The energy metabolism of *Methanomicrococcus blatticola*: physiological and biochemical aspects

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**Abstract**

*Methanomicrococcus blatticola*, a methanogenic archaeon isolated from the cockroach *Periplaneta americana*, is specialised in methane formation by the hydrogen-dependent reduction of methanol, monomethyl-, dimethyl- or trimethylamine. Experiments with resting cells demonstrated that the capability to utilise the methylated one-carbon compounds was growth substrate dependent. Methanol-grown cells were incapable of methylamine conversion, while cells cultured on one of the methylated amines did not metabolise methanol. Unlike trimethylamine, monomethyl- and dimethylamine metabolism appeared to be co-regulated. The central reaction in the energy metabolism of all methanogens studied so far, the reduction of CoM-S-S-CoB, was catalysed with high specific activity by a cell-free system. Activity was associated with the membrane fraction. Phenazine was an efficient artificial substrate in partial reactions, suggesting that the recently discovered methanophenazine might act in the organism as the physiological intermediary electron carrier. Our experiments also showed that *M. blatticola* apparently lacks the pathway for methyl-coenzyme oxidation to CO₂, explaining the strict requirement for hydrogen in methanogenesis and the obligately heterotrophic character of the organism.

**Abbreviations:** coenzyme M (HS-CoM) – 2-mercaptoethane sulfonate; CoB-S-S-CoM – heterodisulfide of HS-CoM and 7-mercaptoheptanoylthreonine phosphate (HS-CoB); F₄₂₀, – (N-lactyl-L-glutamyl)-L-glutamic acid phosphodiester of 7,8-didemethyl-8-hydroxy-5-deazariboflavine-5’-phosphate; F₄₂₀H₂ – 1,5-dihydro-F₄₂₀⁺; phenazineH₂ – reduced phenazine

**Introduction**

The methanogenic archaeon *Methanomicrococcus blatticola* is abundantly present in the hindgut of the cockroach *Periplaneta americana* (Sprenger et al. 2000). The micro-organism derives its energy for growth from reduction of methylated one-carbon compounds (methanol, monomethyl-, dimethyl- and trimethylamine) to methane with hydrogen as the obligatory electron donor.
Whereas most methanogens are autotrophs, *M. blatticola* is strictly dependent on the presence of complex organic nutrients including acetate, yeast extract and even the methanogenic coenzyme M, indicating its limited biosynthetic capabilities. The organism is a member of the order *Methanosarcinales*, which comprises the metabolically most versatile and phylogenetically most complex and advanced type of methanogens.

The ability to metabolise methylated one-carbon compounds is a common property of the *Methanosarcinales*. The biochemistry of the process is well understood for *Methanosarcina* (Figure 1) (see reviews: Thauer 1998; Deppenmeier et al. 1999; Ferry 1999; Deppenmeier 2003). The metabolism starts with the methyl group transfer from the substrate to coenzyme M (HS-CoM) to form methyl-coenzyme M (CH$_3$-S-CoM). The reactions are catalysed by multicomponent enzyme systems, each substrate requiring its dedicated methyltransferase system (Figure 1) (see for instance: Daas et al. 1996; Paul and Krzycki 1996; Wassenaar et al. 1996, 1998; Burke and Krzycki 1997; Ferguson and Krzycki 1997; Burke et al. 1998; Ferguson et al. 2000). Methane is produced by the reduction of CH$_3$-S-CoM with 7-mercaptoheptanoylthreonine phosphate (HS-CoB) as the electron donor. The heterodisulfide of HS-CoM and HS-CoB (CoM-S-S-CoB) is formed as the oxidised product. Reduction of the latter not only regenerates HS-CoM and HS-CoB for the next reaction cycle, but it is also a central step in the methanogenic energy metabolism (Figure 1). In *Methanosarcina*, CoB-S-S-CoB reduction takes place through a sophisticated membrane-bound electron transport system in which b-type cytochromes and methanophenazine, a lipophilic coenzyme, act as intermediary electron carriers (Abken et al. 1998; Deppenmeier et al. 1999; Deppenmeier 2003). Electron transfer reactions are arranged such that protons are translocated out of the cell. Hereby, a proton motive force is built up which drives ATP synthesis. The composition of the system and the role of methanophenazine seem to be typical for *Methanosarcinales*. Hydrogen may serve as the ultimate reductant of CoB-S-S-CoM and *M. blatticola* is strictly dependent on it. In the absence of

![Figure 1. Pathway of methane formation from methylated one-carbon compounds. Reactions involving the methyl group transfer from the substrates and the (membrane-bound) reduction of CoM-S-S-CoB are shown at the bottom left and bottom right parts of the Figure, respectively. The pathway of methyl group oxidation, which is apparently absent in *M. blatticola*, is shown by dashed reaction arrows. The grey area represents the cellular membrane; ovals indicate putative transporters. Abbreviations of coenzymes: HS-CoM, coenzyme M; CH$_3$-S-CoM, methyl-coenzyme M; HS-CoB, coenzyme B, 7-mercaptoheptanoylthreonine phosphate; CoM-S-S-CoM, heterodisulfide of HS-CoM and HS-CoB; MP, methanophenazine; F$_{420}$, coenzyme F$_{420}$; CH$_2$=H$_4$SPT, 5,10-methylene-5,6,7,8-tetrahydro-sarcinapterin. Enzymes (italics): MeOH-MT, MMA-MT, DMA-MT, TMA-MT, methyltransferase systems involved in CH$_3$-S-CoM synthesis from methanol, monomethyl-, dimethyl- and trimethylamine, respectively; MCR, methyl-coenzyme M reductase; HDR, heterodisulfide reductase; H$_2$ase, viologen/methanophenazine reducing hydrogenase.](image-url)