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Molecular characterization of methanogenic N^5-methyl-tetrahydromethanopterin: Coenzyme M methyltransferase

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ABSTRACT

Methanogenic archaea share one ion gradient forming reaction in their energy metabolism catalyzed by the membrane-spanning multisubunit complex N^5-methyl-tetrahydromethanopterin: coenzyme M methyltransferase (MtrABCDEFGH or simply Mtr). In this reaction the methyl group transfer from methyl-tetrahydromethanopterin to coenzyme M mediated by cobalamin is coupled with the vectorial translocation of Na^+ across the cytoplasmic membrane. No detailed structural and mechanistic data are reported about this process. In the present work we describe a procedure to provide a highly pure and homogenous Mtr complex on the basis of a selective removal of the only soluble subunit MtrH with the membrane perturbing agent dimethyl maleic anhydride and a subsequent two-step chromatographic purification. A molecular mass determination of the Mtr complex by laser induced liquid bead ion desorption mass spectrometry (LILBID-MS) and size exclusion chromatography coupled with multi-angle light scattering (SEC-MALS) resulted in a $(MtrABCDEFG)_3$ heterotrimeric complex of ca. 430 kDa with both techniques. Taking into account that the membrane protein complex contains various firmly bound small molecules, predominantly detergent molecules, the stoichiometry of the subunits is most likely 1:1. A schematic model for the subunit arrangement within the MtrABCDEFG protomer was deduced from the mass of Mtr subcomplexes obtained by harsh IR-laser LILBID-MS.

1. Introduction

Methanogenic archaea annually produce ca. 1 billion tons of methane - an important greenhouse gas and energy resource - in anoxic habitats, such as water sediments, rice fields, hydrothermal vent peripheries as well as intestinal tracts of animals [1]. Acetate, CO_2, methanol and few other one-carbon substrates are covalently bound in different oxidation states to the three one-carbon carriers methanofuran, tetrahydromethanopterin (H_4MPT) and coenzyme M (HS-CoM) and reduced to methane by various electron donors [1]. The only ion-gradient forming process universally applied in the energy metabolism of methanogenic archaea is accomplished by N^5-methyl-H_4MPT: HS-CoM methyltransferase (Mtr). This multi-subunit membrane protein complex catalyzes the transfer of the methyl group from methyl-H_4MPT to coenzyme M coupled with the vectorial translocation of Na^+ across the cytoplasmic membrane [2–4].

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

The Mtr complex was first isolated ca. 20 years ago from *Methanothermobacter marburgensis* (formerly known as *Methanobacterium thermoautotrophicum* strain Marburg) [5] and *Methanosarcina mazei* strain Gö1 [6]. The membrane fraction of *M. marburgensis* was extracted with 2.5% dodecyl-β-D-maltoside and the solubilized Mtr complex purified via chromatography on DEAE sepharose, Q sepharose, Superose 6 and Mono Q. The membrane-spanning complex is composed of eight subunits with molecular masses (M_r) of 25.6 kDa for MtrA, 10.7 kDa for MtrB, 27.1 kDa for MtrC, 22.8 kDa for MtrD, 31.2 kDa for MtrE, 7.3 kDa for MtrF, 9.5 kDa for MtrG and 33.5 kDa for MtrH of *M. marburgensis*. The apparent M_r of the whole enzyme complex was determined to be 670 kDa by size exclusion chromatography [7]. This finding led to the conclusion

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that the Mtr complex is a tetramer of heterooctamers Mtr(ABCEDFGH)$_4$. Primary structure analysis indicates that MtrC, MtrD and MtrE are integral membrane proteins with seven, six and six membrane-spanning helices, respectively. MtrE potentially hosts a highly conserved zinc binding motif Asp-X-Glu-X_{22}-His-X_9-Glu [8] which is characteristic for all known Zn^{2+} binding enzymes catalyzing thiol alkylation [9,10] and an invariant aspartate in the membrane matrix which has been already reported as essential residue in several Na^+ or H^+ pumping protein complexes [4,11,12]. MtrA, MtrB, MtrF and MtrG only contain one transmembrane helix anchor. The soluble domain of MtrA is directed to the cytoplasm and contains an 5-hydroxy-benzimidazolyl-cobamide cofactor [5], also called factor III, which is a homologue to vitamin B_{12} (5, 6-dimethyl-benzimidazolyl-cobamide). Active Mtr requires cobalt of 5-hydroxy-benzimidazolyl-cobamide in the oxidation state I. MtrH is the only subunit of the Mtr complex without a membrane-spanning region and can be easily lost during cell lysis and solubilization [5]. Based on the obtained molecular and kinetic data, the reaction proceeds by a methyl transfer of methyl-H_4MPT bound to MtrH to cob(I)alamin bound to MtrA [13] thereby forming methyl-cob(III)alamin containing the methyl group and a histidine as axial ligands. Methyl-cob(III)alamin transfers its methyl group to coenzyme M forming methyl-coenzyme M and returns to the cob(I)alamin state. The latter reactions were shown to be dependent on Na^+ [14,15] and might be therefore coupled with its vectorial translocation. However, for unravelling its complicated catalytic mechanism, larger amounts of a homogeneous protein sample and more detailed structural information are required.

As a first step we developed a procedure to isolate a highly pure and homogeneous MtrA-G complex from *M. marburgensis* and characterized the prepared multisubunit membrane complex in terms of the absolute molecular mass and the oligomeric state.

2. Materials and methods

2.1. Materials

M. marburgensis was obtained from the Deutsche Sammlung für Microorganismen (Braunschweig, Germany). The archaeon was grown as described previously [16]. Dodecyl β-D-maltoside (DDM) and dodecyl tricosaoxyethylene glycol ether (Brij-35) was purchased from Anatrace and dimethyl maleic anhydride (DMMA) from Sigma-Aldrich. All the chromatographic columns and column materials were acquired from GE Healthcare Lifesciences.

2.2. Protein production

2.2.1. Protein isolation

The MtrA-G complex was isolated from the membrane fraction of *M. marburgensis* cell as described earlier [16] with some modifications. All the steps were performed at 4 °C, if not stated otherwise. 10 g of frozen cells were thawed and resuspended in 50 ml lysis buffer (50 mM MOPS-NaOH pH 7.0, 10 mM $MgCl_2$ and 2 mM dithiothreitol) containing 5 mg bovine pancreatic DNase I (Roche). Cells were disrupted by passing them through a microfluidizer (Microfluidics) for 4 times at 16,000 psi pressure. The cell lysates were centrifuged twice at 10,000 ×*g* for 30 min to remove cell debris. The membrane was pelleted from the supernatant by centrifugation at 100,000 ×*g* for 1.5 h, resuspended in the lysis buffer and pelleted again. After resuspending in the lysis buffer to the final volume of 5 ml, the membrane pellet was either used immediately for MtrA-G preparation or shock frozen in liquid nitrogen before storing it at −80 °C till further use.

2.2.2. Protein preparation of the MtrA-G complex after DMMA treatment

The membranes from 10 g cells were resuspended at room temperature in extraction buffer (50 mM HEPES-NaOH pH 8.0, 10 mM $MgCl_2$, 150 mM NaCl and 2 mM dithiothreitol) to adjust the final protein concentration of 200 μg/ml followed by adding solid DMMA (2 mg/ml) to the suspension. The pH of the suspension was kept at 8.0 by adding 1 M NaOH solution while continuously stirring. When the pH became constant and almost all DMMA was dissolved, the membrane was pelleted by centrifugation at 100,000 ×*g* for 1.5 h. The membranes were then resuspended in 14 ml solubilization buffer (50 mM MOPS-NaOH pH 7.0, 10 mM $MgCl_2$, 2 mM dithiothreitol and 2.5% w/v DDM) and incubated at 4 °C for overnight. The non-solubilized membrane was pelleted by centrifugation at 100,000 ×*g* for 1.5 h. The supernatant containing the solubilized MtrA-G complex was loaded to a DEAE-sepharose column (10 ml) previously equilibrated with purification buffer (50 mM MOPS-NaOH pH 7.0, 100 mM NaCl, 10 mM $MgCl_2$, 2 mM dithiothreitol) and 0.1% w/v DDM. The proteins were eluted with a linear 150 ml gradient of 0.1–1.0 M NaCl. The fractions containing the MtrA-G complex were selected for SDS-PAGE analysis. Size exclusion chromatography on a Superose-6 column was finally performed with the purification buffer supplemented with 0.1% w/v DDM and 10% glycerol for removing any remaining impurities and soluble aggregates and to evaluate the homogeneity of the purified complex. Detergent exchange to Brij-35 was achieved by washing and eluting MtrA-G with purification buffer containing 0.2% Brij-35 on the anion exchange DEAE-sepharose column. Purification was completed with size exclusion chromatography.

2.3. Laser induced liquid beam ion desorption mass spectrometry

MtrA-G was transferred into 20 mM Tris, pH 7.0, 2 mM dithiothreitol and 0.1% w/v DDM with ultrafiltration prior to LILBID-MS analysis [17]. The spectra were recorded twice. The ion source was a commercial droplet dispenser (Microdrop), which injects microdroplets of 50 μm radius and 65 pl volume from pressure reduction apertures into high vacuum. The droplets were irradiated one-by-one by high intensity mid-IR laser pulses (wavelength ca. 3 μm) from a home-built Nd:Yag pumped $LiNbO_3$ optical parametric oscillator. The mass analysis was performed by a home-built time of flight mass spectrometer with a Wiley-McLaren-type acceleration region and an ion reflector (Reflectron). The ions with large m/z (up to ~10^6) were detected by a home-built Daly-type high mass detector. Only 200 droplets were sampled for each mass spectrum. 10 μl of sample (ca. 9.0 mg/ml) was used in each analysis.

2.4. Multi-angle light scattering

Multi-angle light scattering coupled with size exclusion chromatography (SEC-MALS) was performed on a TSKgel G4000SWxl column (Tosoh Biosciences) at a flow rate of 0.4 ml/min on a Jasco HPLC unit (Jasco Labor und Datentechnik) connected to a light scattering detector measuring at three angles (miniDAWN TREOS, Wyatt Technology) and a refractive index detector (Optilab T-rEX, Wyatt Technology). The column was equilibrated for at least 16 h with purification buffer supplemented with 0.1% w/v DDM and 10% glycerol. The buffer was filtered through 0.1 μm pore size VVLP filters (Millipore) before applying to the column. 200 μl of protein samples (ca. 1.6 mg/ml) were separated on the column. Data analysis was accomplished using the ASTRA software package 5.3.4.13 (Wyatt Technology) and data fitting was done with a Zimm's model [18]. The refractive index increment dn/dc values of the protein and of DDM were taken as 0.185 ml/g and 0.133 ml/g respectively from the literature. The extinction coefficients of individual Mtr subunits at 280 nm were calculated using ProtParam server [19] whereas that of corrinoid prosthetic group was taken from literature [20]. The sum of the extinction coefficients of individual subunits and cobalamin (866 ml/g cm^{-1}) was used in the data analysis.