Arch Microbiol (1991) 155:459–465

030289339100061W

Archives of
Microbiology

Activities of formylmethanofuran dehydrogenase, methylenetetrahydromethanopterin dehydrogenase, methylenetetrahydromethanopterin reductase, and heterodisulfide reductase in methanogenic bacteria

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Received October 11, 1990/Accepted December 19, 1990

Abstract. The activities of formylmethanofuran dehydrogenase, methylenetetrahydromethanopterin dehydrogenase, methylenetetrahydromethanopterin reductase, and heterodisulfide reductase were tested in cell extracts of 10 different methanogenic bacteria grown on H_2/CO_2 or on other methanogenic substrates. The four activities were found in all the organisms investigated: *Methanobacterium thermoautotrophicum, M. wolfei, Methanobrevibacter arboriphilus, Methanosphaera stadtmanae, Methanosarcina barkeri* (strains Fusaro and MS), *Methanothrix soehngenii, Methanospirillum hungatei, Methanogenium organophilum,* and *Methanococcus voltae*. Cell extracts of H_2/CO_2 grown *M. barkeri* and of methanol grown *M. barkeri* showed the same specific activities suggesting that the four enzymes are of equal importance in CO_2 reduction to methane and in methanol disproportionation to CO_2 and CH_4. In contrast, cell extracts of acetate grown *M. barkeri* exhibited much lower activities of formylmethanofuran dehydrogenase and methylenetetrahydromethanopterin dehydrogenase suggesting that these two enzymes are not involved in methanogenesis from acetate. In *M. stadtmanae,* which grows on H_2 and methanol, only heterodisulfide reductase was detected in activities sufficient to account for the in *vivo* methane formation rate. This finding is consistent with the view that the three other oxidoreductases are not required for methanol reduction to methane with H_2.

Key words: Methanogenic bacteria – Methanofuran – Coenzyme M – Tetrahydromethanopterin – Coenzyme F_{420} – Disulfide reductase

Methanogenic bacteria are anaerobic archaebacteria. Most of them grow on H_2 and CO_2, some use methanol, methylamine, or acetate as methanogenic substrates (Balch et al. 1979).

The mechanism of methane formation has mainly been resolved with *Methanobacterium thermoautotrophicum* (Zeikus and Wolfe 1972) which reduces 1 CO_2 with 4 H_2 to 1 CH_4 and 2 H_2O ($\Delta G^{\circ\prime} = -131$ kJ/mol). In this organism CO_2 is converted to methane via formylmethanofuran (CHO-MFR), N^5-formyl-H_4MPT, N^5,N^{10}-methenyl-H_4MPT (CH ≡ H_4MPT), N^5,N^{10}-methylene-H_4MPT (CH_2 = H_4MPT), N^5-methyl-H_4MPT (CH_3-H_4MPT), and methyl-coenzyme M ($CH_3-S-CoM$) as intermediates (DiMarco et al. 1990). Four redox reactions are involved:

$$CO_2 + MFR + 2\,[H] \rightleftharpoons CHO-MFR + H_2O \quad (1)$$
$$E^{\circ\prime} = -497\ mV$$

$$CH \equiv H_4MPT^+ + 2\,[H] \rightleftharpoons CH_2 = H_4MPT + H^+ \quad (2)$$
$$E^{\circ\prime} = -386\ mV$$

$$CH_2 = H_4MPT + 2\,[H] \rightleftharpoons CH_3 - H_4MPT \quad (3)$$
$$E^{\circ\prime} = -323\ mV$$

$$CH_3-S-CoM + 2\,[H] \rightarrow CH_4 + H-S-CoM \quad (4)$$
$$E^{\circ\prime} = +26\ mV$$

For thermodynamic data see Thauer (1990). Reaction (1) is catalyzed by formylmethanofuran dehydrogenase, reaction (2) by methylene-H_4MPT dehydrogenase, reaction (3) by methylene-H_4MPT reductase, and reaction (4) by methyl-coenzyme M reductase plus heterodisulfide reductase. The latter two enzymes mediate partial reactions (5) and (6), respectively (Thauer 1990).

$$CH_3-S-CoM + H-S-HTP \rightleftharpoons CH_4 + CoM-S-S-HTP \quad (5)$$
$$\Delta G^{\circ\prime} = -45\ kJ/mol$$

$$CoM-S-S-HTP + 2\,[H] \rightleftharpoons H-S-CoM + H-S-HTP \quad (6)$$
$$E^{\circ\prime} = -210\ mV$$

The physiological electron donor of formylmethanofuran dehydrogenase (Bobik and Wolfe 1988;

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Non-standard abbreviations: MFR, methanofuran; H_4MPT, tetrahydromethanopterin; H-S-CoM, coenzyme M; H-S-HTP, 7-mercaptoheptanoylthreonine phosphate (component B); CoM-S-S-HTP, heterodisulfide of H-S-CoM and H-S-HTP

Börner et al. 1989) and of heterodisulfide reductase (Hedderich et al. 1989) are not yet known. The methylene-H_4MPT reductase is specific for reduced coenzyme F_{420} (te Brömmelstroet et al. 1990) and the methylene-H_4MPT dehydrogenase directly reacts with H_2 (Zirngibl et al. 1990). After incubation of cell extracts under aerobic conditions a coenzyme F_{420}-dependent methylene-H_4MPT dehydrogenase is also detectable (Mukhopadhyay and Daniels 1989). Methyl-coenzyme M reductase was found to be highly specific for H−S−HTP as electron donor (Ellermann et al. 1988).

The five oxidoreductases involved in CO_2 reduction to methane have been purified and characterized from *M. thermoautotrophicum:* Formylmethanofuran dehydrogenase (Börner et al. 1989; Karrasch et al. 1989b); methylene-H_4MPT dehydrogenase (Hartzell et al. 1985; Mukhopadhyay and Daniels 1989; Zirngibl et al. 1990); methylene-H_4MPT reductase (te Brömmelstroet et al. 1990; Ma and Thauer 1990a and b); methyl-coenzyme M reductase (Ellefson et al. 1982; Ellermann et al. 1988, 1989; Rospert et al. 1990); and heterodisulfide reductase (Hedderich et al. 1989, 1990).

We describe here the presence of the 5 oxidoreductases in ten methanogenic bacteria belonging to different taxonomic groups (Woese 1987) and/or grown on substrates other than H_2/CO_2.

Materials and methods

Methanofuran, tetrahydromethanopterin, and coenzyme F_{420} were isolated from *Methanobacterium thermoautotrophicum* (strain Marburg). Formylmethanofuran was prepared from methanofuran by formylation with p-nitrophenylformate (Donnelly and Wolfe 1986). L−CoM−S−S−HTP was synthesized as described by Ellermann et al. 1988.

Methanococcus voltae (DSM 1537), *M. thermoautotrophicum* (strain Marburg) (DSM 2133) (Brandis et al. 1981), *Methanobacterium wolfei* (DSM 2970), *Methanobrevibacter arboriphilus* (strain AZ) (DSM 744) (Zehnder and Wuhrmann 1977), *Methanosphaera stadtmanae* (DSM 3091) (for valid name see Int J Syst Bacteriol, 1985, 35: 535), *Methanosarcina barkeri* (strain Fusaro) (DSM 804) (Kandler and Hippe 1977), *M. barkeri* (strain MS) (DSM 800) (Bryant and Boone 1987), *Methanothrix soehngenii* (DSM 2139), *Methanospirillum hungatei* (DSM 1101), *Methanogenium organophilum* (DSM 3596) were from the Deutsche Sammlung von Mikroorganismen (Göttingen, FRG). Cells of *M. soehngenii* grown on acetate (Jetten et al. 1989a) were a gift from M. Jetten (Wageningen).

The bacteria were grown as described: *M. thermoautotrophicum* (Schönheit et al. 1980); *M. wolfei*, medium of *M. thermoautotrophicum* supplemented with sodium tungstate (1 μM) (Winter et al. 1984); *M. arboriphilus* (Pfaltz et al. 1987); *M. stadtmanae* (Miller and Wolin 1985); *M. barkeri* (Karrasch et al. 1989a); *M. hungatei* (Bott et al. 1985); *M. organophilum* (Widdel 1986) and *M. voltae* (Whitman et al. 1982). The cells were harvested in the exponential growth phase, washed once with 50 mM potassium phosphate pH 7 containing 0.5 mM dithiothreitol, and suspended in the same buffer to a final cell concentration of 20−40 mg (dry weight) per ml. The suspension was then anaerobically passed 3 times through a French pressure cell at 140 MPa. The French pressure lysate was anaerobically centrifuged for 40 min at 27000 × *g* and 4° C. The supernatant, which is designated cell extract, was stored under H_2 as gas phase at 0° C and analyzed for enzyme activities within 5−7 h. When only very little activity was found the activity was determined directly after cell lysis in the repetitive experiment.

Enzyme assays were performed in 1.3-ml cuvettes closed with a rubber stopper. Anaerobic conditions were achieved by repeatedly evacuating and gassing the cuvettes with nitrogen. Additions were made with microliter syringes.

Formylmethanofuran dehydrogenase was measured by following the reduction of methylviologen at 578 nm ($\varepsilon = 9.7\,mM^{-1}cm^{-1}$) (Börner et al. 1989). The 0.7-ml assay mixture contained: 50 mM Tricine/KOH pH 8.0; 2 mM dithiothreitol; 100 mM NaCl; 10 mM methylviologen, and 70 μM formylmethanofuran. The reaction was started with cell extract.

Methylene-H_4MPT dehydrogenase was assayed by following the formation of methenyl-H_4MPT at 340 nm ($\varepsilon = 20.8\,mM^{-1}\,cm^{-1}$) or by following the reduction of coenzyme F_{420} at 420 nm ($\varepsilon = 33.56\,mM^{-1}\,cm^{-1}$ at pH 6.5) (Zirngibl et al. 1990). The 1-ml assay mixture contained: 120 mM potassium phosphate pH 6.5; 3 mM formaldehyde; 40−50 μM H_4MPT; and, where indicated, 40 μM coenzyme F_{420}. The reaction was started with cell extract.

Methylene-H_4MPT reductase was tested by following the oxidation of reduced coenzyme F_{420} with methylene-H_4MPT at 420 nm ($\varepsilon = 43\,mM^{-1}\,cm^{-1}$ at pH 8.5) (Ma and Thauer 1990a). The 1 ml assay mixture contained: 100 mM *Tris*/HCl pH 8.5; 10 mM mercaptoethanol; 16 μM coenzyme F_{420}; and 3 mM sodium dithionite for the reduction of F_{420} to $F_{420}H_2$. After 3−4 min at 55° C 15 mM formaldehyde and 16 μM H_4MPT were added. The gas phase was 40% N_2/60% acetylene. The reaction was started with cell extract.

Heterodisulfide reductase (Hedderich et al. 1989) was measured by following the oxidation of benzylviologen at 578 nm ($\varepsilon = 8.6\,mM^{-1}\,cm^{-1}$). The 0.8 ml assay mixture contained: 50 mM *Tris*/HCl pH 8; 2 mM benzylviologen; 50 mM sodium dithionite; and 0.4 mM CoM−S−S−HTP. The reaction was started with cell extract.

Protein was determined with BCA reagent (bicinchoninic acid) from Pierce (Rockford, Ill., USA) using the standard protocol (Smith et al. 1985).

Results

For the determination of enzyme activities cells were used which were harvested in the exponential growth phase. The specific rates of methane formation (μmol/min · mg protein) in the exponentially growing cultures are given in Table 1. They were calculated from the doubling times t_d (min) and the growth yields Y_{CH_4} (g cells/mol CH_4) assuming that (i) the average amount of cells forming methane during a doubling period ($t_2 - t_1$) is 1.5 times that at t_1 and (ii) that the cells consisted of 50% protein (factor 2).

$$\text{Specific rate of } CH_4 \text{ formation} = 2/1.5 \cdot Y_{CH_4} \cdot t_d$$

The specific rates of CH_4 formation in exponentially growing cultures can be used as a guide line for the evaluation of the specific activities of methanogenic enzymes in cell extracts.

The specific activities of the enzymes were generally determined at the growth temperature (Table 1). Only the methylene-H_4MPT reductase – because of experimental reasons (Ma and Thauer 1990a) – was always tested at 55° C.

The following enzyme activities were determined in extracts of freshly harvested cells: formylmethanofuran dehydrogenase; methylene-H_4MPT dehydrogenase; methylene-H_4MPT reductase; heterodisulfide reductase; and methyl-coenzyme M reductase. The presence of these oxidoreductases in ten methanogenic bacteria and the

Table 1. Specific rate of methane formation in exponentially growing cultures of methanogenic bacteria, from which cell extracts were prepared for the determination of enzyme activities. Bacteria belonging to different taxonomic groups are separated by a horizontal line

Organisms	Grown on	Growth temperature (°C)	Doubling time (t_d) (h)	Specific rate of methane formation[a] (μmol · min^{-1} mg $protein^{-1}$)
Methanobacterium thermoautotrophicum	H_2/CO_2	65	2	5.6
Methanobacterium wolfei	H_2/CO_2	61	4	2.8
Methanobrevibacter arboriphilus	H_2/CO_2	37	8	1.4
Methanosphaera stadtmanae	Methanol/H_2	37	11	0.3
Methanosarcina barkeri (Fusaro)	H_2/CO_2	37	12	0.9
	Methanol		12	0.3
	Acetate		24	0.6
Methanosarcina barkeri (MS)	Acetate	37	24	0.6
Methanothrix soehngenii	Acetate	37	168[b]	0.1
Methanospirillum hungatei	H_2/CO_2	37	13	0.9
Methanogenium organophilum	H_2/CO_2	30	6	1.9
Methanococcus voltae	H_2/CO_2	37	6	1.9

[a] The specific rate was calculated from the doubling time t_d and from the following averaged growth yields Y (g cells/mol methane): Y = 2 g/mol for H_2/CO_2 as substrate (Zehnder and Wuhrmann 1977; Schönheit et al. 1980)), Y = 1.5 g/mol for acetate as substrate (Huser et al. 1982; Weimer and Zeikus 1978a), Y = 6.7 g/mol for methanol as substrate (Weimer and Zeikus 1978b; Hippe et al. 1979). It was assumed that the cells were composed of 50% protein. See also text

[b] From Jetten et al. (1989b)

catalytic properties of these enzymes are successively described.

If not otherwise indicated, the enzyme activities were recovered to over 95% in the 100,000 × *g* (40 min) supernatant.

Formylmethanofuran dehydrogenase

This enzyme was tested in the direction of formylmethanofuran oxidation to methanofuran and CO_2. Methylviologen was used as artificial electron acceptor. The formylmethanofuran was prepared from methanofuran isolated from *M. thermoautotrophicum*. For the interpretation of the data it is important to note that some methanogenic bacteria contain modified methanofurans (White 1988).

Using the described assay relatively high specific activities of formylmethanofuran dehydrogenase were found in *M. thermoautotrophicum, M. wolfei, M. arboriphilus, M. barkeri,* and *M. soehngenii* (Table 2). In the other methanogens formylmethanofuran dehydrogenase activity was also definitely present, however, the specific activity was only very low. In the case of *M. stadtmanae* this can be explained since formylmethanofuran dehydrogenase has no apparent function in methane formation from methanol and H_2. In the case of *M. hungatei* and *M. voltae* a reason could be that the enzyme was assayed with the non-physiological coenzyme. *M. hungatei* and *Methanococcus* species are known to contain a methanofuran which differs from that isolated from *M. thermoautotrophicum* (White 1988).

Formylmethanofuran dehydrogenase form *M. barkeri* (Karrasch et al. 1990a, b) has been purified and shown to catalyze the oxidation of *N*-furfurylformamide as pseudo-substrate (Breitung et al. 1990). Only formylmethanofuran dehydrogenase from *M. wolfei* was found to share this property. The specific activity with *N*-furfurylformamide (100 mM) was 0.1 μmol/min · mg protein in cell extracts of *M. barkeri* and 0.17 μmol/min · mg protein in cell extracts of *M. wolfei.*

Methylene-H_4MPT dehydrogenase

This enzyme was tested in the direction of methylene-H_4MPT oxidation to methenyl-H_4MPT. The activity in the absence of any added electron acceptor is designated the H^+-reducing activity. The activity, which was dependent on coenzyme F_{420}, is designated the F_{420}-reducing activity.

All the bacteria belonging to the methanomicrobium group were found to contain the F_{420}-reducing methylene-H_4MPT dehydrogenase activity. The bacteria belonging to the methanobacterium group generally exhibited only H^+-reducing methylene-H_4MPT dehydrogenase activity. In *M. voltae* both activities were found (Table 2).

The specific activity of methylene-H_4MPT dehydrogenase in cell extracts of *M. stadtmanae* and of *M. soehngenii* was very low. As formylmethanofuran dehydrogenase this enzyme has no apparent function in methanogenesis from methanol and H_2 or from acetate. The specific activity of methylene-H_4MPT dehydrogenase in cell extracts of *M. arboriphilus* was also relatively low. The reason for this is not understood. Different pH and buffers were tried.

Table 2. Specific activity of formylmethanofuran dehydrogenase, methylenetetrahydromethanopterin dehydrogenase, methylenetetrahydromethanopterin reductase, and heterodisulfide reductase in cell extracts of methanogenic bacteria grown on H_2/CO_2 or on other substrates (see Table 1). Bacteria belonging to different taxonomic groups are separated by a horizontal line. Note that the specific activities are not V_{max} values. As of experimental reasons they were determined at relatively low substrate concentrations

Organisms	Grown on	Formyl-MFR dehydrogenase	Methylene-H_4MPT dehydrogenase	Methylene-H_4MPT reductase	Heterodisulfide reductase
		(μmol min^{-1}mg protein^{-1})			
Methanobacterium thermoautotrophicum	H_2/CO_2	1.1	7.0[a]	4.6	0.48
Methanobacterium wolfei	H_2/CO_2	1.7	2.3[a]	5.26	0.2
Methanobrevibacter arboriphilus	H_2/CO_2	0.7	0.04[a]	6.6	0.23
Methanosphaera stadtmanae	Methanol/H_2	0.04	0.01	0.02	0.17
Methanosarcina barkeri (Fusaro)	H_2/CO_2	1.3	2.5[b]	6.9	0.3
	Methanol	1.3	2.8[b]	5.4	0.34
	Acetate	0.27	0.14[b]	2.2	0.25
Methanosarcina barkeri (MS)	Acetate	0.3	0.14[b]	0.72	1.0
Methanothrix soehngenii	Acetate	0.15	0.02[b]	0.1	0.36
Methanospirillum hungatei	H_2/CO_2	0.03	8.0[b]	0.34	1.0
Methanogenium organophilum	H_2/CO_2	0.02	8.0[b]	0.1	1.0
Methanococcus voltae	H_2/CO_2	0.02	0.7[a] 7.8[b]	0.42	0.1

[a] H^+-reducing methylene-H_4MPT dehydrogenase
[b] F_{420}-reducing methylene-H_4MPT dehydrogenase

Methylene-H_4MPT reductase

This enzyme was tested in the direction of methylene-H_4MPT reduction to methyl-H_4MPT with reduced coenzyme F_{420} as electron donor.

All the bacteria tested, with exception of *M. stadtmanae*, contained high specific activity of this enzyme (Table 2). The reductase was always specific for coenzyme F_{420}. Neither NADPH, NADH or reduced viologen dyes could substitute for F_{420}/H_2 in the assay. The enzyme has been purified from *M. thermoautotrophicum* (te Brömmelstroet et al. 1990; Ma and Thauer 1990a, b) and from *M. barkeri* (Ma and Thauer 1990c).

Heterodisulfide reductase

This enzyme was tested by following the oxidation of reduced benzylviologen with CoM–S–S–HTP. As a control the rate of oxidation with CoM–S–S–CoM and HTP–S–S–HTP was assayed. The specific activities given in Table 2 have been corrected for these rates, which were generally very low.

All the methanogens investigated contained heterodisulfide reductase in specific activities above 0.1 μmol/min · mg protein. The highest specific activity (Table 2) relative to the specific rate of methane formation in vivo (Table 1) was found in *M. barkeri* (strain MS) and the lowest in *M. voltae*, where it represents only 5% of the in vivo rate. The reductase in the latter organism was found to rapidly loose activity probably explaining this result.

The heterodisulfide reductase activity in *M. barkeri* (strain MS) differed from the activity in all the other investigated methanogenic bacteria in that it was found to be over 50% associated with the membrane fraction. In *M. soehngenii* it was 20%. In all the other methanogens over 90% of the activity was recovered in the 100,000 $\times g$ supernatant.

Methyl-coenzyme M reductase

This enzyme was tested by measuring the formation of CH_4 from CH_3–S–CoM in the presence of dithiothreitol (15 mM) and aquocobalamin (0.3 mM) (Ellermann et al. 1988). With this assay methyl-coenzyme M reductase activity was found in all the methanogens investigated. The specific activity was, however, always only a few percent or even a few per thousandth of that expected from the observed in vivo methane formation rates (Table 1). Therefore these activities were not included in Table 2. Why the enzyme is relatively inactive in vitro is not yet understood (Ellermann et al. 1989). Most of the investigated bacteria have been shown to contain high concentrations of the enzyme: *M. thermoautotrophicum* (Ellermann et al. 1989; Rospert et al. 1990); *M. arboriphilus* (Ellermann, unpublished data); *M. stadtmanae* (Rouvière and Wolfe 1987); *M. barkeri* (Moura et al. 1983; Rouvière and Wolfe 1987); *M. soehngenii* (Jetten et al. 1990); *M. organophilum* (Widdel et al. 1988); and *M. voltae* (Konheiser et al. 1984) (see also Hartzell and Wolfe 1986).

Discussion

For the determination of enzyme activities in the ten methanogenic bacteria investigated we have always used

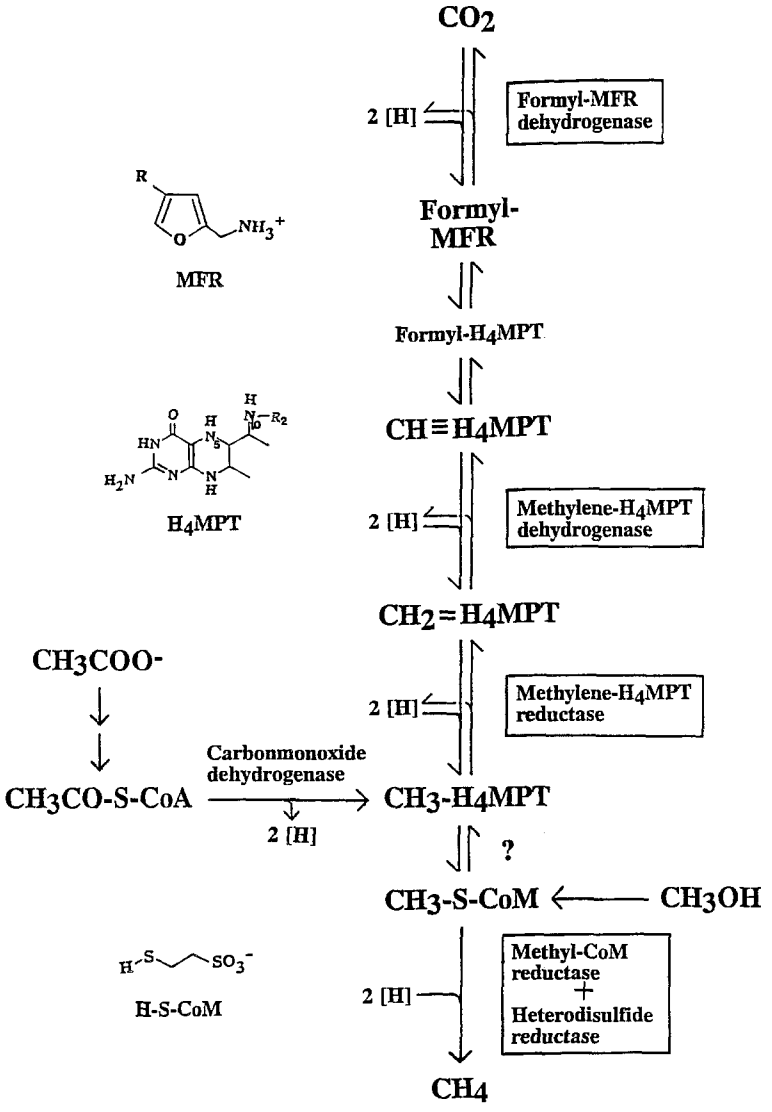

Fig. 1. Scheme of the energy metabolism of methanogenic bacteria. Reactions, intermediates and oxidoreductases involved in CO_2 reduction to CH_4, in CH_3OH disproportionation to CH_4 and CO_2, in CH_3OH reduction with H_2 to CH_4, and in acetate disproportionation to CH_4 and CO_2. The question mark indicates that the reversibility of the CH_3-H_4MPT:coenzyme M methyltransferase reaction remains to be demonstrated. It could also be that CH_3-H_4MPT is directly formed from CH_3OH and H_4MPT rather than indirectly via $CH_3-S-CoM$. $CH \equiv H_4MPT = N^5,N^{10}$-methenyl-$H_4MPT$; $CH_2 = H_4MPT = N^5,N^{10}$-methylene-$H_4MPT$; $CH_3-H_4MPT = N^5$-methyl-H_4MPT. For complete structures of MFR and H_4MPT see DiMarco et al. (1990)

the same protocol: the cells were harvested in the exponential growth phase; cell extracts were prepared in 50 mM potassium phosphate pH 7 containing 0.5 mM dithiothreitol; the cells were lysed by three times passage of the suspensions through a French pressure cell at 140 MPa; and the enzyme activities were assayed directly after cell lysis. This protocol may have been optimal for the determination of the one enzyme but less optimal for the other. This has to be kept in mind when comparing the enzyme activities, especially when only low activities were found.

The results indicate that formylmethanofuran dehydrogenase, methylene-H_4MPT dehydrogenase, methylene-H_4MPT reductase, heterodisulfide reductase, and methyl-coenzyme M reductase are present in all the ten methanogenic bacteria investigated.

In *M. stadtmanae* grown on H_2 and methanol the activities of formylmethanofuran dehydrogenase, methylene-H_4MPT dehydrogenase, and methylene-H_4MPT reductase were very low indicating that the three enzymes are not required in methane formation from H_2 and methanol.

In acetate grown *M. barkeri* the specific activities of formylmethanofuran dehydrogenase and of methylene-H_4MPT dehydrogenase were much lower than in H_2/CO_2 or in methanol grown cells indicating that these two enzymes are not required for acetate fermentation to CO_2 and CH_4. In H_2/CO_2 grown *M. barkeri* and in methanol grown *M. barkeri* the specific activities of the five oxidoreductases were almost identical indicating a role in both CO_2 reduction to methane and in methanol disproportionation to CO_2 and CH_4. Similar results have recently been reported for *Methanosarcina thermophila* grown on acetate, methane, or H_2/CO_2 (Jablonski et al. 1990).

In all the methanogens investigated heterodisulfide reductase was found in significant specific activities (Table 2) and methyl-coenzyme M reductase is reported to be present in high enzyme concentrations (for references see the results section on methylcoenzyme M reductase) indicating that these two oxidoreductases are essential for methanogenesis from all substrates.

A scheme of the energy metabolism of methanogenic bacteria, which takes these and other results into account,

is given in Fig. 1. The scheme shows that in the fermentation of H_2/CO_2, of methanol, and of acetate common reactions are used (see also Fischer et al. 1989; Mahlmann et al. 1989). It also shows that for the fermentation of acetate an additional oxidoreductase, carbon monoxide dehydrogenase, is essential, which is not required in the fermentation of the other substrates. Carbon monoxide dehydrogenase is involved in the catalysis of the following reaction:

$$CH_3CO-S-CoA + H_4MPT \rightleftharpoons CH_3-H_4MPT + CO_2 + CoASH + 2\,[H] \quad (7)$$
$$E^{\circ\prime} = -200\ mV$$

The enzyme has been purified from *M. barkeri* (Krzycki et al. 1989) and from *M. soehngenii* (Jetten et al. 1989a). For recent reviews on the role of carbon dehydrogenase see Thauer et al. (1989) and Thauer (1990).

Acknowledgements. This work was supported by grants from the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie. We thank Sabine Scholz, Gerhard Börner, and Thomas Rösser for the preparation and synthesis of coenzymes.

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