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PROGRESS REPORT

Mechanistic enzymology of CO dehydrogenase from *Clostridium thermoaceticum*
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Progress Report, Mechanistic enzymology of CO dehydrogenase from *Clostridium thermoaceticum*

The final steps in acetyl-CoA biosynthesis by anaerobic bacteria are performed by carbon monoxide dehydrogenase (CODH), a nickel/iron-sulfur protein. Over the past two years, we have focused on characterizing the pathway shown in Figure 1. An important achievement was to establish conditions under which acetyl-CoA synthesis by purified enzymes equals the in vivo rate of acetate synthesis. Under these optimized conditions we established that the rate limiting step in the synthesis of acetyl-CoA from methyl-H_4folate, CO and CoA is likely to be the methylation of CODH by the methylated corrinoid/iron-sulfur protein (C/Fe-SP). We then focused on stopped flow studies of this rate limiting transmethylation reaction and established its mechanism. We have studied the carbonylation of CODH by vibrational spectroscopy (infrared and resonance Raman) and determined that the [Ni-Fe_{3-4}-S_4]-CO species which has been characterized by magnetic resonance methods can be described as [Ni-X-$Fe_{3-4}S_4$]-C≡O. We showed that this species is the catalytically competent precursor of the carbonyl group of acetyl-CoA. We have made progress in the synthesis of seleno-coenzyme A, which we will use to probe the binding of CoA to CODH. We also have compared the CODH from *Methanosarcina thermophila* with the *C. thermoaceticum* enzyme by EPR and electrochemical methods and found that the metal sites of these enzymes are remarkably similar given the evolutionary separation between archaea and bacteria domains.

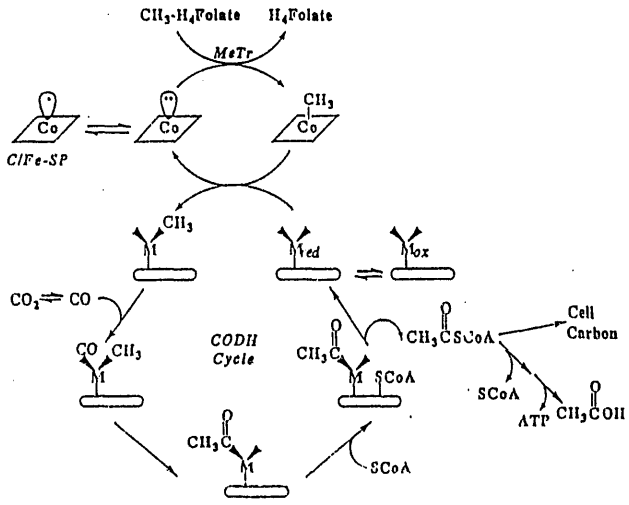

Figure 1

1. Acetyl-CoA Synthesis from Methyltetrahydrofolate, CO and CoA by Enzymes Purified from *C. thermoaceticumm*: Attainment of In Vivo Rates and Identification of Rate Limiting Steps

The work described in this section was recently published (1). Enzymes involved in the synthesis of acetyl-CoA from methyltetrahydrofolate, CO and CoA are CODH, methyltransferase (MeTr), and a corrinoid/iron-sulfur protein (C/Fe-SP) (see Figure 1). Our goals were three-fold: (i) to optimize the method for determining the activity of the synthesis of acetyl-CoA, (ii) to evaluate how closely the rate of synthesis of acetyl-CoA by purified enzymes approaches the rate at which whole cells synthesize acetate, and (iii) to determine which steps limit the rate of acetyl-CoA synthesis. One criticism of schemes describing the mechanism of acetyl-CoA synthesis has been that the rates which had been previously reported of acetyl-CoA synthesis using purified enzymes were 10-20 fold slower than the expected rates based on the *in vivo* rates of synthesis of acetate by whole cells (2). The in vivo rate is ~ 0.1 μmol min^{-1} mg^{-1} of whole cell protein. We determined that the levels of CODH, MeTr, and C/Fe-SP are all ~ 2-3% of cell protein (1); therefore the expected rate of acetyl-CoA synthesis under optimal conditions using purified enzymes is 3-5 μmol min^{-1} mg^{-1}. We optimized conditions for acetyl-CoA synthesis resulting in rates with purified enzymes that are ~ ten-fold faster than those observed previously. These conditions include low pH (see Fig. 4 of Roberts *et al*) and low ionic strength (see Figure 5 of Roberts *et al.*) Under optimal conditions, when MeTr is limiting, the rate of acetyl-CoA synthesis was found to be 5.3 μmol min^{-1} mg^{-1} of MeTr, which equals or surpasses the expected rate based on the rate of acetate synthesis *in vivo* . Thus, CODH, the C/Fe-SP, MeTr, and an electron transfer protein such as FdII are sufficient to perform acetyl-CoA synthesis. When the reaction is dependent upon CODH, the rate of acetyl-CoA synthesis is

~ 0.82 μmol min^{-1} mg^{-1}, ~ ten-fold higher than observed previously, however still lower than the rate of *in vivo* acetate synthesis.

Based on the study of the rates of the overall synthesis and of partial reactions in the synthesis and the dependence of these rates on ionic strength and pH, it appears that at least two steps in the overall synthesis of acetyl-CoA from CH_3-H_4folate, CO and CoA can be partially rate limiting. At optimal conditions of low pH (~ 5.8) and low ionic strength, the rate limiting step involves methylation of CODH by the methylated C/Fe-SP. At higher pH values and/or higher ionic strength, transfer of the methyl group of CH_3-H_4folate to the C/Fe-SP becomes rate limiting.

2. Study of the methylation of CODH

As described above, it appears that methylation of CODH is a rate limiting step in the synthesis of acetyl-CoA from CH_3-H_4folate, CO and CoA. In order to determine the mechanism of this methyl transfer and directly evaluate the dependence of this partial reaction in the overall pathway on reaction conditions, we have performed presteady-state kinetic analyses of the methylation of CODH by the methylated C/Fe-SP (Equation 1). The method involves use of a

$$CH_3\text{-[Co]-Prot} + \text{CODH} \rightleftharpoons \text{[Co]-Prot} + CH_3\text{-CODH} \qquad (1)$$

stopped flow spectrometer which can be kept under strictly anaerobic conditions. Solutions of enzymes and substrates are loaded into tonometers which can be isolated from the atmosphere by stopcocks and the tonometers connected directly to the drive syringes of the stopped flow. These syringes are maintained anaerobically in a temperature controlled bath of anaerobic water. Anaerobicity of the system is monitored routinely by demonstration that the Co^{1+} state of a solution of reduced C/Fe-SP is maintained indefinitely. We have found that the Co^{1+} state of the C/Fe-SP reacts with low levels of O_2 with a rate constant of 1500 s^{-1}.

Two possible mechanisms of methyl-Co bond cleavage include heterolytic or homolytic chemistry. Heterolysis would be inititated by attack of a nucleophilic group of CODH on the electrophilic methyl group of the methylated C/Fe-SP which would generate Co^{1+} and methyl-CODH. Homolytic chemistry would generate Co^{2+} and methyl-CODH. Thus the two mechanisms can be distinguished by observing whether Co^{1+} or Co^{2+} is the product of the transmethylation reaction. We have clearly shown that the mechanism involves the reductive activation of a metal center on CODH (Equation 2a) followed by nucleophilic attack on the electrophilic methyl group generating Co^{1+} and methylated CODH (Equation 2b). Figure 2

$$\text{CODH-}M_{ox} \rightleftharpoons \text{CODH-}M_{red} \quad (2a)$$

$$CH_3\text{-[}Co^{3+}\text{]-Prot} + \text{CODH-}M_{red} \rightarrow \text{[}Co^{1+}\text{]-Prot} + CH_3\text{-M-CODH} \qquad (2b)$$

shows the result of rapidly mixing reduced CODH with the methylated C/Fe-SP and observing the reaction at various wavelengths which report the oxidation state of the cobamide. One observes the decrease in absorbance of the spectrum of methylcobalamin (with a peak at 450 nm) as the spectrum of Co^{1+} increases (with a characteristic peak at 390 nm). The rate of decay of methyl cobalamin and of increase of Co^{1+} are identical and there are clean isosbestic peaks on either side of the major absorption peaks. Therefore, Co^{1+} is the unambiguous product of this transmethylation reaction.

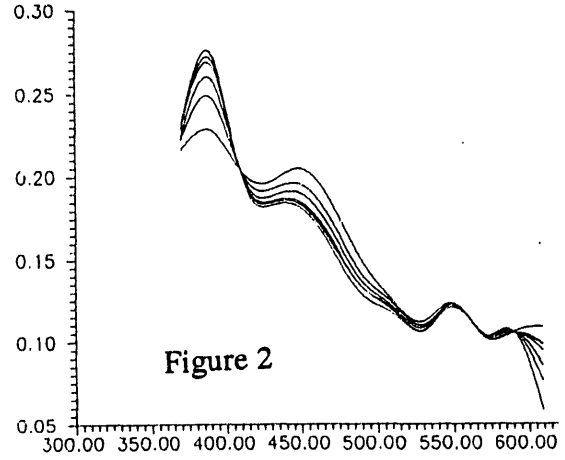

Figure 2

The reaction is biphasic with apparent k_1 and k_2 values which are dependent upon the concentration of CODH but not upon the concentration of methyl-C/Fe-SP. Apparent first order rate constants k_1 and k_2 are 0.029 s^{-1} and 0.0046 s^{-1} at 30 °C. Based on the dependence of k_1 and k_2 on CODH concentration gives second order rate constants of 1074 $M^{-1}s^{-1}$ and 581 $M^{-1}s^{-1}$ at 30 °C and 2460 $M^{-1}s^{-1}$ and 1257 $M^{-1}s^{-1}$ at 40

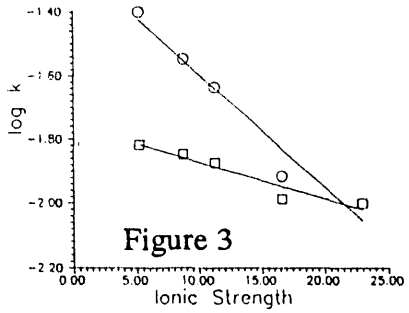

Figure 3

°C. When the ionic strength of the solution is increased from 27 mM to 527 mM (using NaCl), the rate constants k_1 and k_2 decrease 4-5-fold. Plots of the log of k_1 and k_2 versus the square root of ionic strength (Figure 3) are linear indicating that the reaction involves the interaction of opposite charges. We are attempting to determe the cause of this ionic strength effect.

3. Study of the carbonylation of CODH

3a. Determination of the catalytic competence of the Ni-Fe-C intermediate

The work described in this section has been published recently (3). When treated with CO, CODH exhibits an EPR signal which results from an organometallic complex containing nickel, at least 3 iron, and CO and has been referred to as the NiFeC signal and, based on electron nuclear double resonance spectroscopic studies, was postulated to arise from CO bound to an unusual [Ni-Fe_{3-4}-S_4] site (4). Working models of the structure of this species are shown in Figure 4. The ENDOR studies, although not published at the time of the last submission of a progress report, were fully described in the previous report and will not be described here.

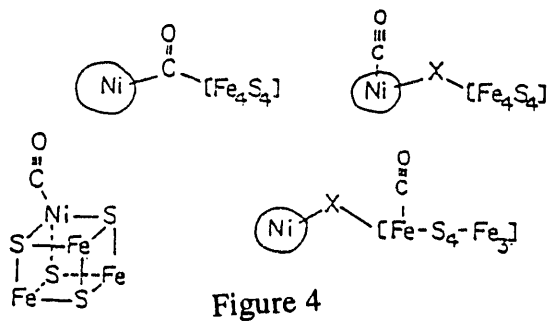

Figure 4

Although this EPR signal has been presumed to be the spectroscopic signature of the enzyme-bound C-1 precursor of the carbonyl group of acetyl-CoA, its catalytic relevance had not previously been rigorously studied. Based on a combination of kinetic, spectroscopic and electrochemical evidence, our work strongly supports the intermediacy of the [Ni-Fe_{3-4}-S_4]-CO species in the synthesis of acetyl-CoA from CO or CO_2 (3). We demonstrated the catalytic competence of this species by showing that the rate of formation of the characteristic EPR signal of the Ni-Fe-C species is approximately ten-fold faster than the rate of an isotope exchange reaction between CO and acetyl-CoA, a partial reaction in the overall synthesis (see Fig. 2 of Gorst and Ragsdale). Thus, the [Ni-Fe_{3-4}-S_4]-CO species satisfies the requirement that an intermediate must be formed at a rate faster than any reaction in which it is proposed to be involved.

Besides kinetic measurements, we obtained direct spectroscopic evidence for the intermediacy of the [Ni-Fe3-4-S4]-CO species. We were successful in generating the NiFeC signal from acetyl-CoA in the absence of CO, demonstrating that the pathway can be run in reverse under certain conditions. The most important condition is that CODH must undergo reductive activation by one-electron at a midpoint potential of -541 mV *vs* the standard hydrogen electrode (SHE) (see Figure 5 of Gorst and Ragsdale). The EPR signal formed under these conditions has the same g values and line shape as the CO-generated signal implying that the structures of the two complexes are very similar. Line-broadening in the 2.028 region of this signal from reaction of CODH with [1-^{13}C] acetyl-CoA (Inset, Figure 4 of Gorst and Ragsdale) clearly demonstrates that the carbonyl from acetyl-CoA is a component of the complex responsible for the EPR signal. Therefore, the EPR-active Ni-Fe-CO species is a product of the cleavage of the C-C and C-S bonds of acetyl-CoA.

Important spectroscopic evidence for intermediacy of the Ni-Fe-C species in acetyl-CoA synthesis was provided by observing and measuring the rate of exchange of label from [1-^{13}C] acetyl-CoA into the NiFeC EPR signal (see Figure 3 of Gorst and Ragsdale). The reaction is reflected in broadening of the NiFeC EPR signal when [1-^{13}C]acetyl-CoA is added to the solution containing CO and CODH. As shown in Figure 3, the amount of hyperfine broadening expected for complete exchange was obtained, resulting from replacement of the NiFe^{12}C signal by a NiFe^{13}C EPR signal (Equation 3). That this isotope replacement occurred at a rate at least

(3) CODH-(NiFe)-CO + CH_3-^{13}CO-SCoA $\rightleftharpoons$ CODH-(NiFe)-^{13}CO + CH_3-CO-SCoA

as fast as complete exchange of the C-1 radioactive label from the carbonyl group of acetyl-CoA by CO, provides further kinetic and spectroscopic evidence for the intermediacy of the NiFeC

species in the CO/acetyl-CoA exchange reaction and thus in the synthesis of acetyl-CoA. In addition, this experiment suggests that the "C" in the NiFeC species is in the form of CO and one can refer to the species as a NiFe-CO intermediate. This would be expected since CO and the carbonyl group of acetyl-CoA are at the same oxidation state.

In summary, the combined kinetic, spectroscopic and electrochemical data strongly support the intermediacy of the NiFe-CO species in the synthesis of acetyl-CoA from CO or CO_2. Based on other electrochemical results, the results in the Gorst/Ragsdale paper are consistent with a model in which the methylation, carbonylation, and acetylation of CODH occur at the same metal center, which is the [$NiFe_{3-4}S_4$] center.

3b. Infrared spectroscopy of the Ni-Fe-C species

As described above, when CODH binds carbon monoxide, an EPR-detectible CODH-CO complex is formed in which the CO group is the precursor of the carbonyl group of acetyl-CoA. We have been trying to characterize this complex and have several models which are consistent with spectroscopic data (Figure 4, above). To further characterize the structure, we have begun studies by vibrational spectroscopy, including resonance Raman and Fourier transform infrared (FTIR). The FTIR studies have been recently published (5). The CO stretching vibration of the CODH-CO complex can be assigned to a single IR peak which is observed at 1995.1 cm^{-1} (see Figure 1 of Kumar and Ragsdale). When ^{13}CO replaces ^{12}CO, this peak predictably shifts to 1950.8 cm^{-1}(see Figure 1B of Kumar and Ragsdale). Assignment of the 1995 cm^{-1} IR band as the signature of a terminally bound carbonyl, *i.e.*, metal-C≡O, complex is unambiguous since the stretching vibration for CODH-CO is much higher than the values for heterobinuclear or homobinuclear metal-CO-metal complexes. The IR absorption band for a terminally bonded carbonyl is found in the range of 2140 to 1800 cm^{-1}, whereas, that for a bridging carbonyl ranges from 1880 to 1700 cm^{-1}.

A measure of the catalytic activity of CODH in acetyl-CoA synthesis is provided by study of an isotopic exchange reaction between CO and the carbonyl group of acetyl CoA (Eq 4).

$$[1\text{-}^{*}C]\text{acetyl-CoA} + CO \rightleftharpoons {}^{*}CO + \text{acetyl-CoA} \quad (4)$$

When CODH was reacted with ^{13}CO in the presence of CH_3-^{12}CO-CoA, both CODH-^{13}CO and CODH-^{12}CO stretching vibrations at 1950 and 1995 cm^{-1}, respectively, are observed with peak height ratios of 4/1 (Figure 1B of Kumar and Ragdale). In the absence of acetyl-CoA, only the 1950 cm^{-1} peak (due to CODH-^{13}CO) was observed. When ^{12}CO and CH_3-^{12}CO-CoA are reacted under similar conditions, only the 1995 cm^{-1} peak was observed. These experiments demonstrate that the metal carbonyl species observed by IR is a catalytically relevant presursor of the carbonyl group of acetyl-CoA. The CO group of the EPR detectable NiFeC complex also was shown to be catalytically competent as the precursor of the carbonyl group of acetyl-CoA (above). Thus, the 1995 cm^{-1} IR peak and the g = 2.08/2.028 EPR signal are spectroscopic signatures of the same complex and the binding site for CO can be described as [Ni-$Fe_{3-4}S_4$]-C≡O. Since all the components of this complex are part of a single cluster and CO is terminally bound, the Ni and Fe components must have an endogenous bridge, X, and the structure of the CO adduct to CODH can be described as [Ni-X-$Fe_{3-4}S_4$]-C≡O.

3c. Resonance Raman spectroscopy

Magnetic resonance methods have and can only establish that Ni, iron, and carbon are part of a spin-coupled cluster. We are performing resonance Raman spectroscopic studies in collaboration with Prof. Tom Spiro to determine whether CO and the methyl group bind to the nickel or iron components of the [Ni-Fe_{3-4}-S_4] center. In preliminary experiments, we observe a spectrum typical of a [4Fe-4S cluster]. When the sample is incubated with ^{12}CO, a new peak can be seen at 354 cm^{-1}. We are in the process of repeating these experiments to obtain better signal to noise and performing similar experiments in the presence of ^{13}CO. Then we will prepare CODH samples which have been purified from cells grown in the presence of ^{64}Ni, ^{58}Ni, ^{57}Fe and

^{54}Fe. The mass difference between ^{64}Ni and ^{58}Ni and between ^{57}Fe and ^{54}Fe is predicted to be sufficient to observe a spectral shift due to a Ni-carbon or Fe-carbon bond. Thus, we will compare the Resonance Raman spectra of the CO-CODH and methyl-CODH adducts.

4. Determination of the CoA binding site of CODH
Synthesis of selenocoenzyme A
CODH apparently binds CoA near the Ni-Fe-C site and one possibility is that the thiol of CoA ligates directly to the metal center. In order to investigate this possibility, we are synthesizing the selenocoenzyme A (Se-CoA) analogue of S-CoA. The reason for using Se is that the properties of selenium are similar to those of S, but should be distinct enough that it would give rise to a modified EPR spectrum if S (or Se) is a ligand to a metal center. In addition, Se-CoA can be prepared with ^{77}Se which has a 1/2 nuclear spin (I) which would result in a splitting of the EPR spectrum of any center to which it is a ligand. In addition, one should be able to perform ^{77}Se NMR spectroscopy to probe the CoA binding site. Synthesis of Se-CoA has been adapted from the procedures of Moffatt and Khorana (6) and Günther and Mautner (7) and is near completion . The synthesis can be divided into three parts.
Part A : Compound 4, Bis(4-morpholino-N,N'-dicyclohexyl-carboxamidium) adenosine 2' 3' cyclic phosphate 5' phosphomorpholidate, was obtained in 19-22 % overall yield. Adenosine was reacted with dibenzyl phosphochloridate, hydrolyzed with 50 % acetic acid, and reduced with Pd/H_2 to give compound 2, adenosine-2'-(3'),5'diphosphate in 60 % yield. Compound 4 was obtained by conversion of 2 to its triethylammonium salt, reaction with dicyclohexyl carbodimide and triethylamine, and finally reaction with morpholine, dicyclohexylcarbodimide and t-butanol. All compounds (2-4) were characterized by NMR spectroscopy.
Part B : A seven step reaction sequence is involved in synthesis of D-4'-phosphoselenopantethine 12. Reaction of dibenzyl diselenide 5 and 2-bromoethylamine hydrochloride 6 yielded 2-benzylselenoethyl amine hydrochloride 7 which was reacted with D-pantothenic acid 8 in its triethylammonium form 9 with ethylchloroformate and dmf to yield D-benzylselenopentethine 10 in 40 % yield. Compound 10 was phosphorylated and partially hydrolysed to give dibenzyloxy phosphorylated D-benzylselenopantethine 11 in 22 % yield. The synthesis of part B is complete up to this step. Sodium reduction of 11 will be performed to yield compound 12.
Part C involves reaction of 12 with 4 of and purification of D-selenocoenzyme A. We are also side by side synthesizing ^{14}C labeled S-CoA and Se-CoA to augment our studies of binding of CoA to CODH.

5. Comparison of the properties of the CODH from *C. thermoaceticum* with those of the methanogenic bacterium, *Methanosarcina thermophila*

Methanosarcina thermophila contains a multienzyme complex called the carbon monoxide dehydrogenase (CODH) complex which has been resolved into a nickel/iron-sulfur and a corrinoid/iron-sulfur component. This complex plays a central role in acetoclastic methanogenesis. The Ni/Fe-S component catalyzes CO oxidation and has been proposed to be involved in cleavage of acetyl-CoA into its methyl, carbonyl, and CoA moieties. James G. Ferry and I feel that since archae and bacteria are so distant evolutionarily, by comparing the properties and sequences of the methanogenic and acetogenic CODHs, we will be able to determine what features of these proteins are essential in catalysis and electron transfer. In work which has been submitted to the Journal of Biological Chemistry (8), four metal centers in the isolated Ni/Fe-S component were characterized by EPR spectroscopy and spectroelectrochemistry. Three fast-relaxing signals recalcitrant to microwave power saturation were observed at temperatures below 20 K. One signal was attributed to a Fe-S center with g values of 2.02, 1.88, and 1.71 (g_{av} = 1.87) and a reduction potential (E^o) of -154 mV. A second EPR signal with g values of 2.04, 1.93 and 1.89 (g_{av} = 1.95) was observed which most likely arises from a $[4Fe\text{-}4S]^{2+/1+}$ center with an E^o of -444 mV. At potentials < -500 mV, a third signal developed with g values of 2.05, 1.95, and 1.90 (g_{av} = 1.97) which also is most likely due to a $[4Fe\text{-}4S]^{2+/1+}$ cluster with an E^o of -540 mV. Based on spectral characteristics, it is likely that the signals of the g_{av} = 1.95 and 1.97 species originate from a single $[4Fe\text{-}4S]^{2+/1+}$ cluster which can exist in two conformations.

Incubation of the Ni/Fe-S component with CO elicited a Ni-Fe-C EPR signal (g = 2.059, 2.051, and 2.029) which was observable at temperatures as high as 150 K and had a half power of saturation of 0.2 mW at 16 K. This signal had previously been observed only in the CODH complex of *M. thermophila* and the acetyl-CoA synthase from acetate-producing bacteria. Incubation of the CO-reduced Ni/Fe-S component with acetyl-CoA resulted in an increase in intensity of the Ni-Fe-C signal which supports the role of the component in the cleavage of acetyl-CoA. In addition, a center with an E^o of -115 mV and a g value of 4.3 was observed.

Based on the similarity between the *C. thermoaceticum* and *M. thermophila* enzymes, we expect that the ligands for the metal sites must be strongly conserved. We are presently comparing the amino acid sequences of the *C. thermoaceticum* (9) and the *Methanothrix* (10) CODHs and eagerly await completion of the sequence of the CODH gene from *M. thermophila.* Recently, Kerby et al compared the sequences of the nickel-containing CODHs (11) and found little sequence homology, but 67% sequence similarity to the small subuit of the *C. thermoaceticum* CODH and 47% similarity to the the large subunit of the *M. soehngenii* CODH (10). Conserved regions include a CXXCXXGPC region and a GXXAHXXHGXH region which would be able to provide S- and N-rich environments, respectively, for metal binding.

The similarity between the metal centers and the reactions catalyzed by both methanogenic and acetogenic proteins is of evolutionary significance and provides biochemical evidence that the acetyl-CoA pathway of autotrophic growth as well as the pathway of acetoclastic methanogenesis may have evolved via a divergent pathway from a progenitor of *archae* and *bacteria.* Could the acetyl-CoA pathway have been present in an organism which predates the evolutionary branching of *archaea* and *bacteria*?

6. Bibliography. The * marks papers or preprints which are enclosed.

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*3. **Gorst, C. M., and S. W. Ragsdale.** 1991. Characterization of a Ni-Fe-CO complex of CO dehydrogenase as a catalytically competent intermediate in the pathway of acetyl-CoA synthesis. J. Biol. Chem. **266**:20687-20693.

*4. **Fan, C., C. M. Gorst, S. W. Ragsdale, and B. M. Hoffman.** 1991. 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. Biochem. **30**:431-435.

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7. Publications resulting from the last two years of DOE support (*, enclosed)

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* 2. Lu, W.P. & RAGSDALE, S.W. (1991) Reductive activation of the coenzyme A/acetyl-CoA isotopic exchange reaction catalyzed by carbon monoxide dehydrogenase from *Clostridium thermoaceticum* and its inhibition by nitrous oxide and carbon monoxide, J. Biol. Chem., 266, 3554-3564.

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* 4. Gorst, C.M. & RAGSDALE, S.W. (1991) Characterization of a Ni-Fe-CO complex of CO dehydrogenase as a catalytically competent intermediate in the pathway of acetyl-CoA synthesis, J. Biol. Chem. 266, 20687-20693.

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* 6. Roberts, J.R., Lu, W.-P., & RAGSDALE, S.W. (1992) Acetyl-CoA synthesis from methyltetrahydrofolate, CO and CoA by enzymes purified from *Clostridium thermoaceticum*: Attainment of *in vivo* rates and identification of rate limiting steps, J. Bacteriol. 174, 4667-4676.

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1. Gorst, C.M., & RAGSDALE, S.W. (1991) Evidence that the NiFe-carbonyl complex of CO dehydrogenase is an intermediate in the pathway of anaerobic acetyl-CoA synthesis. The FASEB Journal 5, A469, Abstract 572, Annual ASBMB meeting, April 21-25, 1991, Atlanta, GA.

2. Baur, J.R., RAGSDALE, S.W., and Lindahl, P.A. (1991) The amino acid sequence and redox properties of the eight-iron ferredoxin II from *Clostridium thermoaceticum*. The FASEB Journal 5, A470, Abstract 574, Annual ASBMB meeting, April 21-25, 1991, Atlanta, GA.

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4. Lu, W.-P., Jablonski, P.E., Ferry, J.G., and RAGSDALE, S.W. Characterization of the metal centers of carbon monoxide dehydrogenase and the corrinoid/iron-sulfur protein from *Methanosarcina thermophila* by EPR spectroscopy and EPR spectroelectrochemistry, International Symposium on Topics in Microbial Diversity Physiology and Metabolism, University of Illinois, May 22-23, 1992.

5. Lu, W.-P., Schiau, I.A., Cunningham, J.R., & RAGSDALE, S.W. (1992) Sequence and expression of the gene encoding the corrinoid/iron-sulfur protein from *Clostridium thermoaceticum* and reconstitution of the recombinant protein to full activity, 35th Annual West Central States Biochemistry Conference, Univ. of Kansas, October 30-31.

6. Zhao, S. and RAGSDALE, S.W.(1992) Study of methyl transfer reactions between the corrinoid/iron-sulfur protein or B_{12} and H_4folate by methyltransferase from *Clostridium thermoaceticum*, 35th Annual West Central States Biochemistry Conference, Univ. of Kansas, October 30-31.

7. Kumar, M. and RAGSDALE, S.W. (1992) Characterization of the CO binding site of carbon monoxide dehydrogenase from *Clostridium thermoaceticum* by infrared spectroscopy, 35th Annual West Central States Biochemistry Conference, Univ. of Kansas, October 30-31.

8. Rajasekharan, S. & RAGSDALE, S.W. (1992) Anaerobic bioremediation of methoxylated aromatics by *Clostridium thermoaceticum*, 35th Annual West Central States Biochemistry Conference, Univ. of Kansas, October 30-31.

8. Related publications resulting from other support

1. Jablonski, P.E., Lu, W.-P., RAGSDALE, S.W., and Ferry, J.G. Characterization of the metal centers of the corrinoid iron sulfur protein from *Methanosarcina thermophila* by EPR spectroscopy and EPR spectroelectrochemistry, J. Biol. Chem., in press.

2. Lu, W.-P., Schiau, I.A., & RAGSDALE, S.W. Sequence and expression of the gene encoding the corrinoid/iron-sulfur protein from *Clostridium thermoaceticum* and reconstitution of the recombinant protein to full activity, J. Biol. Chem., in press.

3. Wirt, M.D., Kumar, M., RAGSDALE, S.W., and Chance, M.R. X-ray absorption spectroscopy of the corrinoid/iron sulfur protein involved in acetyl-CoA synthesis by *Clostridium thermoaceticum*, J. Am. Chem. Soc., submitted, 9-22-95.

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