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Acetate Catabolism by *Methanosarcina barkeri*: Evidence for Involvement of Carbon Monoxide Dehydrogenase, Methyl Coenzyme M, and Methylreductase

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The pathway of acetate catabolism in *Methanosarcina barkeri* strain MS was studied by using a recently developed assay for methanogenesis from acetate by soluble enzymes in cell extracts. Extracts incubated with [2-^{14}C]acetate, hydrogen, and ATP formed $^{14}CH_4$ and [^{14}C]methyl coenzyme M as products. The apparent K_m for acetate conversion to methane was 5 mM. In the presence of excess acetate, both the rate and duration of methane production was dependent on ATP. Acetyl phosphate replaced the cell extract methanogenic requirement for both acetate and ATP (the K_m for ATP was 2 mM). Low concentrations of bromoethanesulfonic acid and cyanide, inhibitors of methylreductase and carbon monoxide dehydrogenase, respectively, greatly reduced the rate of methanogenesis. Precipitation of CO dehydrogenase in cell extracts by antibodies raised to 95% purified enzyme inhibited both CO dehydrogenase and acetate-to-methane conversion activity. The data are consistent with a model of acetate catabolism in which methylreductase, methyl coenzyme M, CO dehydrogenase, and acetate-activating enzymes are components. These results are discussed in relation to acetate uptake and rate-limiting transformation mechanisms in methane formation.

Methanogens can convert compounds such as methanol, H_2 and carbon dioxide, formate, and methylamines, carbon monoxide, and acetate to methane (6, 41). Acetotrophic methanogens convert acetate primarily into methane and carbon dioxide by a mechanism in which the methyl group of acetate is the direct methane precursor (5, 14, 19, 25, 31, 33). This is shown in the following reaction: $^{A}CH_3{}^{0}COOH \rightarrow {}^{A}CH_4 + {}^{0}CO_2$. Methanogenesis from acetate has the lowest available free energy per methane of all methanogenic substrates, very near that of the free energy of ATP hydrolysis (6, 35, 41). Nevertheless, many methanogens are able to conserve ATP by the apparent decarbonylation of an acetyl intermediate (20). This reaction is responsible for the majority of the methane produced in environments that are major sources of biogenic methane (18, 27, 32), although other biochemical mechanisms of acetate conversion to methane exist (19, 43).

At present, there is no proven mechanism of ATP generation during methanogenesis from acetate. In consideration of the low molar growth yields of acetotrophic methanogens and low $\Delta G^{0\prime}$ of aceticlastic methanogenesis, it is probable that only a fraction of a mole of net ATP would be generated per mole of acetate catabolized (6). Presumably, such a fractional ATP yield would be mediated by electron transport phosphorylation, and most hypotheses have focused on how aceticlastic methanogenesis could generate a proton motive force (6, 21, 39, 40).

At present, the most viable hypothesis concerns the possible involvement of carbon monoxide dehydrogenase in acetate catabolism (17, 19–21, 23, 34, 35, 42, 42a; G. Diekert, G. Fuchs, and R. K. Thauer, *in* R. K. Pool, ed., *Microbial Gas Metabolism*, in press). The specific activity of this oxidoreductase is elevated during growth of *Methanosarcina barkeri* strain MS on acetate relative to other substrates (19), and it can comprise up to 5% of the soluble protein of cells grown on acetate (20). This and other data have led to a proposal of ATP generation involving soluble products, such as CO dehydrogenase and methylreductase, as well as membrane-bound components (21).

Recently, we demonstrated that cell-free extracts of *M. barkeri* were capable of transforming acetate to methane in the presence of ATP and hydrogen (21). In this assay, up to 80% of the methane was derived from the methyl group of acetate. The aceticlastic activity was found to be completely soluble and was not membrane bound as in a previously described assay system (1). In this paper, we further explore properties of methanogenesis from acetate in cell-free extracts. We provide evidence that biochemical components of the aceticlastic reaction include carbon monoxide dehydrogenase, methylreductase, methyl coenzyme M (CH_3CoM), and activated forms of acetate.

MATERIALS AND METHODS

Chemicals and radioisotopes. Chemicals were of reagent grade. Gases were obtained from Matheson Scientific, Inc. (Joliet, Ill.). Protein A–Sepharose 4B-Cl was purchased from Sigma Chemical Co. (St. Louis, Mo.). [2-^{14}C]sodium acetate was obtained from New England Nuclear Corp. (Boston, Mass.).

Organism and cultivation. *M. barkeri* neotype strain MS was originally isolated from sewage sludge by M. Bryant, characterized by Weimer and Zeikus (37, 38), and adapted to grow on acetate as previously described (19). It was mass cultured at 37°C in 20-liter carboys in phosphate-buffered medium supplemented with 60 mM sodium acetate and a filter-sterilized vitamin mixture (15).

Preparation of extracts. After 2 weeks of growth, the organism was harvested and washed anaerobically in 50 mM morpholinepropanesulfonic acid (MOPS) buffer (pH 7.1). The following procedures were performed with anaerobic techniques under hydrogen. After washing, the cell pastes were frozen at −80°C. Extracts were prepared in the same

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MOPS buffer by passage through a French pressure cell at 20,000 lb/in^2. The lysate was then centrifuged at 27,000 × *g*, and the supernatant was designated the cell-free extract. The extract was then used immediately or stored under hydrogen at −80°C. Aceticlastic activity was retained by frozen extracts for at least 3 months. The extracts could also be frozen and thawed several times and still retain activity. Protein was assayed by the method of Bradford (4).

Analysis of gases and radioisotopes. CH_4 was analyzed by gas chromatography (20). $^{14}CH_4$ and $^{14}CO_2$ were quantified with a gas proportional counter (24). Radioactivity was quantified in Instagel scintillation cocktail (Packard Instrument Co., Downers Grove, Ill.) with a Packard Prias PLD liquid scintillation counter.

Assays for acetate-dependent methanogenesis. Methane production from acetate was assayed in 8- or 12-ml serum vials that contained 100% hydrogen and were stoppered with black, butyl rubber bungs. Standard reaction mixtures contained from 0.2 to 0.3 ml of MOPS-buffered extract, 0 to 10 μl of an ATP solution adjusted to pH 7.0, and 0 to 10 μl of sodium acetate solution. The concentrations of the solutions were varied to maintain a convenient delivery volume when assay concentrations were varied. The reaction was initiated by adding extract to the vial or by transferring the complete reaction mixture from ice to a vigorous shaking 37°C water bath. The gas phase (0.2 to 0.4 ml) was removed at each time point, and the amount of methane reported at each time point was corrected for previous sample removal. Vials were pressurized to 1.5 atm (ca. 152 kPa) of hydrogen to ensure that hydrogen was not limiting during the reaction.

Radiotracer analysis of [2-^{14}C]acetate metabolites. Reactions were performed in 58-ml serum vials containing either hydrogen or nitrogen gas phases, 1.8 ml of extract (25 mg/ml) in MOPS buffer, 0.18 ml of 200 mM [2-^{14}C]sodium acetate solution (specific activity, 9,800 dpm/nmol), and 80 μl of 250 mM ATP. The reaction was initiated by addition of extract to the vial, followed by incubation at 37°C with vigorous agitation. At 8 min, the reaction was terminated by the addition of 2 ml of ethanol. After 2 h of extraction at room temperature, the mixture was centrifuged for 20 min at 20,000 × *g*. The supernatant was then concentrated 10-fold by evaporation to dryness under air followed by suspension in 0.4 ml of water. The suspension was centrifuged again, and 10 μl was electrophoresed and chromatographed as described by Daniels and Zeikus (7). The location of radioactive spots was determined by autoradiography; they were quantified by scraping the spot and counting in Instagel.

Precipitation of CO dehydrogenase with IgG. CO dehydrogenase was purified to 95% homogeneity (20), and 300 μg was emulsified in Freund incomplete adjuvant injected subcutaneously into two rabbits. The rabbits were boosted again as needed by injection of 500 μg of CO dehydrogenase in Freund incomplete adjuvant. Amounts of 100 μl of the highest-titer serum precipitated CO dehydrogenase activity in 15 μl of cell extract. The immunoglobulin G (IgG) in pooled immune antisera was purified with protein A linked to Sepharose CL-4B (12). This serum (15 to 30 ml) was passed over a 2-ml column equilibrated in 50 mM MOPS–150 mM NaCl (pH 7.1). The column was then washed with 200 to 500 ml of the same buffer. IgG was eluted with 200 mM glycine buffer (pH 7.0) into tubes containing 1 M Tris buffer (pH 8.0). The purified antibody was concentrated by ultrafiltration, desalted by passage over a Sephadex G-25 column equilibrated in 50 mM MOPS–150 mM NaCl (pH 7.0), and concentrated again to 0.3 ml.

Extract used in these experiments was prepared in MOPS-NaCl buffer. It was ultracentrifuged at 150,000 × *g* after the 27,000 × *g* centrifugation to completely remove any nonsoluble material. The extract (0.5 ml) was then mixed with 0.3 ml of concentrated anaerobic IgG solution prepared

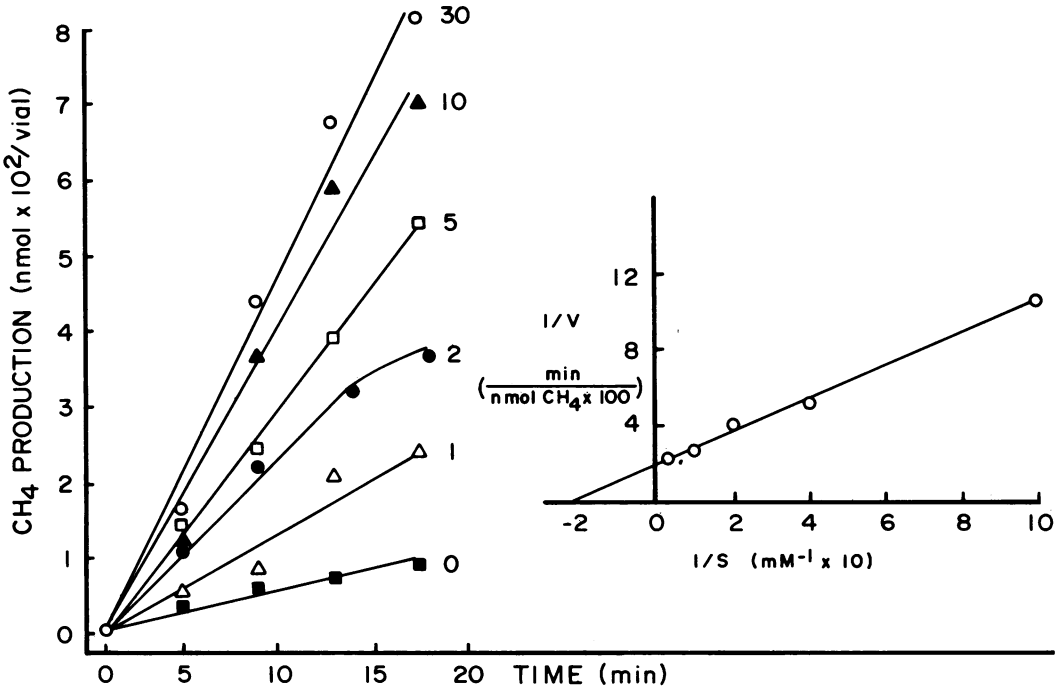

FIG. 1. Effect of acetate concentration on methane production in a cell-free extract assay system. The reactions were conducted in 8-ml vials containing 1.5 atm (ca. 152 kPa) of H_2, 0.2 ml of extract (22 mg of protein per ml), 25 mM ATP, and one of the following concentrations of sodium acetate: 0, 1, 2.5, 5, 10, or 30 mM. The final reaction volume was 210 to 220 μl.

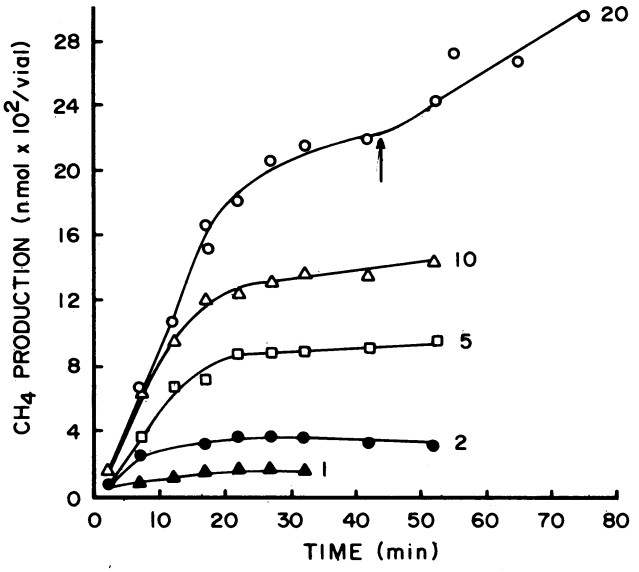

FIG. 2. Effect of ATP concentration on acetate-dependent CH_4 production. The reactions were conducted in 8-ml vials containing 1.5 atm (ca. 152 kPa) of H_2, 0.2 ml of extract (23 mg of protein per ml), 50 mM sodium acetate, and one of the following concentrations of ATP (pH 7.0): 1, 2, 5, 10, or 20 mM. The final reaction volume was 210 to 220 μl. At the arrow, 10 μl of 500 mM ATP was added to the vials originally containing 20 mM ATP.

from immune or nonimmune rabbits. After overnight incubation at 4°C, the mixtures were centrifuged at 25,000 × *g* for 30 min, and the supernatant was removed. The supernatant was tested for CO dehydrogenase activity (20) and for the ability to produce $^{14}CH_4$ from [2-^{14}C]sodium acetate when incubated with hydrogen and ATP.

RESULTS

Effect of sodium acetate and ATP concentration on methanogenesis in cell-free extracts. The rate of methane production in cell-free extracts was dependent on the concentration of acetate present in the assay mixture. In the absence of acetate, extracts incubated under hydrogen produced from 1 to 5% of the total methane produced in extracts supplemented with 50 mM sodium acetate. Figure 1 illustrates the effect of acetate concentration on methane formation in the presence of 25 mM ATP. Estimates of the apparent K_m in different extracts varied from 3 to 8 mM sodium acetate; the average was 5 mM. The apparent V_{max} was approximately 11 nmol/min per mg of protein.

In the presence of 50 mM sodium acetate, the rate of methane formation was dependent on the concentration of ATP (Fig. 2). Controls incubated in the absence of acetate, but with 10 mM ATP, produced negligible methane. The extract produced methane at half-maximal rates when supplemented with 2 to 3 mM ATP. Not only the rate of methanogenesis, but also the duration of methane production, was dependent on the concentration of ATP initially present in the extract. Although extracts supplemented with 10 mM ATP produced methane at initial rates similar to those of extracts with 20 mM ATP, over 800 nmol more total CH_4 was produced under the latter condition. The addition of 20 mM ATP to vials that initially contained 20 mM ATP and whose methanogenic rate had subsided led to a resumption of active methanogenesis.

Methanogenesis from acetyl phosphate in cell-free extracts. An activated form of acetate, acetyl phosphate, served as a methane precursor (Fig. 3). The rate of conversion of acetyl phosphate to methane was independent of ATP concentration. The reaction was linear for at least 40 min, unlike ATP-dependent acetate conversion to methane, which usually ceased after 25 min even in the presence of 20 mM ATP. As with ATP- and acetate-dependent methanogenesis, a lag of variable length was also observed before acetyl phosphate-dependent methanogenesis was initiated. In the absence of acetyl phosphate, significant methanogenesis was not observed from the other reaction mixture components.

Evidence for methylreductase involvement in aceticlastic methanogenesis. To determine whether CH_3CoM is an intermediate of acetate catabolism, extracts were incubated with [2-^{14}C]sodium acetate; the radiolabeled low-molecular-weight compounds were then analyzed by thin-layer electrophoresis and chromatography (Table 1). The methyl group was the precursor of 68% of the methane in this particular experiment. $^{14}CH_3CoM$ was the most prominent intermediate detected in the extract after 8 min. In the 2 ml of total extract employed, 550 nmol of $^{14}CH_3CoM$ was formed from the methyl group of acetate when the reaction was terminated. $^{14}CO_2$ was not produced from [2-^{14}C]acetate in these experiments, excluding the possibility that $^{14}CH_3CoM$ was derived from $^{14}CO_2$. Extracts incubated under nitrogen rather than hydrogen still produced methane from the methyl carbon of acetate, but in much smaller amounts. $^{14}CH_3CoM$ was correspondingly produced at a much lower concentration in these extracts.

Bromoethanesulfonic acid (BCoM), a CH_3CoM analog, is an inhibitor of methylreductase (11, 29). Figure 4 illustrates

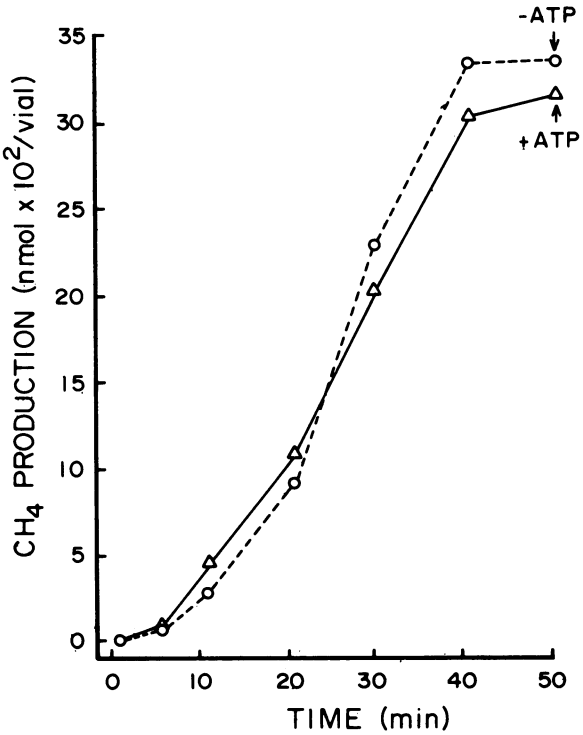

FIG. 3. Acetyl phosphate conversion to methane in the cell extract assay system. The reactions were conducted in 8-ml vials that contained 1.5 atm (ca. 152 kPa) of hydrogen, 0.2 ml of extract (22 mg of protein per ml), and 25 mM acetyl phosphate. The reaction contained either no exogenous ATP or 25 mM ATP.

TABLE 1. $^{14}CH_4$ and $^{14}CH_3CoM$ formation from [2-^{14}C]sodium acetate[a]

Gas phase	nmol of CH_4	dpm of $^{14}CH_4$	% CH_4 from methyl carbon[b]	dpm of $^{14}CH_3CoM$	nmol of CH_3CoM[b]
Nitrogen	130	6.3×10^6	24	2.4×10^5	12
Hydrogen	6,060	4.0×10^8	67	5.4×10^6	550

[a] All values are the total product formed after 8 min of incubation at 37°C in 2 ml of extract as described in the text.
[b] Calculated from specific activity of sodium acetate (9,800 dpm/nmol).

the effect of BCoM on acetate-dependent methane formation in cell-free extracts. Relative to the controls, BCoM (15 μM) reduced the initial rate of methane formation by 90%. Linear methane evolution continued in the inhibited extract for at least 30 min past the cessation of methane formation in the control. At 150 μM, BCoM reduced the rate of methane synthesis to the level of extracts not supplemented with acetate.

Evidence for carbon monoxide dehydrogenase involvement in acetate catabolism. KCN (10 μM) inhibits 85% of methyl viologen-linked CO dehydrogenase activity in extracts of *M. barkeri* (20), as well as aceticlastic methanogenesis by various acetotrophic methanogens (8, 14; M. R. Smith, M. Hart, and M. Weiss, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, I7, p. 140; J. A. Krzycki and J. G. Zeikus, Abstr. Annu. Meet. Am. Soc. Microbiol. I11, p. 141). In cell extracts of *M. barkeri*, 15 μM KCN almost completely inhibited acetate-dependent methanogenesis, whereas 150 μM KCN reduced methane production to the low rate observed in the absence of acetate (Fig. 5).

To further test the hypothesis that carbon monoxide dehydrogenase is a component of the aceticlastic methanogenic pathway, the extract was treated with IgG purified from nonimmune rabbits or rabbits inoculated with 95% homogenous CO dehydrogenase. Immune sera reacted against cell extract in Ouchterlony plates gave rise to one major and one minor precipitation band, indicating that two proteins reacted with the immune antisera. Control antisera from uninoculated rabbits did not react with cell extract. After incubation and centrifugation of extract-antibody mixtures, the control mixture (IgG from nonimmune rabbits and *M. barkeri* extract) contained 60 U of CO dehydrogenase per ml, whereas the immune IgG extract mixture possessed only 11 U of CO dehydrogenase per ml. Immune IgG therefore removed 80% of CO dehydrogenase activity from the extract.

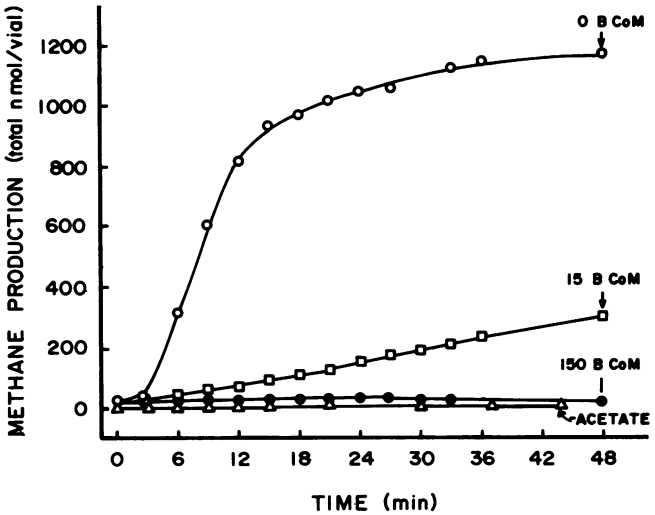

FIG. 4. Effect of BCoM on acetate-dependent methanogenesis. The reaction was conducted in 12-ml vials containing 1 atm (ca. 101 kPa) of hydrogen, 0.3 ml of extract (25 mg/ml), 10 mM ATP, and 30 mM sodium acetate. BCoM was added at concentrations of 0, 15, or 150 μM to initiate the reaction. Controls were incubated without acetate.

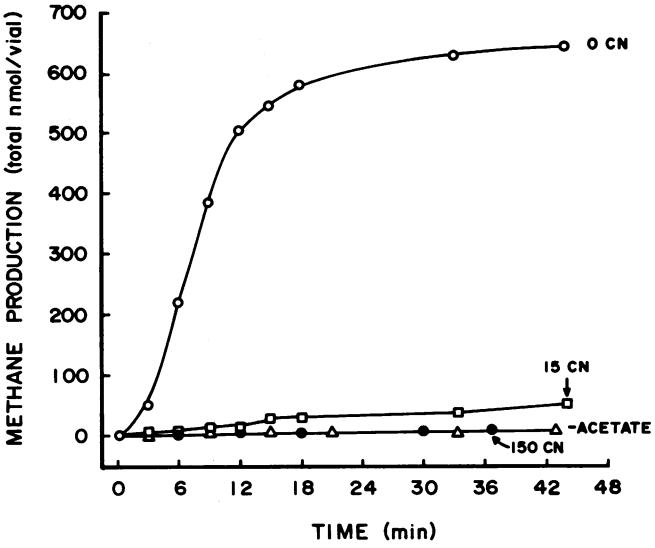

FIG. 5. Effect of KCN on acetate-dependent methanogenesis in cell extracts. The reaction was conducted in 12-ml vials containing 1 atm (ca. 101 kPa) of hydrogen, 0.3 ml of extract (15 mg/ml), 10 mM ATP, and 30 mM sodium acetate. KCN was added in concentrations of 0, 15, or 150 μM to start the reaction. Controls were incubated without acetate.

Figure 6 shows the effect of immunoprecipitation of CO dehydrogenase on methanogenesis from acetate activity. Aceticlastic activity was measured in the extracts by monitoring $^{14}CH_4$ formation from [2-^{14}C]sodium acetate. Methane formation from the methyl group of acetate was 10-fold less in the extract treated with immune IgG.

DISCUSSION

The lack of a suitable assay system for methanogenesis from acetate in cell-free extracts previously impeded the study of this important and intriguing catabolic pathway. A recently described particulate preparation capable of transforming acetate into methane and carbon dioxide (1) may be of use in evaluating membrane function, but its applications in the study of the enzymatic components of the pathway are limited. The completely soluble cell-free extract system described here and in our previous paper (21) is therefore a significant development, since it will allow fractionation and study of the soluble enzymes required for the activation, cleavage, and oxidoreduction reactions of acetate catabolism.

Cell-free extracts displayed on apparent K_m for acetate similar to that found in cell suspensions of *M. barkeri* strain MS (unpublished data), *M. barkeri* strain Fusaro (26), and cultures (31) or cell lysates (1) of *Methanosarcina* sp. strain 227. The enzymes mediating methanogenesis from acetate in cell extracts are not pelleted by centrifugation at 150,000 ×

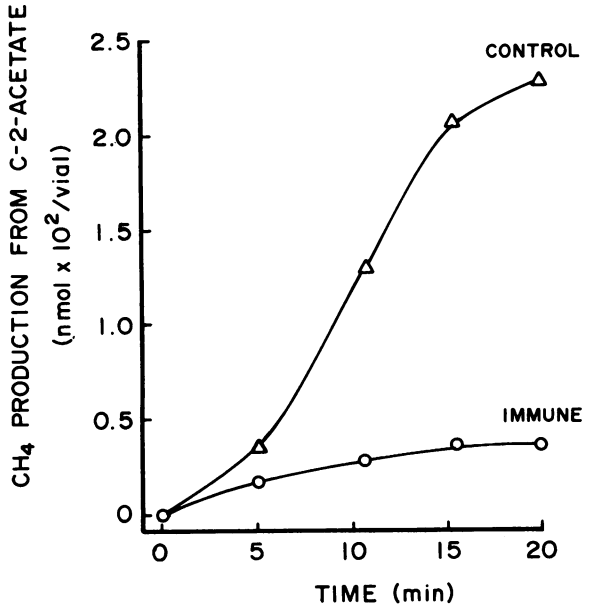

FIG. 6. Effect of immunoprecipitation of CO dehydrogenase on aceticlastic methanogenesis activity. The extract (0.5 ml, 18 mg of protein per ml) was mixed with 0.3 ml of IgG purified from immune (5.8 mg of protein per ml) or non-inoculated (2.4 mg of protein per ml) rabbits and treated as described in the text. The extract-IgG mixture (0.15 ml) was then incubated in 8-ml vials containing 4 μl of 1 M [2-^{14}C]sodium acetate (1,250 dpm/nmol) and 8 μl of 250 mM ATP under 1 atm (ca. 101 kPa) of hydrogen. The headspace was analyzed for $^{14}CH_4$ with time.

g and are therefore probably not associated with membrane vesicles or fragments (21). Therefore, the high apparent K_s for acetate exhibited by whole cells is a reflection of the apparent K_m of the enzymes of the aceticlastic pathway. This implies that transport of acetate across the cell membranes is by either diffusion or facilitated diffusion, since active transport of acetate would impart a lower K_s for acetate to intact cells than the apparent K_m observed for acetate of the catabolic enzyme pathway. Such a surmise is in keeping with general uptake mechanisms for acetate and the low theoretical free energy yield of methanogenesis from acetate (41), which would preclude an energy-dependent mechanism of acetate transport.

As with methanol (28, 36), ATP is required for conversion of acetate to methane in cell-free extracts. Half-maximal rates of methane formation are obtained with approximately 2 mM ATP. ATP is also the limiting factor in the assay; the concentration of ATP influences not only the rate, but also the duration, of methanogenesis. *M. barkeri* possesses an ATPase activity of 30 nmol/min per mg of protein (16), which could hydrolyze the ATP added in as little as 15 min at the protein concentrations employed.

ATP may function in two ways during acetate-dependent methanogenesis in extracts. It may be catalytic, as in the activation of methylreductase (11) or methyltransferase I (36), or it may be required for the production of an activated form of acetate, which is a substrate for enzyme(s) mediating the carbon-carbon bond cleavage. The formation of CH_3CoM, CO_2, and a reduced electron carrier from acetate is a thermodynamically unfavorable process (8). For this reason, this step might require the active input of energy. In this regard, the ATP-independent formation of methane from acetyl phosphate is of interest. Acetate kinase and phosphotransacetylase are very active in extracts of acetate-grown *M. barkeri* (16); acetyl phosphate and acetyl coenzyme A (acetyl-CoA) have been suggested as intermediates of the aceticlastic reaction (35, 42; Diekert et al., in press).

Acetyl-CoA is the direct product of acetate synthesis in the carbon monoxide dehydrogenase-dependent pathways of *Methanobacterium thermoautotrophicum* (10, 34) and *Clostridium thermoaceticum* (13, Diekert et al., in press), which invites speculation that it may also be an intermediate of the acetate catabolic pathway. However, it is not yet proven whether acetyl phosphate (or acetyl-CoA or both) is an activated acetyl intermediate. For example, ATP and acetate might be simply formed from acetyl phosphate by the action of acetate kinase (16). However, the high K_m for acetate and the rate and duration of acetyl phosphate conversion to methane make this speculation seem unlikely.

Hydrogen, although a vital component of the assay for aceticlastic activity in extracts of *M. barkeri*, is not required for methanogenesis by intact cells or in the particulate preparations described by Baresi (1). Because of this, we had previously suggested that the function of hydrogen was to circumvent the disruption of the electron transport chain from CO dehydrogenase to methylreductase caused by the disruption of the membrane (21; Zeikus et al., in press). However, in the absence of hydrogen, no CH_3CoM was formed from the methyl carbon of acetate in cell-free extracts. Therefore, these results suggest that hydrogen may also be required for a reaction before the formation of CH_3CoM. However, it is possible that hydrogen may be needed to reduce CH_3CoM to methane, since evidence for a soluble CO-dependent methylreductase activity has not been demonstrated in *M. barkeri*. The rapid production of hydrogen from CO oxidation by *M. barkeri* extracts (20) makes assay of CO-dependent methylreductase difficult. *Methanosarcina acetivorans* appears to possess a CO-dependent methylreductase (23). It is interesting to note that hydrogen is an inhibitor of the aceticlastic reaction in some strains of *Methanosarcina* (8, 9, 30). This is not the case with our acetate-adapted culture of *M. barkeri* strain MS (J. Krzycki, R. Conrad, B. Morgan, and J. G. Zeikus, submitted for publication). It may be that the hydrogen requirement of the aceticlastic reaction in soluble extracts, coupled with the inhibitory effect of hydrogen on some aceticlastic methanogens, is one reason why a cell-free system for acetate-dependent methanogenesis was such an elusive goal for many years.

The data presented here support the hypothesis that CO dehydrogenase is a component of aceticlastic methanogenesis. Low concentrations of cyanide, an inhibitor of *M. barkeri* CO dehydrogenase, also inhibited acetate-dependent methanogenesis. Cyanide effectively inhibits the exchange of CO_2 into acetate by suspensions of *M. barkeri* strain Fusaro (8); this is consistent with the proposed role of CO dehydrogenase in acetate catabolism. Reaction of aceticlastic extracts with antibodies to CO dehydrogenase led to loss of both CO dehydrogenase and aceticlastic activities. Although this is consistent for CO dehydrogenase involvement in methanogenesis from acetate, it must be interpreted with caution because the 95% purified enzyme contained two proteins that reacted with IgG. In addition, other proteins that may aggregate with CO dehydrogenase may have been coprecipitated by antibody. Nevertheless, this technique has provided the most direct evidence to date for involvement of CO dehydrogenase in aceticlastic methanogenesis, because when it was removed, activity disappeared

The first indication of the participation of CO dehydroge-

nase in the acticlastic pathway was the high specific activity of the enzyme in acetate-grown *M. barkeri* (19). Since this time, evidence has continued to accumulate in favor of this hypothesis. CO dehydrogenase comprises 5% of the total soluble protein in extracts of acetate-grown cells such as those used in this study (20). The high K_m of the enzyme for CO (5 mM) suggests that CO oxidation is a fortuitous reaction and is not the physiological function of the enzyme (20). The acetotrophic methanogens *Methanothrix soenghenii* (17), *Methanosarcina* sp. strain TM-1, and *Methanosarcina acetivorans* (23) also possess high specific activity of CO dehydrogenase.

The inhibition of aceticlastic methanogenesis by BCoM in cultures (14, 30) is also observed in cell-free extracts, which is consistent with the involvement of CH_3CoM and methylreductase in the production of methane from acetate. The methyl carbon of acetate can be traced into both methane and CH_3CoM during methanogenesis in extracts. $^{14}CH_3CoM$ was previously detected in cell suspensions of *M. barkeri* generating $^{14}CH_4$ from [2-^{14}C]sodium acetate (Krzycki and Zeikus, Abstr. Annu. Meet. Am. Soc. Microbiol. I11, p. 141). A recent deuterium labeling study, reminiscent of the early work of Pine and Barker (25), demonstrated that CD_3-CoM and CD_2H-CoM were produced from CD_3COO^- in the same proportions as CD_3H and CD_2H by *Methanosarcina* spp. (22). Methylreductase is present in acetate-grown *Methanosarcina* strain 227 (2). CH_3CoM is also an intermediate in the transformation of CH_3OH or CO_2 to methane (6, 28). It thus appears that the position of CH_3CoM, as the central intermediate of methanogenesis first postulated by Barker (3), is assured.

The data now at hand are completely consistent with our proposed model of acetate catabolism in *M. barkeri* (20, 21, 42), namely, that the reduction of a methyl intermediate is linked to the oxidation of a carbonyl intermediate by carbon monoxide dehydrogenase. Both intermediates are generated after the cleavage of the carbon-carbon bond of an activated intermediate, which could be acetyl-CoA, acetyl phosphate, or an unidentified activated form of acetate. This mode of acetate catabolism in *M. barkeri* may be a general model of acetate catabolism in other acetotrophic methanogens (17, 23).

ACKNOWLEDGMENTS

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