl-CoA and [^{3}H]acetyl-CoA the CO dehydrogenase catalyzed carbonyl exchange proceeds to 85%. HPLC analysis of the acetyl-CoA at the end of reaction routinely recovers 85–90% of the tritium radioactivity demonstrating that little destruction of the starting substrate occurs during the time course of the assay. Linear initial velocity kinetics were obtained under these conditions for the [1-^{14}C]acetyl-CoA CO exchange; representative data are shown in Figure 1. The K_m for acetyl-CoA at 55 °C, pH 6.0, ranges from 0.45 to 0.80 mM with a V_{max} of 100–500 nmol min^{-1} mg^{-1}, depending on the history of the enzyme. The highest value observed so far, corrected to perfect protein homogeneity, was 1000 nmol min^{-1} (mg of $\alpha_3\beta_3$ hexamer)$^{-1}$, a turnover number of 440 min^{-1}. The K_m for CO in the carbonyl exchange assay was determined to be 0.010 mM. For comparison the K_m for the CO dehydrogenase assay was analogously 0.025 mM at pH 7.2, with approximately 1000-fold higher V_{max}. The organism is a thermophile, and indeed the maximal rate for the carbonyl exchange was observed at 75 °C, decreasing to half-maximal at 65 °C; the activity is almost undetectable at 25 °C. Correspondingly, the CO dehydrogenase assay peaks at 85 °C falling to half-maximal at 70 °C.

CO dehydrogenase isolated and stored under argon exhibits an axial EPR signal attributable to nickel at g = 2.074 and 2.028 when exposed to CO under anaerobic conditions, as reported in the literature (Ragsdale et al., 1985). We have found this signal shifts to g = 2.056 and 2.028 using enzyme isolated and stored under CO. We do not understand the nature of this effect at the present time, but there appears to be no difference kinetically between these two types of preparations, as K_m(acetyl-CoA) = 0.80 mM, V_{max} = 450 nmol min^{-1} mg^{-1}, and K_I(CoASH) = 7 μM for CO dehydrogenase prepared and stored under argon.

Inhibitors and Alternate Substrates. It has been reported that coenzyme A is an inhibitor of the carbonyl exchange, 3 mM CoASH reducing the extent of exchange by 93%. A reciprocal plot of initial velocities in the presence of micromolar amounts of coenzyme A is shown in Figure 2. Objective analysis of the data (Cleland, 1979) confirmed the competitive nature of the inhibition giving a K_I of 7 μM. This value is quite striking when compared to those obtained with the coenzyme A analogues in Table I tested as inhibitors to probe the active site of the acetyl-CoA synthase activity. Modification of the nucleotide portion of coenzyme A reveals pivaloylpantetheine-SH is a less effective inhibitor by a factor of 150. *However, excision of the sulfur atom from coenzyme A in desulfo-CoA causes nearly a 3 order of magnitude in-*

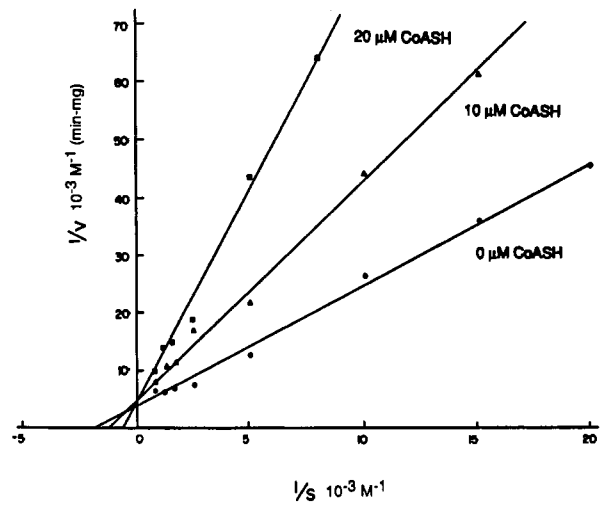

FIGURE 2: Inhibition by coenzyme A of the CO dehydrogenase catalyzed carbonyl exchange under standard conditions of 200 μM acetyl-CoA: [1-^{14}C]acetyl-CoA + CO ⇌ ^{14}CO + acetyl-CoA (●) 0 μM CoASH; (▲) 10 μM CoASH; (■) μM CoASH.

Table I: Substrates and Inhibitors of the Carbonyl Exchange Assay[a]

inhibitors[b]	K_I (μM)	substrates	K_m (μM)
CoASH	7	[1-^{14}C]acetyl-CoA	600
dephospho-CoASH	35	[1-^{14}C]propionyl-CoA	*c*
pivaloylpantetheine-SH	1200		
desulfo-CoAH	6000		
propionyl-CoA	1300		
crotonyl-CoA	1400		
butyryl-CoA	1450		

[a] The CO dehydrogenase catalyzed carbonyl exchange, [1-^{14}C]-acetyl-CoA + CO ⇌ ^{14}CO + acetyl-CoA, was performed under standard conditions as described in the text. [b] Each of the inhibitors listed has no effect on the CO dehydrogenase assay at a concentration of 2 mM. [c] No decarbonylation detected after a 2-h reaction time.

crease in K_I. We suggest sulfur interaction with a metal center to be a major contribution to the high affinity of coenzyme A for acetyl-CoA synthase. Changing this thiol group into a thioester results in a decrease of affinity for the enzyme as K_m(acetyl-CoA)/K_I(CoASH) = 75/1, in accord with a loss of nucleophilicity and electron-donor ability to a metal site. Longer chain acyl-CoA esters are less effective inhibitors, and at this level we cannot unequivocably rule out possible contamination by a minute amount (<1%) of coenzyme A, now known by these results to be a potent inhibitor.

As an initial probe of acyl-CoA specificity, we have noted that while propionyl-CoA is an inhibitor (K_I = 1300 μM) of [1-^{14}C]acetyl-CoA decarbonylation/recarbonylation, there is no detectable enzymic decarbonylation of [1-^{14}C]propionyl-CoA. This isotopic exchange involves the cleavage of both the carbon–sulfur and carbon–carbon bonds of acyl-CoA (at least three steps). In addition, the decarbonylation/recarbonylation reaction requires each one of these to be reversible under the assay conditions. Partial enzymic disassembly of propionyl-CoA could occur, but the enzymic fragments may be trapped kinetically or thermodynamically at stages analogous to I, II, or III (see Scheme I).

It should be possible to ascertain whether the carbon–sulfur bond of propionyl-CoA is cleaved by the enzyme to form an acyl-enzyme intermediate, the first stage of Scheme I. Pezacka and Wood (1986) have demonstrated that CO dehydrogenase in conjunction with a disulfide reductase will catalyze an exchange of [^{3}H]CoASH with acetyl-CoA, indicating the presence of an acetyl-enzyme intermediate. Unfortunately, this second exchange as reported proceeds at best to 10% reaction at 30 min, at approximately 1% the rate of the carbonyl exchange. The kinetic relevance of the CoASH ⇌ acetyl-CoA exchange to acetate biosynthesis has yet to be established. In light of our present data, we ponder whether this second exchange is rate limited by very slow dissociation of CoASH from the enzyme site, given the high affinity of coenzyme A for acetyl-CoA synthase. Further studies delineating the kinetic role of the CoASH ⇌ acetyl-CoA exchange and the fate of propionyl-CoA under the decarbonylation/recarbonylation assay conditions are needed before definite conclusions can be drawn.

Concluding Remarks

Two broadly defined variants of CO dehydrogenase have been isolated from anaerobic bacteria, both containing nickel, iron-sulfur centers, and varying amounts of zinc. The first class possesses only CO ⇌ CO_2 activity, is not involved in acetate metabolism, and shows similarities to the molybdenum-containing CO dehydrogenases of aerobic bacteria (CO inducible, membrane bound). The second class has an additional acetyl-CoA synthase or decarboxylase cleavage activity as the primary function of the enzyme appears to be in assimilative or dissimilative acetate metabolism. Representative of the first class is the CO dehydrogenase from the photosynthetic bacterium *Rhodospirillum rubrum*, extensively characterized by Bonam et al. (1984) as a monomeric protein of 62K (α) containing 0.6 nickel and iron–sulfur clusters and having no detectable acetyl-CoA synthase or acetyl-CoA decarbonylation activity (Bonam & Ludden, 1987). Cells grown under CO in the absence of nickel produce nickel-deficient apo-CO dehydrogenase, which is incapable of CO ⇌ CO_2 conversion. In vitro activation by $NiCl_2$ restores full CO dehydrogenase activity, strongly implicating nickel as the site of CO oxidation. However, no signals due to nickel could be detected by EPR spectroscopy under a variety of conditions; all signals are due to Fe-S centers, and these are identical in both the holo- and apoprotein, leading the authors to conclude that the EPR-silent nickel site is separate and distinct from the Fe-S centers of the protein (Bonam et al., 1988).

CO dehydrogenase/acetyl-CoA synthase from *C. thermoaceticum* is the best characterized from the second class of enzymes. The composition of this enzyme parallels the complexity of its dual functionality being an $\alpha\beta$ trimer containing 2 Ni, 1 Zn, 11 Fe, and 14 S per monomer. Our present work provides additional evidence (differential oxygen sensitivity, selective inhibition) that these two activities occur at different sites on this complex metalloenzyme, even though the K_m(CO) values for the two assays are not distinctly different. Also, inhibition studies of the acetyl-CoA ⇌ CO exchange indicate that the site of acetate assembly has a high affinity for coenzyme A, in particular the thiol group, consistent with an important metal–sulfur ligation. If the findings from the less complex *R. rubrum* enzyme can be extrapolated to the clostridial enzyme, one would anticipate nickel as the site of CO ⇌ CO_2 conversion. Therefore, acetate assemblage should occur elsewhere, possibly a Fe-S center. However, at least one Ni center in the clostridial enzyme is markedly different from the nickel present in the *R. rubrum* congener, being EPR active under CO and assigned to be part of a mixed Ni-Fe center as evidenced by EPR (Ragsdale et al., 1985) and EXAFS studies (Bastian et al., 1988). In addition, coenzyme A has been observed to alter the nickel-site EPR, suggestive of binding at or near the nickel center; thus, an alternative possibility is that the two activities of acetyl-CoA synthase/CO dehydrogenase may be attributed to two distinct nickel sites on the acetogenic enzyme. A similar EPR-active Ni-Fe center

has been characterized in the $\alpha\beta\gamma\delta\epsilon$ CO dehydrogenase from acetate-grown *Methanosarcina thermophila* (Terlesky et al., 1986, 1987). Acetyl-CoA, and not CoASH, has been observed to alter the nickel-site EPR of this methanogenic enzyme. The enzyme has been proposed to function in acetate cleavage; attempts to demonstrate acetyl-CoA $\rightleftharpoons$ CO exchange have been unsuccessful to date.

Recently, Pezacka and Wood (1988) have reported methylation of the clostridial CO dehydrogenase using $^{14}CH_3I$ or $^{14}CH_3$ corrinoid enzyme; 50% of the available label on the CO dehydrogenase can be stoichiometrically converted to acetyl-CoA in the presence of CO and CoASH. The methyl group site of attachment was identified as *S*-methylcysteine located on the β subunit. It may be that the cysteine acts as a shuttle between the corrinoid and the site of carbonylation on CO dehydrogenase. This important finding does not rule in or out the presence of methylnickel or methyliron intermediates on the reaction pathway, but should serve as a starting point for elucidating the detailed mechanism of methyl group carbonylation in acetogenic bacteria.

Finally, we return to the value of K_m(acetyl-CoA)/K_I(CoASH) = 75/1 as a reflection of the substrate/product affinity for CO dehydrogenase active in acetate biosynthesis. If the clostridial acetyl-CoA synthase is indeed designed to run biosynthetically in the direction of acetyl-CoA production, a high affinity for coenzyme A, perhaps as a nickel-site ligand, would facilitate thiolytic capture of an acetyl intermediate and drive carbonylation in the direction of carbon–carbon bond formation. Thus, in acetogenic bacteria, acetyl-CoA synthase may be poised as an enzyme–SCoA complex, set to bind nascent CO generated from CO_2 within the enzyme via its CO dehydrogenase activity and trap any CH_3 transferred from the methyl corrinoid enzyme. Given the similarities already uncovered in the metal centers between the clostridial enzyme and its methanogenic counterpart from *M. thermophila*, (Terlesky et al., 1986, 1987), we speculate that in methanogenic bacteria growing on acetate the reverse might be true; acetyl-CoA synthase operating as a biodegradation catalyst could have a high affinity for acetyl-CoA and a lower one for the free thiol, possibly controlled by the oxidation state of the metal center.

Acknowledgments

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