Binding of Carbon Disulfide to the Site of Acetyl-CoA Synthesis by the Nickel–Iron–Sulfur Protein, Carbon Monoxide Dehydrogenase, from *Clostridium thermoaceticum*†

Manoj Kumar, Wei-Ping Lu, and Stephen W. Ragsdale*

Department of Biochemistry, East Campus, University of Nebraska, Lincoln, Nebraska 68583-0718

Received March 28, 1994; Revised Manuscript Received June 2, 1994®

ABSTRACT: Carbon monoxide dehydrogenase (CODH) is a key enzyme in the pathway of carbon monoxide and carbon dioxide fixation by anaerobic bacteria. It performs the oxidation of CO to CO_2, the reduction of CO_2 to CO, and the synthesis of acetyl-CoA from a methylated corrinoid/iron–sulfur protein, CO, and CoA. These reactions occur at metal centers on CODH and involve metal–carbon bond formation and transformation. There are three iron-containing centers that play distinct roles in CODH: Centers A, B, and C. Center A is the site of synthesis of acetyl-CoA and catalyzes an exchange reaction between CO and acetyl-CoA. Center C is the site of CO oxidation and CO_2 reduction. In the work described here, inhibition of CODH by carbon disulfide was studied. CS_2 was found to serve as a probe of the interaction of CODH with CO at Center A. EPR spectroscopic and steady-state kinetic studies demonstrated that CS_2 mimics the binding of CO to the nickel/iron–sulfur cluster at Center A; however, CS_2 itself does not undergo oxidation–reduction and does not appear to bind to Center C as does CO. In the isotope exchange reaction between acetyl-CoA and CO, CS_2 was found to be a competitive inhibitor with respect to CO (K_i = 0.47 mM) and a mixed inhibitor with respect to acetyl-CoA (K_{i1} = 0.30 and K_{i2} = 1.1 mM). The reaction of dithionite-reduced CODH with CS_2 resulted in an EPR spectrum with *g* values of 2.200, 2.087, and 2.017. This EPR signal from the CS_2 adduct with Center A is similar to that assigned to the Ni(I) state of hydrogenases. EPR spectroelectrochemical titrations demonstrated that the CODH–CS_2 complex has three redox states and that the intermediate state is paramagnetic. A maximum of 0.3–0.4 spins/mol of CODH could be obtained. Fitting this data to the Nernst equation indicated that integral spin intensities could not be obtained because the reduction potentials for the two redox couples were the same (~−455 mV). We suggest that similar redox chemistry may limit the spin intensity of the adduct between Center A and CO. Although CS_2 did not bind to Center C, it inhibited reactions that occur at Center C. CS_2 was a noncompetitive inhibitor *vs* CO_2 in CO_2 reduction and *vs* CO in CO oxidation.

A pathway has been discovered relatively recently that is distinct from most other metabolic pathways [reviewed in Ragsdale (1991)]. Unique aspects include a preponderance of enzyme-bound one-carbon and two-carbon intermediates, the involvement of metal–carbon bonds at key steps, and a strategy of carbon–carbon bond formation that is similar to some well-studied organometallic reactions in solution. It is called the reductive acetyl-CoA pathway or the Wood/Ljungdahl pathway and is the major mechanism of CO_2 fixation in anaerobic environments. Serving an important role in the global carbon cycle, this pathway allows a number of anaerobic bacteria, including sulfate reducers, methanogens, and acetogens, to convert CO or CO_2 to cell carbon (Fuchs, 1986; Ljungdahl, 1986; Ragsdale, 1991). It appears that some of the key enzymes involved in the acetyl-CoA pathway operate in reverse in some methanogens to convert acetic acid to methane (Ferry, 1992a,b; Thauer *et al.*, 1989).

A key enzyme in the acetyl-CoA pathway is the metalloenzyme, carbon monoxide dehydrogenase (CODH). It interconverts CO and CO_2 (Diekert & Ritter, 1983; Drake *et al.*, 1980; Ragsdale *et al.*, 1983) and performs the final steps in the synthesis of acetyl-CoA (Ragsdale & Wood, 1985). CODH has been most extensively studied from *Clostridium thermoaceticum*. It contains 2 Ni, 11–14 Fe, 14 inorganic sulfides, and variable amounts of zinc (Ragsdale *et al.*, 1983). These metals are organized into at least three distinct iron-containing Centers A, B, and C (Kumar *et al.*, 1993). Center A is a nickel/iron–sulfur cluster that elicits an EPR spectrum with *g* values at 2.08, 2.07, and 2.03 when CODH is reacted with CO (Ragsdale *et al.*, 1983a,b, 1985). In acetyl-CoA synthesis, this Ni/Fe-S cluster binds the methyl group of a methylated corrinoid/iron–sulfur protein (C/Fe-SP) and CO and, through a series of organometallic intermediates, catalyzes carbon–carbon bond formation (Ragsdale & Wood, 1985). CODH then catalyzes the condensation of the enzyme-bound acetyl group with CoA to form acetyl-CoA. On the basis of magnetic resonance and infrared spectroscopic results, the minimal working model for the structure of the adduct between CO and Center A is [Ni-X-$Fe_{3-4}S_4$]–C≡O, where X is an unknown bridging ligand between the Ni site and the [4Fe-4S] cluster (Fan *et al.*, 1991; Kumar & Ragsdale, 1992; Lindahl *et al.*, 1990a). CO has been shown to bind as a terminal carbonyl (Kumar & Ragsdale, 1992) to an iron site in the cluster (Qiu *et al.*, 1994). Center B is a [4Fe-4S]$^{2+/1+}$ cluster that serves as an electron carrier in the oxidation of CO or reduction of CO_2 (Kumar *et al.*, 1993). Center C is an iron-containing center of unknown structure and is the site of catalysis of CO oxidation/CO_2 reduction (Kumar *et al.*, 1993). Center A is a relatively slow relaxing center that can

† This manuscript has been assigned Journal Series No. 10673, Agricultural Research Division, University of Nebraska. This work was supported by DOE research (DE-FG02-91ER20053) and equipment (DE-FG05-92ER79133) grants to S.W.R.

* Corresponding author. Phone: 402-472-2943; Facsimile: 402-472-7842; Internet: sragsdale@crcvms.unl.edu.

® Abstract published in *Advance ACS Abstracts,* July 15, 1994.

0006-2960/94/0433-9769$04.50/0

be observed by EPR spectroscopy at temperatures up to 150 K, whereas Centers B and C are fast relaxing and can only be observed by EPR spectroscopy at temperatures below 50 K (Lindahl *et al.*, 1990a; Ragsdale *et al.*, 1982).

In order to better understand the reaction mechanism of CODH, inhibitors have been sought which are specific for each of the reactions catalyzed by CODH. Cyanide is an inhibitor of CO oxidation for all CODHs which have been studied (Abbanat & Ferry, 1990; Diekert & Thauer, 1978; Drake *et al.*, 1981; Ensign *et al.*, 1989a; Grahame & Stadtman, 1987; Ragsdale *et al.*, 1983a; Stephens *et al.*, 1989) and, for the *C. thermoaceticum* enzyme, exerts its effect by binding to Center C (Anderson *et al.*, 1993). CN appears to inhibit CO oxidation in *Rhodospirillum rubrum* by binding to a center that has spectroscopic properties similar to those of Center C (Ensign *et al.*, 1989b). We began to investigate CS_2 as a possible inhibitor of CO oxidation/CO_2 reduction because it is a structural analogue of CO_2. CO_2 has weakly basic oxygen atoms and weakly acidic carbon atoms and can form complexes with metals; several of these are well characterized (Aresta *et al.*, 1975; Behr, 1988; Fujita *et al.*, 1991; Ito *et al.*, 1992). Transition metal–CO_2 complexes are known that can react to form carbon–carbon bonds (Aresta *et al.*, 1992; Braunstein *et al.*, 1988; Tanase *et al.*, 1992; Tsuda *et al.*, 1992) involving chemistry that is relevant to CODH. In the work reported here, carbon disulfide was found to inhibit CODH by competing with CO for binding to Center A. CS_2 is a versatile ligand and, relative to CO_2, forms a variety of strong metal–CS_2 complexes (Butler & Fenster, 1974; Ibers, 1982; Yaneff, 1977). Except for η_1-CS_2 complexes, all involve simultaneous bonding with both carbon and sulfur (Doeff, 1986). The versatility of CS_2 is due to the nucleophilic character of its sulfur atoms and the availability of nonbonding d-orbitals. CS_2 is known to undergo insertion reactions between M–H and M–R bonds (Butler & Fenster, 1974; Mealli *et al.*, 1984). CS_2 is generated during the manufacture of rayon and the vulcanization of rubber, and some studies on its toxicity have been reported (Beauchamp *et al.*, 1981; Vanhoorne *et al.*, 1993).

EXPERIMENTAL PROCEDURES

Materials

CO (99.99%) and N_2 (99.998%) were purchased from Matheson Gas (Joliet, IL). N_2 (99.998%, Linde, Lincoln, NE) and other inert gases were purified from traces of oxygen by passage over heated BASF catalyst. CS_2 (Aldrich, Milwaukee, WI) was distilled under nitrogen at reduced pressure and temperature, sparged with nitrogen, and stored in an anaerobic chamber. **Caution:** *All procedures involving neat CS_2 were performed under a fume hood since it is poisonous and is a fire and explosion hazard.* Solutions containing CS_2 were transferred by gas-tight syringes between butyl rubber-stoppered vials. Solutions containing CS_2 must be prepared fresh since it is volatile and we have observed a decrease in the concentration within 2 h of preparation even when the solution was maintained on ice. $^{13}CS_2$ was purchased from Cambridge Isotope Labs (Woburn, MA); ^{61}Ni and ^{57}Fe were purchased from Advanced Material & Technology (New York, NY). All other chemicals were of analytical grade and were used as purchased.

Methods

Growth of the Organism and Enzyme Purification. C. thermoaceticum strain ATCC 39073 was grown with glucose as the carbon source at 55 °C as previously described (Andreesen *et al.*, 1973). Isolation and purification of CODH was done under strictly anaerobic conditions (Ragsdale *et al.*, 1983a) in a Vacuum Atmospheres (Hawthorne, CA) anaerobic chamber maintained at 16 °C and below 1 ppm oxygen. Oxygen levels were monitored continuously with a Model 317 trace oxygen analyzer (Teledyne Analytical Instruments, City of Industry, CA). CODH was stored in liquid nitrogen. Purified CODH had an average specific activity of 320 units/mg (1 unit = 1 μmol of CO oxidized/min) at 55 °C and pH 7.6 when assayed for CO oxidation with 10 mM methyl viologen as electron acceptor (Ragsdale *et al.*, 1983a,b). The average specific activity of the CODH in the isotopic exchange reaction between CO and [1-^{14}C]-acetyl-CoA was 224 nmol min^{-1} mg^{-1} at 55 °C (28 nmol min^{-1} mg^{-1} at 25 °C) using 340 μM acetyl-CoA, 1 mM CO (solubility of 1 atm of CO), 100 mM pH 5.5 Tris–maleate buffer, and 0.1 mM dithiothreitol. Protein concentrations were determined by the Rose Bengal method (Elliott & Brewer, 1978).

Assay of CO Oxidation Activity. CO oxidation assays were performed at pH 7.6 in 50 mM Tris-HCl containing 1.25 or 10 mM methyl viologen (ϵ_{604} = 13.9 mM^{-1} cm^{-1}), and 2 mM dithiothreitol (Ragsdale *et al.*, 1983a,b). One milliliter of assay mix was sparged with gas mixtures containing CO in N_2 for 5 min at 25 °C. The assay was initiated with CODH. For the inhibition studies, CS_2 was added to assay cuvettes prior to addition of enzyme.

Assay of CO_2 Reductase Activity. Reduction of CO_2 was measured by exploiting the tight and rapid binding of CO to ferrous hemoglobin. A difference spectrum is generated with a decrease in absorption at 419 nm ($\Delta\epsilon$ = +82 $mM^{-1}cm^{-1}$) and 430 nm ($\Delta\epsilon$ = −80 mM^{-1} cm^{-1}). A similar assay was developed earlier to measure the concentration of CO in solution (Bonam & Ludden, 1987). For the assay, a stock solution containing 1 M sodium bicarbonate at pH 8.5 was prepared in anaerobic water. CO_2 reduction was performed at 25 °C in 1 mL of reaction mixture containing 4 μM hemoglobin, 2 mM dithionite, and 150 mM 2-(*N*-morpholino)-ethanesulfonic acid (MES) buffer. CS_2 and bicarbonate were added to the cuvette before initiating the reaction with enzyme. In a series of experiments, the pH was varied from 5.7 to 6.6 and checked after the reaction was complete. The pH-dependent concentration of CO_2 at each pH value was determined from the Henderson–Hasselbalch equation using a pK_a = 6.3. The results were the same whether the CO_2 was added from a stock solution of bicarbonate or from a CO_2-saturated buffer solution.

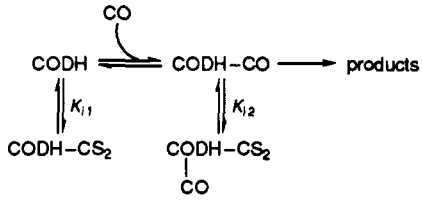
CO/Acetyl-CoA Exchange Activity. The ability of CODH to catalyze the synthesis of acetyl-CoA was measured by determining its activity in an isotopic exchange reaction between CO and [1-^{14}C]acetyl-CoA (Ragsdale & Wood, 1985; Raybuck *et al.*, 1988). This reaction was determined at 25 °C in an standard assay mix containing 580 μL of 100 mM Tris–maleate, pH 5.5, 0.1 mM methyl viologen, 0.1 mM DTT, and 1.5 mg of CODH to initiate the reaction. In one set of reactions the acetyl-CoA concentration was maintained at 344 μM using [1-^{14}C]acetyl-CoA (specific activity = 196 dpm $nmol^{-1}$) and the concentration of CO was varied by bubbing the reaction vial from cylinders containing mixtures of N_2/CO. In another set of reactions, CO was present at 1.0 mM concentration and the concentration of acetyl-CoA was varied. For all the inhibition studies, CS_2 was added prior to CODH. Aliquots (50 μL) were removed at zero time and after every 2 min. The solubility of CS_2 in water under saturating

Table 1

	mode of inhibition	K_m for CO or CO_2, mM	K_i for CS_2, mM	K_i' for CS_2, mM	turnover no., s^{-1} [a,d]
CO oxidation	noncompetitive *vs* CO and MV	0.183 ± 0.03[b] 0.043 ± 0.008[c,e]	12.1 ● 1.1[b] 12.1 ± 1.4[c]	12.1 ± 1.1[b] 12.1 ± 1.4[c]	180 (25) 1500 (55)
CO_2 reduction	noncompetitive *vs* CO_2	7.4 ± 2.1	3.2 ± 0.5	3.2 ± 0.5	1.3 (25) 10.8 (55)
CO/acetyl-CoA exchange	competitive *vs* CO	0.28 ± 0.07	0.47 ± 0.09	∞	0.056 (25) 2.3 (55)
	mixed *vs* acetyl-CoA		0.30 ± 0.05	1.1 ± 0.2	0.28 (25) 11.5 (55)

[a] The values at 55 °C are extrapolated from the values determined at 25 °C and at saturating concentrations of substrates. See details in the text. [b] Determined with 10.0 mM MV. [c] Determined with 1.25 mM MV. [d] Numbers in parentheses are temperatures in °C. [e] The K_m for MV was 6.8 mM.

Scheme 1



conditions is 38.6 mM at 25 °C, and the solubility of CO in water under 100% CO is 1.0 mM at 25 °C (Budavari, 1989). The molar concentration of CODH is expressed as the α,β dimeric form (M_r = 149 000). Samples were counted in a Packard Tri Carb 1600TR scintillation counter in a Biosafe scintillation cocktail.

Regression Analysis of Inhibition Kinetics. The kinetic results were analyzed by nonlinear regression analysis using Sigma Plot (Jandel Scientific, Corte Madera, CA) with eq 1 which describes Scheme 1.

$$v = \frac{V_{max}[CO]}{K_m(1 + [CS_2]/K_{i1}) + [CO](1 + [CS_2]/K_{i2})} \quad (1)$$

EPR Spectroscopy and Spectroelectrochemistry. EPR spectra were recorded on a Bruker ESP 300E spectrometer equipped with an Oxford ITC4 temperature controller, automatic frequency counter (Hewlett Packard, Model 5340A), and gaussmeter (Bruker). Spin concentrations were measured by comparing the double integrals (using supplied Bruker software) of the spectra with those of a 1 mM copper perchlorate standard. Spectroscopic parameters are stated in the figure legends. EPR spectroelectrochemistry was performed as described (Harder *et al.*, 1989b) using an EPR spectroelectrochemical titrator (Harder *et al.*, 1989a). The redox dyes used were 4,4′-dimethyl-*N*,*N*′-trimethylene-2,2′-dipyridinium dibromide (dimethyl-TRIQUAT) (standard reduction potential E_0' = –680 mV), *N*,*N*′-trimethylene-2,2′-dipyridinium dibromide (TRIQUAT) (E_0' = –540 mV), methyl viologen (E_0' = –440 mV), benzyl viologen and (E_0' = –359 mV). Data were analyzed by a Nernst plot to describe the intensity of the EPR signal (expressed as spins/mol of dimer) as a function of potential. Equation 2 describes a center that undergoes two consecutive one-electron reductions described by the reduction potentials for the low potential

$$\text{intensity} = k/\{1 + \exp[(E_1' - E)(nF/RT)] + \exp[(E - E_2')(nF/RT)]\} \quad (2)$$

couple, E_1', and the high potential couple, E_2', and is EPR active in the intermediate redox state. The value of k is 1.0 spin/mol, the expected spin quantitation for an $S = {}^1/_2$ system when E_1' and E_2' are widely spaced. Potentials are given relative to the standard hydrogen electrode (SHE).

RESULTS

Inhibition of CODH by CS_2 (see Table 1). (1) Inhibition of the CO Oxidation Activity. CS_2 inhibits the oxidation of CO. Since preincubation of the enzyme with CS_2 before the reaction was not required, it appears that CS_2 is a rapid binding reversible inhibitor. It behaves like a noncompetitive or mixed inhibitor since both the $1/V^{app}$ and the $(K/V)^{app}$ decrease with increasing concentrations of CS_2. Reciprocal plots of 1/velocity *vs* the concentration of CS_2 at different concentrations of CO (data not shown) intersect in the second quadrant, characteristic of mixed inhibition (Cornish-Bowden, 1979). The kinetic parameters obtained from replots of $1/V^{app}$ and the $(K/V)^{app}$ *vs* the concentration of CS_2 were used as the input values for a nonlinear least-squares fitting procedure. At 25 °C and 1.25 mM methyl viologen (Figure 1A), the kinetic constants were as follows: V_{max}^{app} = 49.5 ± 1.9 units (mL of CODH)$^{-1}$ (12.1 μmol min^{-1} mg^{-1}), K_m^{CO} = 0.043 ± 0.008 mM; $K_{i1} = K_{i2}$ = 12.1 ± 1.3 mM. At 25 °C and 10 mM methyl viologen (Figure 1B), the values were as follows: V_{max}^{app} = 190 ± 10 units (mL of CODH)$^{-1}$ (46 μmol min^{-1} mg^{-1}), K_m^{CO} = 0.18 ± 0.03 mM; $K_{i1} = K_{i2}$ = 12.1 ± 1.1 mM.

Spectroscopic studies show that CO can bind to Center C in the absence of an electron carrier (Kumar *et al.*, 1993). Plots of $1/v$ *vs* 1/[CO] at two concentrations of methyl viologen are consistent with a ping-pong mechanism of binding of CO and electron acceptor (not shown). The V_{max} at saturating concentrations of methyl viologen was estimated 380 units (mL of CODH)$^{-1}$ (93 μmol min^{-1} mg^{-1}), and the K_m for methyl viologen was 6.8 mM. Using 57.4 kJ/mol as the activation energy for the CO oxidation reaction (Kumar *et al.*, 1993), the V_{max} at 55 °C, pH 7.6, is estimated to be 2410 units mL^{-1} (590 μmol CO oxidized min^{-1} mg^{-1}). This value is equivalent to a k_{cat} of 1500 s^{-1} at 55 °C. The specific activity for CO oxidation measured at pH 7.6, 55 °C, and 10 mM methyl viologen (MV) earlier (Ragsdale *et al.*, 1983a) was found to be similar (675 μmol of CO oxidized min^{-1} mg^{-1}).

(2) Inhibition of the Reduction of CO_2 by CS_2. A continuous assay for CO_2 reduction was developed in which production of CO is detected as it forms a complex with hemoglobin. Hemoglobin binds free CO very tightly ($K_d \sim 5 \times 10^{-8}$ M) and rapidly ($k \sim 2 \times 10^5$ M^{-1} s^{-1}) (Antonini & Brunori, 1971), causing a decrease in the intensity of the heme absorption band at 430 nm. In this reaction, dithionite serves two purposes: it reduces ferric to ferrous hemoglobin, the form that can bind CO, and provides electrons to CODH for the reduction of CO_2. Therefore, the CO that is produced by CODH is removed as the hemoglobin–CO complex.

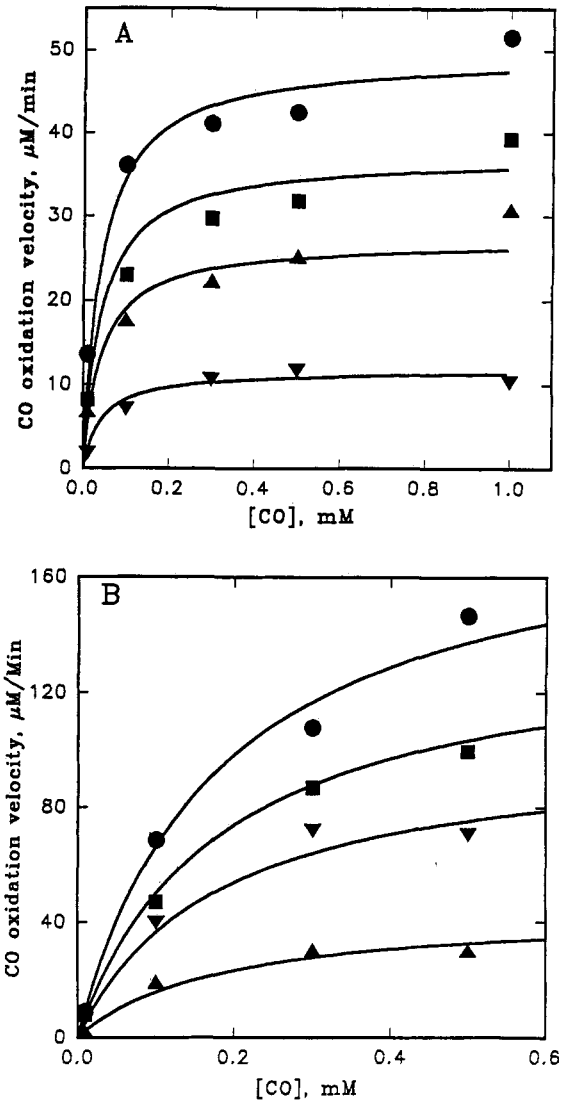


FIGURE 1: Inhibition of the oxidation of CO by CS_2. The reaction mixture contained 50 mM Tris-HCl, pH 7.6, 2 mM dithiothreitol, and either 1.25 (A) or 10 (B) mM methyl viologen and varying amounts of CO and CS_2 at 25 °C. The reaction was initiated by addition of 1 μL of a 4.1 mg/mL solution of CODH. (A) Concentrations of CS_2 were (●) 0 mM, (■) 4 mM, (▲) 10 mM, and (▼) 39 mM. (B) Concentrations of CS_2 were (●) 0 mM, (■) 4 mM, (▼) 10 mM, and (▲) 39 mM. The solid lines were generated from eq 1 with the following values determined by a nonlinear least-squares fitting procedure: (A) $K_{i1} = K_{i2} = 12.1$ mM, $K_m^{CO} = 0.183$ mM, V_{max} = 49.5 units (mL of CODH)$^{-1}$; (B) $K_{i1} = K_{i2} = 12.1$, $K_m^{CO} = 0.043$ mM, V_{max} = 190 units (mL of CODH)$^{-1}$.

CO_2, not bicarbonate, has been shown to be the product of CO oxidation by CODH (Bott & Thauer, 1989). It was therefore necessary to work in a narrow pH range around pH 6.0, since there was no observable reaction above pH 6.6, where the ratio of $NaHCO_3/CO_2$ is high, or below pH 5.6, where the enzyme is unstable. Phosphate and MES were found to be satisfactory buffers. Tris–maleate buffer was found to strongly inhibit CO_2 reduction. The slopes of plots of log V/K and log K_m versus pH were 1.0 ± 0.3 and −1.0 ± 0.5 (data not shown). The log V_{max} was independent of pH. The narrow pH range in which we were able to work did not allow us to evaluate the pK_a of the ionizable group. The results indicate that protonation of a group(s) on the free enzyme results in either tighter binding of CO_2 or increased activity.

CS_2 was found to inhibit the reduction of CO_2 to CO. Five concentrations of CS_2 and five concentrations of CO_2 were

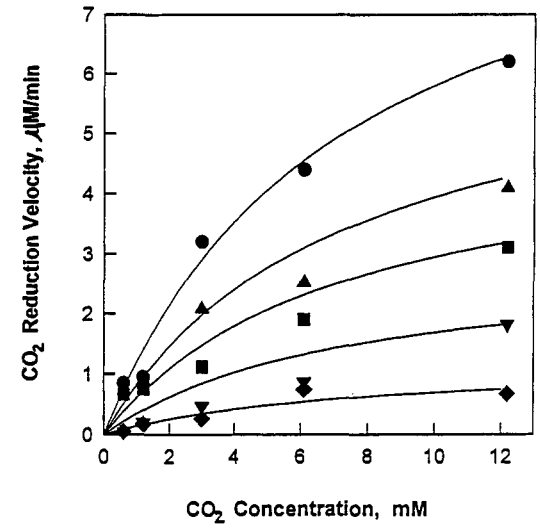


FIGURE 2: Inhibition of the reduction of CO_2 by CS_2. The reaction mixture contained 4 μM hemoglobin, 2 mM dithionite, 150 mM MES buffer, pH 5.90, and varying amounts of CS_2 and bicarbonate at 25 °C. The reaction was initiated by addition of 20 μg of CODH. Concentrations of CS_2 were (●) 0 mM, (▲), 1.6 mM, (■) 3.3 mM, (▼) 8.3 mM, and (◆) 25 mM. The solid lines were generated from eq 1 with the following values determined by a nonlinear least-squares fitting procedure: V_{max} = 10.1 nmol min^{-1} (mL of reaction mix)$^{-1}$, $K2_m^{CO}$ = 7.4 mM, $K_{i1} = K_{i2} = 3.4$ mM.

used. Analysis by nonlinear regression using eq 1 indicates that CS_2 inhibits this reaction noncompetitively relative to CO_2 (Figure 2). As with the CO oxidation reaction, a model in which CS_2 binds equally well to free enzyme and to the enzyme–substrate complex (i.e., $K_{i1} = K_{i2}$) best fits the data. The solid lines were generated with the parameters: V_{max} = 10.1 (±1.4) nmol min^{-1} (mL of reaction mixture)$^{-1}$, K_m for CO_2 = 7.4 (±2.1) mM, $K_{i1} = K_{i2}$ = 3.4 (±0.5) mM. Using 57.4 kJ/mol as the activation energy (determined for the CO oxidation reaction), the V_{max} at 55 °C is calculated to be 8.32-fold greater, or 84 nmol min^{-1} mL^{-1}. This is equivalent to a 4.2 μmol min^{-1} (mg of CODH)$^{-1}$ or a k_{cat} of 10.8 s^{-1} at 55 °C. Although this rate is ~140-fold slower than that of CO oxidation, it is similar to the rate of the CO/acetyl-CoA exchange reaction and of acetyl-CoA synthesis from CH_3-H_4folate, CO, and CoA.

(3) Inhibition of the CO/Acetyl-CoA Exchange Reaction by CS_2. CS_2 inhibits the synthesis of acetyl-CoA by CODH. This reaction can be conveniently monitored by an isotope exchange reaction between CO and the carbonyl group of [1-^{14}C]acetyl-CoA (Hu *et al.*, 1982). In one set of reactions at a constant CO concentration, CS_2 and acetyl-CoA were varied (Figure 3B). The solid lines were generated with the values obtained from the nonlinear fitting procedure: V_{max} = 225 (±6) μM min^{-1}; K_m for acetyl-CoA = 0.98 (±0.07) mM, K_{i1} = 0.30 (±0.05) mM, and K_{i2} = 1.1 (±0.2) mM. The K_m at 55 °C was determined previously to be 0.6 mM (Raybuck *et al.*, 1988). Extrapolating to saturating concentrations of CO (using a K_m for CO of 0.28 mM, see below) and 100.8 kJ/mol as the activation energy (Ramer *et al.*, 1989), the V_{max} at 55 °C is calculated to be 11.9 mM min^{-1} (4.8 μmol min^{-1} mg^{-1}), which corresponds to a turnover number of 11.5 s^{-1}.

In another set of reactions at constant acetyl-CoA (0.344 mM), seven different CS_2 concentrations between 0 and 38.6 mM and three concentrations of CO were used. Nonlinear regression analysis of the data using eq 1 indicated that K_{i2} is infinitely large. Therefore, eq 1 could be reduced to eq 3,

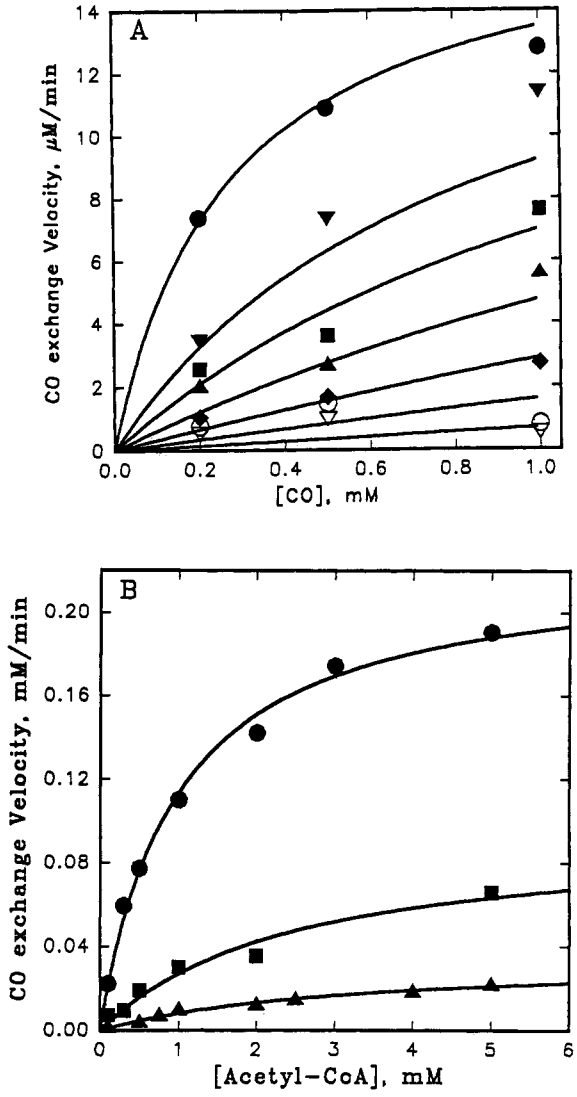


FIGURE 3: Inhibition of the isotopic exchange reaction between CO and [1-^{14}C]acetyl-CoA by CS_2. (A) The reaction mixture contained 100 mM Tris–maleate, pH 5.5, 0.2 mM methyl viologen, 0.2 mM DTT, 344 μM [1-^{14}C]acetyl-CoA, and varying amounts of CO and CS_2 at 25 °C and was initiated by adding 1.5 mg of CODH. Concentrations of CS_2 were (●) 0 mM, (▼), 0.8 mM, (■), 1.66 mM, (▲) 3.3 mM, (◆) 6.6 mM, (○) 13.3 mM, and (▽) 39 mM. The solid lines were generated from eq 1 with the following values determined by a nonlinear least-squares fitting procedure: V_{max} = 14.9 nmol min^{-1} (mL of reaction)$^{-1}$; K_m for CO = 0.28 mM and K_i = 0.47 mM. In (B) the concentration of CO was maintained at 1.0 mM and acetyl-CoA and CS_2 were varied. Concentrations of CS_2 were (●) 0 mM, (■), 1.5 mM, and (▲) 6.0 mM. The best fit values for kinetic parameters were as follows: V_{max} = 225 nmol min^{-1} (mL of reaction mix)$^{-1}$; K_m for acetyl-CoA = 0.98 mM; K_{i1} = 0.30 mM; and K_{i2} = 1.1 mM.

which describes competitive inhibition. Plots of velocity versus

$$v = \frac{V_{max}[CO]}{K_m(1 + [CS_2]/K_{i1}) + [CO]} \quad (3)$$

CO concentration at various concentrations of CS_2 are shown in Figure 3A. The solid lines were generated with the values obtained from the nonlinear fitting procedure: V_{max} = 14.9 (±1.58) μM min^{-1}; K_m for CO = 0.28 (±0.07) mM and K_{i1} = 0.47 (±0.09) mM. Extrapolating to saturating concentrations of acetyl-CoA (using a K_m for acetyl-CoA of 0.98 mM) and 100.8 kJ/mol as the activation energy (Ramer *et al.*, 1989), the V_{max} at 55 °C is calculated to be 2.36 mM min^{-1} (0.94 μmol min^{-1} mg^{-1}), which corresponds to a turnover number of 2.3 s^{-1}. A turnover number of 1.2 s^{-1} was reported earlier (Ramer *et al.*, 1989).

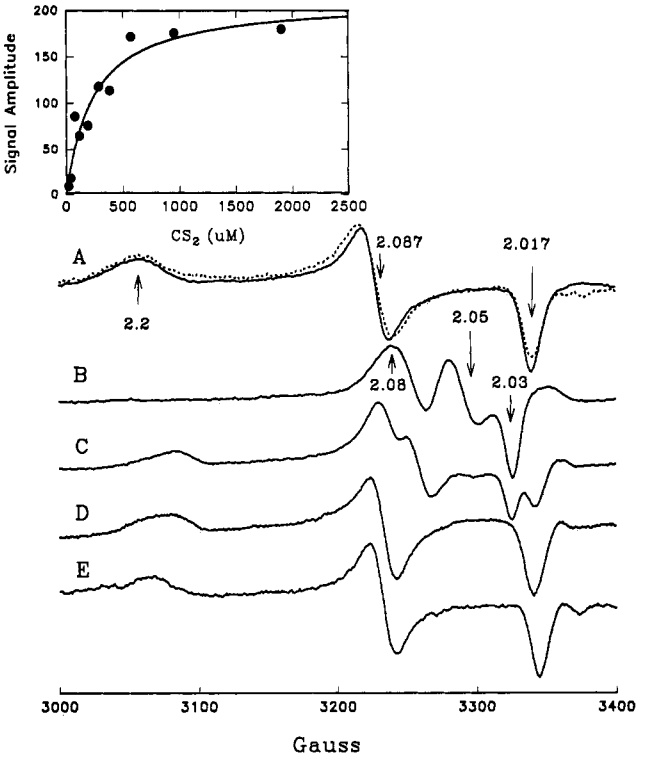


FIGURE 4: EPR spectra of the CODH–CS_2 adduct. (A) ^{61}Ni (- - -) or natural abundance (—) CODH (50 μM final) in 0.5 mM dithionite and 50 mM Tris-HCl, pH 7.6, was reacted with 0.4 mM CS_2 at 25 °C for 10 min before freezing and storing in liquid nitrogen. EPR parameters: temperature, 100 K; microwave power, 40 mW; microwave frequency, 9.4414 GHz; gain , 2 × 10^4; modulation amplitude, 10 G; modulation frequency, 100 KHz. (B) The sample in (A) was reacted with 1 atm of CO. (C) The atmosphere in the sample in (B) was replaced with N_2 by vacuum/gas cycling and reacted with 0.4 mM CS_2. (D) $^{13}CS_2$-treated CODH. EPR conditions as in (A), except that 2 mM CS_2 was used. (E) ^{57}Fe-CODH reacted with CS_2. EPR conditions as in (A) except that the microwave frequency was 9.4424 GHz. Inset: The intensity of the resonance at g = 2.088 was plotted as a function of CS_2 concentration. The solid line was calculated from the equation: $I = I_{max}([CS_2]/K_d) + [CS_2]$, where I and I_{max} represent the observed and maximal signal intensity. The value of K_d was 0.23 mM.

Effect of CS_2 on the Metal Centers in CODH. When CS_2 was reacted with samples of CODH that have been reduced by dithionite or by electrochemical methods, an EPR spectrum (Figure 4A) is observed at 90 K with g values at 2.2, 2.087, and 2.017 (g_{av} = 2.15). The maximum spin intensity observed is 0.3–0.4 spin/mol of CODH, which is similar to that observed for the Ni-Fe-C EPR signal from Center A (Gorst & Ragsdale, 1991; Lindahl *et al.*, 1990a; Ragsdale *et al.*, 1985). It appears that the signal arises from a complex between CS_2 and CODH since varying the concentration of CS_2 (Figure 4, inset) gives a saturation binding curve with a K_d of 0.23 ± 0.07 mM. In addition, the g_{av} = 2.15 signal is completely lost when the sample is placed under vacuum and the atmosphere is replaced with nitrogen to remove the CS_2. The signal returns upon readdition of CS_2. We also assessed the reversibility of formation of the CODH–CS_2 adduct by reacting the CS_2-treated enzyme with CO (Figure 4B). The EPR signal of the CODH–CS_2 complex was replaced with that characteristic of the adduct between CO and Center A (Ragsdale *et al.*, 1982). When the CO-reacted enzyme was again treated with CS_2 after removal of CO, a mixture of the g_{av} = 2.15 and Ni-Fe-C EPR signals was observed (Figure 4C).

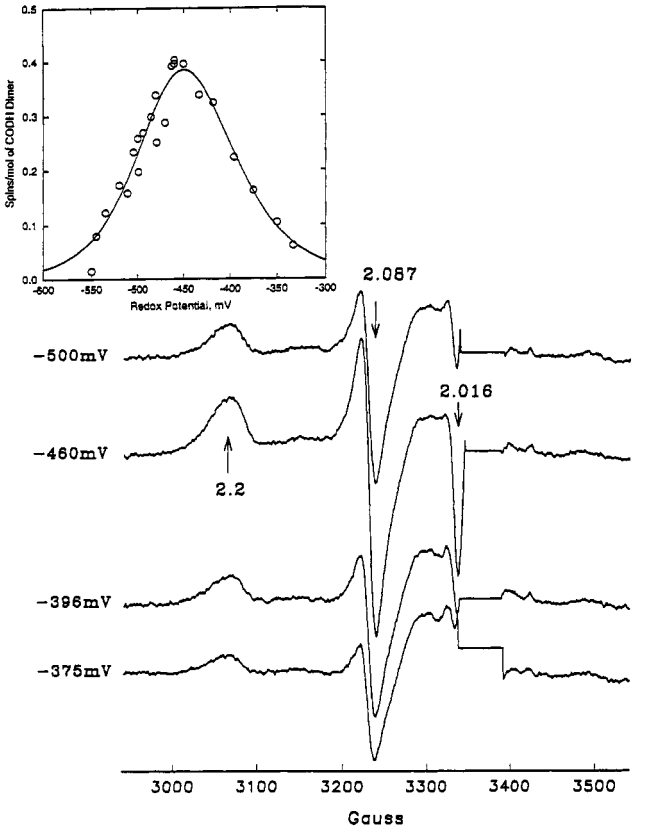


FIGURE 5: EPR spectroelectrochemical titration of the CODH–CS_2 adduct. A 500-μL solution containing CODH (40 μM final), 50 mM Tris-HCl, pH 7.6, 0.78 mM CS_2 (10 μL of CS_2-saturated water), and 40 μM each of four redox mediators was poised at redox potentials from –300 to –550 mV *vs* SHE. The redox dyes used were dimethyl-TRIQUAT, TRIQUAT, methyl viologen, and benzyl viologen. EPR conditions were the same as those listed in Figure 4A. Inset: The intensity of the resonance at $g = 2.087$ was plotted versus the potential. The solid lines were calculated using the Nernst equation with $E_1' = -454 \pm 8$ mV ($n = 1.7 \pm 0.2$) and $E_2' = -455 \pm 11$ mV ($n = 1.3 \pm 0.2$).

When ^{61}Ni ($I = {}^3/_2$)-enriched enzyme was reacted with CS_2, there is a small degree (4–5 G) of hyperfine broadening of the $g = 2.2$ and 2.087 components relative to those of the natural abundance enzyme (Figure 4A). When CODH was treated with $^{13}CS_2$ ($I = {}^1/_2$), the EPR signal was unchanged in morphology relative to that of the $^{12}CS_2$-reacted enzyme (Figure 4D). The linewidth and morphology of the EPR signal of the ^{57}Fe ($I = {}^1/_2$)-enriched enzyme after reaction with CS_2 were identical to those of the natural abundance enzyme (Figure 4E). In Figure 4, spectra C and D, the g ~ 2.2 signal has moved slightly upfield. The reason for this shift is unknown, but it is preparation dependent.

The EPR signal of the Center A–CS_2 complex is observed only from enzyme which has been reduced. This complex is stable enough that an electrochemical titration could be performed. The $g_{av} = 2.15$ EPR signal from the CODH–CS_2 adduct followed a bell-shaped curve that developed as the redox potential was lowered ($E_0' = -454 \pm 8$ mV; $n = 1.7 \pm 0.2$) and then declined at lower potentials ($E_0' = -455 \pm 11$ mV; $n = 1.3 \pm 0.2$) (Figure 5). Similar redox behavior has been observed for the nickel centers in hydrogenases (Coremans *et al.*, 1989; Teixeira *et al.*, 1985). The maximum signal intensity was obtained at ~–450 mV and amounted to 0.3–0.4 spin/mol of CODH. The bell-shaped distribution was simulated by equation 2 describing two successive one-electron reductions with the intermediate redox state giving rise to an EPR signal. Both redox reactions are fully reversible, and even after poising the potential for 30–60 min at –650 mV, the $g_{av} = 2.15$ signal can be regained by reoxidation to –450 mV.

DISCUSSION

Carbon disulfide was found to inhibit the CODH from *C. thermoaceticum*. The nucleophilic character of the sulfur atoms of CS_2 and the availability of nonbonding d-orbitals could allow it to react with a number of groups on CODH. CS_2 is a versatile ligand that forms a variety of metal–CS_2 complexes (Butler & Fenster, 1974; Ibers, 1982; Yaneff, 1977), undergoes insertion reactions between M–H and M–R bonds (Butler & Fenster, 1974; Mealli *et al.*, 1984), and reacts with alcohols, amines, and thiols to give dithio adducts [reviewed in Yokoyama and Imamoto (1984)]. Since CS_2 is a structural analogue of CO_2, it was expected that it would interact with Center C, the site of CO_2 reduction and CO oxidation. However, the following observations indicate that CS_2 instead interacts with Center A, the site of acetyl-CoA synthesis. (i) Reaction of CODH with CS_2 does not change the morphologies, intensities, or relaxation properties of the EPR signals arising from Centers B or C. (ii) The EPR signal from the CODH–CS_2 complex has slow relaxation properties similar to those of Center A, whereas Centers B and C are fast relaxing and can only be observed below 50 and 15 K, respectively. (iii) The g_{av} value of the CODH–CS_2 complex (2.15) is similar to that of Center A ($g_{av} = 2.06$), and not to that of Center B ($g_{av} = 1.94$) or Center C ($g_{av} = 1.86$). (iv) The EPR signal from the CODH–CS_2 complex has a maximum spin intensity of 0.4 spin/mol, similar to that of the Ni-Fe-C EPR signal generated when CODH is reacted with CO. (v) As an inhibitor, CS_2 is competitive with CO in reactions that take place at Center A and noncompetitive with CO at reactions that occur at Center C. (vi) CS_2 is approximately a 10-fold stronger inhibitor of the CO/acetyl-CoA exchange reaction than of CO oxidation or CO_2 reduction. (vii) The dissociation constant determined by EPR titrations (0.23 mM) of the CODH–CS_2 adduct is similar to the inhibition constant for CS_2 in the CO/acetyl-CoA exchange reaction. The only compound which had previously been shown to react with Center A is *o*-phenanthroline, which removes Ni from the protein (Shin *et al.*, 1993).

Figure 6 is a model to describe the interaction between CS_2 and Center A. Inhibition of reactions that occur at Center A by CS_2 is competitive with respect to CO and mixed relative to acetyl-CoA. Thus, CS_2 apparently can bind the free enzyme (K_i = 0.3–0.5 mM) and a form of Center A in which the methyl group and/or CoA are bound and CO is absent (K_i = 1.1 mM). Since CO and CS_2 compete in a mutually exclusive manner, formation of a metal–CS_2 adduct analogous to the adduct between CO and Center A is likely to be responsible for the inhibition. The CODH–CO adduct has been studied by EPR (Gorst & Ragsdale, 1991; Ragsdale *et al.*, 1985), ENDOR (Fan *et al.*, 1991), Mössbauer (Lindahl *et al.*, 1990b), infrared (Kumar & Ragsdale, 1992), and resonance Raman (Qiu *et al.*, 1994) spectroscopies and by electrochemistry (Gorst & Ragsdale, 1991). The carbonyl group in the adduct has been shown to serve as the precursor of the carbonyl group of acetyl-CoA (Gorst & Ragsdale, 1991). On the basis of our working model for the structure of the adduct between CO and Center A, we propose a minimal model for the CODH–CS_2 adduct as [Ni-X-$Fe_{3-4}S_4$]–CS_2. Since resonance Raman spectroscopic results have shown that CO binds to an iron site in Center A (Qiu *et al.*, 1994), we

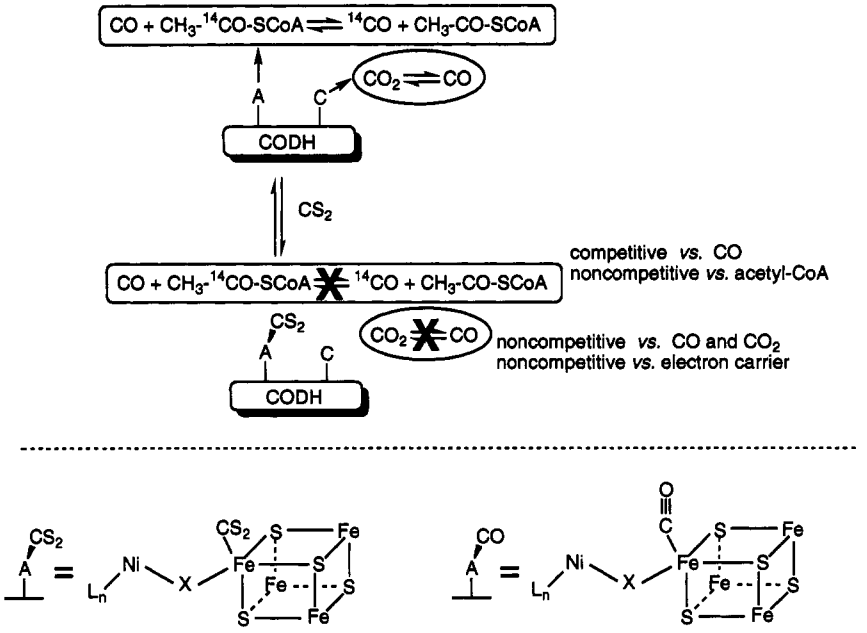


FIGURE 6: Model describing the effect of CS_2 on CODH.

suggest that CS_2 may occupy the same site. Thus, in our model, binding of CO or CS_2 increases the coordination number of an iron site in the cluster by 1 (the actual coordination number is not known). An explanation for the noncompetitive mode of inhibition *vs* acetyl-CoA follows from recent resonance Raman studies in which we suggested that the methyl group precursor of the C-2 of acetyl-CoA binds to Ni (Qiu *et al.*, 1994). CS_2 may bind to a form of Center A in which the carbonyl group has dissociated from a specific iron site and the methyl group remains bound to the Ni site. It appears that CS_2 preferentially binds the reduced form of CODH since the inhibition constant determined for CO oxidation (~12 mM) is greater than that measured for CO_2 reduction (~3 mM).

One major difference between the the EPR signals of the CODH–CO and CODH–CS_2 adducts is that, for the CS_2–CODH adduct, we observed a small degree of hyperfine broadening from the ^{61}Ni isotope replacement, but no increase in linewidth when $^{13}CS_2$ or ^{57}Fe isotope substitutions were made. In contrast, in the CO adduct with Center A, ^{61}Ni, ^{57}Fe, and ^{13}CO substitutions resulted in a significant degree of hyperfine broadening (Fan *et al.*, 1991; Ragsdale *et al.*, 1985). One explanation for these results is that binding of CS_2 to the iron site isolates the Ni and the Fe-S cluster components of Center A. CS_2 is known to insert between metal–ligand bonds (Doeff, 1986; Mealli *et al.*, 1984). However, since reaction with CS_2 is fully reversible, it is likely that the bridge between Ni and Fe-S cluster components of Center A remains intact and that the two components only become magnetically isolated.

What is the correct description of the $S = {}^1/_2$ EPR signal in the CODH–CS_2 adduct? Although the EPR signal resembles those which have been assigned to the Ni(I) state in hydrogenase (Coremans *et al.*, 1989, 1992) and several inorganic nickel complexes (Jubran *et al.*, 1985; Kumar *et al.*, 1989; Lovecchio *et al.*, 1974), the line broadening due to ^{61}Ni replacement is much less than has been observed in these protein-bound and model nickel centers. It is likely that, in the CODH–CS_2 complex, most of the spin density resides on ligands (including possibly the sulfurs of CS_2) and not on nickel. Even in hydrogenase, it is controversial whether the unpaired spin density in the $S = {}^1/_2$ EPR signal in the reduced state of hydrogenase rests primarily on nickel [giving rise to the Ni(I) state] or on ligands (Bagyinka *et al.*, 1993).

A puzzle that has plagued interpretation of the spectroscopy of CODH is why the maximum EPR signal intensity from the CODH–CO complex is only 0.3–0.4 spin/mol of dimeric enzyme. One should observe integral spin intensity in an $S = {}^1/_2$ center. The finding that ~15% of the nickel in CODH (0.3 Ni/mol of dimer) can be removed with *o*-phenanthroline led Lindahl and co-workers to propose that the reason for low spin intensity is that the Ni sites in Center A of CODH are heterogeneous, with only 15% of the Ni (30% of the enzyme) in an active state (Shin *et al.*, 1993). Our studies of the redox chemistry of the complex between CS_2 and CODH suggest another possibility. The CODH–CS_2 complex is similar to the CODH–CO complex; however, it is difficult to perform redox titrations with CO because it is a strongly reducing substrate that establishes a low redox potential. In addition, CO not only forms a stable metal–carbonyl bond at Center A, but also undergoes oxidation at Center C. CS_2, however, is not a substrate and forms a stable complex with CODH that can be electrochemically titrated. Instead of obtaining a sigmoidal dependence of EPR signal intensity *vs* redox potential as expected for a single electron-transfer process, we observed a bell-shaped profile. These results indicate that the CS_2-treated enzyme can undergo two successive one-electron reductions and that the intermediate state is EPR active (eqs 4 and 5). A fit to the appropriate Nernst equation

$$1e^- + \text{Center A}^{(n+2)+} \rightleftharpoons \text{Center A}^{(n+1)+} \quad (4)$$

$$1e^- + \text{Center A}^{(n+1)+} \rightleftharpoons \text{Center A}^{(n)+} \quad (5)$$

demonstrates that the EPR signal from the CODH–CS_2 adduct reaches a maximum of 0.3–0.4 spin/mol because the standard reduction potentials for the two redox processes are the same. In essence, as the Ni/Fe-S cluster becomes reduced by the second electron, the intermediate EPR-active state is converted into a diamagnetic EPR-silent state. By analogy, we suggest that the EPR signal from the CODH–CO complex does not reach above 0.3–0.4 spin/mol because it also

undergoes two isopotential one-electron transfers. The apparent reduction potential for formation of the CODH–CO complex (–540 mV) was measured indirectly by titrating the EPR signal observed when CODH is treated with acetyl-CoA (Gorst & Ragsdale, 1991). Unfortunately, this value is too low to have been able to observe the decrease in the EPR signal intensity that would correspond to the denouement in the bell-shaped distribution. That two-electrons can be transferred to the Ni/Fe-S cluster at Center A is consistent with its structure. Center A could potentially accommodate at least three electrons since Ni has three physiologically relevant redox states (Ni^{3+}, Ni^{2+}, and Ni^{1+}) and the iron–sulfur cluster has at least two readily available redox states.

In reactions that take place at Center C (Anderson *et al.*, 1993; Kumar *et al.*, 1993), CS_2 was found to be a noncompetitive inhibitor. Results presented here and previous kinetic studies of the partially purified CODH from *C. thermoaceticum* (Diekert & Thauer, 1978) and of the CODH from *R. rubrum* (Hyman *et al.*, 1989) have indicated a ping-pong mechanism for the CO oxidation reaction. Spectroscopic studies gave direct evidence for such a mechanism. Binding of CO to Center C was shown to occur rapidly ($k = 10^8$ M^{-1} s^{-1}, 55 °C) before electron transfer to the [4Fe-4S] cluster at Center B ($k = 2000$ s^{-1}, 55 °C) occurs (Kumar *et al.*, 1993). Thus, CO binds to the oxidized form of Center C and CO_2 is released before methyl viologen or other electron acceptors reoxidize CODH. The principle of microscopic reversibility would suggest that the CO_2 reduction reaction also follows a ping-pong kinetic mechanism. In this case, dithionite or other electron donors would be required to reduce CODH before CO_2 binds to the reduced form of Center C. CS_2 was found to be a noncompetitive inhibitor relative to CO and methyl viologen in the CO oxidation reaction and noncompetitive with respect to CO_2 in the reduction of CO_2 to CO. The CS_2–CODH complex was shown to be nonproductive both for CO oxidation/CO_2 reduction and for acetyl-CoA synthesis. Another cumulene, COS, has been found to undergo reduction to CO and H_2S by the *C. thermoaceticum* CODH (Gorst, 1991) and thus appears to serve as an analogue of CO_2 by binding at Center C. COS was found to bind tightly to the CODH from *R. rubrum* ($K_d = 2.2$ μM) and to be competitive *vs* CO in CO oxidation (Hyman *et al.*, 1989). In the only other study (to the knowledge of the authors) of CS_2 binding to an enzyme, a high concentration of CS_2 (28.9 mM) was found to slightly reverse the inhibition of the *R. rubrum* CODH by cyanide (Hyman *et al.*, 1989).

In summary, we have studied the interaction between CODH and CS_2. CS_2 binds to Center A, the site of acetyl-CoA synthesis, in a manner similar to and competitive with CO binding. The reduced form of the CODH–CS_2 complex exhibits EPR spectra similar to those assigned to Ni(I) in other enzymes and model complexes. This complex can undergo two one-electron processes. The narrow spacing between the two reduction potentials can explain why nonintegral EPR signals are obtained in the CODH–CO and CODH–CS_2 complexes at Center A. Analogous to the binding of CO, we propose that CS_2 binds to an iron site in Center A.

ACKNOWLEDGMENT

We thank Lifei Liu, who performed the purification of CODH, and Dr. Javier Seravalli for his insights during the extensive discussions regarding the inhibition experiments.

REFERENCES

Abbanat, D. R., & Ferry, J. G. (1990) *J. Bacteriol. 172*, 7145–7150.
Anderson, M. E., DeRose, V. J., Hoffman, B. M., & Lindahl, P. A. (1993) *J. Am. Chem. Soc. 115*, 12204–12205.
Andreesen, J. R., Schaupp, A., Neurater, C., Brown, A., & Ljungdahl, L. G. (1973) *J. Bacteriol. 114*, 743–751.
Antonini, E., & Brunori, M. (1971) in *Hemoglobin and myoglobin in their reactions with ligands*, North-Holland Publishing Co., Amsterdam, The Netherlands.
Aresta, M., Nobile, C. F., Albano, V. G., Forni, E., & Manassero, M. (1975) *J. Chem. Soc., Chem. Commun.*, 636–637.
Aresta, M., Fragle, C., Quarante, E., & Tommasi, I. (1992) *J. Chem. Soc., Chem. Commun.*, 315–316.
Bagyinka, C., Whitehead, J. P., & Maroney, M. J. (1993) *J. Am. Chem. Soc. 115*, 3576–3585.
Beauchamp, R. O., Bus, J. S., Popp, J. A., Boreiko, C. J., & Goldberg, I. (1981) *CRC Crit. Rev. Toxicol. 11*, 169–278.
Behr, A. (1988) *Angew Chem., Int. Ed. Engl. 27*, 661–678.
Bonam, D., & Ludden, P. W. (1987) *J. Biol. Chem. 262*, 2980–2987.
Bott, M., & Thauer, R. K. (1989) *Z. Naturforsch. 44*, 392–396.
Braunstein, P., Matt, D., & Nobel, D. (1988) *Chem. Rev. 88*, 747–764.
Budavari, S., Ed. (1989) *The Merck Index*, Edition 11, pp 274–275, Merck & Co., Rahway, NJ.
Butler, I. S., & Fenster, A. E. (1974) *J. Organomet. Chem. 101*, 161–194.
Coremans, J. M. C. C., van der Zwaan, J. W., & Albracht, S. P. J. (1989) *Biochim. Biophys. Acta 997*, 256–267.
Coremans, J. M. C. C., van Garderen, C. J., & Albracht, S. P. J. (1992) *Biochim. Biophys. Acta 1119*, 148–156.
Cornish-Bowden, A. (1979) in *Fundamentals of enzyme kinetics*, Butterworths & Co. Ltd., London.
Diekert, G. B., & Thauer, R. K. (1978) *J. Bacteriol. 136*, 597–606.
Diekert, G., & Ritter, M. (1983) *FEBS Lett. 151*, 41–44.
Doeff, M. M. (1986) *J. Am. Chem. Soc. 25*, 2474–2476.
Drake, H. L., Hu, S.-I., & Wood, H. G. (1980) *J. Biol. Chem. 255*, 7174–7180.
Drake, H. L., Hu, S.-I., & Wood, H. G. (1981) *J. Biol. Chem. 256*, 11137–11144.
Elliott, J. I., & Brewer, J. M. (1978) *Arch. Biochem. Biophys. 190*, 351–357.
Ensign, S. A., Bonam, D., & Ludden, P. W. (1989a) *Biochemistry 28*, 4968–4973.
Ensign, S. A., Hyman, M. R., & Ludden, P. W. (1989b) *Biochemistry 28*, 4973–4979.
Fan, C., Gorst, C. M., Ragsdale, S. W., & Hoffman, B. M. (1991) *Biochem. 30*, 431–435.
Ferry, J. G. (1992a) *Crit. Rev. Biochem. Mol. Biol. 27*, 473–502.
Ferry, J. G. (1992b) *J. Bacteriol. 174*, 5489–5495.
Fuchs, G. (1986) *FEMS Microbiol. Rev. 39*, 181–213.
Fujita, E., Creutz, C., Sutin, N., & Szalda, D. J. (1991) *J. Am. Chem. Soc. 113*, 343–353.
Gorst, C. M. (1991) Characterization of the metal centers of carbon monoxide dehydrogenase from *Clostridium thermoaceticum*, Ph.D. Thesis, University of Wisconsin— Milwaukee.
Gorst, C. M., & Ragsdale, S. W. (1991) *J. Biol. Chem. 266*, 20687–20693.
Grahame, D. A., & Stadtman, T. C. (1987) *J. Biol. Chem. 262*, 3706–3712.
Harder, S. A., Feinberg, B. F., & Ragsdale, S. W. (1989a) *Anal. Biochem. 181*, 283–287.
Harder, S. A., Lu, W.-P., Feinberg, B. F., & Ragsdale, S. W. (1989b) *Biochemistry 28*, 9080–9087.
Hu, S.-I., Drake, H. L., & Wood, H. G. (1982) *J. Bacteriol. 149*, 440–448.
Hyman, M. R., Ensign, S. A., Arp, D. J., & Ludden, P. W. (1989) *Biochemistry 28*, 6821–6826.

Ibers, J. A. (1982) *Am. Chem. Soc. Rev. 11*, 57–73.
Ito, T., Sugimoto, S., Ohki, T., Nakano, T., & Osakada, K. (1992) *J. Organomet. Chem. 428*, 69–83.
Jubran, N., Ginzburg, G., Cohen, H., Koresh, Y., & Meterstein, D. (1985) *Inorg. Chem. 24*, 251–258.
Kumar, M., & Ragsdale, S. W. (1992) *J. Am. Chem. Soc. 114*, 8713–8715.
Kumar, M., Day, R. O., Colpas, G. J., & Maroney, M. J. (1989) *J. Am. Chem. Soc. 111*, 5974–5976.
Kumar, M., Lu, W.-P., Liu, L., & Ragsdale, S. W. (1993) *J. Am. Chem. Soc. 115*, 11646–11647.
Lindahl, P. A., Münck, E., & Ragsdale, S. W. (1990a) *J. Biol. Chem. 265*, 3873–3879.
Lindahl, P. A., Ragsdale, S. W., & Münck, E. (1990b) *J. Biol. Chem. 265*, 3880–3888.
Ljungdahl, L. G. (1986) *Annu. Rev. Microbiol. 40*, 415–450.
Lovecchio, F. V., Gore, E. S., & Busch, D. H. (1974) *J. Am. Chem. Soc. 96*, 3109–3118.
Mealli, C., Hoffman., R., & Stockis, A. (1984) *Inorg. Chem. 23*, 56–65.
Qiu, D., Kumar, M., Ragsdale, S. W., & Spiro, T. G. (1994) *Science 264*, 817–819.
Ragsdale, S. W. (1991) *CRC Crit. Rev. Biochem. Mol. Biol. 26*, 261–300.
Ragsdale, S. W., & Wood, H. G. (1985) *J. Biol. Chem. 260*, 3970–3977.
Ragsdale, S. W., Ljungdahl, L. G., & DerVartanian, D. V. (1982) *Biochem. Biophys. Res. Commun. 108*, 658–663.
Ragsdale, S. W., Clark, J. E., Ljungdahl, L. G., Lundie, L. L., & Drake, H. L. (1983a) *J. Biol. Chem. 258*, 2364–2369.
Ragsdale, S. W., Ljungdahl, L. G., & DerVartanian, D. V. (1983b) *Biochem. Biophys. Res. Commun. 115*, 658–665.
Ragsdale, S. W., Wood, H. G., & Antholine, W. E. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 6811–6814.
Ramer, S. E., Raybuck, S. A., Orme-Johnson, W. H., & Walsh, C. T. (1989) *Biochemistry 28*, 4675–4680.
Raybuck, S. A., Bastian, N. R., Orne-Johnson, W. H., & Walsh, C. T. (1988) *Biochemistry 27*, 7698–7702.
Shin, W., Anderson, M. E., & Lindahl, P. A. (1993) *J. Am. Chem. Soc. 115*, 5522–5526.
Stephens, P. J., McKenna, M.-C., Ensign, S. A., Bonam, D., & Ludden, P. W. (1989) *J. Biol. Chem. 264*, 16347–16350.
Tanase, T., Nitta, S., Yoshtikawa, S., Kobayashi, K., Sakurai, T., & Yano, S. (1992) *Inorg. Chem. 31*, 1058–1062.
Teixeira, M., Moura, I., Xavier, A. V., Huynh, B. H., DerVartanian, D. V., Peck, H. D., Jr., LeGall, J., & Moura, J. J. G. (1985) *J. Biol. Chem. 260*, 8942–8950.
Thauer, R. K., Möller-Zinkhan, D., & Spormann, A. M. (1989) *Annu. Rev. Microbiol. 43*, 43–67.
Tsuda, T., Maruta, K.-I., & Kitaike, Y. (1992) *J. Am. Chem. Soc. 114*, 1498–1499.
Vanhoorne, M., Vermeulen, A., & de Bacquer, D. (1993) *Arch. Environ. Health 48*, 370–375.
Yaneff, P. V. (1977) *Coord. Chem. Rev. 23*, 183–220.
Yokoyama, M., & Imamoto, T. (1984) *Synthesis 10*, 797–823.