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Review

The Na^+-translocating methyltransferase complex from methanogenic archaea

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Abstract

Methanogenic archaea are dependent on sodium ions for methane formation. A sodium ion-dependent step has been shown to be methyl transfer from N^5-methyltetrahydromethanopterin to coenzyme M. This exergonic reaction ($\Delta G^{\circ\prime} = -30$ kJ/mol) is catalyzed by a Na^+-translocating membrane-associated multienzyme complex composed of eight different subunits, MtrA–H. Subunit MtrA harbors a cob(I)amide prosthetic group which is methylated and demethylated in the catalytic cycle, demethylation being sodium ion-dependent. Based on the finding that in the cob(II)amide oxidation state the corrinoid is bound in a base-off/His-on configuration it is proposed that methyl transfer from MtrA to coenzyme M is associated with a conformational change of the protein and that this change drives the electrogenic translocation of the sodium ions.

Keywords: Methyltransferase; Sodium ion translocation; Corrinoid protein; Methane formation; Methanogenic archaeon

1. Introduction

Methanogenic archaea are strictly anaerobic unicellular organisms originally thought to be bacteria but now recognized as belonging to a separate phylogenetic domain, the Archaea. They are most numerous in anaerobic freshwater environments such as lake sediments and the digestive tract of animals. In these habitats methanogens play an important role in the degradation of complex organic compounds by consortia of anaerobic microorganisms. Methanogens produce $>10^9$ tons of methane per year. About half is oxidized to CO_2 by methanotrophic aerobic bacteria but most of the rest escapes to the atmosphere where it is a potent greenhouse gas [1].

Methanogenesis is the only way that methanogenic archaea can obtain energy for growth and these are the only organisms known to produce CH_4 as a catabolic product. They can only use acetate, H_2 and CO_2, formate and/or other C_1 compounds such as methanol, methylthiols and methylamines as energy substrates, the reduced C_1 compounds being disproportionated to CH_4 and CO_2 or reduced to methane with H_2 as exemplified for methanol.

$$CH_3COO^- + H^+ \rightarrow CO_2 + CH_4 \quad \Delta G^{\circ\prime} = -36 \text{ kJ/mol} \tag{1}$$

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$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O\ \Delta G^{\circ\prime} = -131\ \text{kJ/mol} \quad (2)$$

$$4\ CH_3OH \rightarrow 3\ CH_4 + 1\ CO_2 + 2\ H_2O\ \Delta G^{\circ\prime} = -106.5\ \text{kJ/mol}\ CH_4 \quad (3)$$

$$CH_3OH + H_2 \rightarrow CH_4 + H_2O\ \Delta G^{\circ\prime} = -112.5\ \text{kJ/mol} \quad (4)$$

Despite the high specialization most methanogens are phylogenetically not closely related. They are classified in five orders, Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales, and Methanopyrales, each phylogenetically related to the others as distantly as the Cyanobacteriales to the Proteobacteriales [2].

All methanogenic archaea have in common that their growth is dependent on the presence of sodium ions (>1 mM). Methanogenesis from acetate, from H_2 and CO_2, and from methanol by cell suspensions has been shown to be Na^+-dependent [3,4]. A clue to the understanding was the finding that only methanol reduction with H_2 to CH_4 did not require the presence of the cation [5]. It led to the discovery that the methyl transfer from N^5-methyltetrahydromethanopterin (CH_3-H_4MPT) to coenzyme M (H-S-CoM) is a sodium ion-dependent step in methanogenesis [6,7]. (In some methanogens N^5-methyltetrahydrosarcinapterin instead of CH_3-H_4MPT is the methyl donor [8]).

$$CH_3 - H_4MPT + H - S - CoM \xrightarrow{Na^+} CH_3 - S - CoM + H_4MPT\ \Delta G^{\circ\prime} = -30\ \text{kJ/mol} \quad (5)$$

This reaction is catalyzed by a sodium-translocating membrane-associated methyltransferase. It is not involved in methanol reduction with H_2 to CH_4 explaining why reaction 4 is not sodium ion-dependent.

In the following minireview we first summarize the evidence that the methyltransferase complex in methanogenic archaea is a site of sodium ion translocation. We then describe the structural and catalytic properties of the complex. For a more detailed introduction to the topic the reader is referred to reviews on the 'pathways of energy conservation in methanogenic archaea' [9], the 'biochemistry of methanogenesis' [8], the 'enzymology of one-carbon metabolism in methanogenic pathways' [10], and the 'role of corrinoids in methanogenesis' [11].

2. The methyltransferase complex as the site of sodium ion translocation

It was already mentioned that CH_4 formation from CH_3OH+H_2 is not sodium ion-dependent, but methanogenesis from formaldehyde and H_2 according to $CH_2O+2\ H_2 \rightarrow CH_4+H_2O$ shows such a dependence. It could, therefore, be concluded that the sodium ion-dependent step was located between intermediates of the formaldehyde and methanol redox state. A comparison of the sodium ion concentration in cells of *Methanosarcina barkeri* performing methanogenesis from either CH_2O+H_2 or CH_3OH+H_2 provided the first evidence that there was not simply a sodium ion-dependent reaction located between the intermediates of the redox state of formaldehyde and methanol but a primary sodium ion pump: sodium ions were extruded from the cytoplasm during methanogenesis from CH_2O+H_2 but not during methanogenesis from CH_3OH+H_2 [12]. These and other results finally led to the identification of the methyltransferase as the sodium ion-requiring enzyme.

The sodium ion-pumping activity of the methyltransferase was demonstrated in two types of experiments. In one set of experiments inside-out vesicles of *Methanosarcina mazei* strain Gö1 were employed. These vesicles were prepared by pronase treatment of the cells yielding protoplasts [13] and a gentle French press treatment of the latter. Such vesicle preparations had already been successfully employed for the study of proton-translocating reactions in this organism [14]. With these vesicles it was shown that methyltransferase activity was stimulated by ATP and by a reducing agent and that sodium ions were pumped into the lumen of the vesicles strictly dependent of methyltransfer from N^5-methyltetrahydromethanopterin to coenzyme M [6,7].

The methyltransferase was purified from membrane fractions of *Methanosarcina mazei* strain Gö1. The purification scheme was quite similar to the one for the enzyme *Methanothermobacter mar-*

burgensis, which will be outlined in Section 3.1. The purified enzyme exhibiting specific activity of 7.0 μmol/min mg was then used for reconstitution experiments with ether lipid liposomes, which were prepared from ether lipids isolated from the same microorganism. The liposomal methyltransferase pumped sodium ions strictly dependent on the availability of the substrates. A ratio of 1.7 mol sodium ions translocated per mol of methyl group transferred could be calculated [15].

3. Structural and catalytic properties of the methyltransferase complex

Methyltransfer from N^5-methyltetrahydromethanopterin to coenzyme M (reaction 5) had until 1993 been studied only in cell extracts of methanogenic archaea [16–18] or with partially purified preparations [19]. The activity was found to be associated with the membrane fraction, to be dependent on the presence of ATP and to require strong reducing conditions. ATP could not be replaced by other nucleoside triphosphates or non-hydrolyzable analogues of ATP; it exhibited a strong effect on the redox potential at which activation of the methyltransferase took place (half-maximum activity of $E^{\circ\prime} = -235$ mV as compared to -450 mV in the absence of ATP) [20]. In the absence of coenzyme M, a methylated corrinoid protein accumulated which was demethylated upon the addition of coenzyme M [19,21–23]. Complete purification was hampered by the fact that the methyl transfer activity was irreversibly lost within minutes under oxic conditions.

3.1. Properties of the purified enzyme complex

N^5-Methyltetrahydromethanopterin:coenzyme M methyltransferase (MtrA–H) was first purified from *M. marburgensis* (formerly *M. marburgensis* strain Marburg [24,25]. The membrane fraction was extracted with 2.5% of the detergent dodecyl-β-D-maltoside and the methyl transfer activity separated from other proteins via chromatography on DEAE Sepharose, Q-Sepharose, Superose 6 and Mono Q. From 60 g wet cells approximately 20 mg protein with a specific activity of 3 U/mg was obtained. The purified enzyme had an apparent molecular mass of 670 kDa and was composed of seven different subunits with apparent molecular masses of 34 kDa (MtrH), 28 kDa (MtrE), 24 kDa (MtrC), 23 kDa (MtrA), 21 kDa (MtrD), 13 kDa (MtrG), and 12 kDa (MtrF). The complex contained 7.6 ± 0.4 mol 5-hydroxybenzimidazolyl cobamide, 37 mol nonheme iron and 34 mol acid-labile sulfur per 670 kDa protein complex. MtrA was found to harbor the corrinoid prosthetic group and to be identical to the corrinoid protein purified from the membrane fraction of *M. marburgensis* by Fuchs and collaborators [26–29]. In a later study it was shown that the complex contained an additional 12.5 kDa subunit (MtrB) [30] and the iron–sulfur content was most probably due to contamination of the preparation with a 45 kDa polyferredoxin [31].

When reconstituted in liposomes the enzyme complex purified from *Methanosarcina mazei* was shown to catalyze vectorial Na^+ translocation [15].

With the purified enzyme from *M. marburgensis* it was shown that the methyltransferase is active only in the cob(I)amide oxidation state and that MtrA-bound co-methyl-5-hydroxybenzimidazolyl cob(III)amide is an intermediate in the formation of methyl-coenzyme M from CH_3-H_4MPT and coenzyme M [32]. The purified methyltransferase complex also catalyzes the reversible formation of CH_3-cob(III)alamine from free cob(I)alamine and CH_3-H_4MPT and the formation of methyl-coenzyme M from free methyl-cob(III)alamine and coenzyme M [33].

$$CH_3 - H_4MPT + \text{cob(I)alamine} \rightleftharpoons CH_3 - \text{cob(III)alamine} + H_4MPT \quad (6)$$

$$CH_3 - \text{cob(III)alamine} + H - S - CoM \overset{Na^+}{\rightleftharpoons} CH_3 - CoM + \text{cob(I)alamine} \quad (7)$$

Both reactions are associated with a free energy change of approximately 15 kJ/mol [33].

The formation of methyl-coenzyme M from CH_3-H_4MPT and coenzyme M (reaction 5) [32] and from free CH_3-cob(III)alamine and coenzyme M (reaction 7) [33] catalyzed by the purified methyltransferase complex is stimulated five-fold by sodium ions with

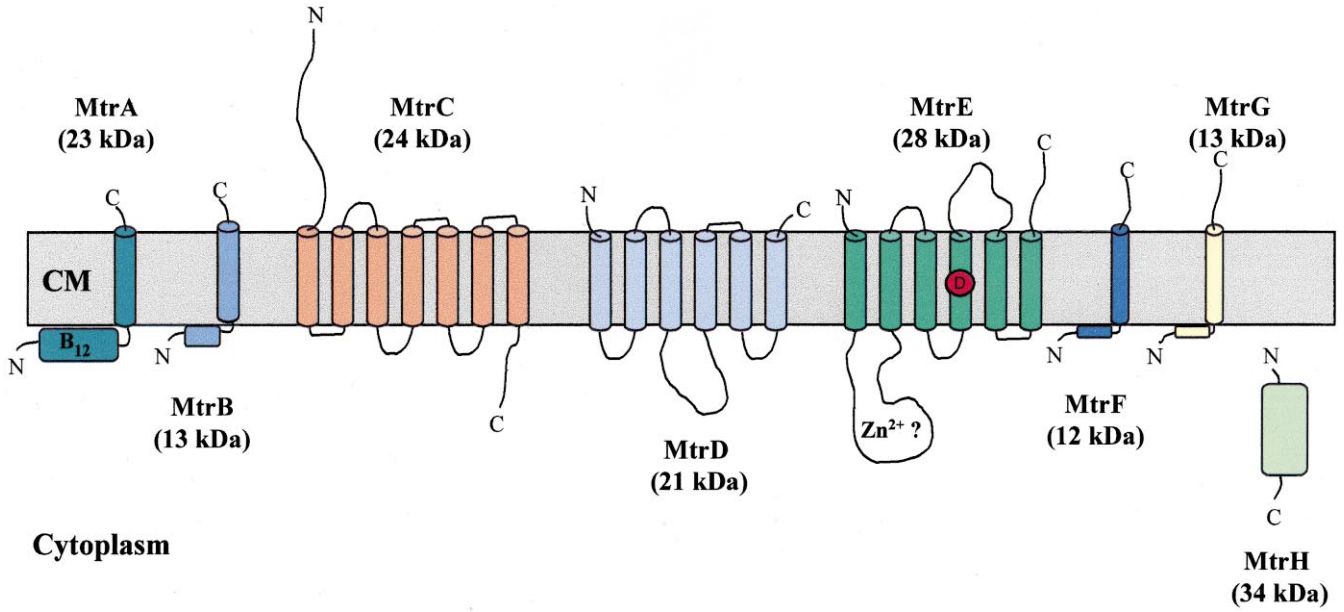

Fig. 1. Topology of the eight Mtr subunits of the membrane-associated sodium ion-translocating methyltransferase complex from methanogenic archaea. The transmembrane helices were predicted by the program TMHMM 1.0 (prediction server, Center for Biological Sequence Analysis (CBS)). The cytoplasmic domains and the loops are not drawn in correct size relative to the transmembrane helices. CM, cytoplasmic membrane; C, C-terminal end; N, N-terminal end.

a K_d of 50 μM. However, the formation of CH_3-cob(III)alamine from CH_3-H_4MPT and cob(I)alamine (reaction 6) is not stimulated by sodium ions [33]. These results indicate that reaction 7 rather than reaction 6 is coupled with sodium ion translocation.

In dodecyl-β-D-maltoside the MtrA–H complex partially dissociates into MtrA–G and MtrH which can be separated. The MtrA–G complex was found to still catalyze reaction 7 but not reactions 5 and 6 whereas subunit MtrH was found to catalyze reaction 6 rather than reactions 5 and 7 [34].

3.2. Encoding genes and phylogenetic diversity

The genes encoding the eight different subunits of MtrA–H from *M. marburgensis* were shown to be organized in a transcription unit *mtrEDCBAFGH* [30]. The same number of genes and the same organization were later found in *Methanococcus jannaschii* [35], *Methanobacter thermoautotrophicus* (formerly *Methanobacterium thermoautotrophicum* strain ΔH [24]) [36], *Methanopyrus kandleri* (with *mtrF* lacking) [37], *Methanosarcina mazei* [38], and *Methanosarcina barkeri* [34]. The respective *mtr* genes in the different methanogens encode proteins of almost the same size and with significant sequence similarity indicating a common phylogenetic origin. The *mtr* genes are not found in the genome of *Archaeoglobus fulgidus* [39], which is a relatively close relative to the Methanosarcinales and which uses the same enzymes and coenzymes as methanogenic archaea in its C_1 metabolism [40–42]. *A. fulgidus* differs from methanogens, however, in that it cannot produce methane as end product of its energy metabolism [43].

3.3. Secondary structure predictions

From the amino acid sequence deduced from the nucleotide sequence of the *mtr* genes prediction with respect to the secondary structure of the eight subunits can be made. MtrC, MtrD, and MtrE are integral membrane proteins with seven, six, and six transmembrane helices, respectively. MtrA, MtrB, MtrF, and MtrG each form only one transmembrane helix and MtrH is devoid of a membrane anchor. The topology of the subunits is shown in Fig. 1 in which the orientation of the subunit termini and hydrophilic loops between transmembrane helices are

drawn towards the inside or outside considering the 'positive inside rule' [44]. The drawing does not accurately reflect the relative size of the subunits or the hydrophobic and hydrophilic domains.

The secondary structure predictions are in good agreement with the calculated hydrophobicities of the subunits which show that MtrC, MtrD, and MtrE are the most hydrophobic and MtrH the most hydrophilic subunits in the MtrA–H complex (Table 1).

Of the eight subunits only the subunits MtrC, MtrD, and MtrE can be considered to be directly involved in sodium ion translocation since only these three subunits have more than one transmembrane helix. More than one transmembrane helix is most probably required for a membrane protein to be able to translocate a solute across the membrane.

3.4. Structure and function of the corrinoid-harboring subunit MtrA

MtrA is the second most hydrophilic subunit in the MtrA–H complex (Table 1). Only the last 20 amino acids of the C-terminal end of MtrA form a hydrophobic domain. Genetic removal of the domain renders MtrA a soluble protein [45], an indication that MtrA is anchored to the cytoplasmic membrane via its C-terminus as shown in Fig. 1 rather than its N-terminal end, as has been proposed [46]. Immunogold labeling studies indicate that the larger hydrophilic part of MtrA is oriented towards the cytoplasm [46].

MtrA is the corrinoid-harboring subunit of the methyltransferase complex. The corrinoid is very tightly bound to the subunit as indicated by the finding that the prosthetic group is not lost during SDS–PAGE if the protein is not heated prior to electrophoresis [25]. Recombinant MtrA expressed in *Escherichia coli* was shown to bind cob(II)alamine in the base-off/His-on configuration [45]. From the three conserved histidines in MtrA His-84 was identified by site-directed mutagenesis to be the active site histidine [37,47]]. MtrA does not contain the consensus sequence DXHXXG-41-42-SXL-26–28-GG exhibited by other corrinoid enzymes such as methionine synthase that have the corrinoid bound in the base-off/His-on configuration [48–50]. Only the HXXG sequence appears to be conserved [47]. On each MtrA subunit there is only one corrinoid binding site [51].

From structural studies it is known that in aqueous solution cob(II)alamine and methylcob(III)alamine contain an axial ligand, whereas cob(I)alamine does not [52]. In unmethylated MtrA cobalt in the bound corrinoid should therefore be without axial ligands (Fig. 2). Methylation of cob(I)amide bound to MtrA should therefore be associated with a conformational change of the protein due to ligation to histidine residue to the methylcob(III)amide. Upon demethylation the conformational change would be reversed. Since demethylation is dependent on sodium ions [32,33], the conformational change associated with this step probably couples with the vectorial translocation of this cation [53].

Table 1
Hydrophobicities of the eight Mtr subunits from six different methanogenic archaea

Methanogenic archaeon	MtrA (23 kDa)	MtrB (13 kDa)	MtrC (24 kDa)	MtrD (21 kDa)	MtrE (28 kDa)	MtrF (12kDa)	MtrG (13 kDa)	MtrH (34 kDa)
M. marburgensis	+13	−54	−287	−241	−172	−53	−21	+26
M. thermoautotrophicus	+11	−46	−270	−241	−170	−52	−21	+26
M. kandleri	−23	−47	−272	−189	−143	–	−8	+47
M. jannaschii	−22	−45	−259	−228	−185	−26	−21	+20
M. mazei	−14	−13	−288	−259	−147	−51	−19	+24
M. barkeri	−15	−19	−259	−244	−149	−45	−20	+13

M. marburgensis: *Methanothermobacter marburgensis* (formerly *Methanobacterium thermoautotrophicum* strain Marburg) [24]; *M. thermoautotrophicus*: *Methanothermobacter thermoautotrophicus* (formerly *Methanobacterium thermoautotrophicum* strain ΔH [24]); *M. kandleri*: *Methanopyrus kandleri*; *M. jannaschii*: *Methanococcus jannaschii*; *M. mazei*: *Methanosarcina mazei* strain Gö; *M. barkeri*: *Methanosarcina barkeri* strain Fusaro. The hydrophobicities of the subunits were calculated from the hydropathies of their amino acid side chains according to [65].

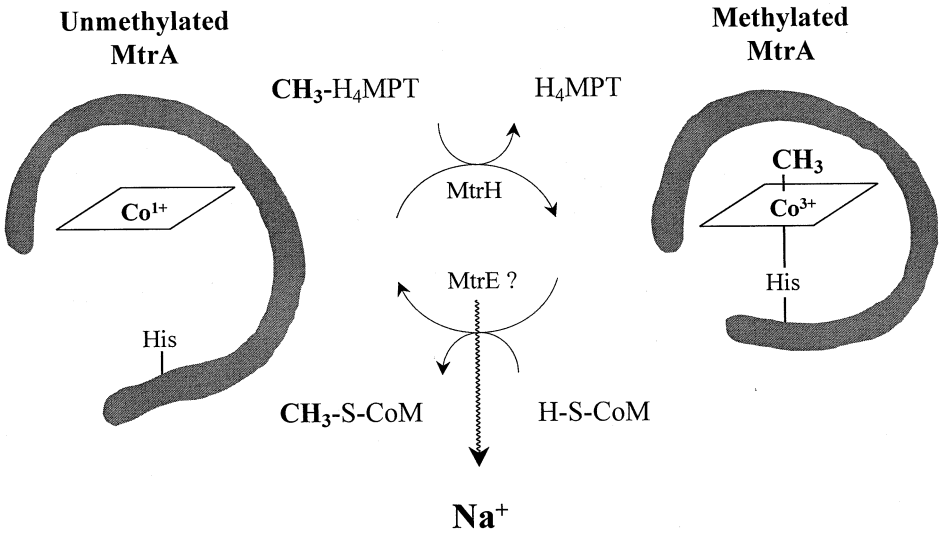

Fig. 2. Proposed conformational change of MtrA upon methylation and demethylation of its corrinoid prosthetic group. The demethylation step, which is probably catalyzed by MtrE, is drawn as a reaction coupled with vectorial sodium ion translocation, since demethylation rather than methylation has been shown to be dependent on sodium ions. After [53].

3.5. *Structure and function of the subunits MtrH and MtrE*

MtrH is the most hydrophilic subunit in the MtrA–H complex and the only subunit to lack a hydrophobic membrane anchor (Fig. 1). It is the only subunit that can be partially separated from the MtrA–H complex by chromatography after incubation in 2.5% dodecyl-β-D-maltoside. The separated 34 kDa MtrH subunit can catalyze the methylation of cob(I)alamine with CH_3-H_4MPT (reaction 6) indicating that the subunit mediates the transfer of the methyl group of CH_3-H_4MPT to the corrinoid prosthetic group of MtrA [34]. MtrH shows sequence similarity to the methyltransferase AcsE from *Clostridium thermoaceticum* [54], which catalyzes the methyl group transfer from N^5-methyltetrahydrofolate to the corrinoid iron–sulfur protein, to the methyltetrahydrofolate binding domain of methionine synthase from *E. coli* [55], and to the protein CMUB from a *Methylobacter* sp. [56] which is involved in methyltetrahydrofolate formation from methylchloride and tetrahydrofolate. The crystal structure of AcsE from *C. thermoaceticum* has recently been elucidated [57].

MtrE is one of the three most hydrophobic subunits in the MtrA–H complex (Fig. 1, Table 1). Its function is still speculative. It is proposed to catalyze the transfer of the methyl group from the corrinoid prosthetic group of MtrA to coenzyme M which is the sodium ion-dependent partial reaction and which therefore should be coupled with Na^+ translocation [33]. One reason for the proposal is that of the three subunits with more than one transmembrane helix MtrE has the largest cytoplasmic domain; the loop between the first and second transmembrane helices (Fig. 1) is by far the largest with 62 amino acids (Fig. 1). This loop harbors the sequence motif Asp^{26}-X-Glu^{28}-X_{22}-His^{51}-X_9-Glu^{61} which is highly conserved in the MtrE subunit from all methanogens and which is a candidate for a zinc binding site [58]. All enzymes known to date that catalyze the alkylation of a thiol group have been shown to be zinc proteins (for literature see [59]). This includes the proteins MtaA and MtbA from *Methanosarcina barkeri*, which are soluble zinc proteins [59,60] that catalyze the methylation of coenzyme M with methylcob(III)alamine (reaction 7) as proposed for MtrE but without coupling this reaction with sodium ion translocation [53]. Whereas MtaA and MtbA are phylogenetically closely related proteins [61] they show no sequence similarity to MtrE. An explanation for this could be their different function: the cytoplasmic proteins MtaA and MtbA catalyze only the scalar reaction whereas the integral membrane protein MtrE is proposed to additionally cou-

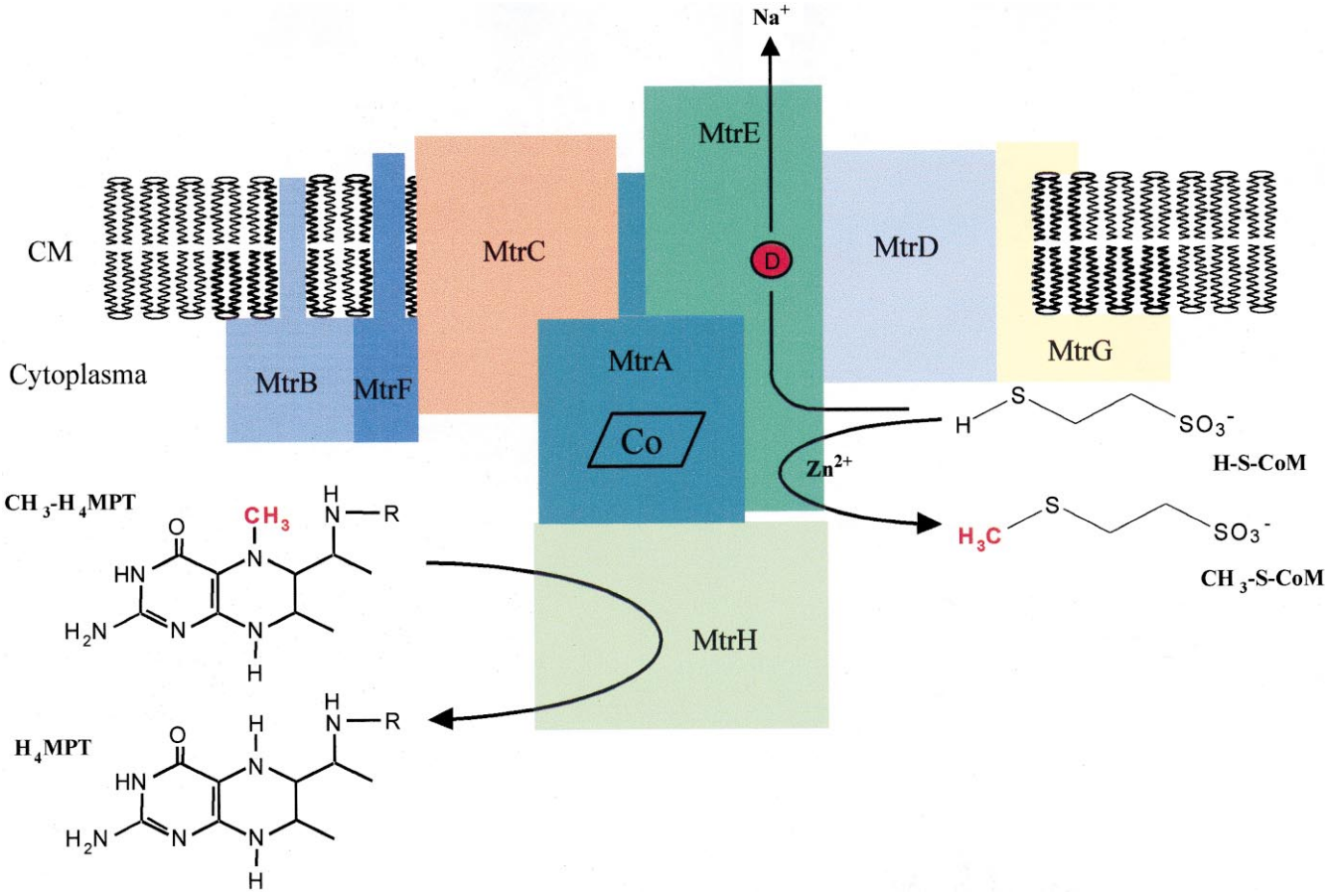

Fig. 3. Two-dimensional model of the membrane-associated sodium ion-translocating methyltransferase complex from methanogenic archaea. The subunits were drawn such that their areas correspond to their molecular masses. Co: cobalt 1+ in 5-hydroxybenzimidazolyl cobamide; D: aspartate 183 in the fourth transmembrane helix of MtrA (Fig. 1); R: rest.

ple the scalar reaction with vectorial Na^+ translocation.

The second reason for the proposal is that only MtrE has a transmembrane helix with an aspartate residue (Fig. 1), the sequence of this helix in the MtrE subunit from all methanogens being highly conserved: 168-IWGITIGAIGSSTGDVHYGAER-191. An aspartate residue in a transmembrane helix has been shown to be essential for sodium ion translocation as catalyzed by the β-subunit of oxaloacetate decarboxylase from *Klebsiella pneumoniae* [62]. An aspartate residue is also conserved in the transmembrane helix of the sodium ion-translocating glutaconyl-CoA decarboxylase from *Acidaminococcus fermentans* and of the sodium ion-translocating methylmalonyl-CoA decarboxylase from *Veillonella parva* and *Propionigenium modestum* [63]. An aspartate residue in a transmembrane helix of the C-subunit of the *E. coli* ATPase has been shown to be crucial for H^+ translocation [64].

The function of the five other subunits is less clear. MtrF appears not to be essential since it is lacking in the MtrA–H complex from *Methanopyrus kandleri* [37].

3.6. A model of the MtrA–H complex

The apparent molecular mass of the purified MtrA–H complex of 670 kDa is consistent with a complex in which each subunit is present four times. The determined corrinoid content of 7.6 ± 0.4 mol per 670 kDa complex indicates, however, that the MtrA subunit is present eight times. The corrinoid content is based on a protein determination with bicinchoninic acid [25]; it could easily be incorrect by a factor of two. At present it is therefore justifi-

able to assume that the purified methyltransferase complex is a tetramer of a heterooctamer and that the heterooctamer is the active unit catalyzing methyl transfer from N^5-methyltetrahydromethanopterin to coenzyme M and the translocation of the sodium ions. A two-dimensional model considering the size, hydrophobicities, topology and function of the subunits is shown in Fig. 3. The relative locations of the subunits MtrC, MtrD, MtrG, MtrB, and MtrF with unknown function were chosen arbitrarily. In the real three-dimensional complex these five subunits are definitely much closer to each other.

In the model (Fig. 3) MtrA is drawn in the center of the MtrA–H complex. MtrA is the subunit which harbors the corrinoid prosthetic group that is methylated and demethylated in the catalytic cycle, methylation being catalyzed by MtrH and demethylation most probably by MtrE. MtrA cannot be the subunit that mediates sodium ion translocation since MtrA is anchored to the membrane via only one C-terminal transmembrane helix. This function is assigned to MtrE which is drawn to be in an extended transmembrane contact with MtrA. Via the contact the conformational changes induced in MtrA by methylation and demethylation of its prosthetic group (Fig. 2) are assumed to be transmitted to MtrE which couples the conformational change with sodium ion translocation.

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