JOURNAL OF BACTERIOLOGY, Nov. 1997, p. 6902–6911 Vol. 179, No. 22
0021-9193/97/$04.00+0

Methylthiol:Coenzyme M Methyltransferase from *Methanosarcina barkeri*, an Enzyme of Methanogenesis from Dimethylsulfide and Methylmercaptopropionate

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Received 20 May 1997/Accepted 3 September 1997

During growth on acetate, *Methanosarcina barkeri* expresses catabolic enzymes for other methanogenic substrates such as monomethylamine. The range of substrates used by cells grown on acetate was further explored, and it was found that cells grown on acetate also converted dimethylsulfide (DMS) and methylmercaptopropionate (MMPA) to methane. Cells or extracts of cells grown on trimethylamine or methanol did not utilize either DMS or MMPA. During growth on acetate, cultures demethylated MMPA, producing methane and mercaptopropionate. Extracts of acetate-grown cells possessed DMS- and MMPA-dependent coenzyme M (CoM) methylation activities. The activity peaks of CoM methylation with either DMS or MMPA coeluted upon gel permeation chromatography of extracts of acetate-grown cells consistent with an apparent molecular mass of 470 kDa. A 480-kDa corrinoid protein, previously demonstrated to be a CoM methylase but otherwise of unknown physiological function, was found to methylate CoM with either DMS or MMPA. MMPA was demethylated by the purified 480-kDa CoM methylase, consuming 1 mol of CoM and producing 1 mol of mercaptopropionate. DMS was demethylated by the purified protein, consuming 1 mol of CoM and producing 1 mol of methanethiol. The methylthiol:CoM methyltransferase reaction could be initiated only with the enzyme-bound corrinoid in the methylated state. CoM could demethylate, and DMS and MMPA could remethylate, the corrinoid cofactor. The monomethylamine corrinoid protein and the A isozyme of methylcobamide:CoM methyltransferase (proteins homologous to the two subunits comprising the 480-kDa CoM methylase) did not catalyze CoM methylation with methylated thiols. These results indicate that the 480-kDa corrinoid protein functions as a CoM methylase during methanogenesis from DMS or MMPA.

Dimethylsulfide (DMS) and related methylated thiols play significant roles in the ecosystem. DMS generated in the open ocean has been implicated as the major source of cloud condensation nuclei present in the marine atmosphere, with subsequent global effects on climate (9). A major source of DMS and other methylated thiols is dimethylsulfoniopropionate (DMSP), a compatible solute found in a diverse array of organisms including algae (29), reef corals, diatoms (21), and some plants (44). Concentrations of DMSP (56) and DMS (57) can be in the micromolar range in cyanobacterial mats and salt marsh sediments. DMSP can be directly metabolized by two major routes in the environment (56). It can be directly cleaved to DMS and acrylic acid by bacteria and algae, using DMSP lyase (11, 12, 35). DMSP can also be sequentially demethylated under aerobic or anaerobic conditions to yield 3-methylmercaptopropionate (MMPA) and 3-mercaptopropionic acid (MPA) (52, 56). Aerobic bacteria producing methanethiol (MSH) from either DMSP or MMPA have been described (52).

DMS is also formed from several other processes. Methionine added to anaerobic sediments produces MSH and lesser amounts of DMS (28). DMS and MSH are also made from methoxylated aromatics and sulfide by some homoacetogens (1, 30). Dimethyl sulfoxide, an oxidation product of DMSP, is reduced to DMS by several marine sulfate-reducing bacteria (25). Phototrophic nonsulfur bacteria produce DMS during photosynthesis (38).

Given the variety of reactions generating different methylated thiols from abundant environmental precursors, it is not surprising that methanogenic bacteria have been found to produce methane from these compounds. DMS and MSH are active methane precursors in freshwater sediments and sewage sludge (61), as well as in estuarine and alkaline or hypersaline sediments (27). Pure cultures of methanogens that grow on DMS and/or MSH include *Methanolobus taylorii* GS-16 (27, 42, 43), *Methanolobus bombayensis* (26), *Methanosarcina siciliae* (40, 41), *Methanosarcina* sp. strain MTP4 (19), *Methanosarcina acetivorans* (41, 49), *Methanohalophilus oregonense* (36), and *Methanohalophilus zhilinae* (37). Recently, the three *Methanosarcina* spp. in this list were found to also grow on MMPA as well as DMS (53, 54).

Methanogens which can produce methane from methylated thiols typically consume other methylotrophic methanogenic substrates such as methylamines and methanol. Coenzyme M (CoM) is methylated by methylotrophic substrates as an intermediate step to both methane and carbon dioxide formation (17). Proteins mediating the methylation of CoM with trimethylamine (TMA) (15, 16), monomethylamine (MMA) (4, 6), and methanol (47, 55) have been isolated. In each pathway, a distinct corrinoid protein is methylated with the methylotrophic substrate by a substrate-specific polypeptide. The methylated corrinoid protein then serves as the substrate for one of two isoforms of methylcobamide:CoM methyltransferase (MT2) termed MT2-M and MT2-A. MT2-A is used for methylamine metabolism, while MT2-M is used for methanol metabolism and in vitro has limited ability to function in CoM methylation by TMA (15, 16).

In contrast, little information concerning the pathways for methanogenesis from methylated thiols has been available. No proteins which can mediate CoM methylation with DMS, MSH, and MMPA have been identified. It is known that meth-

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ylated thiols are metabolized similarly to other methylotrophic substrates, in that 1 mol of methyl group is oxidized to CO_2 in order to generate the reducing equivalents for reduction of 3 mol of methyl group to methane (19, 27, 54). Methanogenesis from DMS, MSH, and MMPA is inhibited by bromoethanesulfonate (BES) (27, 54). BES is a specific inhibitor of methyl-CoM reductase, the final enzyme of methane formation, indicating that methylation of CoM is necessary for the metabolism of methylated thiols by methanogens. The DMS and MSH CoM methylation pathways are distinct. Some methanogens produce stoichiometric amounts of MSH and methane during DMS consumption (28), while others can further convert MSH to methane (19). The pathways of DMS- and MSH-dependent CoM methylation are also regulated. For example, cell suspensions of *Methanolobus taylori* grown on TMA do not metabolize DMS (43).

Methanosarcina barkeri MS is the neotype strain of the species and has been shown to produce methane from all known methylotrophic substrates except methylated thiols (2). During growth on acetate, *M. barkeri* expresses pathways for methanogenesis from other catabolic substrates. For example, cells grown on acetate synthesize the corrinoid protein and two methyltransferases required for CoM methylation with MMA (4, 6). In this work we document that cells grown on acetate express proteins required for methanogenesis from DMS or MMPA. A single corrinoid protein which carried out CoM methylation with either DMS or MMPA was identified. This enzyme, which had been previously identified and characterized as a 480-kDa CoM methylase (46, 51), was first detected following methylation of the protein-bound corrinoid with radiolabeled acetate in cell extracts in which methylreductase was inhibited (7), presumably due to methylation of the corrinoid by $^{14}CH_3$-CoM (51). The 480-kDa CoM methylase is most easily isolated as the $^{14}CH_3$-corrinoid protein (32) and is composed of two subunits in equimolar amounts, with one corrinoid cofactor bound per $\alpha\beta$ dimer. The corrinoid is probably bound by the β subunit, which is homologous to the cobalamin-binding domain of methionine synthase (46). The α subunit is homologous to MT2-A and MT2-M (46) and possesses MT2 activity (51). Together both subunits mediate an active methyl iodide:CoM methyltransferase reaction, but a physiologically relevant methyl donor had not been identified. Here we present data demonstrating that the purified 480-kDa CoM methylase uses either DMS or MMPA as the methyl donor and that this protein can act in methanogenesis from either of these substrates.

MATERIALS AND METHODS

Materials. Gases were purchased from Linde Specialty Gases (Columbus, Ohio) and passed through catalyst R3-11 (Chemical Dynamics Corp., South Plainfield, N.J.) to remove O_2 prior to use. Morpholinopropanesulfonic acid (MOPS), 2-mercaptoethanesulfonate (CoM; $HSCH_2CH_2SO_3^-$), TMA (trimethylamine hydrochloride) MMA (monomethylamine hydrochloride), BES, methylviologen, dithiothreitol, and 5,5′-dithio-bis(2-nitrobenzoate) were purchased from Sigma (St. Louis, Mo.). Titanium(III) chloride (10% aqueous solution), 2-methylmercaptoacetate ($CH_3SCH_2COO^-$), 2-methylmercaptoethanol ($CH_3SCH_2CH_2OH$), and 3-methylmercapto-1-propanol ($CH_3SCH_2CH_2CH_2OH$) were purchased from Aldrich (Milwaukee, Wis.). MSH (CH_3SH) was purchased from Fluka Chemical Corp. (Ronkonkoma N.Y.). Methyl iodide was purchased from J. T. Baker Inc. (Phillipsburg, N.J.). Monobromobimane was purchased from Calbiochem (La Jolla, Calif.). DMS (CH_3SCH_3) was purchased from Spectrum Chemical (Gardenia, Calif.). Polyacrylamide gel supplies were purchased from Bio-Rad (Hercules, Calif.). MMPA ($CH_3SCH_2CH_2COO^-$) was prepared by alkaline hydrolysis of methyl-3-(methylthio)propionate ($CH_3SCH_2CH_2COOCH_3$) (58), obtained from Aldrich. DMSP [$(CH_3)_2SCH_2CH_2COO^-$] was prepared from acrylic acid and DMS (8). All conjugate bases listed above were used as the sodium salts.

Preparation of cell suspensions and extracts. *M. barkeri* MS (DMS 800) was cultured in 15- to 40-liter carboys under anaerobic conditions in phosphate-buffered medium (33) supplemented with 80 mM sodium acetate, methanol, or TMA. Cells were harvested just after entering stationary growth phase but while still actively methanogenic. Collected cells were washed three times with 50 mM anoxic MOPS (pH 7.0) and used immediately for cell suspension studies or frozen for use as cell extracts. Cell extracts in 50 mM MOPs buffer were prepared under anaerobic conditions by being broken with a French pressure cell and then subjected to ultracentrifugation at 150,000 × *g* as described previously (7). Cell extracts were stored under H_2 at −70°C in serum vials.

Gas chromatography. DMS, MSH, and methane were quantitated with a model 8A gas chromatograph (Shimadzu Scientific Instruments, Columbia, Md.) equipped with a 0.5-m Poropak R column (Alltech Associates, Inc., Deerfield, Ill.) and a flame ionization detector. The column, injector, and detector were at 130°C, and the nitrogen flow rate was 20 ml/min.

Purified enzymes. The corrinoid bound to the 480-kDa CoM methylase was methylated in cell extracts incubated with ^{14}C-2 acetate and BES as described previously (7). Isolation of the $^{14}CH_3$-corrinoid protein was monitored by the radiotracer, using the procedure of Kremer et al. (32). The final purified $^{14}CH_3$-corrinoid protein was found to have a specific activity of 4.8×10^4 dpm/mg of protein. The "as-isolated" methyl-corrinoid protein was used in all experiments described here unless indicated otherwise. MMAMT (monomethylamine:monomethylamine corrinoid protein methyltransferase), MMCP (monomethylamine corrinoid protein), and MT2-A were isolated as described previously (4, 6).

Gel electrophoresis. Denaturing polyacrylamide gel electrophoresis (12% acrylamide) was performed with the buffer system of Laemmli (34) in the presence of 0.1% sodium dodecyl sulfate, using a Mini-slab electrophoresis system (Idea Scientific Co., Minneapolis, Minn.). Samples for electrophoresis were prepared with 5% 2-mercaptoethanol–0.1% sodium dodecyl sulfate–10% glycerol–0.002% bromothymol blue and were heated to 80°C for 15 min prior to electrophoresis. Molecular size markers (Bio-Rad) were phosphorylase *b* (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and hen egg white lysozyme (14.4 kDa). Gels were stained with colloidal Coomassie G-250 as described previously (51).

Methanogenesis from methylated thiols by cell suspensions and extracts. Freshly harvested cell suspensions (1 ml, 8 to 14 mg [dry weight]/ml) of acetate- or methanol-grown *M. barkeri* MS in 50 mM MOPS (pH 7.0) were kept on ice in stoppered 13.5-ml serum vials with a headspace of 100% H_2 or N_2. Potential methanogenic substrates were added to 10 mM (final concentration) in the suspension, and the reaction was initiated by transfer to a 37°C shaking water bath. Cell extracts (0.2 ml) of acetate (28 to 31 mg of protein/ml)-, methanol (22 to 26 mg of protein/ml)-, or TMA (23 to 28 mg of protein/ml)-grown *M. barkeri* MS in 13.5-ml stoppered serum vials were kept on ice with a headspace of H_2 and supplemented with 12.5 mM $MgCl_2$, 10 mM ATP, and 10 mM potential methanogenic substrate. Methanogenesis by cell extracts was initiated by transfer to a 37°C shaking water bath. In the case of DMS or MSH, sufficient amounts were added to the vial that the final concentration in solution at 37°C would be 10 mM. The approximate partitioning was 50% total DMS and 10% total MSH in the liquid phase at 37°C. Samples (30 μl) of headspace gas were removed periodically and analyzed for methane or MSH by gas chromatography. Liquid samples (10 μl) were removed periodically and for quantitation of MPA by determination of free thiol concentration with 5,5′-dithio-bis(2-nitrobenzoate), using the method described previously (4, 13).

Dry weights of cell suspensions were determined after filtration followed by heating at 120°C for 5 days. Protein concentrations for extracts were determined by Coomassie binding (3), using bovine serum albumin as the standard.

The partitioning coefficients of DMS and MSH between liquid and gas phases were determined by five or more trials in stoppered 13.5-ml serum vials containing 0.2 to 1.0 ml of 50 mM MOPS (pH 7.0) under 1 atm of H_2. DMS (from 67 to 670 nmol) and MSH (from 135 to 2,700 nmol) were added to vials and placed at 37°C. The gas and liquid phases were sampled periodically to determine DMS and MSH concentrations in each phase until equilibrium was reached. The partitioning coefficients did not vary significantly with the different concentrations of MSH and DMS tested.

HPLC analysis of CoM and MPA by monobromobimane derivatization. A method for quantitation of CoM and MPA was developed based on published procedures for separation of bimane-derivatized low-molecular-weight thiols (14, 39). Samples were diluted 1:20 in 50 mM K_2HPO_4 containing 1.5 mM monobromobimane, incubated in the dark for 30 min, and then frozen at −70°C prior to analysis by reverse-phase high-performance liquid chromatography (HPLC) on a 25-cm Microsorb MV C_{18} column (Rainin, Woburn, Mass.) with a 3-cm ODS-5S C_{18} guard column (Bio-Rad) at 20°C. The column was eluted with various concentrations of methanol in 50 mM sodium acetate buffer (pH 3.5). The thiol-bimane adducts were injected onto the column equilibrated with 15% methanol and then eluted at 1.0 ml/min with an 8.0-ml linear gradient of 15 to 100% methanol followed by elution for 2 min with 100% methanol. Elution of derivatized thiols was monitored at 245 nm.

Detection of DMS:CoM and MMPA:CoM methyltransferase activity in extracts. Aliquots of cell extracts (0.2 ml) of acetate-grown *M. barkeri* MS were prepared as described above for methanogenesis assays but were also supplemented with 1 mM BES, 10 mM CoM, and 10 mM DMS or MMPA. The loss of the free thiol of CoM due to methylation by DMS or MMPA was monitored by removing liquid-phase samples (10 μl) at 1-min intervals. CoM and MPA concentrations were then quantified by bimane derivatization and HPLC analysis.

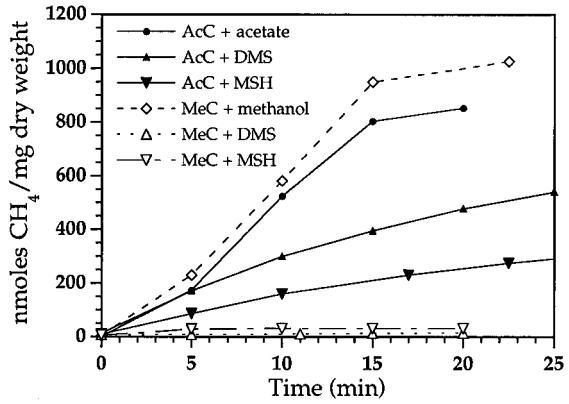

FIG. 1. Methanogenesis by cell suspensions of *M. barkeri* grown on acetate (AcC) (9 mg [dry weight]/ml) or on methanol (MeC) (12 mg [dry weight]/ml) and supplemented with 10 mM acetate, methanol, DMS, or MSH as indicated. Details are described in Materials and Methods. Cell suspensions were incubated under an H_2 headspace, except for acetate-grown cells supplemented with acetate, which were incubated under N_2.

Gel permeation chromatography of DMS- and MMPA-dependent CoM methylation activities. Corrinoid proteins in cell extracts were activated by methylation using the previously established protocol (7). Methylation of the corrinoid proteins allows their direct entry into the methyl-Co/Co(I) catalytic cycle characteristic of corrinoid-dependent methyltransferases independent of a cellular reductive activation system (10) or chemical reductants. Acetate-grown cell extract (29 mg of protein/ml) in an H_2-flushed vial was supplemented with 12.5 mM $MgCl_2$, 10 mM ATP, 1 mM BES, and 5 mM acetate and incubated at 37°C for 5 min with agitation. All manipulations of methylated extracts were performed under dim red light in an anaerobic chamber (Coy Laboratories, Ann Arbor, Mich.) maintained with an atmosphere of 97% N_2 and 3% H_2. The cell extract (5.5 mg of protein) was fractionated in a Biologic medium pressure chromatography unit (Bio-Rad) equipped with a Superose 6HR column (Pharmacia LKB Inc., Pleasant Hill, Calif.) and eluted with anaerobic 100 mM NaCl–50 mM MOPS (pH 7.0) at a flow rate of 0.4 ml/min. Fractions (0.4 ml) were collected and analyzed for DMS- or MMPA-dependent CoM methylation. Aliquots of each fraction (0.1 ml) were supplemented with 10 mM CoM and either 20 mM MMPA or 20 mM DMS in stoppered N_2-flushed vials. After 5 min, the fractions were sampled for the production of MPA (from MMPA) or MSH (from DMS). Molecular mass size standards for the Superose 6HR column included thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), and ovalbumin (45 kDa).

Methylthiol:CoM methyltransferase activity of the 480-kDa CoM methylase. Methyltransferase reactions were performed in anoxic 2-ml stoppered vials under 1 atm of H_2. Reactions mixtures (100 μl) were prepared on ice and included 2.3 mM Ti(III) citrate (48), 0.5 mM methylviologen, 8.5 or 17 μg of 480-kDa CoM methylase in the methyl-corrinoid form, 2 or 10 mM CoM unless otherwise specified, and the indicated concentrations of methyl donor in MOPS buffer (pH 7.0). Reactions were initiated by the addition of CoM and transfer of the assay mixture to a 37°C shaking water bath. Liquid samples (10 μl) were removed anaerobically at the indicated time points and derivatized with monobromobimane for quantitation of CoM and/or MPA. Gas samples (30 μl) were taken anaerobically when indicated for the quantitation of MSH concentration by gas chromatography. All reported reactions required the 480-kDa CoM methylase, CoM, and a methyl donor.

Assay of MT2 activity. MT2 activity was measured in N_2-flushed vials under dim red light. Samples were prepared at 4°C and contained 17.0 μg of 480-kDa CoM methylase supplemented with 1 mM methylcobalamin and 10 mM CoM or MMPA. Reactions were initiated by transfer of samples to 37°C, and methyltransferase activity was quantified by cyanide derivatization (20).

DMS- or MMPA-dependent methylation of the corrinoid cofactor bound to the 480-kDa CoM methylase. The 480-kDa CoM methylase was isolated in the $^{14}CH_3$-corrinoid cofactor form as described above. The protein (0.42 mg, 5.2 nmol of corrin, 2×10^4 dpm) was incubated with 2.3 mM Ti(III) citrate and 0.5 mM methylviologen in 50 mM MOPS (pH 7.0). Samples were then reacted with 25 nmol of CoM alone or with 25 nmol of CoM and 10 mM MMA, MMPA, DMS, or methyl iodide. All samples plus an untreated protein control were then incubated at 37°C with agitation for 5 min. Reactions were halted by the addition of an equal volume of 95% ethanol followed by heating at 80°C for 10 min. Samples were dried under vacuum and resuspended in aerobic 50 mM potassium phosphate buffer (pH 5.5) for analysis of hydroxylated and/or methylated corrinoid cofactor by reverse-phase HPLC on a 25-cm Microsorb MV C_{18} column with a 3-cm ODS-5S C_{18} guard column at 20°C. The column was equilibrated with 50 mM potassium phosphate (pH 5.5) in 30% methanol. Corrinoid cofactors were eluted at 1.0 ml/min with a 30-ml gradient of 50 mM potassium phosphate (pH 5.5) in 30 to 100% methanol followed by a elution with 100% methanol. The column eluate was monitored for absorbance and radioactivity by using a model 166 UV-visible detector and a model 171 radioisotope detector (Beckman Instruments, Arlington Heights, Ill.).

UV-visible spectroscopy of the 480-kDa CoM methylase with a Hewlett-Packard 8453 photo diode array spectrophotometer was performed under dim red light to prevent photolysis of the methylated corrinoid cofactor. A protein solution of 1 ml (0.68 mg of protein, 7.4 nmol of corrinoid cofactor) was prepared in 20 μM dithiothreitol and 50 mM MOPS (pH 7.0) at 22°C in an anaerobic cuvette with a nitrogen gas phase. A spectrum of the as-isolated methyl-corrinoid protein was collected, and the cuvette was scanned immediately after the addition of 35 μM CoM and then again after the subsequent addition of 1 mM MMPA or DMS.

RESULTS

Methanogenesis from methylated thiols by whole cells. Cell suspensions were prepared from cultures of *M. barkeri* grown on acetate or methanol to early stationary phase. These cells were tested for the presence of methyltransferases capable of methane formation from the growth substrate and from DMS and MSH under a hydrogen atmosphere (Fig. 1). Cells grown on methanol rapidly converted their growth substrate to methane, but MSH and DMS were not used as methanogenic substrates at detectable rates (less than 0.1 nmol/min/mg [dry weight]). Similar results were found with cells cultured on TMA (not shown). In contrast, cells grown on acetate converted both DMS and MSH to methane at 34 and 12% of the rate of acetate-dependent methanogenesis (Fig. 1 and Table 1). MMPA was also a methanogenic substrate, and suspensions of cells grown on acetate produced methane from MMPA and DMS at very similar rates.

DMS and MSH could be oxidized by acetate-grown cells to provide reducing equivalents for methane formation from the methylated thiol. This was evidenced by formation of methane by acetate-grown cell suspensions incubated with these methylated thiols under nitrogen rather than hydrogen (Table 1).

Methanogenesis from MMPA during growth on acetate. To determine if methylated thiols could serve as methane precursors under physiological conditions, triplicate cultures (10 ml) on 20 mM sodium acetate were incubated with and without 20 mM MMPA. In the absence of MMPA, 158.8 ± 15.4 μmol of methane were produced (throughout this report, "±" values are 1 standard deviation [SD]). However, cultures supplemented with MMPA produced 214.3 ± 8.5 μmol of methane during the same time period. MPA produced from the demethylation of MMPA was quantitated by bimane derivatization and HPLC analysis of the culture supernatant, 49 ± 17 μmol of MPA had been produced. These results demonstrated that MMPA can serve as a significant methanogenic substrate during culture of *M. barkeri.*

TABLE 1. Rates of methanogenesis from various substrates by cell suspensions of acetate grown *M. barkeri*

Methanogenic substrate	Rate (nmol of CH_4 produced/min/mg [dry weight])[a]	
	H_2 atmosphere	N_2 atmosphere
Acetate	2.5 ± 1.1	63.1 ± 8.2
Methanol	7.3 ± 2.9	5.0 ± 1.7
MMA	48.8 ± 6.9	25.4 ± 3.8
DMS	21.2 ± 5.6	11.3 ± 2.9
MSH	7.6 ± 2.5	2.4 ± 0.5
MMPA	17.4	ND[b]

[a] Average ± SD of three or more separate cell suspensions except for MMPA (average of two separate suspensions).

[b] ND, not determined.

TABLE 2. Rates of methane formation from various substrates by extracts of cells grown on acetate, methanol, or TMA

Substrate	Rate (nmol of CH_4 produced min/mg of cell extract)[a]		
	Acetate-grown cell extract	Methanol-grown cell extract	TMA-grown cell extract
Acetylphosphate	14.8 ± 3.2	ND[b]	ND
Methanol	3.2 ± 1.3	49.4 ± 6.7	0.2 ± 0.1
MMA	35.7 ± 4.1	12.8 ± 4.9	66.6 ± 10.4
TMA	ND	ND	70.1 ± 11.9
DMS	12.3 ± 2.4	0.4 ± 0.1	0.2 ± 0.1
MSH	2.9 ± 0.2	0.2 ± 0.1	0.1 ± 0.1
MMPA	14.1	0.3	0.2

[a] Averages ± SD from three or more separate extracts except for MMPA (averages of two separate extracts).
[b] ND, not determined.

Methylated thiol-dependent methanogenesis in cell extract is a function of the growth substrate. Extracts were prepared from cells grown on TMA, methanol, or acetate in order to further establish the range of compounds which could be utilized as methanogenic precursors by cells grown on these substrates (Table 2). Extracts of cells grown on acetate produced methane from DMS, MMPA, MMA, and acetylphosphate. Although acetylphosphate is an intermediate of methanogenesis from acetate (18), MMA was converted to methane at a higher rate than acetylphosphate. Methane was made from DMS, MMPA, and acetylphosphate at very similar rates. Methanogenesis from MSH and methanol occurred approximately fourfold more slowly than from acetylphosphate, DMS, or MMPA. In contrast, methanogenesis from DMS, MSH, or MMPA by extracts of cells grown on methanol or TMA was not detectable. These results indicate that a specific enzyme system or systems is induced (or derepressed) in acetate-grown cells which can utilize methylated thiols as methanogenic substrates.

Methanogenesis from either DMS or MMPA in cell extracts was coupled with the formation of MSH or MPA, respectively (Fig. 2). Stoichiometric amounts of methane and MPA were produced in extracts supplemented with MMPA, indicating that 1 mol of MMPA was converted to 1 mol of methane and 1 mol of MPA. In contrast, in extracts incubated with DMS, the rate of methane formation was slightly faster than the rate of

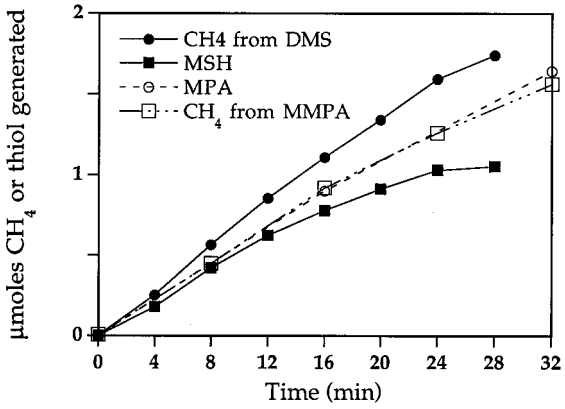

FIG. 2. Production of MSH or MPA during methanogenesis from DMS and MMPA, respectively, by extracts of acetate-grown cells. Extract (28.9 mg of protein/ml) was incubated under H_2 in the presence of 10 mM ATP, 12 mM $MgCl_2$, and either 10 mM DMS or 10 mM MMPA.

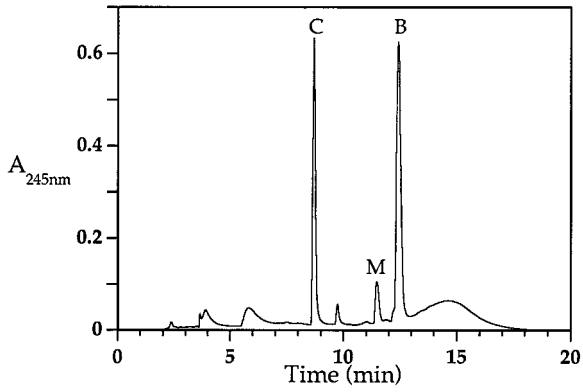

FIG. 3. Reverse-phase HPLC separation of bimane-derivatized thiols from a typical MMPA:CoM methyl transfer reaction. Derivatization of thiols and HPLC were done as described in Materials and Methods. Relevant peaks are labeled; C, CoM-bimane; M, mercaptopropionate-bimane; and B, unreacted monobromobimane. MSH derivatized with bimane eluted at the same position as unreacted monobromobimane. All other peaks were detected in reaction mixtures where CoM and MMPA were omitted and did not change during the course of the assay.

MSH production. In part this is due to consumption of MSH itself for methane formation. However, it is clear that DMS is converted to methane by an initial demethylation which results in the formation of MSH.

Extracts of acetate-grown cells incubated under hydrogen were also tested for the ability to produce methane from a variety of methylated compounds not listed in Table 2. Several compounds were converted to methane at low rates; these included dimethylamine (1.1 nmol/min/mg), 2-methylmercaptoethanol (0.4 nmol/min/mg), and 2-methylmercaptoacetate (2.1 nmol/min/mg). A number of other compounds, including DMSP, alanine, methionine, glycine, sarcoscine, *N*,*N*-dimethyl glycine, betaine, TMA, choline, creatinine, and acetone, were not converted to methane at significant rates (above 0.1 nmol/min/mg).

CoM methylation by DMS and MMPA in cell extracts. BES is an inhibitor of methanogenesis from DMS and MMPA, indicating that methyl-CoM is a likely intermediate of methanogenesis. Detection of CoM methylation by DMS and MMPA presented a problem. Typically, CoM methylation is followed by loss of the free thiol of CoM, using Ellman's reagent. However, the MMPA:CoM and DMS:CoM methyl transfer reactions do not result in a change in the net concentration of free thiol since MPA and MSH are made as products. Therefore, monobromobimane derivatization followed by HPLC analysis was used to monitor the course of methylthiol:CoM methyltransferase reactions. A typical example of a chromatogram from an MMPA:CoM methyl transfer reaction is presented in Fig. 3. Thiols were derivatized with an excess of monobromobimane to generate the corresponding thiol-bimane derivatives. The bimane derivatives of CoM (retention time of 8.8 min) and MPA (11.4 min) were well separated by reverse-phase HPLC. The bimane derivative of MSH eluted with the same retention time as unreacted monobromobimane (12.5 min). Other peaks found in the chromatogram arose from other components (protein and reducing agents) of the methyltransferase assay and were present in the absence of added free thiols. Only those peaks derived from the thiols involved in the reactions were observed to change in intensity during any of the reactions described here.

The rate of CoM methylation by DMS in cell extracts of acetate-grown cells was 19 nmol of CoM consumed/min/mg of protein. The same rate of CoM methylation by MMPA was

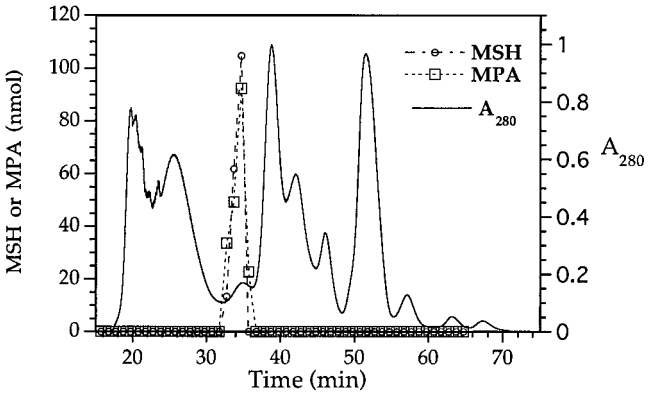

FIG. 4. Elution profile of MMPA- and DMS-dependent CoM methylation activities obtained by chromatography of an extract of acetate-grown cells on a Superose 6HR column. Fractions were assayed for the CoM-dependent production of MSH from DMS by gas chromatography and then for the CoM-dependent production of MPA from MMPA by HPLC analysis of the MPA-bimane derivative.

given by measuring either disappearance of CoM or appearance of MPA and was 17 nmol/min/mg of protein.

Gel permeation reveals a coincident activity peak for both DMS- and MMPA-dependent CoM methylation. Fractionation of an extract of cells grown on acetate was initiated to identify proteins involved in methyl transfer between methylated thiols and CoM. Corrinoid-dependent enzymes have been implicated in methanogenesis from methylamines, CO_2, methanol, and acetate. It was therefore deemed a strong possibility that a corrinoid-dependent methyltransferase is also involved in CoM methylation by methylated thiols. To circumvent the need for a reductive activation system such as required for methanol:CoM methyl transfer (10), corrinoid proteins in an extract of acetate-grown cells were methylated by using a previously published protocol (7). This allows corrinoid-dependent methyltransferases to directly enter the methyl-Co/Co(I) catalytic cycle.

The extract was applied to a medium-pressure gel permeation column. Individual fractions were collected at the start of the separation and assayed for CoM methylation that was dependent on either DMS or MMPA (Fig. 4). The activity peaks of CoM methylation in assays using DMS or MMPA as the methyl donor were absolutely coincident and eluted with an apparent molecular mass of approximately 470 kDa. Of the DMS:CoM and MMPA:CoM methyl transfer activities originally present in the extract, 43 and 34%, respectively, were recovered in the pooled fractions of this one CoM methylation activity peak. The absence of any other activity peak, along with the simultaneous elution of CoM methylation activities with either DMS and MMPA, indicated that a single protein could be responsible for CoM methylation by either methylated thiol.

The purified 480-kDa corrinoid protein mediates CoM methylation with either MMPA or DMS. The apparent molecular mass of the methylthiol:CoM methyltransferase activities, along with the utilization of DMS and MMPA as methanogenic substrates only in cells grown on acetate, were consistent with the involvement of the 480-kDa CoM methylase in this reaction. Levels of the transcript encoding the 480-kDa CoM methylase are highest in cells grown on acetate; little transcript is detectable in cells grown on TMA or methanol (46). The 480-kDa CoM methylase was therefore purified in its methylated form (32) and tested for participation in CoM methylation with either DMS or MMPA. Figure 5A illustrates the purity of the CoM methylase preparation used in these experiments.

In an initial experiment, 0.28 mg of purified 480-kDa CoM methylase was added to 0.1 ml of extract (2.9 mg of protein) of cells grown on acetate. This stimulated the rate of methane formation by the extract from DMS or MMPA 2.5-fold, while the rate of methane formation from methanol or MMA was unchanged. This result further implicated the 480-kDa CoM methylase in methanogenesis from methylated thiols.

In subsequent experiments, it was demonstrated that the stimulation of methanogenesis in cell extract was due to the inherent ability of the purified 480-kDa CoM methylase to function as a methylthiol:CoM methyltransferase (Fig. 5B and Table 3). DMS- or MMPA-dependent methylation of CoM by highly purified 480-kDa corrinoid protein was not stimulated by the addition of small amounts of extract of acetate-grown cells, indicating that the two subunits which comprise the 480-kDa CoM methylase are sufficient to mediate the methylation of CoM with DMS or MMPA.

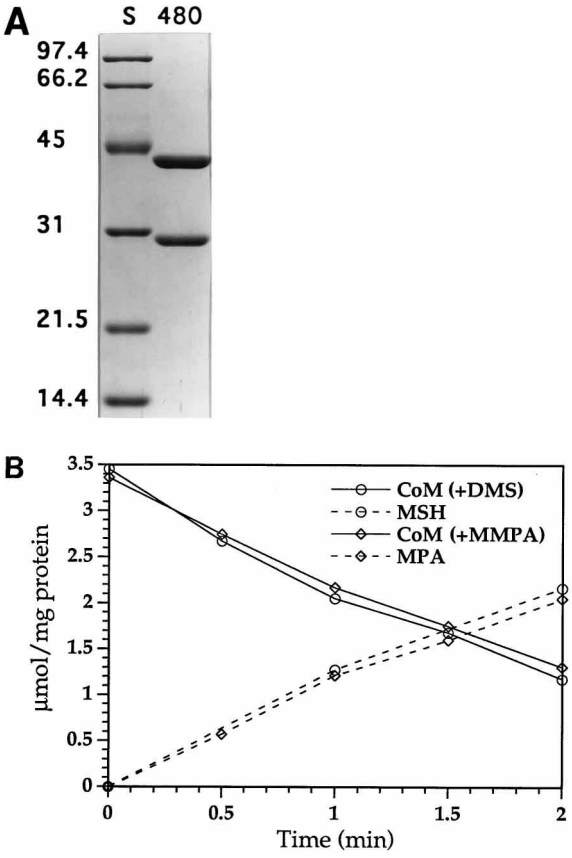

FIG. 5. Methylthiol:CoM methyl transfer activity is mediated by the purified 480-kDa CoM methylase. (A) Denaturing polyacrylamide electrophoretic gel of purified 480-kDa CoM methylase. Protein samples loaded prior to electrophoresis are indicated at the top: S, molecular mass standards (masses in kilodaltons are indicated to the left); and 480, 480-kDa CoM methylase (5 μg). The gel was stained with Coomassie G-250. (B) Catalysis of DMS- or MMPA-dependent methylation of CoM by the 480-kDa CoM methylase. CoM methylation by the protein was monitored by formation of the bimane derivative and HPLC analysis in the presence of DMS [CoM (+DMS)] or MMPA [CoM (+MMPA)]. The formation of MSH from DMS was monitored by gas chromatography, while the formation of MPA from MMPA was monitored by HPLC of the bimane derivative. The reaction mixtures contained 26 μg of the purified protein with 2.3 mM Ti(III) citrate, 1 mM CoM, and 10 mM DMS or MMPA in an initial volume of 0.1 ml. The product formed or substrate consumed is reported as micromoles per milligram of protein present in the reaction mixture.

TABLE 3. Rates of CoM methylation by the purified 480-kDa CoM methylase with different methyl donors

Methyl donors	Observed rate (μmol of CoM methylated/min/mg of protein)[a]
DMS	1.6
MMPA	1.3
Methylmercaptopropanol	1.5
Methyl iodide	2.3
MSH	0.17
Methylmercaptoethanol	0.13
Methylmercaptoacetate	0.04
DMSP	<0.02[b]

[a] Each value is the average of duplicate vials. The liquid-phase concentration of methyl donors was 10 mM except for methyl iodide, which was added to 25 mM. The same concentration of CoM (2 mM) was used with all methyl donors. CoM consumption was monitored by bimane derivatization and HPLC analysis.
[b] The lower limit of detection for this assay.

When MMPA was used as the methyl donor, the disappearance of CoM coincided with the generation an equivalent amount of MPA (Fig. 5B), indicating that 1 mol each of CoM and MMPA was converted to 1 mol each of methyl-CoM and MPA. The appearance of MPA depended on the presence of the protein, CoM, and MMPA. The K_m for MMPA was estimated to be approximately 10 mM. ATP (10 mM) and/or $MgCl_2$ (12 mM) did not stimulate the MMPA:CoM methyltransferase reactions mediated by the purified methylthiol:CoM methyltransferase. The presence of Ti(III) citrate (2.3 mM) extended the linearity of the reaction but was not essential.

The MMPA:CoM methyltransferase reaction was found to be reversible. In the presence of 20 mM methyl-CoM and 10 mM MPA, the protein catalyzed the methylation of MPA at the rate of 0.47 μmol/min/mg. This result was surprising, since the protein did not catalyze a methyl iodide:MPA methyltransfer reaction (51). However, it was found that the 480-kDa protein did mediate a relatively slow methylcobalamin:MPA methyltransfer reaction at 26 nmol/min/mg of protein with 1 mM methylcobalamin and 10 mM MPA.

When DMS:CoM methyltransfer mediated by the purified methylthiol:CoM methyltransferase was examined, the disappearance of CoM was seen to coincide with the generation of an equivalent amount of MSH (Fig. 5B), indicating that 1 mol each of DMS and CoM was converted to 1 mol each of MSH and methyl-CoM. The disappearance of CoM was dependent on the presence of DMS and protein, while the appearance of MSH was dependent on CoM and protein.

Several methylated thiols were tested to determine the methyl donor range of the methylthiol:CoM methyltransferase (Table 3). DMS and MMPA were most effective in donating methyl groups to CoM. Only 3-methylmercapto-1-propanol, in which the carboxyl group of MMPA is replaced by an alcohol, was as effective as DMS and MMPA in the methylation of CoM. Other methylated thiols such as MSH, 2-methylmercaptoethanol, and 2-methylmercaptoacetate were 10- to 40-fold less effective than methyl donors to CoM when the reaction was measured with 2 mM CoM and 10 mM methyl donor. No CoM methylation was detectable when DMSP was tested as a methyl donor.

Catalysis by the methylthiol:CoM methyltransferase requires the methylated form of the enzyme-bound corrinoid. Corrinoid-dependent methyltransferases cycle between methylated corrinoid in the Co(III) redox state and the highly nucleophilic, reducing, and oxygen-sensitive Co(I) state (22). In the Co(II) form, these methyltransferases are inactive (10). However, Ti(III) citrate in the presence of methylviologen will reductively activate corrinoid proteins binding Co(II) corrinoid mediating CoM methylation by MMA (6) or TMA (16). We found that the methylthiol:CoM methyltransferase obligately required the bound methylated corrinoid cofactor for activity and that the Co(II) corrinoid form of the enzyme could not be activated by Ti(III) citrate. To test the activity of Co(II) corrinoid protein in methylthiol:CoM methyl transfer, the as-isolated $^{14}CH_3$-corrinoid protein (0.34 mg of protein, 4.25 nmol of corrin, 1.6×10^4 dpm) was demethylated by reaction with 50 nmol CoM. Methyl-CoM and CoM were then removed with a Sephadex G-25 column. This treatment resulted in the complete demethylation of the protein-bound $^{14}CH_3$-corrinoid, as evidenced by loss of all radioactivity from the protein, and oxidation to the Co(II) state. The orange Co(II) methylthiol:CoM methyltransferase was completely inactive in MMPA:CoM and DMS:CoM methyl transfer. Incubation with 2.3 to 10 mM Ti(III) citrate and 0.5 mM methylviologen could not activate the Co(II) protein for methyltransfer between DMS or MMPA and CoM, suggesting inability of these reducing compounds to convert the protein-bound corrinoid to the Co(I) state. The as-isolated $^{14}CH_3$-corrinoid protein which was not reacted with CoM but was treated with Sephadex G-25 and subjected to the same assay conditions was fully active. These results are consistent with our previous observation that methyl iodide:CoM methyltransfer by this protein was catalyzed only when the enzyme-bound corrinoid was methylated (51).

UV-visible spectroscopy and HPLC analysis reveal corrinoid demethylation by CoM and methylation by MMPA or DMS. The inactivation of the 480-kDa methylthiol:CoM methyltransferase by demethylation of the protein-bound CH_3-corrinoid indicates a role for the cofactor in methyl transfer between these substrates. The interaction of the protein-bound cofactor with CoM, DMS, and MMPA was therefore monitored by UV-visible spectroscopy (Fig. 6) and reverse-phase HPLC (Table 4). The as-isolated 480 kDa $^{14}CH_3$-corrinoid methylthiol:CoM methyltransferase had the characteristic spectrum observed previously (32) for this protein in the methylated form (Fig. 6, trace A). The amount of radioactivity

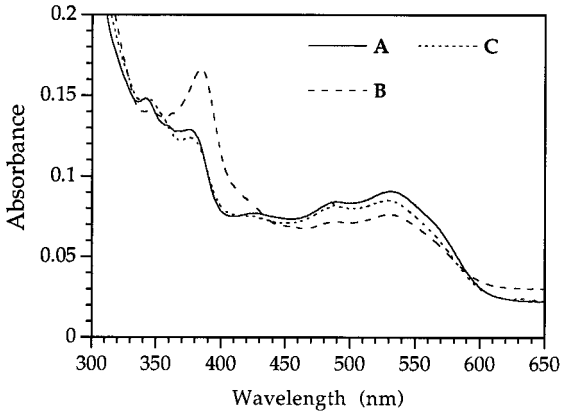

FIG. 6. Demethylation of the methylated corrinoid cofactor bound to the methylthiol:CoM methyltransferase by CoM and subsequent remethylation by DMS. UV-visible spectra were taken of 0.68 mg of protein (7.4 nmol of corrinoid cofactor) per ml in MOPS buffer (pH 7.0) under a nitrogen atmosphere. A single cuvette that contained the protein in the as-isolated methyl-corrinoid state was scanned initially (trace A), immediately after the addition of 35 nmol of CoM (trace B), and immediately after the subsequent addition of 1,000 nmol of DMS (trace C).

TABLE 4. Methylation of corrinoid cofactor bound to the 480-kDa CoM methylase with methylated thiols

Addition to protein[a]	% Corrin recovered[b]	nmol of hydroxy corrinoid[c]	nmol of methyl corrinoid[c]	Specific radioactivity (dpm/nmol of methylated corrinoid eluting from reverse-phase column)
None	88.8	0	4.62	3,690
CoM	57.5	2.99	0	
CoM + MMA	84.2	4.38	0	
CoM + MMPA	91	0.96	3.77	0
CoM + DMS	94.2	0.05	4.85	36
CoM + MeI	91.3	0.11	4.64	45

[a] The $^{14}CH_3$-corrinoid protein was incubated for 5 min with the indicated additions before extraction of protein bound corrinoid with ethanol and subsequent analysis by HPLC.

[b] Summed total of hydroxylated and methylated corrinoid recovered from HPLC analysis. Percentage recovered is based on 5.2 nmol of corrinoid (0.42 mg of protein, 6 nmol of corrin per nmol of 480-kDa protein) per sample.

[c] As determined by reverse-phase HPLC of ethanol-extracted protein following reaction.

associated with the $^{14}CH_3$-corrinoid protein indicated that the enzyme-bound $^{14}CH_3$-corrinoid had a specific radioactivity of 3,800 dpm/nmol, using the stoichiometry of 6 mol of corrinoid cofactor bound per mol of 480-kDa CoM methylase (32). Following ethanol extraction of the $^{14}CH_3$-protein, $^{14}CH_3$-corrinoid, but not hydroxylated corrinoid, was detected by HPLC. The specific radioactivity of the $^{14}CH_3$-corrinoid cofactor was near the predicted value (Table 4, line 1). Addition to the protein of a fivefold molar excess of CoM over the $^{14}CH_3$-corrinoid bound to the protein resulted in the demethylation of the $^{14}CH_3$-corrinoid and the formation of Co(I) corrinoid, as indicated by the formation of an intense peak at 384 nm in the UV-visible spectrum (Fig. 6, trace B). HPLC analysis of the corrinoid cofactor aerobically extracted from the protein reacted with CoM revealed that all corrinoid cofactor was now in the hydroxylated state, as expected following aerobic extraction of Co(I) corrinoid (Table 4, line 2). No CH_3-corrinoid was detected in protein treated with CoM alone.

Without addition of a suitable methyl donor, the UV-visible Co(I) signal was observed to decay over several minutes and the protein displayed the spectrum of Co(II) protein (not shown). However, if DMS was added to the Co(I) corrinoid protein, the protein was remethylated, as indicated by the immediate change of the UV-visible spectrum to that of methylated corrinoid protein (Fig. 6, trace C). Co(I) corrinoid protein reacted with MMPA also displayed a spectrum similar to that of a methylated corrinoid protein (not shown).

HPLC analysis of the as-isolated $^{14}CH_3$-corrinoid methylthiol:CoM methyltransferase reacted with a fivefold molar excess of CoM (relative to the enzyme-bound $^{14}CH_3$-corrinoid), and a 1,000-fold molar excess of ^{12}C-DMS or ^{12}C-MMPA confirmed methylation of the corrinoid cofactor by methylthiols (Table 4, lines 4 to 6). As discussed above, treatment of the $^{14}CH_3$-corrinoid methylthiol:CoM methyltransferase with CoM alone resulted in completely demethylated corrinoid (Fig. 6, trace B; Table 4, line 1). However, in the presence of these methyl donors, most of the corrinoid cofactor was recovered in the methylated form after reaction with CoM but was no longer significantly radiolabeled. This finding indicated that the methyl group on the corrinoid had undergone several turnovers in the presence of CoM and was finally remethylated by the excess of ^{12}C-methyl donor. The specific radioactivity of the methylated corrinoid isolated from the protein after reaction with CoM and nonradioactive DMS, MMPA, or methyl iodide was much lower than the specific radioactivity of the $^{14}CH_3$-corrinoid extracted from the as-isolated protein and indicated that 99 to 100% of the methyl group on the protein-bound corrinoid was now derived from DMS, methyl iodide, or MMPA. The small amount of the radioactivity remaining with the CH_3-corrinoid may have resulted from catalytically inactivate $^{14}CH_3$-corrinoid protein. Small amounts of hydroxylated corrinoid were detected as well following the reaction with CoM and the methyl donors, indicating that some oxidative inactivation of the enzyme-bound corrinoid occurred during turnover. In any case, the results outlined in Fig. 6 and Table 4 indicate that CoM demethylates the protein, resulting in protein-bound Co(I) corrinoid, and that DMS or MMPA can methylate the enzyme-bound Co(I) corrinoid.

As a negative control, the as-isolated $^{14}CH_3$-corrinoid protein was incubated with limiting CoM but with excess MMA as a potential methyl donor (Table 4, line 3). The 480-kDa CoM methylase does not mediate MMA:CoM methyl transfer (51), and MMA did not remethylate the corrinoid cofactor following demethylation by CoM. Only hydroxylated corrinoid was detectable in aerobic ethanol extracts of the protein reacted with CoM in both the presence and absence of MMA.

Methylthiol:CoM methyltransferase activity is not a general property of methylotrophic corrinoid proteins and MT2 homologs. DMS and MMPA did not serve as methane precursors in extracts of cells grown on TMA or methanol, indicating that methyltransferases involved in CoM methylation with these substrates do not convert methylated thiols to methane. This was tested directly with the purified MMA:CoM methyltransferases (6). A reaction mixture (100 μl) containing 90 μg of MMAMT, 30 μg of MMCP, and 10 μg of MT2-A was incubated under nitrogen in the presence of 2 mM Ti(III) citrate, 0.5 mM methylviologen, and 2 mM CoM. MMPA or MMA was then incubated with the methyltransferases, and CoM methylation was determined by bimane derivatization of the remaining free thiol after 20 min of incubation. Incubation with MMA resulted in the methylation of 176 nmol of CoM. However, incubation with 10 mM MMPA resulted in neither methylation of CoM nor production of MPA. This finding indicates that MMCP and MT2-A do not mediate a methylthiol:CoM methyltransferase reaction at detectable rates.

DISCUSSION

The first reports of methylated thiols as substrates for pure cultures of methanogens are little more than a decade old (27). Methanogenesis from MMPA by pure cultures was documented only recently (53, 54). Thus, little information concerning the biochemistry of methanogenesis from methylated thiols has been forthcoming. This is the first report of an enzyme which can effect the methylation of CoM with methylated thiols such as MMPA and DMS.

This enzyme comes from an unexpected source. Although other *Methanosarcina* spp., such as *M. acetivorans*, are known to use DMS or MMPA as methane precursors, strains of *M. barkeri* had not been observed to produce methane from these substrates (53, 54, 61). This is understandable since previous attempts to demonstrate growth or cometabolism of MMPA or DMS were made with *M. barkeri* MS grown on methanol, and our results indicate that this activity is repressed during growth on this substrate. Only cells grown on acetate converted DMS or MMPA to methane at significant rates. We have not yet been able to establish cultures of aggregated *M. barkeri* with DMS or MMPA as the sole substrate, but cultures do convert MMPA to methane and MPA during growth on acetate. Recently, we were able to establish growth of a disaggregated

culture of *M. barkeri* (50) (the kind gift of Kevin Sowers) with MMPA or DMS. This culture also grows on another substrate of the methylthiol:CoM methylase, 3-methylmercapto-1-propanol (45).

The phenomenon of induction of multiple catabolic pathways during growth on acetate has been observed previously. *M. barkeri* Fusaro induces pathways of methanogenesis from TMA following growth on acetate (60). Cells of *M. barkeri* MS did not use TMA following growth on acetate, but they do express the proteins required to convert MMA to methane. MMCP, the corrinoid protein involved in the MMA-dependent methylation of CoM (4, 6), was first identified in cells grown on acetate (7, 32). Similarly, it appears that *M. barkeri* MS expresses an enzyme required for methanogenesis from either MMPA or DMS during growth on acetate. The rationale for the expression of catabolic enzymes other than those for growth on acetate is unclear, but several theories can be advanced. Of all substrates, methanogenesis from acetate provides the least available free energy (18). The induction of the methylamine or methylated thiol pathway may represent a response to energy limitation. Alternatively, *M. barkeri* in brackish or marine environments may encounter high concentrations of acetate most often in conjunction with high concentrations of methylamines and methylated thiols, and a selective advantage has been gain by the evolution of a mechanism for simultaneous expression of these catabolic pathways. For example, DMSP lyase produces DMS and acrylic acid (12). Acrylate is converted to acetate by some clostridial species (24, 59). Similarly, the degradation of glycine betaine by some anaerobes results in formation of TMA and acetate (23).

The 480-kDa CoM methylase is the only enzyme detectable in extracts of cells grown on acetate which is capable of methylating CoM with either methylated thiol. Gel permeation chromatography of extracts of cells grown on acetate revealed only a single peak of methylthiol:CoM methyltransferase activity, and this had the approximate molecular mass of the 480-kDa CoM methylase. The highly purified protein itself carries out CoM methylation with either methylated thiol. In addition, expression of the 480-kDa CoM methylase is regulated in a manner similar to that observed for expression of the methylthiol:CoM methyltransferase activity. Levels of the 480-kDa methylthiol:CoM methyltransferase protein (31) and transcript (46) are much higher in cells grown on acetate than in those grown on methanol or TMA.

Involvement of the 480-kDa CoM methylase in a methanogenic pathway is not unexpected. The CoM methylase activity of the protein is catalyzed by the α subunit, which is homologous to MT2-M and MT2-A (46, 51). These methylcobamide:CoM methyltransferases are involved in methanogenesis from methanol and the methylamines, respectively (16). The β subunit of the 480-kDa CoM methylase is homologous to the cobalamin-binding domain of methionine synthase (46). This motif has also been identified in the corrinoid proteins involved in methanogenesis from methanol (47) and methylamine (5). These corrinoid proteins are in turn homologous to the β subunit of the 480-kDa CoM methylase (47). The 480-kDa protein is thus composed of a corrinoid-binding subunit and a CoM-methylating subunit, which both are homologous to polypeptides involved in methanogenesis from other methylotrophic substrates.

However, there are key differences between the methylthiol:CoM methyltransferase and the proteins mediating CoM methylation with other methylotrophic substrates. CoM methylation with either methanol, TMA, or MMA requires three polypeptides (6, 15, 47). For example, during the MMA-dependent reaction, a 52-kDa polypeptide, MMAMT, is required to methylate MMCP, which in turn is demethylated by MT2-A to methylate CoM (6). The equivalent of MMAMT is not found in the 480-kDa methylthiol:CoM methylase; i.e., a separate polypeptide which catalyzes the methylation of the corrinoid-binding β subunit with DMS or MMPA is not present. Nevertheless, methylation of the corrinoid bound to the 480-kDa methylthiol:CoM methylase by DMS or MMPA does occur. It is possible that this function is catalyzed by a domain on one or the other subunits of the methylthiol:CoM methylase. For example, short sequences at the N terminus of the β subunit and the C terminus of the α subunit are not homologous to corresponding portions of MMCP or MT2-A (5, 46). Another possibility is that the methylated thiol and CoM share elements of the same binding site on the α subunit, and from there each substrate methylates or demethylates the corrinoid bound to the β subunit. Either hypothesis is consistent with the stable association of the two subunits. The α subunit is unusual compared to the other MT2 homologs, which do not form tight complexes with their respective corrinoid proteins. It is also apparent that CoM methylation with methylated thiols is not simply a property of MT2 homologs and their corrinoid proteins. This is evidenced by the lack of methylthiol:CoM methyltransferase activity in cells or extracts of cells grown on TMA or methanol. As we show here, no methylthiol:CoM methyltransferase activity is detectable with the three polypeptides of the reconstituted MMA:CoM methyl transfer reaction.

In the case of CoM methylation by TMA, DMA, MMA, or methanol, separate proteins specific for each substrate exist (4, 6, 10, 15, 16, 47). In contrast, the methylthiol:CoM methylase activity of the 480-kDa corrinoid protein can utilize multiple methyl donors. However, the methylthiol:CoM methyltransferase does display the selectivity which is indicative of physiological function. Several methylated thiols were found to be 10-fold less effective as methyl donors than DMS, MMPA, or 3-methylthio-1-propanol at equivalent concentrations. For example, 2-methylmercaptoacetate, which differs from MMPA by one methylene group, was a poor methyl donor. Similarly, 2-methylmercaptoethanol, which differs from 3-methylthio-1-propanol by only one methylene group, was also a poor substrate. The methyl groups of the sulfonium ion DMSP should be excellent methyl donors to a corrinoid, but this compound was completely inactive as a substrate of the methylthiol:CoM methyltransferase. The differences in reactivity between these methyl donors indicate that CoM methylation is not merely a chemical methylation of the enzyme-bound corrinoid by methylated thiols. Due to the labor-intensive nature of the bimane derivatization assay, we did not perform detailed kinetic studies. However, an apparent K_m value for MMPA of approximately 10 mM was determined. This value is comparable to the K_m of 50 mM recently published for the reconstituted methanol:CoM methyl transfer reaction (47).

The ability of the 480-kDa methylthiol:CoM methyltransferase to catalyze the methylation of CoM with either MMPA or DMS suggests that methanogens which utilize these compounds may do so with a single methyltransferase composed of an MT2 homolog and a corrinoid-binding polypeptide. It is notable that organisms which utilize MMPA also utilize DMS (54). We are currently investigating this possibility by comparing the properties of the methylthiol:CoM methyltransferase of *M. acetivorans* to those of the enzyme from *M. barkeri.*

ACKNOWLEDGMENT

This work was supported by a grant from the Department of Energy (DE-FGoz-91ERZ00042).

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