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Characterization and Purification of Carbon Monoxide Dehydrogenase from *Methanosarcina barkeri*

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Carbon monoxide-dependent production of H_2, CO_2, and CH_4 was detected in crude cell extracts of acetate-grown *Methanosarcina barkeri*. This metabolic transformation was associated with an active methyl viologen-linked CO dehydrogenase activity (5 to 10 U/mg of protein). Carbon monoxide dehydrogenase activity was inhibited 85% by 10 μM KCN and was rapidly inactivated by O_2. The enzyme was nearly homogenous after 20-fold purification, indicating that a significant proportion of soluble cell protein was CO dehydrogenase (ca. 5%). The native purified enzyme displayed a molecular weight of 232,000 and a two-subunit composition of 92,000 and 18,000 daltons. The enzyme was shown to contain nickel by isolation of radioactive CO dehydrogenase from cells grown in ^{63}Ni. Analysis of enzyme kinetic properties revealed an apparent K_m of 5 mM for CO and a V_{max} of 1,300 U/mg of protein. The spectral properties of the enzyme were similar to those published for CO dehydrogenase from acetogenic anaerobes. The physiological functions of the enzyme are discussed.

An early indication of carbon monoxide metabolism by anaerobic bacteria came in 1931 when Fisher et al. (12) reported that CO was converted to CH_4 and CO_2 by sewage sludge and mixed cultures of methanogens. Kluyver and Schnellen (26) later demonstrated that cell suspensions of *Methanosarcina barkeri* and *Methanobacterium formicicum* transformed CO into CH_4 and CO_2. Daniels et al. (4) showed that the slow growth of *Methanobacterium thermoautotrophicum* on CO as energy source was associated with a deazaflavin-linked CO dehydrogenase activity in cell extracts. Recently, hydrogen production and consumption were shown to be associated with growth of *Methanosarcina barkeri* on CO as the sole carbon and energy source (30). When compared with aerobic or phototrophic bacteria that grow on CO, methanogens are considered to be non-utilitarian or adventitious consumers of the gas (16, 25, 36) because the CO-metabolizing system is constitutive (4, 27) and growth on CO is slow in comparison to growth on other energy sources consumed (4, 30).

Acetate synthesis by cell extracts of *Clostridium thermoaceticum* grown on glucose was shown to require a protein fraction containing the nickel enzyme CO dehydrogenase (9, 10). CO dehydrogenase-dependent acetyl-coenzyme A synthesis is now supported in other species of H_2-consuming acetogens (5, 6, 17, 23). Acetate is an important cell carbon precursor of methanogenic bacteria (13, 14, 20, 33, 34, 39). Synthesis of acetyl-coenzyme A or acetate from one-carbon compounds by *Methanosarcina barkeri* (22) and *Methanobacterium thermoautotrophicum* (33, 34) involves the carbonylation of a methyl group, a reaction presumably catalyzed by CO dehydrogenase.

We previously reported (27) that CO dehydrogenase activity in cell extracts of *Methanosarcina barkeri* increased fivefold when this organism was grown on acetate versus on one-carbon substrates. This finding implied that methanogens may utilize CO dehydrogenase to decarboxylate acetate in a manner mechanistically analogous to the reverse direction of the enzyme during acetate synthesis. The purpose of this paper is to more carefully characterize CO dehydrogenase activity in extracts from acetate-grown cells and to compare the properties of the enzyme from *Methanosarcina barkeri* with those published for CO dehydrogenase in H_2-consuming acetogenic bacteria.

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MATERIALS AND METHODS

Chemicals, gases, and purification materials. All chemicals were reagent grade. Gases were purchased from Matheson Scientific, Inc., Joliet, Ill. DEAE-Sephadex A-25, Sephadex G-25, Sepharose CL-6B, and molecular weight standards for gel filtration were obtained from Pharmacia Fine Chemicals, Piscataway, N.J. Bio-Gel HTP hydroxylapatite was purchased from Bio-Rad Laboratories, Richmond, Calif. ^{63}Ni (6.2 mCi/mg) was purchased from New England Nuclear Corp., Boston, Mass.

Organism and cultivation. *Methanosarcina barkeri* MS was originally provided by M. Bryant, University of Illinois, and its general physiological properties are described elsewhere (20, 21, 27, 30, 39, 40). It was mass cultured on 60 mM sodium acetate in 15 liters of the PBB medium described previously (20), except that twice the concentration of filter-sterilized vitamins were added after autoclaving. Cultures were incubated at 37°C without agitation under an N_2 gas phase. During later stages of logarithmic methane production, cells were harvested by anoxic centrifugation and washed anaerobically three times in buffer A (30 mM Tris-hydrochloride [pH 8.0], 2 mM dithiothreitol, and 2 mM $Na_2S_2O_4$) and then stored at −80°C under hydrogen until use.

Cells were mass cultured for the isolation of ^{63}Ni-labeled CO dehydrogenase as described above, except that 400 μCi of $^{63}NiCl_2$ was added after autoclaving.

Analysis of gases and radioisotopes. CH_4 was analyzed with a model 600D Aerograph gas chromatograph (Varian Associates, Palo Alto, Calif.) equipped with a Poropak R column (Anspec Co., Inc., Warrenville, Ill.) and a flame ionization detector. H_2 was detected with a model 802 gas chromatograph (Packard Instrument Co., Inc., Downers Grove, Ill.), containing a Poropak N column and a thermal conductivity detector. CO_2 was analyzed by a Packard model 417 gas chromatograph equipped with a thermal conductivity detector and a carbosieve B column (2.7 m by 0.3 cm).

TABLE 1. Purification procedure used for carbon monoxide dehydrogenase of *Methanosarcina barkeri*[a]

Fraction	Vol (ml)	Total protein (mg)	Total activity (U)[b]	Sp act (U/mg of protein)	Yield (%)	Fold purification
Cell-extract supernatant (150,000 × *g*)	7.4	130	1,400	10.8	100	1
Sepharose CL-6B	38	69	1,180	17.0	84	1.6
DEAE-Sephadex	46	7.7	703	91	50	8.4
Bio-Gel HTP hydroxylapatite	12	2.3	505	216	36	20

[a] Activity was determined by methyl viologen reduction according to details provided in the text.
[b] One unit = 1 μmol of CO oxidized per min.

^{63}Ni was quantified in Instagel (Packard) with a Packard PLD Prias liquid scintillation counter. ^{63}Ni in acrylamide gels was analyzed by slicing frozen gels in 0.3-cm sections, digesting the gel slice in 30% H_2O_2 at 80°C for 4 h, and counting in Instagel.

Preparation of extracts and purification of CO dehydrogenase. Crude cell extracts were prepared by suspending washed cells in an equal volume of buffer A (for purification) or anaerobic water (for inhibitor and gas production studies), followed by disruption in a French pressure cell and centrifugation at 30,000 × *g* as described earlier (21).

The cell extract was ultracentrifuged anaerobically in sealed Spinco tubes (Beckman Instruments, Inc.) at 150,000 × *g* for 90 min to remove membrane fragments before protein purification. The following procedures were all performed in an anaerobic glovebag (Coy Laboratory Products, Inc., Ann Arbor, Mich.) with a 5% H_2-95% N_2 atmosphere at 30°C. The ultracentrifuged supernatant (5 to 15 ml) was loaded on a Sepharose CL-6B column (57.5 by 2.6 cm) equilibrated with buffer A and then eluted at a flow rate of 20 ml/h. Individual fractions were collected in tubes which were then stoppered and removed from the glovebag for analysis. Fractions containing CO dehydrogenase were returned to the glovebag, pooled, and applied to a DEAE-Sephadex A-25 column (26 by 2.6 cm) equilibrated in buffer A. The protein was then eluted with a linear gradient (500 ml) of 0.1 to 0.4 M NaCl dissolved in buffer A at a flow rate of 50 to 60 ml/h. CO dehydrogenase activity eluted as a single peak. The pooled fractions were dialyzed overnight against 4 liters of 30 mM K_2HPO_4-HCl buffer, pH 7.0, containing 2 mM $Na_2S_2O_4$–2 mM dithiothreitol (buffer B). The dialyzed solution was then applied to a hydroxylapatite column (20 by 0.76 cm) equilibrated in buffer B and eluted at 20 ml/h with a linear gradient (80 ml) of 0.03 to 0.3 M K_2HPO_4 adjusted to pH 7.0 with HCl and containing 2 mM $Na_2S_2O_4$–dithiothreitol. The enzyme was either used directly or dialyzed against buffer A. Purified enzyme was extremely oxygen sensitive and could be manipulated only with syringes flushed with dithionite solutions or CO.

Before electrophoresis or spectral analysis, CO dehydrogenase was concentrated with Centriflo type CF25 ultrafiltration cones (Amicon Corp.). Dilute solutions of the enzyme were placed into cones and loaded into a rotor in the glovebag. Tubes containing $Na_2S_2O_4$ were also placed into the rotor to scavenge oxygen, and the rotor was sealed with vacuum grease before removal from the glovebag. After centrifugation, the rotor was returned to the glovebag, where samples were removed.

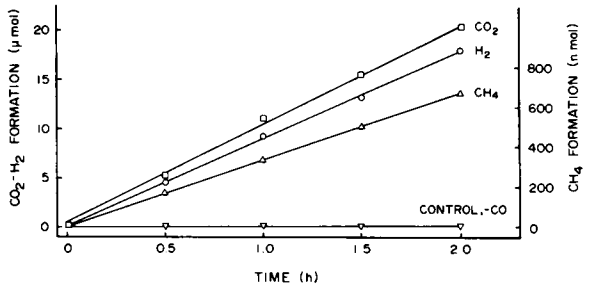

FIG. 1. CO-dependent gas production of H_2, CO_2, and CH_4 by cell extracts of *Methanosarcina barkeri*. Extracts were prepared from acetate-grown cells and were incubated in vials under 55% CO at 37°C without agitation. N_2 served as the headspace in control experiments.

Protein content of the crude cell extract and the purified enzyme fractions was estimated by the method of Bradford (2), with bovine serum albumin in the appropriate buffer as standard.

Enzymatic analysis. CO dehydrogenase was assayed in 1 ml of 60 mM KPO_4 buffer (pH 7.0)–5 mM methyl viologen, contained in glass cuvettes (1.4 ml) that were stoppered with grey rubber bungs. The cuvettes were flushed and evacuated with N_2, and 0.4 cc of 100% CO was added. After equilibration for at least 30 min at 37°C, enough $Na_2S_2O_4$ was added to turn the reaction mixture light blue. The assay was initiated by injection of enzyme, and dye reduction was monitored at 578 nm with an Eppendorf Spectrophotometer (Brinkmann Instruments, Inc., Wesbury, N.Y.). Inhibitor studies were performed with 30,000 × *g* cell extract diluted 10-fold with 55 mM KPO_4, pH 7.0. The dilute extract (2 to 3 mg of protein/ml) was incubated with inhibitors under a 5% H_2-95% N_2 gas phase at room temperature before assay in the presence of the same concentration of inhibitor. A similarly diluted crude extract was used to assess oxygen sensitivity. It was stirred under air at 1°C, samples were removed and reduced with 1 mM dithionite under H_2, and activity was assayed.

The apparent K_m for purified CO dehydrogenase was determined at 37°C by injecting up to 2 atm (ca. 203 kPa) of 100% CO into cuvettes with the bung taped into place. A Bunsen coefficient of 0.0173, obtained by interpolation of values at 30°C and 40°C (1), was used to calculate the concentration of CO in solution. These assays used 20 mM methyl viologen, at this concentration no increase in CO dehydrogenase activity was observed under 1 atm of CO.

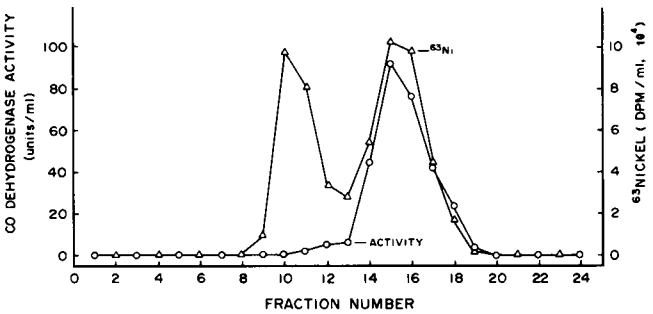

FIG. 2. Elution of ^{63}Ni proteins and CO dehydrogenase on hydroxylapatite. Extract was prepared from cells grown in ^{63}Ni. 700 U of CO dehydrogenase (8 mg of protein) from the DEAE-Sephadex step was applied to the column, and 2.5-ml fractions were collected.

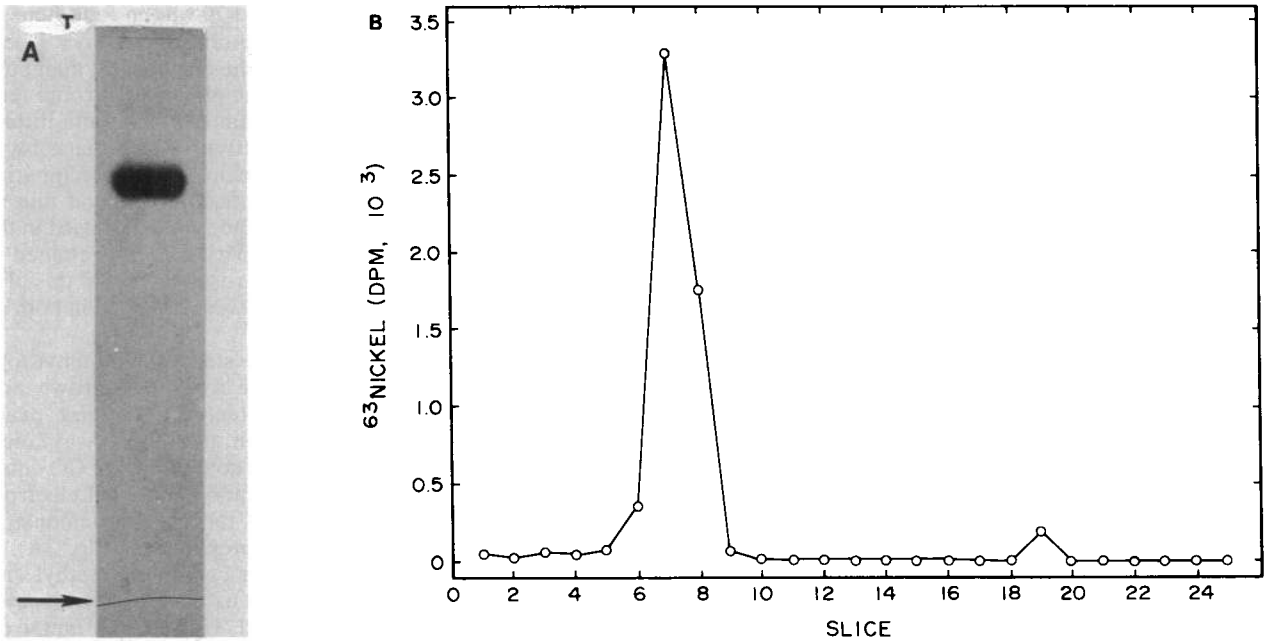

FIG. 3. Native gel electrophoresis of purified ^{63}Ni-labeled CO dehydrogenase. A 20-ug portion of 20-fold purified enzyme was applied to the gels. (A) Staining of proteins with Coomassie brilliant blue. T, Top of resolving gel; arrow, wire marking location of dye front. The R_f value of the CO dehydrogenase band was 0.29. (B) Location of ^{63}Ni in 0.3-cm sections of an activity-stained gel. The first slice is the top of the resolving gel. The large peak of ^{63}Ni had an R_f value of 0.29 and corresponded to the CO dehydrogenase activity-stained band.

Gas production from CO was measured in 12-ml vials, containing 0.5 ml of 120 mM KPO_4 buffer (pH 7.0) and an N_2 gas phase. N_2 or CO was injected (6 ml) and allowed to equilibrate at 37°C without shaking. The reaction was then initiated by injection of 0.5 ml of a 20-mg of protein per ml crude extract. CO_2 was corrected for its solubility as described previously (27).

Electrophoresis and molecular weight determination. Native discontinuous polyacrylamide slab gel electrophoresis was performed anaerobically in Tris-hydrochloride buffer (pH 8.9), according to a standard procedure (3). After electrophoresis, gels were placed into anaerobic 30-mM Tris-hydrochloride (pH 8.0) solution, removed from the glovebag, and gassed extensively with N_2 to remove H_2. Afterward, 1 mM methyl viologen was added, and the solution was reduced with enough $Na_2S_2O_4$ to turn the dye light blue. The gel was gassed with CO for detection of CO dehydrogenase activity. After development of the blue band, triphenyltetrazolium chloride was added to make the color permanent (31). Sodium dodecyl sulfate electrophoresis was performed by the procedures of Laemmli (28) in either 10 or 11.5% acrylamide gels. Protein standards (Sigma Chemical Co., St. Louis, Mo.) employed for molecular weight determinations were: myosin (205,000), β-galactoside (116,000), phosphorylase B (97,400), bovine serum albumin (66,000), ovalbumin (45,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), carbonic anhydrase (29,000), trypsinogen (24,000), trypsin inhibitor (21,100), and α-lactalbumin (14,200). Gels were scanned at 633 nm with a model 2202 Ultroscan laser densitometer (LKB Instruments, Inc., Rockville, Md.). Native enzyme molecular weights were determined on a Sepharose CL-6B column (57.5 by 2.6 cm) equilibrated with 50 mM NaCl in 30 mM Tris-hydrochloride (pH 8.0). Purified CO dehydrogenase (3 ml) or standard protein was loaded and eluted with the same buffer.

Spectra. Concentrated enzyme was passed over a G-25 Sephadex column equilibrated in 30 mM Tris-hydrochloride (pH 8.0) to remove dithionite and dithiothreitol. After this procedure, the enzyme retained 10% activity. The enzyme was scanned with a model 25 dual-beam spectrophotometer (Beckman Instruments, Inc.) first under H_2, and then the same solution was scanned after injection of 1 atm overpressure of CO.

RESULTS

Analysis of CO dehydrogenase in cell extracts. Figure 1 illustrates the influence of CO on gaseous product formation by cell extracts of *Methanosarcina barkeri* grown on ace-

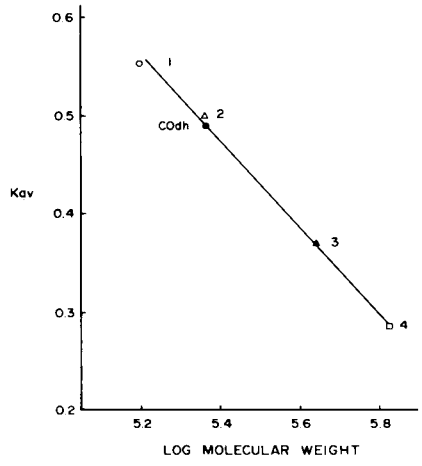

FIG. 4. Molecular weight determination of native CO dehydrogenase on Sepharose CL-6B. Standards: (1) aldolase, 158,000; (2) catalase, 232,000; (3) ferritin, 444,000; and (4) thyroglobulin, 669,000. CO dehydrogenase is indicated by the solid circle.

FIG. 5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of 20-fold purified CO dehydrogenase. A 10-μg portion of protein was applied to a 10% gel. T, Top of resolving gel; arrow, location of dye front. Lane A contains the following molecular weight standards (top to bottom): bovine serum albumin, ovalbumin, glyceraldehyde-3-phosphate dehydrogenase, carbonic anhydrase, trypsinogen, trypsin inhibitor, and alpha-lactalbumin. Lane B is the purified CO dehydrogenase, showing alpha and beta subunits (top and bottom, respectively).

tate. Cell extracts produced CO_2, H_2, and CH_4 when incubated under a 55% CO gas phase. Apparently, reducing equivalents produced by the action of CO dehydrogenase were channeled into both H_2 and CH_4 production, which occurred linearly for 2 h. Significant production of H_2, CH_4, or CO_2 was not observed in control experiments with cell extracts incubated under N_2.

The effects of various enzyme inhibitors on CO dehydrogenase activity in cell extracts were examined. Air and KCN were the most potent inhibitors tested. Under 1-atm CO and with 5 mM methyl viologen, only 10 μM KCN was required to decrease enzyme activity by 85% of control values. Oxygen treatment resulted in an activity loss of 90% within 30 min. Treatment of oxygen-inactivated enzyme with CO, H_2, or dithionite did not restore activity. The following potential inhibitors were also tested at the indicated concentrations: 10 mM $NaAsO_2$, 10 mM NaN_3, 10 mM NaI, 900 μM iodopropane, 10 mM Na_2SO_3, and 0.5 atm of N_2O. Na_2SO_3 inhibited enzyme activity by 40%, whereas the other compounds did not cause significant inhibition.

The crude cell extract was ultracentrifuged at 150,000 × *g*. Only 4 to 8% of the CO dehydrogenase activity was associated with the pellet, whereas the remaining activity was in the supernatant. Similar specific activities were observed in both fractions. Although this finding suggests that the enzyme is not strongly associated with the membrane, a weak association such as that observed for the CO dehydrogenase of *Rhodopseudomonas gelatinosa* (38) could exist.

Enzyme purification. The CO dehydrogenase from *Methanosarcina barkeri* was purified 20-fold in four steps (Table 1). The procedure generally required 3 to 4 days. Due to the extreme oxygen sensitivity of the enzyme, all manipulations were performed in an anaerobic glovebag at room temperature in buffers containing sodium dithionite and dithiothreitol. The CO dehydrogenase activity was remarkably stable under these conditions. Ammonium sulfate precipitation was an unsatisfactory primary purification method due to low enzyme recovery. DEAE-Sephadex was not used in the first step because 30% enzyme activity was not retained by the column. Gel filtration on Sepharose eliminated this phenomenon, and single activity peaks were obtained in both steps 2 and 3.

Figure 2 illustrates the last step of CO dehydrogenase purification on hydroxylapatite from cells grown on ^{63}Ni. Two peaks of ^{63}Ni were detected. The first peak was coincident with a yellow protein, the second was coincident with a yellowish-brown protein containing the CO dehydrogenase activity. Anaerobic polyacrylamide gel electrophoresis of the pooled fractions 13 through 19 demonstrated a nearly homogeneous protein preparation (Fig. 3A). When the gel was incubated in buffer containing methyl viologen under CO, the vast majority of enzyme activity was associated with the major protein band. However, a minor protein contaminant was present, which migrated near the top of the resolving gel. The preparation appeared to be 95% homogeneous when scanned by laser densitometer. ^{63}Ni migrated with CO dehydrogenase activity in the gel (Fig. 3B). The slices of gel with the highest amount of ^{63}Ni were those retaining the most color when methyl viologen activity staining was made permanent by the addition of triphenyltetrazolium chloride. ^{63}Ni was tightly bound to the enzyme and was not removed by anaerobic dialysis or gel permeation.

Characterization of the purified enzyme. The elution volume of CO dehydrogenase from the Sepharose column was determined with ^{63}Ni-labeled protein because it was very difficult to maintain active CO dehydrogenase in the purified

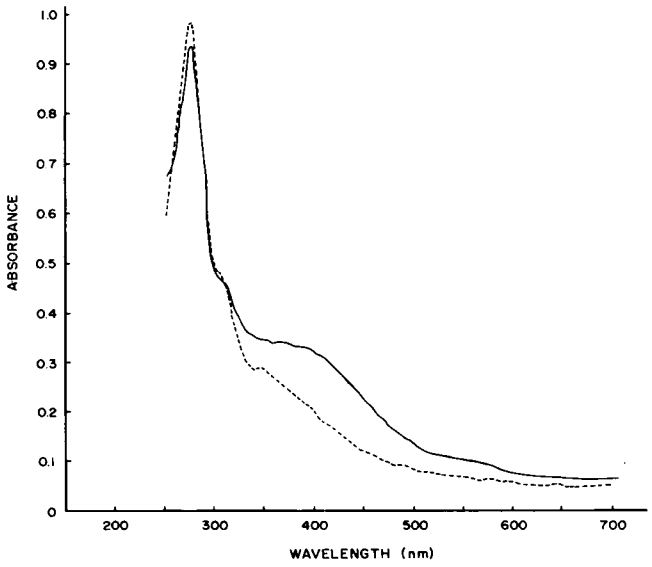

FIG. 6. Spectral analysis of purified CO dehydrogenase. Reductants were removed from purified enzyme by gel filtration. Protein (1 mg/ml) in 30 mM Tris-hydrochloride was scanned under H_2 (solid line) or after injection of 1 atm of CO into the same cuvette (broken line).

state without reducing agents. Nearly 100% of the radioactivity applied eluted at a volume indicating a molecular weight for the native enzyme of 232,000, when compared with standards of known molecular weight (Fig. 4).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the purified CO dehydrogenase revealed two subunits, with molecular weights equal to 92,000 and 18,000 (Fig. 5). Densiometric estimates of the ratio of dye retained by the large and small subunits ranged from 4.0 to 4.3. This gives a molar ratio of 0.78 to 0.84 for the two subunits, which suggests an $\alpha_2\beta_2$ subunit stoichiometry for the native enzyme.

Figure 6 illustrates the UV-visible spectrum of CO dehydrogenase as isolated under H_2 or after exposure to CO. Under H_2, the enzyme displayed a peak at 280 nm, with a slight shoulder at 310 nm and a second very broad shoulder extending from 340 to 420 nm. The broad shoulder underwent a dramatic decrease in absorbance upon injection of CO into the cuvette. The purified enzyme exhibited high specific activity but also a high apparent K_m for CO (Fig. 7). It was not possible to demonstrate saturation kinetics for CO at 37°C with 20 mM methyl viologen and 2 atm of CO. The data allowed estimation of an apparent K_m for dissolved carbon monoxide of 5 mM and an apparent V_{max} of 1,300 U/mg. The purified enzyme did not exhibit methyl viologen-linked hydrogen oxidation, nor did it evolve hydrogen when incubated under CO.

DISCUSSION

The CO dehydrogenase purified from *Methanosarcina barkeri* is similar to CO dehydrogenases from the acetogenic bacterial species *C. thermoaceticum* (7–9, 31) and *Acetobacterium woodii* (32) in that it is a nickel enzyme, it displays nearly identical spectral properties when incubated under CO, and it is strongly inhibited by cyanide and oxygen. The CO dehydrogenase from *Methanosarcina barkeri*, however, differs markedly from acetogenic bacterial enzymes in subunit composition. The CO dehydrogenase of *C. thermoaceticum* apparently exists as either a dimer or hexamer of two subunits with similar molecular weights (7, 31); whereas, the *Methanosarcina barkeri* enzyme has a large and small subunit in an apparent $\alpha_2\beta_2$ configuration.

CO dehydrogenase of *Methanosarcina barkeri* represents the third nickel-containing enzyme isolated from methanogenic bacteria. Hydrogenase (15, 18) and methyl reductase (19) purified from *Methanobacterium* species are also nickel

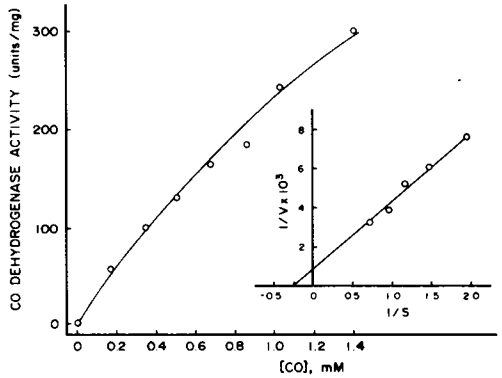

FIG. 7. Relationship between CO concentration and specific activity of purified CO dehydrogenase from *Methanosarcina barkeri*. Conditions and calculations were as described in the text. Inset is a Lineweaver-Burke plot of the same data.

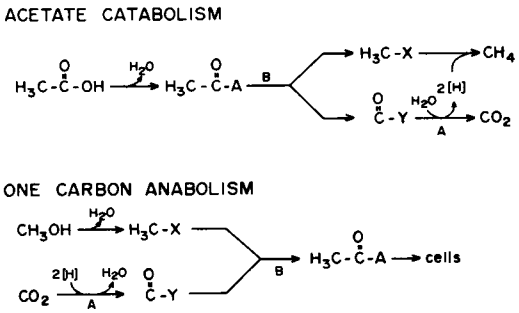

FIG. 8. Proposed functions for CO dehydrogenase in acetate metabolism of *Methanosarcina barkeri*. A and B, Sites for enzyme activity, H_3CO-A, CH_3-X, and O = C-Y, Intermediary carbon carriers not yet identified. O=C-Y, Intermediate metabolite that is an in vivo substrate for CO dehydrogenase. It may either be a free coenzyme or an enzyme-bound one-carbon unit.

proteins. Currently, CO dehydrogenase from *Methanobrevibacter arboriphilus* has been partially purified and ^{63}Ni coelutes with the enzyme activity (R. Thauer, personal communication).

Carbon monoxide dehydrogenase is a major enzymatic component of *Methanosarcina barkeri* cultured on acetate. The near homogeneity of the purified enzyme after a 20-fold increase in specific activity implies that up to 5% of the soluble cellular protein is CO dehydrogenase. In comparison to the catabolic enzymes so far examined, hydrogenase constitutes 2 to 3% (18) and methyl reductase constitutes (12%) (11) of the soluble protein in hydrogen-grown *Methanobacterium* species. The extremely low affinity of *Methanosarcina barkeri* enzyme for CO suggests that its primary function is other than to consume environmental CO, although this methanogen can grow poorly on CO as the sole carbon and energy source (30). The apparent affinity constants for CO dehydrogenase activity in aerobic bacteria (50 to 60 μM CO) and phototrophic anaerobes (12 μM CO) that grow readily on CO (24, 29, 35, 37) are much lower than that reported here for *Methanosarcina barkeri* (5 mM CO). These findings support the previous suggestions that CO dehydrogenase activities in the methanogens may function in acetate metabolism (22, 27, 34, 41).

The physiological role supported here for *Methanosarcina barkeri* CO dehydrogenase is its function in carbonylation or decarbonylation reactions associated with acetate metabolism (Fig. 8). The proposed catabolic function for CO dehydrogenase in the acetate metabolism of *Methanosarcina barkeri* is supported by the following lines of evidence: the high amount of CO dehydrogenase protein present during growth on acetate, the low affinity of the enzyme for CO and its general similarity to acetogen CO dehydrogenase (present data), and the correlation between a fivefold increase in enzyme specific activity and a two- to threefold increase in the doubling time during growth on acetate versus H_2-CO_2 or methanol (27). The enzyme is proposed, in Fig. 8I, to participate in the catabolic decarbonylation (site B) of an acetyl moiety into a methyl intermediate and a carbonyl intermediate, which is specifically dehydrogenated by the enzyme at site A to provide electrons for methyl reduction to methane. Since this model involves a redox reaction, it allows for a mechanism of ATP generation during acetate cleavage by electron transport-mediated phosphorylation. During growth on acetate, the function of CO dehydrogenase

is catabolic, and its role is certainly not to synthesize acetate or acetyl-coenzyme A as has been proposed for growth of both acetogens and methanogens on one-carbon substrates (6, 9, 17, 22, 23, 34, 41). The suggested anabolic function of CO dehydrogenase in *Methanosarcina barkeri* is shown in Fig. 8II, and this proposal is supported by our previous report of direct acetate synthesis from CO and methyl B_{12} in cell extracts (22). The anabolic role of CO dehydrogenase during growth on one-carbon substrates is the reverse of that proposed here for the enzyme during acetate catabolism. Namely, the enzyme functions at site A to synthesize a carbonyl intermediate from CO_2 and may participates in the synthesis of an acetyl moiety by carbonylating a methyl intermediate.

ACKNOWLEDGMENTS

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