The energy conserving methyltetrahydromethanopterin:coenzyme M methyltransferase complex from methanogenic archaea: function of the subunit MtrH

Birgit Hippler, Rudolf K. Thauer*

Max-Planck-Institut für terrestrische Mikrobiologie and Laboratorium für Mikrobiologie des Fachbereichs Biologie der Philipps-Universität, Karl-von-Frisch-Strasse, D-35043 Marburg, Germany

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Abstract In methanogenic archaea the transfer of the methyl group of N^5-methyltetrahydromethanopterin to coenzyme M is coupled with energy conservation. The reaction is catalyzed by a membrane associated multienzyme complex composed of eight different subunits MtrA–H. The 23 kDa subunit MtrA harbors a corrinoid prosthetic group which is methylated and demethylated in the catalytic cycle. We report here that the 34 kDa subunit MtrH catalyzes the methylation reaction. MtrH was purified and shown to exhibit methyltetrahydromethanopterin:cob(I)alamin methyltransferase activity. Sequence comparison revealed similarity of MtrH with MetH from *Escherichia coli* and AcsE from *Clostridium thermoaceticum*: both enzymes exhibit methyltetrahydrofolate:cob(I)alamin methyltransferase activity.

Key words: Methyltransferase; Energy conservation; Sodium motive force; Corrinoid; Tetrahydromethanopterin; Tetrahydrofolate; Methanogenic archaeon

1. Introduction

Methanogenesis from CO_2 and from acetate proceeds via N^5-methyltetrahydromethanopterin and methyl-coenzyme M as intermediates [1]. The formation of methyl-coenzyme M from N^5-methyltetrahydromethanopterin (CH_3-H_4MPT) and coenzyme M (H-S-CoM) is catalyzed by a methyltransferase, which is membrane associated and which couples the exergonic methyl transfer reaction with the electrogenic translocation of sodium ions across the cytoplasmic membrane [2]. The methyltransferase reaction is thus coupled with energy conservation.

The methyltetrahydromethanopterin:coenzyme M methyltransferase is an enzyme complex composed of eight different subunits MtrA–H [3]. The complex contains tightly bound the corrinoid 5′-hydroxybenzimidazolyl-cobamide (factor III), which in the active enzyme is in the cob(I)amide oxidation state [4–6]. The complex catalyzes the methylation of its corrinoid prosthetic group with CH_3-H_4MPT and its demethylation with coenzyme M [6]. The complex also catalyzes the methylation and demethylation of free cob(I)alamin [5].

$$CH_3-H_4MPT + \text{cob(I)alamin} \rightarrow H_4MPT + CH_3-\text{cob(III)alamin} \quad (1)$$

$$CH_3-\text{cob(III)alamin} + H-S-CoM \rightarrow \text{cob(I)alamin} + CH_3-S-CoM \quad (2)$$

Of the eight subunits in the methyltransferase complex only the function of MtrA has been studied so far. The 23 kDa subunit harbors the corrinoid prosthetic group [4] which is bound in the nucleotide loop-off form [7]. In the cob(II)amide and cob(III)amide oxidation states, a histidine residue of MtrA is the axial ligand to cobalt. The active site histidine has been determined to be His-84 by site directed mutagenesis [8]. The MtrA amino acid sequence lacks the corrinoid binding motif DxHxxG-X_{41-42}-SxL-x_{24-28}-GG [9] characteristic of all other corrinoid proteins containing the corrinoid bound in the nucleotide loop-off/His-on configuration [10,11] such as cobalamin-dependent methionine synthase [12], methylmalonyl-CoA mutase [13], glutamate mutase [14,15], methylene glutarate mutase [16], methanol:coenzyme M methyltransferase [17], and methylamines:coenzyme M methyltransferases [18,19].

This report deals with the function of MtrH. Evidence is presented that the 34 kDa subunit harbors the CH_3-H_4MPT binding site and catalyzes the transfer of the methyl group from CH_3-H_4MPT to the corrinoid prosthetic group (Eq. 1).

2. Materials and methods

Methanobacterium thermoautotrophicum strain Marburg DSM 2133 was from the Deutsche Sammlung für Mikroorganismen und Zellkulturen. The cells were grown at 65°C and harvested as described by Schönheit et al. [20]. Hydroxocob(III)alamin and methylcob(III)alamin were from Sigma. Sodium 2-mercaptoethanesulfonate and the thin layer chromatography (TLC) aluminum sheets 20×20 cm^2 silica gel 60 were from Merck. Dodecyl-β-D-maltoside was from Fluka. Titanium(III)citrate was prepared anaerobically from $TiCl_3$ [21]. Tetrahydromethanopterin was purified from *M. thermoautotrophicum* [22]. [^{14}C]Formaldehyde (0.37 MBq/μmol; 1.85 MBq) was from DuPont. N^5-[^{14}C]Methyltetrahydromethanopterin was synthesized from [^{14}C]formaldehyde (74 Bq/nmol) and tetrahydromethanopterin by enzymatic reduction [23]. The fast liquid chromatography (FPLC) equipment was from Pharmacia. The λ ZAP Express gene library was that described by Vorholt et al. [24]. The cloning vector λ ZAP Express (*Bam*HI/CIAP treated), the helper phage ExAssist and *Escherichia coli* strains XLOLR and XL1-Blue MRF′ were from Stratagene. The DIG oligonucleotide tailing kit and the DIG luminescent detection kit for nucleic acids were from Boehringer Mannheim.

2.1. Purification of MtrH

The subunit was prepared from *M. thermoautotrophicum* under strictly anaerobic conditions. Except for the centrifugation steps, all steps were performed in an anaerobic chamber filled with 95% N_2 and 5% H_2.

*Corresponding author. Fax: (49) (6421) 178209.
E-mail: thauer@mailer.uni-marburg.de

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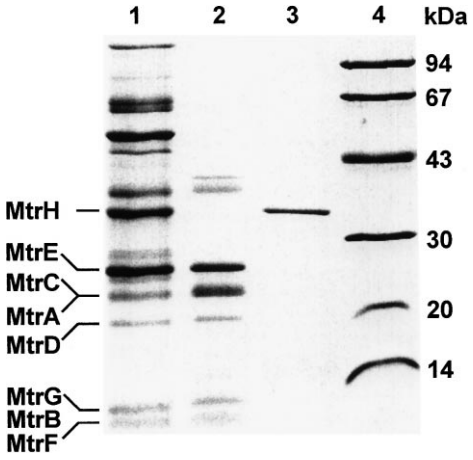

Fig. 1. Separation of MtrH from the MtrA–H complex and the MtrA–G complex by chromatography on Q-Sepharose as analyzed by SDS-PAGE. Chromatographic separation was performed as described in Section 2. For analysis 15% polyacrylamide gels were used which after electrophoresis were stained with Coomassie brilliant blue R250. Lane 1, MtrA–H complex (7 μg protein) which was eluted from the Q-Sepharose column in fractions 53 and 54; lane 2, MtrA–G complex (4 μg protein) which was eluted in fractions 29–33; lane 3, MtrH (1 μg protein) which was eluted in fractions 55–58; lane 4, molecular mass standards.

Cell extract of *M. thermoautotrophicum* was prepared by suspending 47 g (wet mass) cells at 4°C in 80 ml 50 mM Tris-HCl pH 7.6 containing 2 mM dithiothreitol (referred to as Tris buffer). The cells were disrupted at 0°C by sonification for 3×8 min using a Branson sonifier at an energy output of 200 W. Unbroken cells and cell debris were removed by centrifugation at 10 000×*g* and 4°C for 30 min. To obtain the membrane fraction the supernatant was centrifuged at 200 000×*g* and 4°C for 90 min. The pellet was washed once with Tris buffer and was then resuspended in 20 ml Tris buffer containing 1.1% (w/v) dodecyl-β-D-maltoside. After incubation at 0°C overnight, non-solubilized membranes were sedimented by centrifugation at 200 000×*g* and 4°C for 60 min. The solubilized membranes were applied to a Q-Sepharose column HiLoad 16/10 (flow rate 3 ml/min) equilibrated with Tris buffer supplemented with 4 mM dodecyl-β-D-maltoside (buffer A). The column was eluted with NaCl in buffer A: 60 ml 0.20 M, 60 ml 0.26 M, 60 ml 0.30 M, 60 ml 0.34 M, 60 ml 0.38 M, 60 ml 0.44 M and 60 ml 2 M. Fractions of 5 ml were collected. The enzyme complex MtrA–G lacking the MtrH subunit was eluted at 0.30 M (fractions 29–33). The complex MtrA–H and the MtrH subunit were eluted in different fractions at 0.38 M (MtrA-H: fractions 53 and 54; MtrH: fractions 55–58). The chromatographic steps were performed at 18°C which is the temperature within the anaerobic chamber.

Protein was determined by the method of Bradford [25] using reagents from Bio-Rad Laboratories. Bovine serum albumin was used as standard.

2.2. Methyltransferase activity assays

The radioactive assays were performed in 1.5 ml plastic tubes in an anaerobic chamber. The cob(I)alamin and methylcob(III)alamin containing assays were carried out under dim red light.

2.2.1. Methyltetrahydromethanopterin:coenzyme M methyltransferase (MT). The 30 μl assay mixture contained 50 mM Tris-HCl pH 7.6 containing 2 mM dithiothreitol, 10 mM $MgCl_2$, 2.5 mM ATP, 0.5 mM Ti(III)citrate, 0.3 mM $^{14}CH_3$-H_4MPT (70 Bq/nmol) and 0.5 mM H-S-CoM. These components were mixed at room temperature before placing the reaction tubes at 60°C. After a short incubation time the reaction was started by the addition of enzyme (0.5–10 μg protein). In time intervals of 30–60 s, samples of 2 μl were withdrawn and analyzed for $^{14}CH_3$-H_4MPT and $^{14}CH_3$-S-CoM by thin layer chromatography on silica gel 60. The sheets were developed at 18°C in an anaerobic chamber for 60–90 min in *n*-butanol/acetic acid/ water (2:1:1, by volume) [6]. The TLC sheets were exposed overnight on a phosphor storage screen and analyzed using a PhosphorImager (Molecular Dynamics). The R_f value for $^{14}CH_3$-H_4MPT was about 0.2 and that for $^{14}CH_3$-S-CoM about 0.7.

2.2.2. Methyltetrahydromethanopterin:cob(I)alamin methyltransferase (MT1). The 30 μl assay mixture contained 50 mM Tris-HCl pH 7.6 containing 2 mM dithiothreitol, 10 mM $MgCl_2$, 2.5 mM ATP, 0.5 mM Ti(III)citrate, 0.3 mM $^{14}CH_3$-H_4MPT (70 Bq/nmol) and 0.5 mM cob(I)alamin. These components were mixed at room temperature before placing the reaction tubes at 60°C. After a short incubation time the reaction was started by the addition of enzyme (0.5–10 μg protein). In time intervals of 30–60 s, samples of 2 μl were withdrawn and analyzed for $^{14}CH_3$-H_4MPT and $^{14}CH_3$-cob(III)alamin by thin layer chromatography on silica gel 60. The sheets were developed at 18°C in an anaerobic chamber for 60–90 min in *n*-butanol/acetic acid/ water (2:1:1, by volume) [6]. The TLC sheets were exposed overnight on a phosphor storage screen and analyzed using a PhosphorImager (Molecular Dynamics). The R_f value for $^{14}CH_3$-H_4MPT was about 0.2 and that for $^{14}CH_3$-Cob(III)alamin about 0.6.

2.2.3. Methylcob(III)alamin:coenzyme M methyltransferase activity (MT2). The activity assay was performed in 1.5 ml quartz cuvettes under anaerobic conditions. The 0.8 ml assay mixture contained 50 mM Tris-HCl pH 7.6, 0.25 mM CH_3-cob(III)alamin and 2 mM H-S-CoM. After incubation at 60°C enzyme (1–100 μg) was added and the formation of cob(II)alamin from methylcob(III)alamin was followed by measuring the decrease in absorbance at 520 nm spectrometrically ($\Delta\varepsilon_{520} = 6.2\ mM^{-1}\ cm^{-1}$).

2.3. Cloning and sequencing of the mtrH gene from Methanosarcina barkeri (strain Fusaro)

A heterologous probe was derived from the sequence of the *mtrD* gene from *Methanosarcina mazei* (strain Gö1) [26]. The synthetic oligonucleotide 5′-AACATAGGAGGTACAATTGAAGGGTTCCAC-3′ was labelled with digoxigenin-UTP and used to screen a λ ZAP Express genomic library (*Sau*3AI) of *M. barkeri* [24]. From one of the positive clones the phagemid pMB6 was generated by excision and recircularization of pBK-CMV carrying a 8 kbp *Sau*3AI fragment including the *mtrH* gene. This gene was sequenced using the dideoxynucleotide method of Sanger et al. [27]. Both strands of the *mtrH* gene were sequenced independently and completely. The nucleotide sequence has been submitted to the EMBL Nucleotide Sequence Data Base with the accession number AJ132817.

3. Results and discussion

Methyltetrahydromethanopterin:coenzyme M methyltransferase was purified from the membrane fraction of *Methanobacterium thermoautotrophicum* by extraction with dodecyl-β-D-maltoside followed by chromatography on Q-Sepharose. During chromatography fractions with the complex lacking the 34 kDa MtrH subunit and fractions containing only the 34 kDa MtrH subunit were obtained (Fig. 1). The MtrA–G complex no longer catalyzed the overall reaction but still exhibited methylcobalamin:coenzyme M methyltransferase

Table 1
Specific methyltransferase activities of the MtrA–H complex, of the MtrA–G complex and of the MtrH subunit separated by chromatography on Q-Sepharose

Subunit	Specific activity (U/mg)		
	MT	MT1	MT2
MtrA–H	1	1	0.1
MtrA–G	< 0.001	< 0.001	0.2
MtrH	< 0.001	0.07	< 0.001

The following methyltransferase activities were tested: methyltetrahydromethanopterin:coenzyme M methyltransferase (MT); methyltetrahydromethanopterin:cob(I)alamin methyltransferase (MT1); and methylcob(III)alamin:coenzyme M methyltransferase (MT2). Chromatographic separation was performed as described in Section 2. The fractions analyzed were those also analyzed by SDS-PAGE in Fig. 1. The MtrA–H complex was only approximately 50% pure.

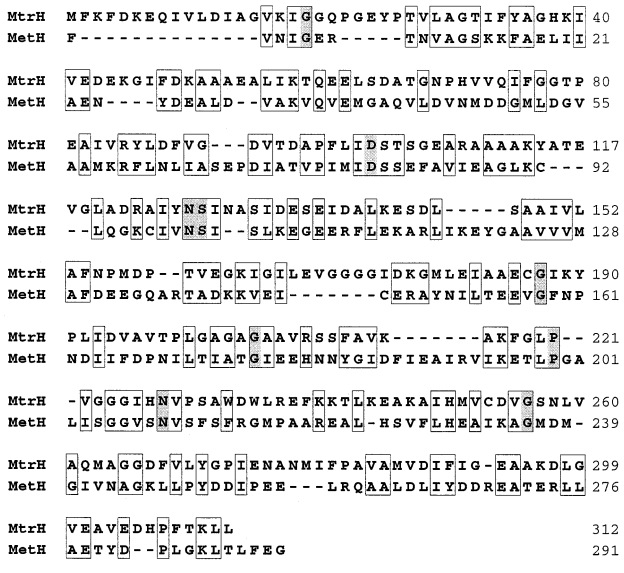

Fig. 2. Alignment of the amino acid sequence of MtrH with that of MetH. The sequences shown represent majority sequences of six MtrH sequences and eight MetH sequences (see text). Identical and chemically similar amino acids are boxed. Amino acids which are identical in all 14 sequences are shaded in gray. The alignment was performed with the Lasergene computer DNAStar program (MEGALIGN) using the clustal method.

activity. The subunit MtrH catalyzed the methylation of cob(I)alamin with CH_3-H_4MPT to methylcob(III)alamin but lacked methyltetrahydromethanopterin:coenzyme M methyltransferase activity and methylcob(III)alamin:coenzyme M methyltransferase activity (Table 1).

The results summarized in Table 1 indicate that MtrH in the MtrA–H complex is the subunit which harbors the CH_3-H_4MPT binding site and which catalyzes the methyl transfer to the corrinoid prosthetic group. This conclusion is substantiated by the finding that MtrH shows sequence similarity to AcsE [28], which catalyzes the transfer of the methyl group from N^5-methyltetrahydrofolate (CH_3-H_4F) to a corrinoid-iron-sulfur protein in *Clostridium thermoaceticum* and which also exhibits methyltetrahydrofolate:cob(I)alamin methyltransferase activity. Tetrahydromethanopterin is a tetrahydrofolate analogue [29]. Both MtrH and AcsE share sequence similarity to the N-terminal third of cobalamin-dependent methionine synthase MetH assumed to harbor the CH_3-H_4F binding site [30].

In Fig. 2 the amino acid sequence of MtrH is aligned with that of the putative CH_3-H_4F binding domain of MetH. The sequences shown represent majority sequences of the MtrH sequence from *M. thermoautotrophicum* [3,31], *Methanococcus jannaschii* [32], *Methanopyrus kandleri* [8], *Methanosarcina mazei* [26] and *Methanosarcina barkeri* (see Section 2) and of the MetH sequence from *Escherichia coli* [33], *Pseudomonas putida* [34], *Mycobacterium tuberculosis* [35], *Mycobacterium leprae* [36], *Synechocystis* sp. [37], *Caenorhabditis elegans* [38], *Rattus norvegicus* [39] and *Homo sapiens* [40]. The two majority sequences are 21.3% identical and 37.2% similar.

The purified MtrH catalyzed methylcobalamin formation from CH_3-H_4MPT and cob(I)alamin at significantly lower rates than the MtrA–H complex. A possible explanation could be that MtrH has to be in contact with the other subunits for optimal catalytic efficiency. An example of such a cooperative effect has recently been reported for another corrinoid containing enzyme complex. The methanol:coenzyme M methyltransferase from *Methanosarcina barkeri* is composed of the subunits MtaA, MtaB and MtaC of which MtaC harbors the corrinoid prosthetic group [17]. MtaB catalyzes the methylation of the corrinoid and MtaA its demethylation. The presence of MtaA was shown to positively affect MtaB activity and vice versa [41].

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