Metabolism of Reduced Methylated Sulfur Compounds in Anaerobic Sediments and by a Pure Culture of an Estuarine Methanogen†

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Addition of dimethylsulfide (DMS), dimethyldisulfide (DMDS), or methane thiol (MSH) to a diversity of anoxic aquatic sediments (e.g., fresh water, estuarine, alkaline/hypersaline) stimulated methane production. The yield of methane recovered from DMS was often 52 to 63%, although high concentrations of DMS (as well as MSH and DMDS) inhibited methanogenesis in some types of sediments. Production of methane from these reduced methylated sulfur compounds was blocked by 2-bromoethanesulfonic acid. Sulfate did not influence the metabolism of millimolar levels of DMS, DMDS, or MSH added to sediments. However, when DMS was added at ~2-μM levels as [14C]DMS, metabolism by sediments resulted in a [14CH4/14CO2] ratio of only 0.06. Addition of molybdate increased the ratio to 1.8, while 2-bromoethanesulfonic acid decreased it to 0, but did not block [14CO2] production. These results indicate the methanogens and sulfate reducers compete for DMS when it is present at low concentrations. However, at high concentrations, DMS is a “noncompetitive” substrate for methanogens. Metabolism of DMS by sediments resulted in the appearance of MSH as a transient intermediate. A pure culture of an obligately methylotrophic estuarine methanogen was isolated which was capable of growth on DMS. Metabolism of DMS by the culture also resulted in the transient appearance of MSH, but the organism could grow on neither MSH nor DMDS. The culture metabolized [14C]DMS to yield a [14CH4/14CO2] ratio of ~2.8. Reduced methylated sulfur compounds represent a new class of substrates for methanogens and may be potential precursors of methane in a variety of aquatic habitats.

Dimethylsulfide (DMS), dimethyldisulfide (DMDS), and methane thiol (MSH) are methylated reduced sulfur compounds which have been detected in sediments (1, 9, 25), brackish waters (27), and in the water column of the open ocean (2, 4, 14). DMS and MSH are formed during aerobic decomposition of cruciferous plants (13) and by anaerobic degradation of microbial mats (31) and sulfur-containing amino acids such as methionine (23, 29; R. P. Kiene and J. Visscher, manuscript in preparation). DMS can also arise as an algal excretory product associated with the breakdown of dimethylsulfonopropanoate (9, 26). Methylated reduced sulfur compounds are thought to play an important role in the transfer of sulfur from aquatic and terrestrial ecosystems to the atmosphere (2, 4, 14). However, microbial decomposition of these volatile compounds may decrease their outward flux.

Zinder and Brock (29, 30) reported that both DMS and MSH were degraded to methane and carbon dioxide by anoxic freshwater lake sediments and sewage sludge. Inhibition of this activity by chloroform suggested the involvement of methanogenic bacteria. However, pure cultures of Methanobacterium ruminantium, M. thermoautotrophicum, and Methanosarcina barkeri were incapable of forming [14CH4 from added [14C]-labeled DMS or MSH. In addition, their attempts at obtaining enrichment cultures with MSH or DMS as substrate were unsuccessful.

We now report that addition of DMS, DMDS, or MSH to anoxic sediments from a variety of aquatic habitats (e.g., fresh water, brackish salt marsh, and alkaline saline or hypersaline conditions) stimulates production of methane and that this activity is blocked by 2-bromoethanesulfonic acid (BES), a specific inhibitor of methanogenic bacteria (6). In addition, our data suggest that some degree of competition exists between methanogens and sulfate respirers for DMS in anoxic sediments when the compound is present at micromolar levels (<10 μM). Furthermore, we have isolated a methylotrophic methanogen from estuarine sediments which grows on DMS.

MATERIALS AND METHODS

Sediment types and sampling. To determine if stimulation of methanogenesis by DMS, DMDS, and MSH was a common feature of anoxic sediments, surface sediments were collected from a diverse array of aquatic environments. These included two estuarine salt marshes (Flax Pond, N.Y., and San Francisco Bay, Calif.), a freshwater lake (Searsville Lake, Calif.), and two hypersaline, alkaline lakes: Mono Lake, Calif. (salinity, 100%; pH 9.7; sulfate, 100 mM) and the pelagic sediments from the monimolimnion of Big Soda Lake, Nev. (salinity, 89%; pH 9.7; sulfate, 68 mM; sulfide, 7 mM) as well as the littoral zone sediments from the same lake (salinity, 27%; pH 9.7; sulfate, 58 mM). The littoral zone sediments from Big Soda Lake were rich in decomposing cyanobacteria (18) and had a foul mercaptan odor. In most cases, experiments were initiated within 1 h of sampling. However, in certain cases (Mono Lake and some Big Soda Lake samples) experiments were not set up until 2 to 3 weeks after sample collection (19). In these circum-

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stances, sediments were stored at ~10°C in completely filled screw-cap jars until experiments commenced.

Experiments with sediment slurries. Slurries were prepared by homogenization with an equal volume of water taken from the same collection site. Anaerobic procedures were used for the preparation as well as any subsequent manipulations (21). Slurries (volume, 15 to 30 ml) were dispensed into serum bottles (50 or 100 ml) and crimp-sealed under N2 with black butyl rubber stoppers (Bellco Glass, Inc., Vineland, N.J.). Selected bottles received substrate additions of DMS, DMDS, MSH, or the methanogenic inhibitor BES (6) or all of these at the concentrations indicated. Bottles were incubated in the dark at ~22°C with constant shaking (300 rpm). Incubation times lasted for 3 to 6 weeks. Methane in the headspace of the bottles was analyzed by gas chromatography (22), which also proved suitable for monitoring DMS and MSH.

To test the influence of sulfate upon methane production from reduced methylated sulfur compounds, sediments were collected from an intertidal mudflat in San Francisco Bay (21). Slurries were prepared by 1:1 homogenization with sulfate-free artificial bay water (21) as delineated above. The homogenate was dispensed (15 ml) into serum bottles (50 ml) containing 15 ml of artificial bay water with either sulfate (0.42 mmol per bottle, corresponding to ~15 mM final concentration in slurry) or an equivalent concentration of NaCl. We estimate the sulfate content of the “sulfate-free” bottles caused by carry-over of interstitial waters to be <1 mM (21). Slurries were then given additions of DMS (150 µmol per bottle or ~5 mM), DMDS (150 µmol per bottle or ~0.5 mM), or MSH (22 µmol per bottle or ~0.75 mM). Controls did not receive substrate additions.

San Francisco Bay salt marsh slurries were used to study the metabolism of [14C]DMS. Slurries (50 ml in 160-ml serum bottles) received 5 µCi of [U-14C]DMS (specific activity, 22 mCi/mmol; purity, 96%) (Amersham Corp., Arlington Heights, Ill.). The final added DMS concentration due to isotope addition was ~4.5 µM. Selected slurries were killed by autoclaving (121°C, 250 kPa for 30 min) or received treatments of BES (~40 mM) or NaMoO4 (~20 mM) to serve as inhibited controls. Analysis of headspace and slurry phases for 14CH4 and 14CO2 were performed by gas chromatography–gas-proportional counting (5).

Enrichment and isolation of pure cultures capable of growth on DMS. Estuarine basin salts plus vitamins (EBSV) medium (19) supplemented with DMS as the sole substrate (~10 mM) was used to enrich for DMS-degrading methanogens. After the basal salts were boiled under N2/CO2, the flask was sealed and injections of nutrient supplements (vitamin mix, dilute fatty acids, coenzyme M), cysteine-sulfide reducing agent, and sodium carbonate were made. The medium was then dispensed in 10-ml portions into Balch tubes (18 by 150 mm; Bellco Glass) or serum bottles (liquid volume, 20 ml) and crimp-sealed under N2/CO2 with black butyl rubber stoppers. Stoppers were previously treated by soaking in boiling 0.1 N NaOH to remove inhibitory volatiles. The medium was autoclaved (121°C, 250 kPa, 20 min) and cooled. DMS (ice cold) was injected into one of the tubes (chilled to ice cold) to yield a working stock solution. The stock was subsequently dispensed into medium tubes by aseptic injection through a sterile, 0.2-µm filter. All additions of test substrates (DMDS, methanol, trimethylamine, sodium acetate, sodium formate) were made in this fashion, with the exception of MSH, which was added aseptically by gaseous injection. The final pH was ~7.7. Roll tubes were prepared with addition of 1.5% Ionagar (Oxoid Ltd., London, England) to EBSV/DMS medium, following by dispensing into streak tubes (24 by 140 mm).

Enrichment cultures were obtained by inoculating EBSV/DMS medium with 0.5 ml of slurry previously incubated for ~3 weeks with DMS. The tube was placed on a rocker platform and headspace methane was monitored with time. Transfers were made every 2 weeks from methane-positive tubes. After several transfers sediment was diluted out and consistent growth occurred. The enrichment was cleansed of eubacteria by two successive transfers into EBSV/DMS medium containing an antibiotic mix (tetracycline, penicillin G, kanamycin, and vancomycin; 20 µg/ml each). This was followed by serial dilution into EBSV/DMS medium. The highest methane-positive dilution (10−5) was streaked onto roll tubes and incubated for ~4 weeks. A colony was picked from a methane-positive tube and transferred back to EBSV/DMS broth. Purity was ascertained by the uniform morphology (regular cocci, 0.7 µm in diameter) and the inability of the culture to grow in the absence of DMS on a rich medium (EBSV supplemented with 0.2% each yeast extract and tryptone, plus 1% glucose). Growth of the culture was monitored by increases in headspace methane (21), epifluorescence microscopy with acridine orange for direct counts (8), and A595 (path length, 18 mm). Thermodynamic calculations based on stoichiometries ob-
served during growth of this culture on DMS were made by using published values of free energies (28).

**Experiments with sulfate-respiring bacteria.** Two pure cultures of sulfate-respiring bacteria were examined for their ability to metabolize \([^{14}C]DMS\). *Desulfovibrio desulfuricans* (aestuarii) (ATCC 17990) and *D. salinitribens* (ATCC 14822) were grown in the lactate-yeast extract medium of Mara and Williams (15) in the presence of 10 \(\mu\)Ci of \([^{14}C]DMS\). Cultures were grown under \(N_2\) in test tubes (18 by 150 mm; see above), and the gas phase was monitored for \(^{14}CO_2\) as described previously.

**Reagents.** DMS and DMDS were obtained as liquids of 99% purity (Aldrich Chemical Co., Milwaukee, Wis.) and were stored at 5°C under \(N_2\). Odor problems were minimized by storing the reagent bottles within larger sealed bottles which contained activated charcoal. MSH was obtained as a gas in a lecture bottle (purity, 96%; Matheson Scientific Inc., E. Rutherford, N.J.). All other chemicals were of standard reagent grade.

**RESULTS**

**Sediment slurry incubations.** The results for Mono Lake were typical of most of the environments studied and are therefore presented as a representative example. Addition of 10 mM DMS or 1 mM MSH to Mono Lake sediments stimulated methanogenesis after the endogenous production ceased (Fig. 1). Stimulation by DMS was about 25-fold, and that by MSH was about 3.5-fold. By contrast, these sediments were strongly inhibited by 10 mM DMDS. Addition of BES (10 mM) plus DMS (10 mM) caused a 99% inhibition of methane formation. Similar results were obtained with BES alone (e.g., final \(CH_4 = 8\) nmol inhibited versus 800 nmol endogenous). The yield of methane recovered in substrate-amended slurries varied as a function of concentration of the substrate. In the case of Mono Lake sediment metabolism of DMS, optimal yield (20 \(\mu\)mol of \(CH_4\)) was found at 0.026 mmol of DMS per bottle (equivalent to ~1 mM). Assuming that 1 mol of DMS yields 1.5 mol of \(CH_4\) (30), this was equivalent to ~52% conversion efficiency. At higher levels of DMS, yields of methane decreased (Fig. 2).

All six sediment types were tested for their ability to produce methane from DMS, DMDS, or MSH and the results are summarized in Table 1. In the cases of DMS and DMDS, a concentration range of four orders of magnitude was examined, while a much narrower range was chosen for MSH (for practical reasons). All three methylated reduced sulfur compounds were capable of stimulating methanogenesis in each of the six sediment types tested (Table 1). The kinetics of methane production from these compounds were similar to that observed in Mono Lake (Fig. 1). The only exception was the inhibition observed for MSH in the Big Soda Lake sediments. In general, DMS produced the greatest stimulation, and estuarine salt marsh sediments responded most favorably to additions of increasingly higher levels of the compound. Conversion efficiencies of >60%

**TABLE 1.** Stimulation or inhibition of methanogenesis in sediments slurries from a variety of environments

<table>
<thead>
<tr>
<th>Substrate</th>
<th>µmol per bottle</th>
<th>% Stimulation or inhibition of methanogenesis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mono Lake</td>
<td>Big Soda Lake</td>
</tr>
<tr>
<td></td>
<td>Littoral</td>
<td>Pelagic</td>
</tr>
<tr>
<td>DMS</td>
<td>2,600–3,000</td>
<td>360</td>
</tr>
<tr>
<td></td>
<td>135–300</td>
<td>1,160</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>2,618</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>252</td>
</tr>
<tr>
<td>DMDS</td>
<td>2,600–3,000</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>26–56</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>ND</td>
</tr>
<tr>
<td>MSH</td>
<td>20–52</td>
<td>352</td>
</tr>
</tbody>
</table>

* Incubation time was 3 to 6 weeks at room temperature (22°C). Values represent the mean of three samples, and minus signs indicate inhibition.

b \[([\text{micro moles of } CH_4 \text{ formed} - \text{endogenous } CH_4 \text{ formed (micro moles)}]/\text{endogenous } CH_4 \text{ formed (micro moles)}) \times 100 = \text{percent stimulation or inhibition.}\]

^c ND, Not determined.
were sometimes observed (e.g., 63% for Flax Pond). By contrast, sediments from the alkaline lakes responded to higher DMS additions with either total inhibition or decreasing yields. Similar results were obtained for DMDS; however, this compound was highly inhibitory when added in the millimolar range. Therefore, the sediment microflora was more sensitive to DMDS than to DMS. Formation of methane from added noninhibitory levels of DMS, DMDS, or MSH was blocked when BES was included with each substrate (e.g., Fig. 1 for Mono Lake; others not shown).

The kinetics of methane formation from millimolar levels of methylated reduced sulfur compounds by San Francisco Bay sediment slurries was not affected by the presence or absence of ~15 mM sulfate (Table 2). Little endogenous activity was present in these mudflat sediments (in contrast to those from the salt marshes) and this agrees with previous observations (21). Addition of DMS, DMDS, or MSH to the slurries stimulated methane formation above the endogenous controls. However, methane production rates in slurries containing ~5 mM DMS, ~0.5 mM DMDS, or ~0.75 mM MSH were essentially the same whether or not significant amounts of sulfate were present. These results contrast with the way these sediments respond to amendment with competitive substrates, such as acetate or hydrogen (21).

To delineate the mechanism(s) by which methanogens metabolize methylated reduced sulfur compounds, we studied the degradation of DMS by sediment slurries from the littoral zone of Big Soda Lake. These sediments were rich in organic matter derived from decomposing macrophytes and cyanobacteria (18) and contained abundant gas bubbles. The

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TABLE 2. Influence of sulfate ions upon methane production from DMS, DMDS, and MSH in intertidal San Francisco Bay sediment slurries*

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Amt of methane (μmol/bottle) after incubation for (days):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Endogenous + SO₄²⁻</td>
<td>0.026 (0.001)</td>
</tr>
<tr>
<td>Endogenous − SO₄²⁻</td>
<td>0.025 (0.002)</td>
</tr>
<tr>
<td>DMS + SO₄²⁻</td>
<td>0.064 (0.012)</td>
</tr>
<tr>
<td>DMS − SO₄²⁻</td>
<td>0.075 (0.004)</td>
</tr>
<tr>
<td>DMDS + SO₄²⁻</td>
<td>0.013 (0.002)</td>
</tr>
<tr>
<td>DMDS − SO₄²⁻</td>
<td>0.014 (0.001)</td>
</tr>
<tr>
<td>MSH + SO₄²⁻</td>
<td>0.18⁴ (0.09)</td>
</tr>
<tr>
<td>MSH − SO₄²⁻</td>
<td>0.15⁵ (0.04)</td>
</tr>
</tbody>
</table>

*Results represent the mean of three experimental bottles and values in parentheses indicate one standard deviation. Slurry volume, 30 ml.

†Higher initial levels in MSH bottles were due to the presence of methane in the MSH cylinder.
generally linear of "4CO2. respiring (-68% and \( ^{14}C \)) methane. \( ^{14}C \) methanogenesis was demonstrated that this activity was stimulated by addition of DMS and inhibited by BES or autoclaving (Fig. 3A). DMS disappeared in experimental flasks over the course of the incubation; however, significant loss of DMS occurred during the first 60 h in autoclaved or BES-inhibited control flasks incubated with DMS (Fig. 3B). These findings indicate that the loss of nearly half of the added DMS can be accounted for by abiological processes, presumably by absorption into the rubber stopper (Kiene, unpublished data) as well as complexation with the sediment. No detectable quantities of DMS were found in flasks incubated without the compound. MSH had a large, transient accumulation in DMS-amended flasks (after which it eventually disappeared), while flasks containing BES plus DMS demonstrated a steady accumulation of lower levels of MSH (Fig. 3C). Flasks incubated only with the inhibitor BES also accumulated MSH.

The metabolism of DMS by the microflora of anoxic sediments was followed in short-term experiments with \([U-{^{14}C}]DMS\). Sediment slurries from a San Francisco Bay salt marsh produced both \( ^{14}CH_{4} \) and \( ^{14}CO_{2} \) from \([^{14}C]DMS\), and production of these gases occurred immediately upon addition of the radioisotope (Fig. 4). Production rates were generally linear and continued for over 48 h. Autoclaved controls did not produce any radiolabeled gases or methane, while addition of BES inhibited production of both methane and \([^{14}C]methane\). However, BES did not block production of \( ^{14}CO_{2} \). Furthermore, molybdate, an inhibitor of sulfate-respiring bacteria (22), stimulated both methane and \([^{14}C]methane\) production, while \( ^{14}CO_{2} \) was significantly inhibited (-68% at 50 h). Ratios of \( ^{14}CH_{4}/^{14}CO_{2} \) in the uninhibited flasks were quite low (0.06 at 50 h), while those containing molybdate were much higher (1.8 at 50 h). When we incubated slurries under an \( H_{2} \) rather than \( N_{2} \) atmosphere without inhibitors, we achieved ratios of ~1.4 and \( CH_{4} \) and \( ^{14}CH_{4} \) were stimulated over the endogenous (data not shown).

Experiments with pure cultures. The isolated pure culture consisted of regular cocci which grew singly, in multiple packets of cells, and in large "clumps." The cells autofluoresced when examined under UV microscopy, implying the presence of \( F_{420} \) (16). Scanning electron micrographs indicated that the culture was composed of only small (0.7 \( \mu m \)), regular-shaped cocci (C. Culbertson, unpublished data). No growth occurred when the culture was inoculated into basal salts medium supplemented with glucose (1%), yeast extract (0.2%), and tryptone (0.2%), but lacking DMS. This demonstrated that the culture did not contain contaminant eubacteria.

Methane production occurred during growth of the culture with concurrent loss of DMS and the appearance of MSH as a transient intermediate (Fig. 5A, B, and C). We calculated that levels of DMS and MSH in the gas phase represented about 21 and 28%, respectively, of the total quantity contained in the assay bottles (the difference being contained in the 20-mL liquid phase). Therefore, addition of ~15 \( \mu \)mol of DMS (-0.8 mM) to the growth culture achieved the production of 15 \( \mu \)mol of methane and the transient appearance of about 4.3 \( \mu \)mol of MSH. Due to an error in addition, the levels of DMS in the BES treatment was about five times higher than in the uninhibited culture. However, because the culture is routinely grown at ~10 mM DMS, the inhibition observed was due to BES and not to inhibitory levels of substrate. Uninoculated or BES-containing controls produced neither methane nor MSH and did not demonstrate a significant loss of DMS.
Growing cultures metabolized [14C]DMS (10 μCi added) to 14CH4 (366,630 dpm/ml) and 14CO2 (129,062 dpm/ml), achieving final ratios of ~2.83. Controls containing BES did not form any radiolabeled gaseous products and demonstrated no increase in methane or turbidity with incubation. Growth of the culture was confirmed by performing acridine orange direct counts. Cell numbers increased from 1.5 × 10⁶/ml at the time of inoculation to 2.27 × 10⁸/ml after 7 days of growth on DMS in media supplemented with 0.2% each yeast extract and tryptone. We never observed the evolution of H2 during growth on DMS.

Growth on DMS was enhanced by supplementation of the basal salts media with either 0.1 μM Selenite or yeast extract plus tryptone (Table 3). The culture was also able to grow on trimethylamine or methanol, but no growth was observed on acetate, H2 plus CO2, or formate (Table 3) or on DMDS or methionine (not shown). In addition, we were unable to obtain growth on MSH, even when we incubated the culture under an H2-plus-CO2 atmosphere.

Growth did not occur on diethylsulfide, but traces of methane were evolved (R. Oremland, unpublished data). The cultures of sulfate-respiring bacteria did not produce any 14CO2 from [14C]DMS at any phase of their growth (data not shown).

**DISCUSSION**

Methanogenic bacteria are known to metabolize only a restricted suite of growth substrates. These include hydrogen plus carbon dioxide, acetate, methanol, and formate (3). More recently, methylated amines were identified as a growth substrate for certain methanogens (7), and it was subsequently demonstrated that these compounds represent important methanogenic substrates in environments containing high levels of sulfate (20). Although DMS and MSH were previously implicated as methane precursors in sludge and freshwater sediments, attempts to confirm these experiments with pure cultures were unsuccessful (30). Subsequently, there have been no reports concerning the ability of methanogens to utilize reduced methylated sulfur compounds. However, methionine addition to sediments from Big Soda Lake (19) and San Francisco Bay (21) stimulated methanogenesis, and this activity was blocked by BES. This suggested the involvement of methanogens at some step in the conversion of methionine to methane. The methiol group of methionine has been suggested as a possible source of DMS and MSH in anoxic environments (29; Kiene and Visscher, in preparation), although these compounds can also arise from breakdown of the osmoregulant dimethylsul-
The observed and the production that indicate absorption. However, BES-inhibited unsupplemented bottles, thereby sediments. Thus, these in the syntheses. This readily showed that difficulties range of ability to degrade DMS, DMDS, and MSH to methane. However, inhibition by BES does not prove that these compounds were directly attacked by methanogenic bacteria. This question was examined more closely by further experiments with inhibitors and radioisotopes and by isolation of a methanogen capable of growing on DMS (see below).

An interesting observation was that while DMS, DMDS, and MSH could stimulate methanogenesis when added at some concentrations, they were effective inhibitors when applied at higher levels (Fig. 1; Table 1). The sensitivity of these systems to inhibition varied with the type of compound (e.g., DMDS tended to inhibit at lower levels than DMS did), as well as the type of environment (e.g., alkaline lake sediments were more sensitive than estuarine salt marsh sediments). The alkaline sediments are generally poor in metals like iron (11) which would bind $S^{2-}$ or sulphydryl groups. Thus, when testing sediments (or cultures) for their ability to degrade DMS, DMDS, or MSH to methane, a range of concentrations should be examined. It should be noted that previous studies used micromolar concentrations of $^{14}$C[DMS and $^{14}$CJSMH and thus did not encounter these difficulties (30). Unfortunately, these radioisotopes are not readily available from manufacturers without expensive custom syntheses.

Degradation of DMS to methane by Big Soda Lake sediment slurry was a function of the metabolism of methanogenic bacteria (Fig. 3). Although significant loss of DMS occurred in the BES plus DMS controls, similar loss was observed in the autoclaved sediments (Fig. 3B). This loss was therefore due to nonbiological complexation and absorption. However, DMS totally disappeared from the experimental bottles, thereby implicating methanogens in the removal process. Metabolism of DMS resulted in the appearance of MSH as an intermediate (Fig. 3C). Formation of MSH was much lower in the BES plus DMS controls and did not decline as did the uninhibited samples. These results indicate that methanogens are responsible for both the production and the consumption of MSH during metabolism of DMS. Finally, the appearance of some MSH in the unsupplemented, BES-inhibited bottles indicates that MSH may be a natural precursor of some of the methane formed in these sediments. Thus, about 0.9 μmol of MSH was detected in the BES-inhibited flasks, while endogenous production yielded about 1.5 μmol of methane. Assuming that 4 mol of MSH yields 3 mol of methane (30), this results in at least 30% of the observed methane arising from MSH.

The lack of any noticeable influence of sulfate ions upon the metabolism of DMS, DMDS, or MSH to methane (Table 2) indicates that they are candidates for noncompetitive methanogenic precursors. These results closely resemble those obtained for trimethylamine, methanol, and methionine additions to these same, low endogenous activity sediments (21). However, experiments with $^{14}$C[DMS yielded results which implicated that sulfate-respiring bacteria may also compete with methanogens for DMS (Fig. 4). Although addition of BES stopped $^{14}$CH$_4$ formation, it did not block that of $^{14}$CO$_2$. Furthermore, use of molybdate to inhibit sulfate respirers enhanced $^{14}$CH$_4$ production while lowering that of $^{14}$CO$_2$. Finally, the low ratio of $^{14}$CH$_4$/$^{14}$CO$_2$ (~0.06) produced in the uninhibited samples was far below the 3:1 ratio predicted for DMS metabolism by methanogens (30). These results are consistent with the notion that DMS is ecologically similar to methanol, in that at high concentrations (approximately millimolar) it is a noncompetitive methanogenic precursor (21), but at lower environmental levels (micromolar), sulfate reducers have increased affinity (a lower $K_{m}$) for it (12). This explains the high conversion efficiencies we observed in some of our substrate addition experiments (Fig. 2; Table 1) as well as the low $^{14}$CH$_4$/$^{14}$CO$_2$ ratios in the radioisotope experiments (Fig. 4).

A note of caution must be added to the $^{14}$C[DMS experiments with inhibitors. Because sulfate reducers are also the primary hydrogen sink in these sediments (22), the enhanced $^{14}$CH$_4$/$^{14}$CO$_2$ ratio (1.8) may have been due in part to increased CO$_2$ reduction on the part of the methanogens taking place in the absence of sulfate reduction. Indeed, when we incubated slurry under $H_2$ instead of N$_2$, we achieved ratios of ~1.4. Freshwater sediments yielded 7:1 ratios for DMS metabolism (30), and we have observed 2:1 ratios for the littoral zone sediments of Big Soda Lake (R. Kiene and R. Oremland, unpublished data). To further unravel this question, it was necessary to work with pure cultures of methanogens and sulfate respirers. When we added $^{14}$C[DMS to pure cultures of sulfate respirers growing on lactate, we never observed formation of $^{14}$CO$_2$. Therefore, these organisms are incapable of metabolizing DMS under the conditions attempted. However, since this represents a very limited screening, the possibility still remains that some type of sulfate respirer can metabolize DMS.

The methanogenic isolate from San Francisco Bay was able to grow on DMS as a sole energy source. The organism was also capable of growth on methanol or trimethylamine, but not $H_2$ plus CO$_2$, acetate, or formate (Table 3). These substrate affinities classify the organism as an obligately methylotrophic methanogen, similar to Methanococoides methylutens (24). However, preliminary physiological studies indicate that our culture represents a novel species, and we are conducting further work to define this organism's characteristics (Kiene and Oremland, unpublished data). Growth was stimulated by 0.1 μM selenite, as has been shown for other methanogens (10), as well as by addition of yeast extract plus tryptone (Table 3).

Growth of the culture on DMS resulted in the consumption of this substrate as well as the transient appearance of MSH as an intermediate (Fig. 5). Therefore, MSH must be both produced and consumed as a consequence of DMS metabolism. These results reinforce the interpretation of the sediment DMS experiments with BES (Fig. 3). However, the culture was unable to grow on MSH (or on MSH plus $H_2$), but nonetheless was capable of metabolizing it during growth
on DMS. We propose the following reaction sequence for the methanogenic conversion of DMS by the pure culture:

\[(CH_3)_2S + 2H^+ + 2e^- → CH_4 + CH_3SH \]

\[ΔG^0_f = -72.8 \text{ kJ/mol} \]

\[CH_3SH + H_2O → 0.5CH_4 + 0.5CO_2 + H_2S + 2H^+ + 2e^- \]

\[ΔG^0_f = -1.0 \text{ kJ/mol} \]

\[\text{Sum: } (CH_3)_2S + H_2O → 1.5CH_4 + 0.5CO_2 + H_2S \]

\[ΔG^0_f = -73.8 \text{ kJ/mol} \]

This sequence allows for the appearance of MSH as a transient intermediate and achieves a final CH_4/CO_2 ratio of 3.0, which is close to the value we observed in the culture radioisotope experiments. At this time we are uncertain as to why we attained only a 2.8 ratio, but it is possible that the excess reducing power was used for cell synthesis. Because of the poor energy yield associated with the second reaction, the proposed reactions explain the inability of the organism to grow on MSH. Therefore, the organism can convert MSH to methane, provided there is a growth substrate present. The question then arises as to why the organism should metabolize MSH at all. One possibility is that the cells require the generated reducing equivalents to carry out the first reaction, or they must rid themselves of MSH before it accumulates to toxic levels. The biochemistry of methanogenesis from methylated reduced sulfur compounds should be an interesting area for future research. Biochemical studies will help to test the validity of our proposed reaction sequence.

The stimulation of methanogenesis by addition of either DMS or MSH to sediments (Fig. 1; Table 1) therefore appears to be a consequence of direct attack by methanogens on these compounds. However, the culture was incapable of growth on DMDS or MSH, even though these compounds stimulated methane formation in sediments. Most likely, DMDS must first be converted to MSH or DMS by either chemical or microbiological reactions occurring in the sediment which do not involve methanogens (Kiene, manuscript in preparation). In addition, MSH can be converted to DMS in anoxic sediments (Kiene, in preparation), thereby complicating the picture of the microbial pathways these compounds enter within sediments.

In summary, we have extended the earlier observations of Zinder and Brock (29, 30) and have demonstrated that reduced methylated sulfur compounds stimulate the formation of methane by a wide variety of sediment types. At least one of these compounds (DMS) can support growth of a pure culture of a methanogenic bacterium, a fact which was previously unreported. Furthermore, our study suggests that sulfate-respiring bacteria may contribute to the metabolism of DMS in sediments. Our results imply that, in certain environments such as Big Soda Lake, MSH and DMS may be of significance as in situ methanogenic precursors. Future studies directed at ascertaining the significance of reduced, methylated sulfur compounds as methane precursors in the environment should prove to be of interest.

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