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Function of coenzyme F_{420}-dependent NADP reductase in methanogenic archaea containing an NADP-dependent alcohol dehydrogenase

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Abstract Methanogenic archaea growing on ethanol or isopropanol as the electron donor for CO_2 reduction to CH_4 contain either an NADP-dependent or a coenzyme F_{420}-dependent alcohol dehydrogenase. We report here that in both groups of methanogens, the N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenase and the N^5,N^{10}-methylenetetrahydromethanopterin reductase, two enzymes involved in CO_2 reduction to CH_4, are specific for F_{420}. This raised the question how $F_{420}H_2$ is regenerated in the methanogens with an NADP-dependent alcohol dehydrogenase. We found that these organisms contain catabolic activities of an enzyme catalyzing the reduction of F_{420} with NADPH. The F_{420}-dependent NADP reductase from *Methanogenium organophilum* was purified and characterized. The N-terminal amino acid sequence showed 42% sequence identity to a putative gene product in *Methanococcus jannaschii*, the total genome of which has recently been sequenced.

Key words Coenzyme F_{420} · F_{420}-dependent NADP reductase · F_{420}-dependent N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenase · F_{420}-dependent N^5,N^{10}-methylenetetrahydromethanopterin reductase · Methanogenic archaea · *Methanogenium organophilum* · *Methanobacterium palustre* · *Methanogenium liminatans* · *Methanoculleus thermophilicus*

Abbreviations t_d Doubling time · F_{420} Coenzyme F_{420} · H_4MPT Tetrahydromethanopterin · $CH_2 = H_4MPT$ N^5, N^{10}-methylene-H_4MPT

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Introduction

Until recently, it was assumed that the process of methane formation in methanogenic archaea does not involve pyridine nucleotides (Wolfe 1991; Weiss and Thauer 1993; Thauer 1997). None of the enzymes involved in methanogenesis from CO_2, acetate, or methanol was found to utilize NAD or NADP as coenzyme. Instead, some of the enzymes use the 5-deazaflavin F_{420} as coenzyme; it functions like NAD(P) by transferring only two electrons with one proton (a hydride ion). The redox potential E'_0 of the $F_{420}/F_{420}H_2$ couple is –360 mV as compared to –320 mV for the $NAD(P)^+$/NAD(P)H couple (Walsh 1986; Gloss and Hausinger 1987).

Recently, methanogenic archaea that can grow on CO_2 and ethanol or isopropanol as sole energy sources have been isolated; CH_4 and acetic acid or acetone, respectively, are thereby generated as catabolic end products (Widdel 1986; Zellner and Winter 1987; Widdel et al. 1988). These methanogens have been found to contain either an NADP-dependent alcohol dehydrogenase or an F_{420}-dependent alcohol dehydrogenase (Bleicher et al. 1989; Frimmer and Widdel 1989; Zellner et al. 1989). Both types of enzymes have been purified and characterized (Widdel and Wolfe 1989; Bleicher and Winter 1991; Widdel and Frimmer 1995; Berk et al. 1996). The finding of an NADP-dependent alcohol dehydrogenase in methanogens utilizing alcohols as electron donors for CO_2 reduction to CH_4 clearly indicates an involvement of NADP in methanogenesis from CO_2 and alcohols.

In methanogenesis from CO_2, generally two enzymes that are F_{420}-specific are involved. These are the F_{420}-dependent N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenase and the F_{420}-dependent N^5,N^{10}-methylenetetrahydromethanopterin reductase. These two enzymes catalyze reactions analogous to those catalyzed by N^5,N^{10}-methylenetetrahydrofolate dehydrogenase and N^5,N^{10}-methylenetetrahydrofolate reductase, which in most organisms are pyridine-nucleotide-dependent enzymes (Thauer et al. 1993). Therefore, we first investigated whether the

two tetrahydromethanopterin-dependent enzymes in methanogens with an NADP-dependent alcohol dehydrogenase utilize NAD(P) as coenzyme, but found that in the two organisms investigated in this respect, both enzymes were highly specific for F_{420}. We then looked for an enzyme activity catalyzing the reduction of F_{420} with NADPH and found that the methanogens with an NADP-dependent alcohol dehydrogenase contained such an enzyme in activities sufficient to account for the observed rates of methane formation. This enzyme was purified and characterized from *Methanogenium organophilum*, one of these organisms.

$$NADPH + H^+ + F_{420} \rightleftharpoons NADP^+ + F_{420}H_2$$

$$\Delta G^{0\prime} = +7.7 \text{ kJ/mol}$$

Materials and methods

Coenzyme F_{420} (F_{420}) and tetrahydromethanopterin (H_4MPT) were purified from *Methanobacterium thermoautotrophicum* as described previously (Breitung et al. 1992). N^5,N^{10}-methylenetetrahydromethanopterin (CH_2=H_4MPT) was synthesized from H_4MPT and formaldehyde by spontaneous reaction (Escalante-Semerena et al. 1984). $F_{420}H_2$ was generated by reduction of F_{420} with H_2 at 60°C in the presence of F_{420}-reducing hydrogenase from *Methanobacterium thermoautotrophicum* (Jacobson et al. 1982). After completion of the reaction, the enzyme was separated from the reduced coenzyme by ultrafiltration with a 30-kDa Centricon microconcentrator (Amicon, Beverley, Mass., USA). The 2′,5′-ADP-agarose resin was obtained from Sigma (Deisenhofen, Germany). The Resource Q column and the FPLC equipment was from Pharmacia (Freiburg, Germany).

Cultures and growth conditions

Methanogenium organophilum (DSM 3596; Widdel 1986), *Methanobacterium palustre* (DSM 3108; Zellner et al. 1989), *Methanogenium liminatans* (DSM 4140; Zellner et al. 1990), and *Methanoculleus thermophilicus* (DSM 3915; Widdel et al. 1988) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). The organisms were grown as described in the indicated references: *Methanogenium organophilum* on ethanol (50 mM), and *Methanobacterium palustre*, *Methanogenium liminatans*, and *Methanoculleus thermophilicus* first on 80% H_2/20% CO_2 and then on isopropanol (65 mM).

Preparation of cell extracts

Cells from a 3–l culture were harvested at the end of linear growth ($OD_{578} \approx 0.5$) by centrifugation for 30 min at 5,000 × *g*, and the pellet (1.5 g, wet mass) was suspended in 4 ml 50 mM Tris/HCl (pH 7.5). Subsequently the cell suspension was passed three times through a French pressure cell at 140 MPa. Unbroken cells and cell debris were removed by centrifugation for 30 min at 30,000 × *g*. The supernatant is referred to as cell extract. All the steps were performed at 4°C under anoxic conditions.

Determination of enzyme activities

Activities were routinely assayed in 1.5-ml glass cuvettes containing N_2 or, where indicated, H_2 as gas phase and were sealed with rubber stoppers. Anoxic conditions were achieved by repeatedly evacuating and flushing the cuvettes with the respective gas. Additions were made with microliter syringes. Changes in absorbance were monitored photometrically. Unless otherwise stated, the enzyme activities were determined at the temperature for optimal growth of the organisms, which is 30°C for *Methanogenium organophilum*, 37°C for *Methanobacterium palustre* and *Methanogenium liminatans*, and 55°C for *Methanoculleus thermophilicus*. One unit (U) is defined as 1 μmol substrate transformed per min under the standard assay conditions.

NADP-dependent alcohol dehydrogenase activity was determined by following the reduction of $NADP^+$ (1 mM) at 366 nm (ε = 3.3 mM^{-1} cm^{-1}) with ethanol (170 mM) at 30°C in 100 mM glycine/KOH (pH 9.5) (*Methanogenium organophilum*) or with isopropanol (50 mM) at 37°C in 50 mM Tricine/KOH (pH 8.0) (*Methanobacterium palustre*) as described by Frimmer and Widdel (1989) and Bleicher and Winter (1991), respectively.

F_{420}-dependent alcohol dehydrogenase activity was determined by following the reduction of F_{420} (30 μM) at 401 nm (ε = 25.9 mM^{-1} cm^{-1}) with isopropanol (50 mM) at 37°C in 100 mM citric acid/NaOH (pH 6.0) (*Methanogenium liminatans*) or with isopropanol (130 mM) at 40°C in succinic acid/KOH (pH 4.2) (*Methanoculleus thermophilicus*) as described by Bleicher and Winter (1991) and Widdel and Wolfe (1989), respectively.

F_{420}-dependent methylenetetrahydromethanopterin dehydrogenase activity was determined in 50 mM citric acid/NaOH (pH 6.0) by following the formation of methenyltetrahydromethanopterin from methylenetetrahydromethanopterin (40 μM) and $F_{420}H_2$ (25 μM) at 340 nm (ε = 20.8 mM^{-1} cm^{-1}) (Schwörer et al. 1993).

F_{420}-dependent methylenetetrahydromethanopterin reductase activity was determined in 100 mM Tris/HCl (pH 8.0) by following the oxidation of $F_{420}H_2$ (50 μM) with methylenetetrahydromethanopterin (40 μM) at 420 nm (ε = 40 mM^{-1} cm^{-1}) (Ma and Thauer 1990).

F_{420}-reducing hydrogenase activity was determined in 100 mM Tris/HCl (pH 7.5) by following the reduction of coenzyme F_{420} (30 μM) with H_2 (100%) at 420 nm (ε = 36 mM^{-1} cm^{-1}) (Setzke et al. 1994).

F_{420}-dependent NADP reductase activity was assayed in both directions. The standard assay mixture for the determination of F_{420} reduction with NADPH contained 70 μM F_{420} and 1 mM NADPH in 1 ml 50 mM citric acid/NaOH (pH 6.0). The standard assay mixture for the determination of NADP reduction with reduced coenzyme F_{420} contained 50 mM Tricine/KOH (pH 8.0), 1 mM $NADP^+$, and 100 μM $F_{420}H_2$. Cuvettes with a 0.5-cm light path were used. The rates were determined by following either the change in absorbance at 366 nm (NADPH; ε = 3.3 mM^{-1} cm^{-1}) or at 401 nm (F_{420}; ε = 25.9 mM^{-1} cm^{-1}) (DiMarco et al. 1990).

Determination of protein

Protein was quantified by using the bicinchoninic acid (BCA) reagent from Pierce (Rockford, Ill., USA) with bovine serum albumin as standard.

Purification of the F_{420}-dependent NADP reductase from *Methanogenium organophilum*

Cell extract from 1.5 g cells (wet mass) was subjected to an $(NH_4)_2SO_4$ precipitation [60%, in 50 mM Tricine/KOH (pH 8.0) containing 0.5 mM $ZnCl_2$] and then centrifuged at 30,000 × *g*. The supernatant, which contained 79% of the total activity, was desalted using a 30-kDa Centricon microconcentrator and subsequently applied to a 2′,5′-ADP-agarose column (1 × 5 cm) equilibrated with 50 mM Tricine/KOH (pH 8.0) containing 0.5 mM $ZnCl_2$. Active fractions were eluted in the same buffer containing 200 mM KCl. After desalting in a 30-kDa microconcentrator, the active fractions were subjected to a Resource Q column equilibrated with the aforementioned buffer to which a linear gradient from 0 to 400 mM KCl in the elution buffer was applied. Active fractions eluted at a concentration of KCl from 200 to 220 mM. The fractions (8 ml) were concentrated and desalted with a 30-kDa microconcentrator. The enzyme solution could be stored at 4°C or at –20°C under oxic conditions for several weeks without significant loss of activity.

Table 1 Coenzyme F_{420}-dependent enzymes in cell extracts of methanogenic archaea with either an NADP-dependent or an F_{420}-dependent alcohol dehydrogenase. The specific activities were determined under the standard assay conditions described in Materials and methods (1 U = 1 µmol substrate per min). The specific activity of F_{420}-dependent NADP reductase is given for the specific rate of F_{420} reduction with NADPH [t_d the doubling time during growth on alcohol and CO_2, *CH_2=H_4MPT* N^5,N^{10}-methylenetetrahydromethanopterin, *nd* not detected (lower limit of detection: 0.01 U/mg)]

Enzyme	Specific activity (U/mg protein)			
	Methanogenium organophilum ($t_d \approx 25$ h)	*Methanogenium palustre* ($t_d > 50$ h)	*Methanogenium liminatans* ($t_d > 50$ h)	*Methanoculleus thermophilicus* ($t_d > 50$ h)
NADP-dependent alcohol dehydrogenase	1.1	0.6	nd	nd
F_{420} dependet-alcohol dehydrogenase	nd	nd	0.4	1.2
F_{420}-dependent CH_2=H_4MPT dehydrogenase	5.3	9.8	26.5	28.3
F_{420}-dependent CH_2=H_4MPT reductase	2.3	6.5	2.5	6.4
F_{420}-reducing hydrogenase	1.6	1.6	1.6	2.7
F_{420}-dependent NADP reductase	15	0.3	0.06	0.04

Results

Five methanogens that can grow on alcohols and CO_2 and contain an NADP-dependent alcohol dehydrogenase are known to date: *Methanobacterium palustre*, *Methanobacterium bryantii* M.o.H.G. (Bleicher et al. 1989), *Methanogenium organophilum* (Frimmer and Widdel 1989), *Methanocorpusculum bavaricum*, and *Methanocorpusculum parvum* (Bleicher et al. 1989). *Methanobacterium palustre* and *Methanobacterium bryantii* M.o.H.G. belong to the order Methanobacteriales, and the three other organisms belong to the order Methanomicrobiales (Boone et al. 1993). For the following studies we therefore chose *Methanobacterium palustre* and *Methanogenium organophilum*. For comparison, we also investigated two alcohol-fermenting methanogens containing an F_{420}-dependent alcohol dehydrogenase: *Methanogenium liminatans* (Bleicher and Winter 1991) and *Methanoculleus thermophilicus* (Widdel and Wolfe 1989). Both belong to the order of Methanomicrobiales (Boone et al. 1993).

Specific activity of F_{420}-dependent enzymes in methanogens growing on alcohols and CO_2

The two methanogens with an NADP-dependent alcohol dehydrogenase and the two with an F_{420}-dependent alcohol dehydrogenase were found to contain high specific activities of F_{420}-dependent methylenetetrahydromethanopterin dehydrogenase and F_{420}-dependent methylenetetrahydromethanopterin reductase (Table 1), two enzymes known to be involved in methanogenesis from CO_2 in all methanogenic archaea investigated so far. It was investigated whether the two methanogens with an NADP-dependent alcohol dehydrogenase (*Methanobacterium palustre* and *Methanogenium organophilum*) might additionally contain an NAD(P)-dependent methylenetetrahydromethanopterin dehydrogenase and methylenetetrahydromethanopterin reductase or an NAD(P)-dependent methylenetetrahydrofolate dehydrogenase and NAD(P)-dependent methylenetetrahydrofolate reductase. However, such activities were not observed (lower limit of detection: 0.01 U/mg protein). Instead, the two organisms were found to contain high specific activities of an enzyme catalyzing the reduction of F_{420} with NADPH. This enzyme, designated F_{420}-dependent NADP reductase, was also detectable in the two methanogens with an F_{420}-dependent alcohol dehydrogenase (*Methanogenium liminatans* and *Methanoculleus thermophilicus*), albeit with much lower specific activity.

In Table 1 the specific activity of the F_{420}-reducing hydrogenase is also given. This enzyme is required during growth of the four methanogens on H_2 and CO_2. An NADP-reducing hydrogenase in these organisms was not found (lower limit of detection: 0.01 U/mg protein).

Table 2 Purification of F_{420}-dependent NADP reductase from *Methanogenium organophilum*. Cell extract was prepared from 1.5 g cells (wet mass). The specific activity was determined under standard assay conditions described in Materials and methods (1 U = 1 µmol NADPH oxidized per min)

Purification step	Protein (mg)	Activity (U)	Specific activity (U/mg protein)	Yield (%)	Purification (-fold)
Cell extract	110	1,667	15.1	100	1
Supernatant of $(NH_4)_2SO_4$ precipitation (60%)	13.2	1,320	100	79	6.6
2′,5′-ADP-agarose	0.96	765	800	46	53
Resource Q	0.52	519	998	31	66

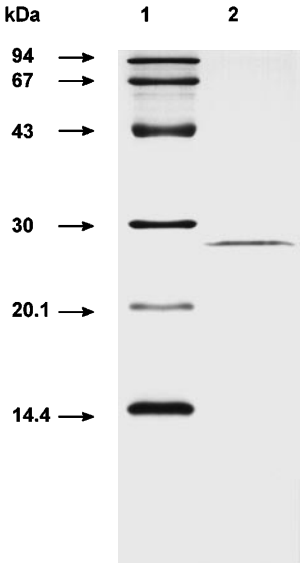

Fig. 1 Analysis of purified F_{420}-dependent NADP reductase from *Methanogenium organophilum* by sodium dodecylsulfate polyacrylamide gel electrophoresis. Protein was separated on 16% polyacrylamide slab gels (8 × 7 cm) (Laemmli, 1970). *Lane 1* 5 µg molecular mass standards (Pharmacia, Freiburg, Germany) consisting of phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa); *lane 2* 1 µg of purified enzyme. The gel was stained with Coomassie Brillant Blue R250

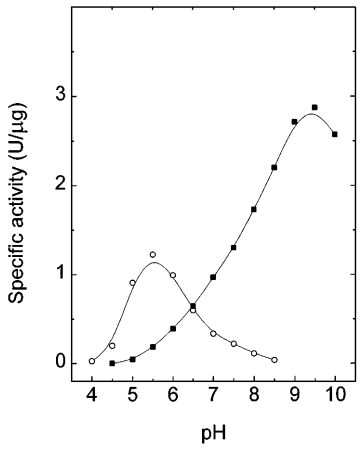

Fig. 2 Effect of pH on the activity of the F_{420}-dependent NADP reductase from *Methanogenium organophilum*. Buffers used for assays at 30°C were: acetic acid/NaOH (pH 4.0–5.5), citric acid NaOH (pH 5.5–6.5), Mops/KOH (pH 6.5–7.5), Tris/HCl (pH 7.5–8.5) and Glycine/KOH (pH 8.5–10.0). ○ Reduction of F_{420} by NADPH. The concentrations of F_{420} and NADPH were 0.07 mM and 1 mM, respectively. ■ Reduction of $NADP^+$ by $F_{420}H_2$. The concentrations of $F_{420}H_2$ and $NADP^+$ were 0.1 mM and 1 mM, respectively. Note that the pK of $F_{420}H_2$ dissociation to $F_{420}H^- + H^+$ is 9.7 (Jacobson and Walsh 1984)

Purification and properties of the F_{420}-dependent NADP reductase from *Methanogenium organophilum*

The F_{420}-dependent NADP reductase was purified 66-fold to apparent homogeneity (Table 2). The most efficient step in the purification procedure was the affinity chromatography on 2′,5′-ADP-agarose. The overall yield was approximately 30%. SDS/PAGE revealed only one band at 28 kDa, indicating that the enzyme is composed of only one type of subunit with this apparent molecular mass (Fig. 1).

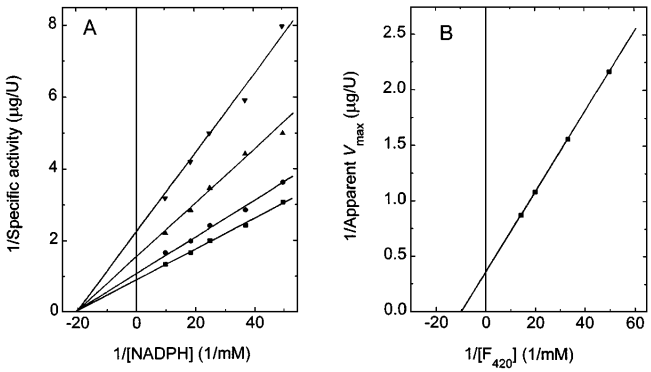

Fig. 3A, B Kinetics of F_{420} reduction with NADPH at pH 6.0 as catalyzed by purified F_{420}-dependent NADP reductase from *Methanogenium organophilum*. **A** Reciprocal plots of the rates of F_{420} reduction versus the NADPH concentration at different fixed concentrations of F_{420}: ■ 70 µM F_{420}; ● 50 µM F_{420}; ▲ 30 µM F_{420}; ▼ 20 µM F_{420}. **B** Reciprocal plot of the apparent V_{max} values (Fig. 3A) versus the F_{420} concentration

The purified enzyme was found to be relatively stable under oxic conditions. It catalyzed both the reduction of F_{420} with $NADP^+$ and the reduction of $NADP^+$ with $F_{420}H_2$ (however, with different relative rates at different pH; Fig. 2). The pH optimum for F_{420} reduction with NADPH was 5.5; for $NADP^+$ reduction with $F_{420}H_2$, it was 9.2. Kinetic analysis revealed that the enzyme exhibits a sequential kinetic mechanism as shown for F_{420} reduction by NADPH in Fig. 3A, suggesting the formation of a ternary complex. From replots such as those shown in Fig. 3B, the V_{max} and K_m values given in Table 3 were obtained. Consistent with a ternary complex catalytic mechanism is the finding that the UV/visible spectrum of the purified enzyme was similar to that of bovine serum albumin, indicating the absence of a redox-active chromophoric prosthetic group.

In Table 3, the N-terminal amino acid sequence of the F_{420}-dependent NADP reductase from *Methanogenium organophilum* is also given. It shows 42% sequence identity to a putative gene product of 24-kDa mol. mass in *Methanococcus jannaschii*, the total genome of which has recently been sequenced (Bult et al. 1996). The predicted coding region *MJ1501* in *Methanococcus jannaschii* thus probably encodes for an F_{420}-dependent NADP reductase. The N-terminal amino acid sequence data published here has been submitted to the EMBL Sequence Data Bank and is available under accession no. P80951.

Discussion

The four methanogens investigated in this work generate methane during growth on alcohols and CO_2 at specific rates between maximally 0.2 µmol min^{-1} (mg protein)$^{-1}$

Table 3 Properties of purified F_{420}-dependent NADP reductase from *Methanogenium organophilum*

Apparent molecular mass	
Native enzyme	90 kDA ± 15 kDa[a]
Subunit	28 kDA ± 1 kDa
Presence of a chromophoric prosthetic group	No
Reduction of $NADP^+$ with $F_{420}H_2$	
V_{max} at pH 8.0	2,000 U/mg protein
K_m for $F_{420}H_2$ at pH 8.0	0.04 mM
K_m for $NADP^+$ at pH 8.0	0.1 mM
pH optimum	9.2
Reduction of F_{420} with NADP	
V_{max} at pH 6.0	2,800 U/mg protein
K_m for $F_{420}H_2$ at pH 6.0	0.1 mM
K_m for $NADP^+$ at pH 6.0	0.05 mM
pH optimum	5.5
Temperature optimum	34° C
Stable in the presence of O_2	Yes
N-Terminus	
Methanogenium organophilum	**MK**VG**I**M**GGTG**NI**G**E**GLA**R**R**I-IGG**K**YDVMIK(G)(R)(D)(**K**)(**A**)[b]
Methanococcus jannaschii	**MK**IA**I**L**GGTG**DQ**G**F**GLA**L**R**L**AKNN**K**IIIGSR K K E **K** **A**[c]

[a] Determined by gradient gel electrophoresis under nondenaturating conditions

[b] Amino acids in brackets were determined with some ambiguity; - indicates that no phenylhydantoin derivative could be identified

[c] N-terminal amino acid sequence of a putative protein encoded by the predicted coding region *MJ1501* of the *Methanococcus jannaschii* genome (Bult et al. 1996)

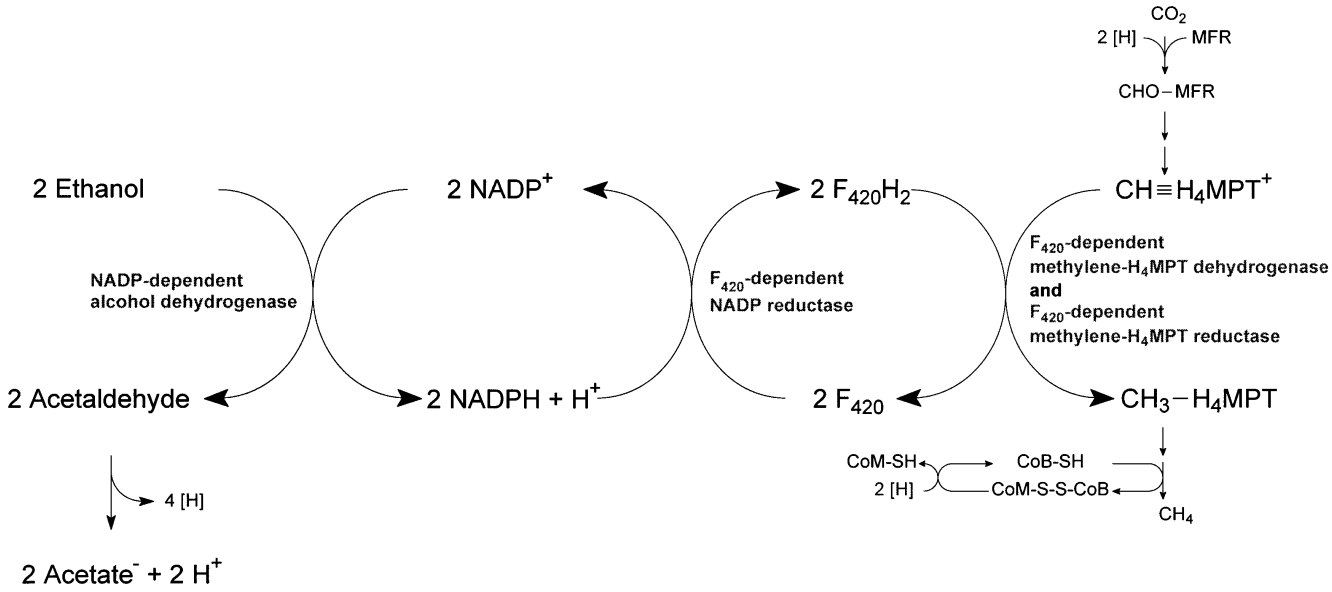

Fig. 4 Metabolic pathway of CO_2 reduction to CH_4 with alcohols as electron donors in methanogens containing an NADP-dependent alcohol dehydrogenase (*MFR* methanofuran, *CHO-MFR* formylmethanofuran, *H_4MPT* tetrahydromethanopterin; *CH≡H_4MPT$^+$* N^5,N^{10}-methenyltetrahydromethanopterin; *CH_3-H_4MPT* N^5-methyltetrahydromethanopterin, *CoM-SH* reduced coenzyme M, *CoB-SH* reduced coenzyme B, *CoM-S-S-CoB* heterodisulfide of coenzyme M and coenzyme B)

(organisms with t_d > 50 h) and 0.4 μmol min^{-1} (mg protein)$^{-1}$ (*Methanogenium organophilum*) as estimated from the doubling times (Table 1) and an assumed growth yield of 2 g cells (dry mass) per mol CH_4. Any enzyme involved in methanogenesis from alcohol and CO_2 in these organisms, therefore, should have a specific activity in the crude extract at least in this range. This is the basis for the following interpretations of the results summarized in Table 1.

Cell extracts of *Methanogenium organophilum* and *Methanobacterium palustre*, the two methanogens with an NADP-dependent alcohol dehydrogenase, catalyzed the reduction of F_{420} with NADPH at specific rates of 15 μmol min^{-1} (mg protein)$^{-1}$ and 0.3 μmol min^{-1} (mg protein)$^{-1}$, respectively. These high specific activities indicate that in the two methanogens during growth on alcohol and CO_2 the F_{420}-dependent NADP reductase probably has the function of coupling the NADP-dependent oxidation of the alcohol to the aldehyde with the F_{420}-dependent reduction of CO_2 to methane as depicted in Fig. 4.

In agreement with this interpretation is the finding that in *Methanogenium liminatans* and *Methanoculleus thermophilicus*, the two methanogens with an F_{420}-dependent alcohol dehydrogenase, the specific activity of the F_{420}-dependent NADP reductase in the cell extracts was very low, indicating an anabolic function probably in the regeneration of NADPH required in biosynthetic reactions. This is the function proposed for the enzyme when it was first found in *Methanococcus vannielii* (Jones and Stadtman 1980; Yamazaki and Tsai 1980) and then in other methanogens growing on H_2 and CO_2 (Eirich and Dugger 1984).

An F_{420}-dependent NADP reductase has also been detected in the non-methanogenic archaea *Halobacterium cutirubrum* (DeWit and Eker 1987), *Archaeoglobus fulgidus* (Kunow et al. 1993), and other *Archaeoglobus* species (Vorholt et al. 1995). The enzyme is also present in bacteria such as *Streptomyces griseus* (Eker et al. 1989). The specific activity of the enzyme in all these microorganisms is very low, indicating an anabolic rather than a catabolic function.

The F_{420}-dependent NADP reductase has, until now, been purified from *Methanococcus vannielii* (Yamazaki and Tsai 1980), *Methanobacterium thermoautotrophicum* (Eirich and Dugger 1984), *Streptomyces griseus* (Eker et al. 1989), *Archaeoglobus fulgidus* (Kunow et al. 1993), and *Methanogenium organophilum* (this work). The enzymes from the different organisms have in common that they are all composed of only one type of subunit with an apparent molecular mass of approximately 30 kDa, that they lack a chromophoric prosthetic group, and that – where investigated – they are *Si*-face specific with respect to C5 of F_{420} and *Si*-face specific with respect to C4 of NADP (Yamazaki et al. 1980, 1985; Jacobson and Walsh 1984; Kunow et al. 1993). It is, therefore, likely that these enzymes are all phylogenetically related. Unfortunately, amino acid sequence information, from which this could be more directly deduced, is only available from the enzyme of *Methanogenium organophilum*.

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