Production and Consumption of Dimethylsulfoniopropionate in Marine Microbial Mats

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The fate of dimethylsulfoniopropionate (DMSP), a major sulfonium compound in marine ecosystems, was examined in *Microcoleus chthonoplastes*-dominated microbial mats. Chemical decomposition of DMSP was observed under laboratory conditions at pH values higher than 10.0. pH profiles measured in situ showed that these highly alkaline conditions occurred in microbial mats. Axenic cultures of *M. chthonoplastes* contained 37.3 μmol of DMSP g of protein⁻¹, which was partially liberated when the cells were subjected to an osmotic shock. DMSP-amended mat slurries showed a rapid turnover of this compound. The addition of glutaraldehyde blocked DMSP decrease, indicating biological consumption. Populations of potential dimethyl sulfide consumers were found in the top 10 mm of the mat.

One of the major sulfonium compounds in marine environments, dimethylsulfoniopropionate (DMSP), is present in macroalgae (4, 28, 40), phytoplankton (17, 40), higher plants (6), and phototrophic prokaryotes (17, 40). It acts as a precursor of methylated sulfur species, such as dimethyl sulfide (DMS) (5, 19, 23) and thiols (21, 22). In the water column, DMSP and DMS are found during blooms of *Phaeocystis pouchetii* (1, 12) and blooms of *Ceratium lineatum*, *Cyclococcus leptoporus*, and *Gyrodinium aureolum* (35). DMS is produced in pure cultures of the phytoplankton species *Hymenomonas carterae* (36) and *Gymnodinium nelsonii* (7). In marine sediments, DMSP might originate from *Spartina* leaves (6) or macroalgae, like *Ulva lactuca* (9, 10) and *Enteromorpha intestinalis* (11), which are occasionally washed onto the sediment. Recently, Keller et al. (17) studied the DMSP content of algae but did not include benthic species. The concentrations of DMSP in the pelagic species surveyed varied significantly among different species, ranging from 0.01 to 2,200 μmol of DMSP per cm³ of cell volume.

Two routes of DMSP degradation have been described. During the first one, DMSP is cleaved into DMS and acrylate (39):

conceivably followed by rapid microbial consumption of the products (21). The second pathway involves two demethylation steps during which 3-methiolpropionate and 3-mercaptopropionate (3-MPA) are formed, respectively (21):

in which XH and YH represent methyl group acceptors.

The first pathway (equation 1) involves either a chemical dehydration at elevated pH (pH > 13) (40) or an enzymatic reaction of DMSP-lyase. This enzyme was found to be

present in both marine phytoplankton (3, 16) and bacteria (19, 39). Experiments with anoxic salt marsh slurries indicated the involvement of both sulfate-reducing bacteria and methanogenic bacteria in the decomposition of DMSP via this pathway. In the second pathway (equations 2 and 3), sulfate-reducing bacteria are involved (22), but methylo-trophic bacteria and methanogenic bacteria are likely candidates as well.

The presence of DMS and DMSP-like compounds has been reported for various marine ecosystems (14, 18, 19, 30) including microbial mats (2, 37). Consumption of DMS was shown for several bacteria (34) which can also be found in these laminated marine ecosystems, including colorless sulfur bacteria (37), phototrophs (38, 41), and methanogens (20, 27). Similarly, acrylate and 3-MPA are anticipated to be metabolized by microbes present in these sediment ecosystems (22). The scope of the present study is to evaluate the production and (biotic and abiotic) consumption of DMSP in microbial mats, both under oxic and anoxic conditions.

MATERIALS AND METHODS

Field measurements and sample collection. Sediment samples were collected in June 1990 and March 1991 from a microbial mat, located in the supralittoral zone at the Frisian Island of Texel, The Netherlands (55°08'N, 4°50'E). pH profiles were measured in situ by using a combination pH needle electrode (Diamond General, Ann Arbor, Mich.) attached to a micromanipulator (MM33; Plato, Amsterdam, The Netherlands) and connected to a Schott meter (CG 817). Depth profiles of chlorophyll *a* (Chl*a*) and most-probable-number counts of sulfate-reducing bacteria, chemolithotrophic sulfur bacteria, and anoxygenic phototrophic bacteria were determined in the upper 50 mm of the mat. Sediment samples for most-probable-number incubations were stored on ice and processed within 1 h, while cores for Chl*a* analysis were stored at -20°C until processed. Viable populations were determined after incubation in liquid carbonate-buffered (19 mmol liter⁻¹) mineral medium (37) to which an equal amount of filtered and autoclaved seawater was added. The mineral medium was supplemented as follows (values indicating the final concentrations). For sulfate reducers, 28 mmol of sulfate liter⁻¹, 5 mmol of lactate liter⁻¹, and 5 mmol of acetate liter⁻¹ were added as sub-

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strates and FeSO_4 ($0.2 \text{ mmol liter}^{-1}$) was added to visualize sulfide production. Inoculated tubes were incubated anoxically. The medium for counting chemolithotrophic bacteria was supplemented with thiosulfate ($10 \text{ mmol liter}^{-1}$), FeSO_4 ($7.2 \text{ } \mu\text{mol liter}^{-1}$) and bromocresol blue to indicate acidification of the medium, while incubation took place in the presence of oxygen. The medium for anoxygenic phototrophs was supplemented with hydrogen sulfide ($1.6 \text{ mmol liter}^{-1}$) and incubated in the light under anoxic conditions. After 6 weeks of incubation at room temperature, growth was determined in three replicates and scored by the method of De Man (8).

Slurry experiments. The upper 20 mm of the mat was used for slurry preparation. The cyanobacterial layer (top 6 mm) was removed, and the remaining sediment was homogenized with sterilized, aged seawater (1 part sediment and 3 parts seawater). Anoxic incubations were handled under a flow of oxygen-free nitrogen gas. The slurries were preincubated for 24 h before the inhibitors were added and then incubated for another 24 h prior to DMSP addition (final concentration, $600 \text{ } \mu\text{mol liter}^{-1}$). Sodium molybdate (MoO_4^{2-} ; final concentration, $20 \text{ mmol liter}^{-1}$) was used to inhibit sulfate-reducing bacteria, and 2-bromoethanesulfonic acid (BES; final concentration, $10 \text{ mmol liter}^{-1}$) was used to inhibit methanogenic bacteria (23, 26), while phosphate-buffered glutaraldehyde (pH 7.5; final concentration, 0.5% [vol/vol]) was used to stop microbial activity. After the addition of DMSP, the slurries (10 ml) were transferred to glass vials (26 ml) and either sealed with Teflon-lined caps (anoxic incubations) or cotton plugs (oxic incubations). Slurries (pH, 7.5 to 7.7; salinity, 30‰) were either incubated in a rotary shaker or stirred continuously. The development of DMS and CH_4 was monitored in two replicate vials by headspace analysis. Subsequently, the residual DMSP was measured after the addition of 4 ml of NaOH ($10 \text{ mol liter}^{-1}$). The increase in DMS was evaluated after hydrolysis for 1 h at room temperature.

Chemical hydrolysis experiments. A stock solution of DMSP was diluted to a final concentration of $270 \text{ } \mu\text{mol liter}^{-1}$ in sterile sodium bicarbonate buffers ($50 \text{ mmol liter}^{-1}$) of pH 9.6, 10.0, and 10.5. Chemical decomposition at 20°C in the dark was monitored over time by headspace analysis of DMS in two replicate bottles.

Experiments with axenic cultures of *M. chthonoplastes*. *Microcoleus chthonoplastes* 11 was isolated by Lucas J. Stal from a laminated microbial ecosystem from the Frisian Island Mellum (Germany). Axenic cultures were obtained by using a combination of antibiotics and single-filament isolation from soft-agar plates (29). Culture conditions applied and composition of the ASN III medium used were described by Stal and Reed (32). Cultures were subjected to osmotic shocks by transfer from a 33‰ medium to an 8‰ medium.

Analytical procedures. Protein was measured by using the Folin phenol reagent (24) in the supernatant after hydrolysis of the cells in NaOH. Sediment cores were sliced and analyzed spectrophotometrically for Chla and bacteriochlorophyll *a* (BChla) in methanol extracts (32). DMS, methane thiol, and methane were determined by headspace analysis on a Packard 427 gas chromatograph with a Porapak R (80/100 mesh) column and flame ionization detector (21, 37). Retention times were 30 s for CH_4 , 170 s for methanethiol (MSH), and 390 s for DMS. Peak areas were obtained with a Shimadzu C-R6A integrator. Detection limits of the analysis were $1 \text{ } \mu\text{mol liter}^{-1}$ for CH_4 and $0.5 \text{ } \mu\text{mol liter}^{-1}$ for MSH and DMS.

FIG. 1. Depth profiles of Chla (indicated by bars) and pH (indicated by symbols) measured in the top 30 mm of a microbial mat from Texel, The Netherlands. Symbols: ●, 0800 h (am) profile; ■, 1800 h (pm) profile.

Reagents. DMSP as a chloride salt (purity of >98%) was obtained through Research Plus (Bayonne, N.J.), DMS (analytical grade) was from Merck (Darmstadt, Germany), and MSH (purity of >98%) was from Fluka (Buchs, Switzerland). All other reagents were analytical grade.

RESULTS

Microbial mat characteristics. In order to characterize the microbial mat, the vertical distribution of photopigments was established and pH profiles were measured at the beginning and end of the light period. The Chla concentration was the greatest in the layer from 2.5 to 5.0 mm ($0.825 \text{ mg per cm}^3$ of sediment) (Fig. 1). BChla peaked at $0.445 \text{ mg per cm}^3$ of sediment (data not shown). Microscopy revealed that *M. chthonoplastes* was the dominant cyanobacterial species, while the distinct purple layer consisted mainly of *Thiocapsa roseopersicina* (37). The morning pH profile (Fig. 1) displayed only a slight elevation in the cyanobacterial layer, but toward the end of the photosynthetic period, a considerable increase was observed (Fig. 1). During the late afternoon, the pH peaked at 10.6 in the *Microcoleus* layer, presumably as a result of extensive CO_2 fixation.

The vertical distribution of functional groups of sulfur bacteria (Fig. 2) showed similar profiles for sulfate reducers, colorless sulfur bacteria, and anoxygenic phototrophs, all of which are potential DMS consumers. These functional groups developed the highest cell number close to the sediment surface; however, the maximum total numbers of cells per cubic centimeter of sediment varied from 2×10^9

FIG. 2. Vertical distribution of anoxyphototrophic bacteria (left), autotrophic colorless sulfur bacteria (center), and sulfate-reducing bacteria (right). Error bars indicate 95% confidence intervals.

for colorless sulfur bacteria, 1×10^8 cells for sulfate reducers, and 1×10^7 for phototrophs.

Chemical decomposition of DMSP. The abiotic release of DMS from DMSP (final concentration, $270 \mu\text{mol liter}^{-1}$) in buffered solutions (pH 9.6, 10.0, and 10.5) at 20°C was monitored over time (Fig. 3). At pH 9.6, approximately 1% of the DMSP was hydrolyzed after 24 h of incubation. At pH 10.0, a slightly larger fraction was converted to DMS ($\approx 4\%$ DMSP after 24 h), but at pH 10.5, a significant amount of the added DMSP was transformed to DMS. After 1 h 0.5% was hydrolyzed, while within 24 h 15% was converted to DMS. At pH 10.0 and 10.5, the release of DMS from DMSP appeared to be linear over time (Fig. 3). The standard deviation between the two replicate was less than 5% at all sampling times.

FIG. 3. Time course of chemical hydrolysis of DMSP (initial concentration, $270 \mu\text{mol/liter}$) at different pH values.

Production of DMSP by *M. chthonoplastes*. The presence of DMSP within *M. chthonoplastes* cells was tested in six replicate axenic cultures, which were treated with NaOH. After DMS was measured, protein was determined and the specific DMSP content, which ranged from 36.4 to $38.1 \mu\text{mol g of protein}^{-1}$, was assessed. In order to investigate the release of intracellular DMSP, two replicate cultures were subjected to an osmotic shock by transfer from a 33‰ medium to an 8‰ medium. Aliquots of the culture were taken by filtration through a $0.45\text{-}\mu\text{m}$ -pore-size membrane filter every hour during 6 h and analyzed for DMSP (Fig. 4). After an initial lag of 1 to 2 h, DMSP was released into the medium and reached a plateau after 4 h, when approximately 50% of the DMSP had been excreted. The standard deviation between the duplicates was less than 8% throughout the experiment.

Consumption of DMSP in microbial mat slurries. The time course of added DMSP (final concentration, $600 \mu\text{mol liter}^{-1}$) consumption was monitored in slurries to which either glutaraldehyde, MoO_4^{2-} , or BES was added, while the control treatments (oxic and anoxic) received only DMSP. All treatments except for the glutaraldehyde-poisoned slurries displayed a rapid decrease of the added DMSP (Fig. 5A), indicating biological activity as the major contributor to DMSP consumption. Glutaraldehyde treatments did not accumulate DMS or CH_4 .

Under anoxic conditions, the DMSP consumption rate did not differ significantly between the control and BES treatment, with a rate of approximately $60 \text{ nmol of DMSP ml of slurry}^{-1} \text{ h}^{-1}$, while consumption in the MoO_4^{2-} -added slurries was much slower (approximately 50%). The addition of BES, which selectively inhibits methanogens (26), caused an initial accumulation of DMS, which disappeared as the experiment continued (Fig. 5B). Methane production was not observed in the BES-added slurries. In slurries to which MoO_4^{2-} was added in order to inhibit sulfate reducers (26)

FIG. 4. Time course of DMSP release by *M. chthonoplastes* 11 after an osmotic shock (33 to 8‰) at $t = 0$. The dashed line indicates the specific DMSP content when the organism was grown at 33‰.

and in controls, DMS did not accumulate (Fig. 5B), but methane did (Fig. 5C).

Under oxic conditions, DMSP consumption was slower and exhibited a rate of $17 \text{ nmol of DMSP ml of slurry}^{-1} \text{ h}^{-1}$, and complete consumption of DMSP was observed after 48 h (data not shown). In the presence of oxygen, DMS accumulated during the initial 18 h of the experiment and disappeared thereafter (Fig. 5B), while methane could not be detected. Methane thiol could not be observed in any of the treatments. At each sampling time, the standard deviations in slurry experiments were less than 12% between replicate vials.

DISCUSSION

Abiotic hydrolysis of DMSP was first described by Challenger and Simpson (4) and was reported to occur in highly alkaline solutions (3, 40). At pH values commonly encountered in the marine environment, DMSP is thought to be relatively stable: a half-life of 8 years was calculated for this sulfonium compound in seawater at pH 8.2 and 10°C (5). However, during photosynthesis, the pH in the microbial mat increased to >10.5 (Fig. 1) as a result of carbon dioxide depletion. At pH values of >10.0 , chemical DMSP hydrolysis was observed in laboratory experiments (Fig. 3). Particularly in combination with the removal of the DMS or acrylate formed, abiotic degradation at elevated pH values could contribute significantly to DMSP turnover in sediment ecosystems. Whether the process of chemical degradation of DMSP due to local pH increases in open ocean systems plays a significant role in the function of a DMSP sink remains obscure, but given the relative low ambient DMSP concentrations (35), this is expected to be of minor importance. Similarly, the internal pH of 9.3 reported for *Phaeocystis* colonies (25) is not sufficient to support significant chemical hydrolysis of DMSP.

Axenic cultures of *M. chthonoplastes* contained significant amounts of DMSP: the specific content of 36.4 to $38.1 \text{ } \mu\text{mol g of protein}^{-1}$ found in this study is substantially higher than reported for *Microcoleus lyngbyaceus* ($0.36 \text{ } \mu\text{mol g of protein}^{-1}$ [40]). Although the specific content could be expected to vary with the culture conditions employed, it is unlikely that this accounts for the observed

FIG. 5. Effects of inhibitors and poison on consumption of DMSP in microbial mat slurries amended with DMSP. Time course of DMSP consumption (A) and production of DMS (B) and CH_4 (C). The solid lines indicate anoxic incubations, and the dashed lines indicate oxic incubations. Each value represents the average of two replicates, and the standard deviation was less than 12%.

difference between these *Microcoleus* species. Other osmolytes, like glucosylglycerol and trehalose, were also reported in *M. chthonoplastes*-dominated mats (31). In this study, $20 \text{ } \mu\text{mol of DMSP g of protein}^{-1}$ was released to the medium, when *M. chthonoplastes* was subjected to a salinity shock from 33 to 8‰. A similar DMSP release can be expected during heavy rainfalls to which microbial mats are exposed regularly, especially those located in the supralittoral zone. In contrast, during wind-induced evaporation,

the salinity of the pore water might increase to $>100\%$ (36a). Hence, under natural conditions, both the specific content and actual release of DMSP can be expected to fluctuate. Earlier studies showed release of DMS and DMSP by macroalgae and phytoplankton species. For example, the DMSP content in *Ulva lactuca* tissue decreased from 110 to $90\ \mu\text{mol ml}^{-1}$ within a few hours when the salinity was decreased from 30 to 10% (10). In cells of the coccolithophorid phytoplankton species *Hymenomonas carterae*, the specific DMSP content increased to a maximum value of 3.80 ng of DMSP per 1,000 cells when the salinity of the culture medium was increased from medium containing 15 to 70% (36). DMS was liberated within 3 to 4 h upon transfer of the cells from 35 to 17.5% .

Metabolism of DMSP in anoxic slurries occurred rapidly and was influenced by the addition of inhibitors. Glutaraldehyde largely inhibited DMSP consumption; biological activity hence played a dominant role in the degradation of this sulfonium compound at pH values of <10.5 . The slight decrease of DMSP in the presence of glutaraldehyde could be due to chemical processes (23). Interestingly, DMSP consumption under anoxic conditions was more rapid than under oxic conditions. In the anoxic BES-treated slurries, DMS accumulated, which suggested that methanogens were important DMS consumers in the mat slurries. However, eventually DMS was consumed completely, even in the presence of BES. DMS accumulation was also observed in controls under oxic conditions in which a slow but steady increase was found for 18 h, after which DMS decreased again, possibly also a result of chemical reaction with oxygen or diffusion through the cotton plug. This observation could imply that not only DMSP but also DMS is consumed more slowly in the presence of oxygen. The slower consumption under these conditions could also be due to removal of the cyanobacterial layer in which an important fraction of the aerobic microorganisms is expected to be present. However, not all aerobes were removed and during the 48 h of preincubation of the slurries, this population could have been restored.

Similar experiments with DMSP additions were performed with anoxic sediment slurries from a *Spartina*-dominated salt marsh (23). Accumulation of DMS from DMSP was observed after 2 h, and the addition of BES or MoO_4^{2-} did not influence the rate of DMS formation. Methane had accumulated significantly after 10 days. From data obtained in comparable inhibitor experiments, it was concluded that consumption of amended DMS was mainly carried out by methanogens and sulfate reducers (18). Kiene et al. (20) suggested that when DMS is present in relatively high concentrations, it might be a noncompetitive substrate for methanogenic bacteria, which is supported by observations in the present study. Degradation of DMSP under anoxic conditions was also observed in sea grass-dominated sediments, in which 3-MPA was formed in addition to DMS and acrylate (21). Consecutively, acrylate might either be metabolized to acetate and propionate (22), presumably by clostridia (39), or react with hydrogen sulfide to form 3-MPA (21). In the present experiment, 3-MPA was not analyzed; hence, further investigations are needed to reveal the contribution of demethylation and DMS cleavage in DMSP degradation in benthic marine ecosystems.

Viable counts of chemolithoautotrophs in the presence of oxygen showed large numbers in the top layer of the microbial mat (Fig. 2). Some of these organisms are capable of metabolizing DMS (37). Methylotrophic hyphomicrobia are also reported to consume DMS (33) and possibly utilize

methyl groups of DMSP. However, these organisms are only sporadically found in marine environments (15). The consumption of DMS in the absence of oxygen could be due to activity of sulfate reducers, methanogens, and phototrophs (18, 23, 27, 38, 41), while DMSP could be consumed by sulfate reducers or acetogens (13). In the anoxic controls and MoO_4^{2-} -treated cells, methane was found to accumulate after 6 h, indicating metabolic activity of methanogens in the microbial mat slurries. The consumption of both DMSP and DMS in the presence of oxygen might be explained by the presence of a potential aerobic DMS-consuming population of 10^5 cells per cm^3 of sediment reported for the same microbial mat (37).

In summary, DMSP was found in axenic cultures of *M. chthonoplastes*, which was the dominant cyanobacterium in the microbial mat studied in this experiment. The release of DMSP was observed after cells were exposed to an osmotic shock. At elevated pH values, chemical hydrolysis of DMSP might occur, but slurry experiments showed that biological consumption played a dominant role in the degradation of this sulfonium compound at pH and salinity values regularly encountered in situ.

Estimates of the contribution of microbial mats to the global sulfur flux are presently lacking and might not be very important. However, the relatively high standing concentrations of DMS and DMSP (37) and large numbers of potential DMS(P)-consuming microorganisms, all occurring on a small scale, make these marine microbial mats a useful system to study.

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