JOURNAL OF BACTERIOLOGY, Aug. 1988, p. 3384–3389
0021-9193/88/083384-05$02.00/0
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Vol. 170, No. 8

Effect of Molybdenum and Tungsten on Synthesis and Composition of Formate Dehydrogenase in *Methanobacterium formicicum*

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The influence of sodium molybdate and sodium tungstate on formate dehydrogenase activity was studied in H_2-CO_2-grown cultures of *Methanobacterium formicicum*. Depletion of molybdate from the growth medium resulted in a 75-fold decrease of intracellular molybdenum and a 35-fold decrease in enzyme activity; however, growth rate and cell yields were not influenced. By using an indirect enzyme-linked immunoassay, the amount of formate dehydrogenase approximated 3% of the total protein in cells grown in the presence of molybdate. Molybdenum-starved cells contained approximately 15-fold less formate dehydrogenase protein; Western blot (immunoblot) analysis revealed that both subunits of the enzyme were synthesized. Molybdenum starvation resulted in an increase in the amount of mRNA that hybridized to *fdh*-specific DNA. The results indicated an inverse relationship between the amount of transcript and the amount of formate dehydrogenase protein detected in response to molybdenum starvation. The addition of 1 mM tungstate to molybdate-containing media resulted in nearly complete loss of enzyme activity and decreased the intracellular concentration of molybdenum 10-fold. Cells grown in the presence of tungstate synthesized high amounts of inactive formate dehydrogenase and contained mRNA that hybridized to *fdh*-specific DNA in amounts similar to that in cells grown with sufficient molybdate. Inactive formate dehydrogenase, purified from cells grown in the presence of tungstate, had the same subunit composition and contained amounts of molybdopterin cofactor, albeit metal-free, comparable to those in the active enzyme.

Methanobacterium formicicum grows with H_2-CO_2 or formate as a source of electrons for the reduction of carbon dioxide to methane (20). Formate dehydrogenase is synthesized at high levels when cells are grown on H_2-CO_2 (20). The enzyme contains iron-sulfur centers (3, 23, 26), flavin adenine dinucleotide (24), and molybdenum (23) liganded with a cyanide-labile sulfur (2). The enzyme also contains a molybdopterin cofactor similar, but not identical, to all other described molybdopterins (15). The flavin adenine dinucleotide is required for reduction of the physiological electron acceptor coenzyme F_{420}, a 5-deazaflavin (22, 24). The genes coding for the two subunits of M_r 85,000 and 35,000 (23) have been cloned and sequenced (26). The cotranscribed genes are present on a large operon, of which the transcriptional start site and promoter region have been characterized (18).

We are interested in the influence of molybdenum and tungsten on the synthesis and stability of the formate dehydrogenase in *M. formicicum*. Here we show the effects of these metals on the composition of the enzyme and report that cells grown in molybdenum-depleted media contain lower levels of formate dehydrogenase protein but higher levels of mRNA that hybridized to *fdh*-specific DNA. The results suggest a molybdenum-sensitive regulation of formate dehydrogenase synthesis in *M. formicicum*.

MATERIALS AND METHODS

Cell growth. *M. formicicum* JF-1 (DSM 2639) was cultured on H_2-CO_2 in 10-liter fermentors at 40°C as described previously (20). The basal medium (no added sodium molybdate) contained the following constituents, in grams per liter (final concentration): NH_4Cl, 1.48; K_2HPO_4, 1.36; KH_2PO_4, 0.90; NaCl, 0.45; $MgSO_4$, 0.045; $CaCl_2 \cdot 2H_2O$, 0.06; CH_3COONa, 2.0; Na_2CO_3, 3.0; cysteine HCl, 0.27; $Na_2S \cdot 9H_2O$, 0.27; Na_2SeO_3, 0.0002; $Fe(NH_4)(SO_4)_2$, 0.01; resazurin, 0.001; sodium formate (0.5%, wt/vol). A vitamin solution and a trace mineral solution (molybdate omitted) were added (both at 1%, vol/vol) (30). Cultures were sparged with H_2-CO_2 (80:20) at 300 ml/min and were harvested during log phase when the A_{550} was near 2.0. Anoxic harvesting was performed by maintaining the culture under an atmosphere of H_2-CO_2 or N_2 and using a continuous-flow centrifuge (Cepa, Lehr-Baden, Federal Republic of Germany). Cell paste was immediately frozen and stored in liquid N_2.

M. formicicum was also grown in tubes (16 by 150 mm; Bellco Glass, Inc., Vineland, N.J.) that contained 5 ml of basal media modified as indicated. Tubes were sealed with a butyl rubber stopper secured with an aluminum crimp collar (1). Cultures were grown under an atmosphere of H_2-CO_2 (80:20) at 40°C. Growth was followed at 550 nm with a Spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.). One optical density unit corresponds to a cell mass of 0.75 g (dry weight)/liter (19).

All glassware for tube or fermentor cultures was acid cleaned in concentrated sulfuric acid before use.

Preparation of cell extracts. Cell extract was prepared anaerobically as described before (20). The O_2-free buffer was 50 mM potassium phosphate (pH 7.5), which also contained 10 mM sodium azide and 2 mM 2-mercaptoethanol to protect the enzyme against inhibition by contaminating amounts of oxygen (20, 21). The cell suspension (10 g of thawed cell past per 20 ml of buffer) was passed through a French pressure cell (SLM-Aminco, Urbana, Ill.) at 1,405 kg/cm^2 followed by centrifugation at 10,000 × *g* for 20 min. The supernatant solution was stored in liquid N_2.

Formate dehydrogenase and molybdopterin purifications. Formate dehydrogenase was purified as described previ-

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ously (23). Activity was assayed by following the formate-dependent reduction of methyl viologen (Sigma Chemical Co., St. Louis, Mo.) or coenzyme F_{420} with a Lambda 1 spectrophotometer (The Perkin-Elmer Corp., Oak Brook, Ill.) as described previously (21). Coenzyme F_{420} was purified from *M. formicicum* as described previously (22). A previously described (26) indirect enzyme-linked immunosorbent assay (ELISA) was used to assay inactive formate dehydrogenase and monitor the purification from cells grown in the presence of sodium tungstate. Protein was assayed with protein dye reagent (Bio-Rad Laboratories, Richmond, Calif.) by the method of Bradford (5), with bovine serum albumin (Sigma) as the protein standard.

Aerobic release and purification of molybdopterins from formate dehydrogenases were as described before (15), which yielded form A (9).

FPLC. Fast protein liquid chromatography (FPLC) was performed in a Coy anaerobic chamber (Coy Laboratory Products, Inc., Ann Arbor, Mich.). All containers and solutions used for anaerobic procedures were made O_2-free by repeated vacuum degassing and replacement with O_2-free N_2. Filtered cell extract (75 to 100 μl) was applied to a Mono Q HR 5/5 ion-exchange column (Pharmacia, Inc., Piscataway, N.J.) equilibrated with 50 mM potassium phosphate, pH 7.6, at 1.5 mP and 0.5 ml/min. During sample loading, the column was washed with equilibration buffer for 6 min. A 0.0 to 1.0 M KCl gradient was then applied for 30 min followed by two 6-min washes with 1.0 and 2.0 M KCl. A Pharmacia model GP-250 gradient programer was used. Formate dehydrogenase was assayed by reduction of methyl viologen as described previously (21).

Western immunoblot analysis. Proteins were denatured and electrophoresed in a sodium dodecyl sulfate-polyacrylamide gel (9%) (14), followed by electroblotting onto a nitrocellulose filter (Bio-Rad). Subsequent procedures were as described previously (29), except ^{125}I-labeled conjugated goat anti-rabbit immunoglobulin G was used. Anti-formate dehydrogenase antiserum, prepared as described previously (26), was diluted 1:5,000.

RNA isolation and dot blots. Total RNA was isolated from cells by the general procedures described previously (13). Approximately 0.1 g of cell paste was suspended in 0.5 ml of lysis buffer (50 mM Tris, pH 8.0, 20 mM EDTA, 1% sodium dodecyl sulfate, 0.2% diethyl pyrocarbonate). An equal volume of phenol–*m*-cresol–8-hydroxyquinoline (1,000:140:0.4, wt/wt/wt) was added to the cell suspension and mixed at 65°C for 5 min. The aqueous phase was reextracted, and the RNA was precipitated with ethanol. The RNA concentration was determined by A_{260}, and the purity was determined by the A_{260}/A_{280} ratio. RNA samples for dot blot analysis were prepared by the general procedures described previously (27). Appropriate dilutions of the glyoxal-denatured RNA were manually spotted onto GeneScreen hybridization membrane (New England Nuclear Corp., Boston, Mass.) and baked for 120 min at 80°C. Prehybridization and hybridization were performed as recommended by the manufacturer. Hybridization was at 45°C for 18 to 24 h. After hybridization, the filters were washed three times at 21°C for 5 min in 2× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate, pH 7.0) containing 0.1% sodium dodecyl sulfate, followed by two 30-min washes at 65°C in the same solution. Finally, the filters were washed with 0.2× SSC. DNA probes were radiolabeled with commercially available kits for random priming (Pharmacia, Inc.). X-ray film was exposed to filters for 18 to 30 h at −70°C.

Analytical determination. Methane was determined by gas chromatography as described previously (17).

Molybdenum was detected by atomic absorption spectrophotometry on a model 475 spectrophotometer (Varian Instruments, Palo Alto, Calif.), as described before (23). Molybdenum standard was purchased from Fisher Scientific Co., Springfield, N.J. Tungsten was assayed as described previously (6). Sodium tungstate (Fisher Scientific) was used as a standard.

Fluorescence spectra were obtained with a Perkin-Elmer model 650-10s fluorescence spectrophotometer; the spectra were not corrected.

Electrophoretic analysis of proteins by sodium dodecyl sulfate-polyacrylamide (9%, wt/vol) slab gel electrophoresis was as previously described (14). The M_r markers (Sigma) were as follows: carbonic anhydrase, 32,000; ovalbumin, 45,000; bovine albumin, 66,200; phosphorylase *b*, 92,500.

RESULTS

Effect of sodium molybdate and sodium tungstate on formate dehydrogenase activity. Formate dehydrogenase is not required for growth on H_2-CO_2 but is synthesized at high

TABLE 1. Metal concentrations and the amounts and activity of formate dehydrogenase in cell extracts of *M. formicicum*

Culture[a]	Metals (nmol/mg of protein)[b]		Formate dehydrogenase		
			Activity (u/mg of protein)[b] assayed with:		Amt (μg/mg of protein)[c]
	Mo	W	Methyl viologen	F_{420}	
1	2.22 ± 0.49	ND[d]	1.80 ± 0.29	1.07 ± 0.06	ND
2	0.51 ± 0.10	ND	1.64 ± 0.36	1.02 ± 0.06	31.3
3	0.29 ± 0.03	ND	1.61 ± 0.30	0.73 ± 0.05	24.3
4	0.18 ± 0.03	ND	0.72 ± 0.01	0.36 ± 0.05	ND
5	0.07 ± 0.01	ND	0.12 ± 0.03	<0.01[e]	2.0
6	0.03 ± 0.01	ND	0.05 ± 0.01	<0.01	1.7
7	0.03 ± 0.01	8.9 ± 0.8	0.03 ± 0.01	<0.01	30.5
8	0.03 ± 0.01	<0.5[e]	0.03 ± 0.01	<0.01	25.5

[a] Cultures 1 to 3 were grown in basal media supplemented with 10^{-4}, 10^{-5}, and 10^{-6} M sodium molybdate, respectively. Cultures 4 to 6 were grown in basal media with no added sodium molybdate; each was grown in succession with the previous culture used as an inoculum (10%). Culture 4 was inoculated with a culture grown in basal medium that contained 10^{-6} M added sodium molybdate. Culture 7 was grown in basal medium supplemented with 10^{-6} M sodium molybdate and 1 mM sodium tungstate. Culture 8 was grown in basal medium supplemented with 1 μM sodium tungstate.

[b] Values are the mean ± standard deviation (minimum of four determinations).

[c] Determined by ELISA after FPLC fractionation of extracts. Values are the sum of all fractions (Fig. 3).

[d] ND, Not done.

[e] Below the limit of detection.

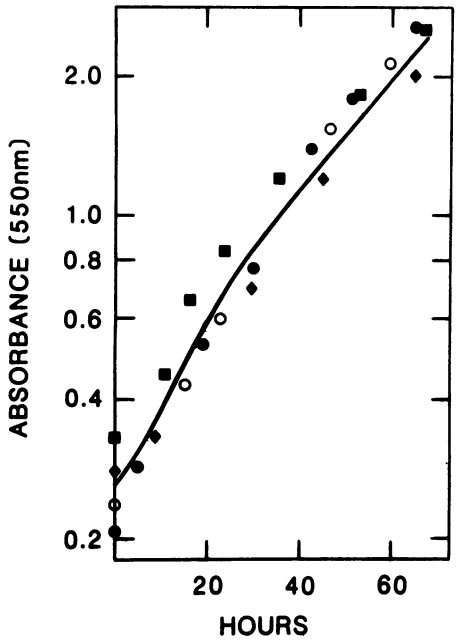

FIG. 1. Growth of *M. formicicum* in the presence of sodium molybdate or sodium tungstate. The fermentor cultures (Table 1) were grown with the indicated amounts (final concentration) of molybdate or tungstate added to the media: ■, 10^{-4} M molybdate; ○, 10^{-6} M molybdate; ●, no additions and after three successive transfers; ◆, 10^{-3} M tungstate. The inocula were cultures grown in basal medium supplemented with 10^{-6} M molybdate.

levels (20); therefore, growth on this substrate was used to study the effect of molybdenum and tungsten on formate dehydrogenase activity. Table 1 shows that, by lowering the molybdate concentration of basal medium, there was nearly a 75-fold decrease in the molybdenum concentrations of cell extracts; however, neither the growth rate nor the cell yield was influenced (Fig. 1). Formate dehydrogenase activity decreased as the concentration of molybdenum detected in extracts decreased (Table 1). An approximately 35-fold decrease in activity (assayed with methyl viologen) occurred when the molybdenum concentration was depleted to 0.03 nmol/mg of protein. When tungstate was added to media instead of molybdate, or at a 1,000-fold-greater concentration than molybdate, the intracellular molybdenum decreased by 10-fold and formate dehydrogenase activity was approximately 50-fold lower (Table 1). These results suggest that tungstate prevents the accumulation of molybdenum and that tungsten is unable to replace the molybdenum requirement for formate dehydrogenase. High levels of tungsten were present in extracts of cells grown in the presence of 10^{-3} M tungstate (Table 1); however, growth was not influenced (Fig. 1).

Figure 2 shows the rate of methanogenesis from formate by cells grown on H_2-CO_2 in the presence of tungstate. The rate decreased in response to increasing amounts of tungstate present during growth (Fig. 2A). The addition of up to 10^{-3} M molybdate to cultures grown in the presence of 10^{-3} M tungstate did not overcome the inhibition after 10 h of incubation in an N_2 atmosphere (data not shown); however, after 72 h of incubation in an H_2-CO_2 atmosphere, the added molybdate overcame the effect of tungstate (Fig. 2B). Growth was inhibited by tungstate in a pattern similar to that in Fig. 2 when formate was the only source of carbon and energy (data not shown). These results suggest that tungsten is an antagonist of molybdenum in the formate dehydrogenase of *M. formicicum*.

Immunological assays. The relative amounts of formate dehydrogenase protein in crude extracts of *M. formicicum* were estimated by an ELISA, using anti-formate dehydrogenase antiserum. The assay detected at least 5 ng of purified enzyme and was linear between 5 and 12 ng. Extracts were fractionated by FPLC to detect any modified forms of the enzyme. Cells with high levels of molybdenum (Fig. 3, top) contained a greater amount of formate dehydrogenase cross-reacting material than molybdenum-starved cells (Fig. 3, middle). Although cells grown in the presence of tungstate contained negligible formate dehydrogenase activity (Table 1), a protein peak and high amounts of cross-reacting material eluted in the same position as the active enzyme (Fig. 3,

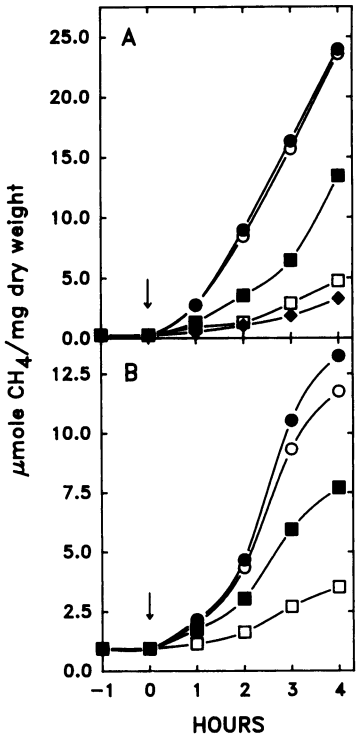

FIG. 2. Rate of methanogenesis from formate. (A) Formate-dependent methanogenesis by *M. formicicum* cultures grown in the presence of sodium tungstate. Each tube culture was pregrown in basal medium containing 10^{-6} M molybdate and the indicated amounts of tungstate to an optical density of 0.25 to 0.30 followed by replacement of the H_2-CO_2 atmosphere with N_2. After methanogenesis had ceased, sodium formate (0.5%, wt/vol, final concentration) was added to each culture (arrow) and methanogenesis was followed. Values are the means of triplicate cultures. The final concentrations in (moles per liter) of tungstate added to media were as follows: ●, zero; ○, 10^{-6}; ■, 10^{-5}; □, 10^{-4}; ◆, 10^{-3}. (B) Ability of sodium molybdate to overcome sodium tungstate inhibition of formate-dependent methanogenesis by H_2-CO_2-grown *M. formicicum*. Each culture was grown as described in panel A, except 1 mM (final concentration) tungstate was added to the media. Molybdate was then added to the cultures at the indicated concentrations and incubated in an H_2-CO_2 atmosphere for an additional 72 h followed by replacement with N_2. After methanogenesis had ceased, sodium formate (0.5%, wt/vol, final concentration) was added to each culture (arrow) and methane production was monitored. Values shown are the means of triplicate cultures. Molybdate (final concentrations in moles per liter) was added to the cultures at: □, 10^{-6}; ■, 10^{-5}; ○, 10^{-4}; ●, 10^{-3}.

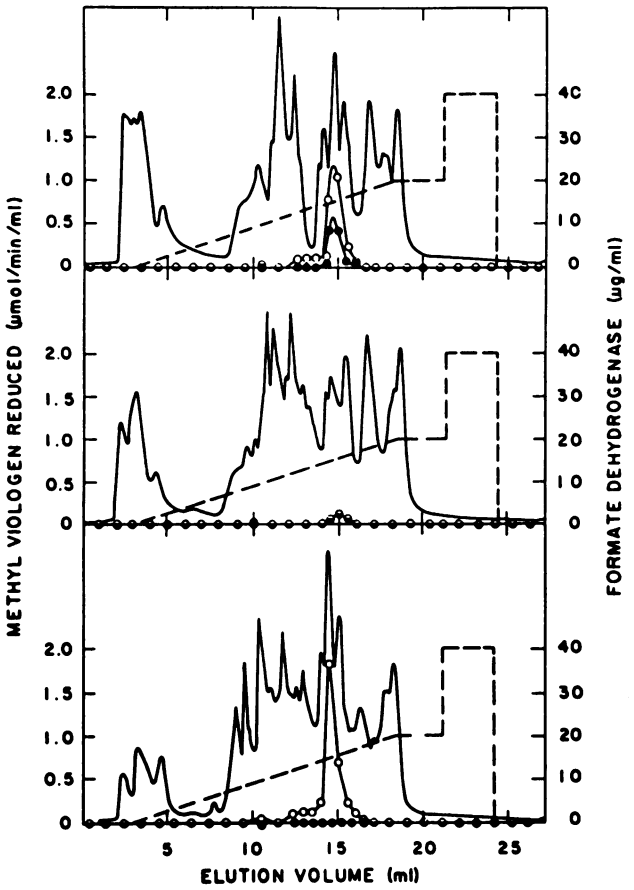

FIG. 3. Activity and amount of formate dehydrogenase in FPLC-fractionated extracts of *M. formicicum*. The extracts applied to the Mono Q column were as follows: (top) 2 mg of protein from cells containing 0.29 nmol of molybdenum per mg of protein (culture 3, Table 1); (middle) 2 mg of protein from cells containing 0.03 nmol of molybdenum per mg of protein (culture 6, Table 1); (bottom) 2 mg of protein from cells containing 8.9 nmol of tungsten per mg of protein (culture 7, Table 1). The fractions were 0.5 ml. Symbols: ●, formate-dependent reduction of methyl viologen; ○, amount of formate dehydrogenase detected in the ELISA; ——, relative A_{280}; ---, KCl gradient (3 ml of 0.0 M, 15 ml of 0.0 to 1.0 M, 3 ml of 1.0 M, 3 ml of 2.0 M).

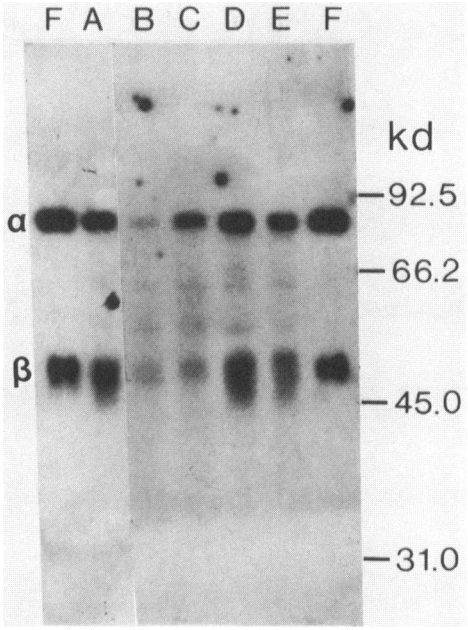

FIG. 4. Western blot analysis of cell extracts of *M. formicicum*. Lane A, 10 μg of extract protein from cells containing 2.22 nmol of molybdenum per mg of protein (culture 1, Table 1); lane B, 10 μg of extract protein from cells containing 0.03 nmol of molybdenum per mg of protein (culture 6, Table 1); lane C, 50 μg of lane B; lane D, 10 μg of extract protein from cells grown in the presence of 1 mM sodium tungstate and containing 8.9 nmol of tungsten per mg of protein (culture 7, Table 1); lane E, 10 μg of extract protein from cells grown in the presence of 1 μM sodium tungstate (culture 8, Table 1); lanes F, 0.5 μg of homogeneous formate dehydrogenase. α, 85,000-M_r subunit; β, 53,000-M_r subunit.

bottom). No other peaks of cross-reacting material were detected in the FPLC profiles, suggesting that there were no significant amounts of modified forms of formate dehydrogenase that cross-reacted with antiserum. The formate dehydrogenase reached approximately 3% of the total cellular protein when grown in the presence of molybdate or tungstate (Table 1). The extracts containing the lowest concentration of molybdenum had at least 15-fold less formate dehydrogenase protein than extracts that contained high concentrations of molybdenum or tungsten (Table 1). These results indicate that decreased amounts of formate dehydrogenase may be responsible for the decreased enzyme activity in molybdenum-starved cells.

Crude extracts were also analyzed by using Western blots probed with anti-formate dehydrogenase antiserum (Fig. 4). The results were consistent with the results obtained with the ELISA, indicating decreased amounts of formate dehydrogenase in molybdenum-starved cells; albeit in low amounts, both subunits were present. The Western blots also showed that cells grown in the presence of tungstate contained high levels of both formate dehydrogenase subunits.

Properties of formate dehydrogenase from *M. formicicum* grown in the presence of sodium tungstate. The inactive formate dehydrogenase synthesized in cells grown on H_2-CO_2, and in the presence of 10^{-3} M tungstate, was purified. Gel electrophoresis showed that the protein was >90% pure (Fig. 5). The inactive enzyme had the same subunit composition as active formate dehydrogenase. The enzyme contained a compound with fluorescence spectra (15) identical to, and in approximately equal amounts as, that of the molybdopterin cofactor from active formate dehydrogenase (data not shown). Analysis for molybdenum and tungsten in the inactive enzyme revealed that neither metal was present above 0.05 mol/mol of formate dehydrogenase. However, it remains possible that tungsten was lost during the purification. Nonetheless, the results show that inactive formate dehydrogenase, containing a molybdenum-free molybdopterin cofactor, was synthesized in cells grown in the presence of tungstate.

Effect of molybdenum starvation or the presence of tungsten on levels of formate dehydrogenase mRNA. The relative amounts of *fdh*-specific message in *M. formicicum* cells starved for molybdenum was studied with a 0.5-kilobase *Sna*BI-*Bgl*II restriction enzyme fragment contained within the *fdhA* structural gene (18) as a probe in dot blot analyses. The amount of transcript increased as the intracellular concentration of molybdenum decreased (Fig. 6). Southern blot analysis indicated that a single region on the chromosome was homologous to the probe used for Northern

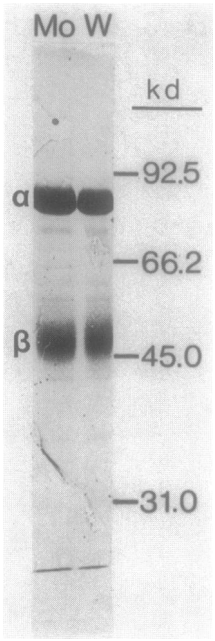

FIG. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of formate dehydrogenases purified from *M. formicicum*. Lane Mo, 40 μg of formate dehydrogenase purified from cells grown in the presence of 10^{-6} M sodium molybdate; lane W, 40 μg of formate dehydrogenase purified from cells grown in the presence of 10^{-6} M sodium tungstate. α, 85,000-M_r subunit; β, 53,000-M_r subunit.

(RNA) dot blot analysis (data not shown). The results indicate an inverse relationship between the amount of *fdh*-specific transcript and formate dehydrogenase protein in response to the molybdenum content of the cells.

The amount of *fdh*-specific transcript in cells grown in media containing no added sodium molybdate but 1 μM sodium tungstate, or 1 μM sodium molybdate and 1 mM sodium tungstate, was also compared (Fig. 6). The amounts of message in tungsten-containing cells (lanes 7 and 8) more closely resembled that of cells with the intermediate concentrations of molybdenum and formate dehydrogenase protein (lanes 2 and 3). Thus, the inverse relationship between the amount of formate dehydrogenase polypeptide and the levels of message also applied to cells grown in the presence of tungstate.

DISCUSSION

Although molybdenum is present in the formate dehydrogenase from *M. formicicum*, no evidence of a requirement for activity has been reported. The results presented here show that this metal is essential for activity. Very little enzyme activity was detected in cells grown in the presence of sodium tungstate, which suggests that this organism is incapable of synthesizing a functional tungsten-containing formate dehydrogenase. Several active tungsten-containing formate dehydrogenases are synthesized in strictly anaerobic organisms, including the methanogen *Methanococcus vannielii*, which also requires tungsten for optimum growth on formate (11, 12). The molybdenum content of the medium was adjusted to limit the synthesis of active formate dehydrogenase in H_2-CO_2-grown *M. formicicum*; however, growth on H_2-CO_2 was not influenced. *Methanobacterium thermoautotrophicum* requires molybdenum for optimum growth on H_2-CO_2 (25), but other methanogens require tungsten (31).

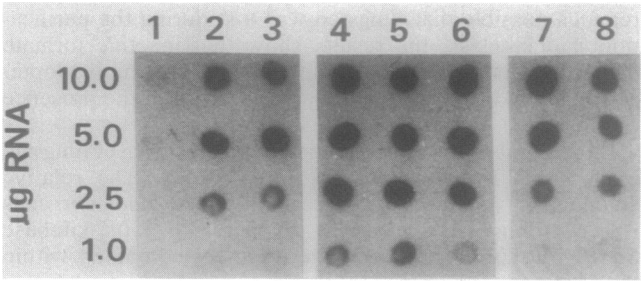

FIG. 6. Levels of *fdh*-specific mRNA in *M. formicicum*. Filters contained the indicated amounts of total cellular RNA per dot. Lanes 1 to 8 contained RNA from cultures 1 to 8 (Table 1), respectively. The hybridization probe was ^{32}P-labeled *Sna*BI-*Bgl*II restriction enzyme fragment described previously (18).

Although the formate dehydrogenase synthesized in tungsten-containing cells was inactive, tungsten did not prevent the synthesis of the molybdopterin cofactor or of either of the two subunits. Inactive formate dehydrogenase is synthesized in *M. formicicum* grown in the presence of sodium tungstate. These results are similar to the inactive nitrate reductase protein synthesized in tungstate-supplemented *Escherichia coli* (7), but differs from the amounts of formate dehydrogenase in *E. coli* (7) and nitrogenase in *Rhodopseudomonas capsulata* (8). The latter two proteins are not synthesized at optimal levels when tungstate is added to the growth medium. Inactive, yet stable forms of rat liver sulfite oxidase are synthesized when tungstate is added to the diet (10). One-third of the sulfite oxidase synthesized contains tungsten and two-thirds is cofactor-free. The inactive formate dehydrogenase synthesized in *M. formicicum* cells supplemented with sodium tungstate contained metal-free molybdopterin cofactor, and the subunits were electrophoretically identical to those of the native enzyme, which suggests little or no degradation of the inactive form. These results suggest that incorporation of molybdenum is not required for enzyme stability. However, the results do not rule out that the presence of metal-free pterin cofactor may be required for stabilization of the enzyme. Further research is needed to determine whether molybdenum or tungsten is required for cofactor synthesis or cofactor insertion into the apoenzyme.

The relative amounts of formate dehydrogenase decreased but *fdh*-specific message increased in molybdenum-starved cells. The results suggest that *fdh*-specific mRNA synthesis or stability is regulated in response to molybdenum. The results also suggest that molybdenum-starved cells may synthesize a formate dehydrogenase apoprotein that may be rapidly degraded, although translational regulation cannot be ruled out at this juncture.

Synthesis of the molybdenum-containing nitrate reductase is autoregulated in *E. coli* (4). Although no direct response to molybdenum is observed, autoregulation of the *E. coli* nitrate reductase requires the molybdopterin cofactor. Similarly, *Neurospora crassa* nitrate reductase negatively controls its own synthesis (28). Autoregulation could also be postulated for the *M. formicicum* formate dehydrogenase since it is synthesized in relatively large amounts (this report) and is located on the cytoplasmic membrane (manuscript in preparation). Thus, a control is possible which limits wasteful overproduction and potential overloading of the cytoplasmic membrane. The results obtained with cells grown in the presence of sodium tungstate are consistent with the hypothesis, but do not prove, that formate dehydrogenase itself may be a factor in controlling transcription

of the *fdh* genes. Tungsten-containing cells were depleted of molybdenum, but the amounts of formate dehydrogenase and *fdh*-specific message were comparable to cells containing sufficient molybdenum. Clearly, these results do not allow conclusions regarding potential autoregulation; further research is necessary to answer this question, which is only mentioned here to offer a testable model.

In the native habitat, *M. formicicum* may seldom encounter the low levels of molybdenum imposed on the molybdenum-starved cells reported here; thus, the severalfold increase in *fdh*-specific mRNA may not be physiologically significant. Derepression of formate dehydrogenase apoprotein synthesis could be a physiologically significant response to molybdenum limitation in an effort to improve the kinetics of molybdenum incorporation. For example, the *chlD* gene of *E. coli* is essential for synthesis of active molybdoenzymes when grown in the presence of low concentrations of molybdate and is repressed by molybdate or tungstate in the growth media (16). Clearly, more research is necessary to determine the mechanisms of the molybdenum-sensitive regulation of formate dehydrogenase synthesis in *M. formicicum*.

ACKNOWLEDGMENTS

We thank David Brown for assistance in obtaining cell material.

This work was supported by grants DMB-8409558 from the National Science Foundation and 5086-260-1255 from the Gas Research Institute.

LITERATURE CITED

1. **Balch, W. E., and R. S. Wolfe.** 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethane-sulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl. Environ. Microbiol. **32**:781–791.
2. **Barber, M. J., H. D. May, and J. G. Ferry.** 1986. Cyanide inactivation of formate dehydrogenase from *Methanobacterium formicicum*. Biochemistry. **25**:8150–8155.
3. **Barber, M. J., L. M. Siegel, N. L. Schauer, H. D. May, and J. G. Ferry.** 1983. Formate dehydrogenase from *Methanobacterium formicicum*: electron paramagnetic resonance spectroscopy of the molybdenum and iron-sulfur centers. J. Biol. Chem. **258**: 10839–10845.
4. **Bonnefoy, V., M.-C. Pascal, J. Ratouchniak, and M. Chippaux.** 1986. Autoregulation of the *nar* operon encoding nitrate reductase in *Escherichia coli*. Mol. Gen. Genet. **204**:180–184.
5. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
6. **Cardenas, J., and L. E. Mortenson.** 1974. Determination of molybdenum and tungsten in biological samples. Anal. Biochem. **60**:372–376.
7. **Giordano, G., B. A. Haddock, and D. H. Boxer.** 1980. Molybdenum-limited growth achieved either phenotypically or genotypically and its effect on the synthesis of formate dehydrogenase and nitrate reductase by *Escherichia coli* K12. FEMS Microbiol. Lett. **8**:229–235.
8. **Hallenbeck, P. C., C. M. Meyer, and P. M. Vignois.** 1982. Regulation of nitrogenase in the photosynthetic bacterium *Rhodopseudomonas capsulata* as studied by two-dimensional gel electrophoresis. J. Bacteriol. **151**:1612–1616.
9. **Johnson, J. L., and K. V. Rajagopalan.** 1982. Structural and metabolic relationship between the molybdenum cofactor and urothione. Proc. Natl. Acad. Sci. USA **79**:6856–6860.
10. **Johnson, J. L., K. V. Rajagopalan, and H. J. Cohen.** 1974. Molecular basis of the biological function of molybdenum. Effect of tungsten and xanthine oxidase and sulfite oxidase in the rat. J. Biol. Chem. **249**:859–866.
11. **Jones, J. B., and T. C. Stadtman.** 1977. *Methanococcus vannielii*: culture and effects of selenium and tungsten on growth. J. Bacteriol. **130**:1404–1406.
12. **Jones, J. B., and T. C. Stadtman.** 1981. Selenium-dependent and selenium-independent formate dehydrogenases of *Methanococcus vannielii*. J. Biol. Chem. **256**:656–663.
13. **Krol, A. J. M., J. G. J. Horntelez, T. Toozendaal, and A. van Kammen.** 1982. On the operon structure of the nitrogenase genes of *Rhizobium leguminosarium* and *Azotobacter vinelandii*. Nucleic Acids Res. **10**:4147–4157.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
15. **May, H. D., N. L. Schauer, and J. G. Ferry.** 1986. Molybdopterin cofactor from *Methanobacterium formicicum* formate dehydrogenase. J. Bacteriol. **166**:500–504.
16. **Miller, J. B., D. J. Scott, and N. K. Amy.** 1987. Molybdenum-sensitive transcriptional regulation of the *chlD* locus of *Escherichia coli*. J. Bacteriol. **169**:1853–1860.
17. **Nelson, M. J. K., and J. G. Ferry.** 1984. Carbon monoxide-dependent methyl coenzyme M methylreductase in acetotrophic *Methanosarcina* spp. J. Bacteriol. **160**:526–532.
18. **Patel, P. S., and J. G. Ferry.** 1988. Characterization of the upstream region of the formate dehydrogenase operon of *Methanobacterium formicicum*. J. Bacteriol. **170**:3390–3395.
19. **Schauer, N. L., D. P. Brown, and J. G. Ferry.** 1982. Kinetics of formate metabolism in *Methanobacterium formicicum* and *Methanospirillum hungatei*. Appl. Environ. Microbiol. **44**:549–554.
20. **Schauer, N. L., and J. G. Ferry.** 1980. Metabolism of formate in *Methanobacterium formicicum*. J. Bacteriol. **142**:800–807.
21. **Schauer, N. L., and J. G. Ferry.** 1982. Properties of formate dehydrogenase in *Methanobacterium formicicum*. J. Bacteriol. **150**:1–7.
22. **Schauer, N. L., and J. G. Ferry.** 1983. Flavin adenine dinucleotide requirement for the reduction of coenzyme F_{420} by formate dehydrogenase from *Methanobacterium formicicum*. J. Bacteriol. **155**:467–472.
23. **Schauer, N. L., and J. G. Ferry.** 1986. Composition of the coenzyme F_{420}-dependent formate dehydrogenase from *Methanobacterium formicicum*. J. Bacteriol. **165**:405–411.
24. **Schauer, N. L., J. G. Ferry, J. F. Honek, W. H. Orme-Johnson, and C. Walsh.** 1986. Mechanistic studies of the coenzyme F_{420} reducing formate dehydrogenase from *Methanobacterium formicicum*. Biochemistry **25**:7163–7168.
25. **Schonheit, P., J. Moll, and R. K. Thauer.** 1979. Nickel, cobalt and molybdenum requirement for growth of *Methanobacterium thermoautotrophicum*. Arch. Microbiol. **123**:105–107.
26. **Shuber, A. P., E. C. Orr, M. A. Recny, P. F. Schendel, H. D. May, and J. G. Ferry.** 1986. Cloning, expression, and nucleotide sequence of the formate dehydrogenase genes from *Methanobacterium formicicum*. J. Biol. Chem. **261**:12942–12947.
27. **Thomas, P. S.** 1983. Hybridization of denatured RNA transferred or dotted to nitrocellulose paper. Methods Enzymol. **100B**:255–266.
28. **Tomsett, A. B., and R. H. Garrett.** 1981. Biochemical analysis of mutants defective in nitrate assimilation in *Neurospora crassa*: evidence for autogenous control by nitrate reductase. Mol. Gen. Genet. **184**:183–190.
29. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
30. **Wolin, E. A., M. J. Wolin, and R. S. Wolfe.** 1963. Formation of methane by bacterial extracts. J. Biol. Chem. **238**:2882–2886.
31. **Zellner, G., and J. Winter.** 1987. Growth promoting effect of tungsten on methanogens and incorporation of tungsten-185 into cells. FEMS Microbiol. Lett. **40**:81–87.