Methyl Sulfide Production by a Novel Carbon Monoxide Metabolism in *Methanosarcina acetivorans*\(^\text{\textregistered}\)

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We observed dimethyl sulfide and methanthiol production in pure incubations of the methanogen *Methanosarcina acetivorans* when carbon monoxide (CO) served as the only electron donor. Energy conservation likely uses sodium ion gradients for ATP synthesis. This novel metabolism permits utilization of CO by the methanogen, resulting in quantitative sulfide methylation.

Rother and Metcalf (18) recently described a CO metabolism for the methanogen *Methanosarcina acetivorans*. Unlike previously described CO methanogenic pathways (5, 12, 17), this metabolism produces acetate, formate, and methane but not hydrogen. We cultured *M. acetivorans C2A* by using CO as a growth substrate and observed the additional production of both dimethyl sulfide (DMS) and methanethiol (MeSH). Culturing (33.5°C) occurred under 300 kPa of CO in medium (150 ml per bottle) similar to that described by Moran et al. (16), but with 1 g/liter sodium bicarbonate and no organic substrate in 600-ml bottles. Standard bottles for analyte quantification were made in parallel but lacked the sulfide addition. Cultures were inoculated (2.0 ml) from a CO-grown preculture. At intervals throughout growth, 1.0-ml liquid samples were collected for sulfide analysis (by a technique adopted from Hach water quality test kits [Hach Company, Loveland, CO], which showed no interference with DMS or MeSH), acetate and formate analysis (by ion chromatography; Dionex LC30 and AS18 analytical column), and cell counting. Headspace samples (200 µl) were analyzed for CH₄, DMS, and MeSH by using gas chromatography (HP 5890 with a GS-Q column and flame ionization detector), and the analyte was quantified by comparison to standard bottles to determine the total moles of analyte in the bottle (combined dissolved and gaseous fractions).

Our results (Fig. 1) show that during growth, microbial DMS and MeSH production effectively scrubbed out free sulfide in the culture medium to less than 4% of the pregrowth values, suggesting sulfide methylation as the pathway for methyl sulfide formation. Enhanced growth (as measured by increased cell densities and methane production) when cultures were exposed to more sulfide suggests energy conservation by sulfide methylation (Fig. 2). Here, we used four sets of triplicate culture bottles containing preinoculation sulfide totaling 0.00, 0.16, 0.31, and 0.63 mmol per bottle.

At standard state, energy yields for MeSH production are consistent with energy conservation (calculated with data from references 1 and 19):

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\Delta G^\circ = -34.4 \text{ kJ/mol CO consumed}
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Free-energy gains by MeSH production are comparable to those previously estimated (18) for acetate formation from CO, −41.2 kJ/mol CO consumed.

CO is a known methanogenesis inhibitor in both *M. acetivorans* (18) and other methanogen species (5, 17). This inhibition likely targets the methanogenic pathway at methyl coenzyme M reductase (MCR) and would restrict carbon flow through this enzyme, leading to methyl-CoM accumulation, which would eventually stop energy production by sequestering all CoM.

Methyl sulfide formation provides a low-energy method for regenerating CoM without MCR activity. Working with *Methanosarcina barkeri*, Tallant et al. (22) demonstrated that direct methylation of MeSH to DMS has a modest energy barrier of only 0.35 kJ per reaction. When MCR is inhibited, the transfer protein (480-kDa corrinoid protein) that normally methylates CoM becomes methylated itself by methyl-CoM (4, 21) and elevated MeSH concentrations promote small-scale methylation of MeSH to DMS (15), suggesting reversibility in the first step of methanogenic DMS consumption. Thus, in instances of MCR inhibition by CO, we hypothesize that methyl sulfide formation is essential for regenerating CoM and maintaining an active metabolism for energy production. Furthermore, this occurrence suggests that the methyltransferase protein identified by Cao and Krzycki (4) is metabolically versatile and can be active in both methanogenic and non-methane-producing energy conservation, depending upon environmental conditions.

The mechanism for energy conservation during sulfide methylation is unclear. When generating methane, methanogens rely largely on reduction of a heterodisulfide bond formed by MCR activity for net energy formation (23). The observed

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methyl sulfide production likely bypasses MCR, suggesting a different pathway for energy conservation. One option is via a sodium gradient (3) linked to ATP production in another methanogen, *Methanococcus voltae* (8). The activation of CO₂, before reduction, however, consumes some of the sodium gradient produced by Mtr (6), making it unclear how effective ATP synthesis via this route would be during methyl sulfide production by *M. acetivorans*. Nevertheless, the ability of methanogens to link methyl transfer to a sodium ion gradient may permit energy conservation by sulfide methylation. In contrast, nonenergetic sulfide methylation from methyl transfer is observed in *Holophaga foetida* (9), a species with no known ability for sodium ion gradient formation.

To the best of our knowledge, the metabolism described here is the first example of both a methanogen producing high concentrations of methyl sulfides and of a CO metabolism resulting in sulfide methylation. *M. acetivorans* was isolated from marine sediments rich in decaying sea grass and kelp deposits (20). The bladders used to keep kelp upright underwater are filled with up to 12% CO (13), and their decay is a significant CO source. Under realistic marine conditions (500 m deep), the atmosphere at that time may have contained elevated CO concentrations (11), permitting sulfide methylation by the metabolism described herein. The quest for life outside our planet depends on searching for chemical signatures of life (7). If similar, early-evolving organisms are present on other planets, then methyl sulfides provide a valuable target in the search for extraterrestrial microbial life.

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