APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1993, p. 2723–2726
0099-2240/93/082723-04$02.00/0

Vol. 59, No. 8

Effects of Methylated, Organic, and Inorganic Substrates on Microbial Consumption of Dimethyl Sulfide in Estuarine Waters

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Received 12 January 1993/Accepted 1 June 1993

We examined the effects of a variety of amendments on the consumption of [U-^{14}C]dimethyl sulfide in a Georgia salt marsh. Methylated compounds, particularly those with dimethyl groups, significantly inhibited dimethyl sulfide consumption, while nonmethylated substrates had little effect. Dimethyl disulfide and dimethyl ether were the most effective inhibitors tested.

Biogenic dimethyl sulfide (DMS) is a major source of sulfur to the marine troposphere and plays a possibly important climatic role via the formation of marine tropospheric aerosol and cloud condensation nuclei (3). While the sea-air flux of DMS determines its climatic impact, it appears that only a small fraction of the DMS produced in the ocean actually escapes to the atmosphere (10, 19). Other sinks for DMS include photooxidation to dimethyl sulfoxide (2) and, in particular, biological consumption of DMS within the marine euphotic zone, which may be the dominant sink for DMS in some instances (10).

Previous measurements of DMS consumption in ocean water samples by several methods have been reported. Kiene (8, 10, 11) used chloroform to inhibit DMS consumption and Wolfe (19) used [^{14}C]DMS to measure its conversion to cell material and CO_2 in whole-seawater samples. These studies showed that DMS is rapidly consumed, with turnover times of hours to days, and recent work (20) suggests that DMS consumption kinetics may be attuned to low nanomolar concentrations, saturating by 10 or 20 nM.

Previously isolated aerobic DMS consumers were either chemolithotrophic *Thiobacillus* spp. (5) or methylotrophic *Hyphomicrobium* spp. (4, 17). Additionally, a *Pseudomonas acidovorans* isolated from a peat biofilter cometabolized DMS but could not use it as a carbon source (21). Methanogens (14, 16) and phototrophs such as a *Thiocapsa* sp. (18) which can grow on DMS anaerobically have been isolated.

In this study, the radioisotope method was used along with a variety of chemical amendments (0.1 to 1,000 μM) to explore which compounds inhibit DMS consumption in whole-water samples from coastal Georgia. Potential methanotrophic and methylotrophic substrates included (i) methylene chloride (CH_2Cl_2), chloroform ($CHCl_3$), and carbon tetrachloride (CCl_4); (ii) methanol, paraformaldehyde, and formate; (iii) mono-, di-, and trimethylamines and trimethylamine *N*-oxide; (iv) the gases acetylene (C_2H_2) and dimethyl ether [$(CH_3)_2O$]; (v) several related sulfur compounds, including dimethyl disulfide [DMDS; $(CH_3)_2S_2$], carbon disulfide (CS_2), and dimethyl sulfoxide [$(CH_3)_2SO$]; and (vi) methylated organic compounds, including dimethylsulfoniopropionate (DMSP), *S*-methyl methionine, DL-methionine, choline, glycine betaine, and *N*,*N*-dimethyl glycine (Fig. 1). Also tested were several common heterotrophic substrates (glucose, acetate, glycine), and several inorganic substrates, including ammonium, nitrate, and thiosulfate.

Samples were collected from the Marsh Landing dock on the Duplin River, a saltwater tidal creek which separates Sapelo Island from mainland Georgia. [U-^{14}C]DMS [$(^{14}CH_3)_2S$, 22.3 mCi mmol^{-1}) was obtained from Amersham Co. (Arlington Heights, Ill.). [^{14}C]DMS stocks were prepared and experimental additions were taken from the headspace fraction with a gastight syringe after equilibration at room temperature (21°C). Injection volumes were 40 μl into a 60-ml sample, for final concentrations of 2.7 nM [^{14}C]DMS, similar to the ambient concentrations of unlabeled DMS in these waters. Short (1- to 3-h) incubations were in 70-ml serum bottles sealed with Teflon-lined septa, which were kept in the dark within 1°C of in situ water temperatures (27 to 31°C). DMS consumption was determined as the production of labeled CO_2 and particulates (cell material). Sample collection, incubation procedures, and processing are described in detail elsewhere (20).

Inhibitor substrates were of the highest purity available (98 to 99.9%) and were prepared either gravimetrically or by dilution. For the chlorinated methanes, DMDS and CS_2, water-saturated solutions were first prepared, and then dilutions were made to give approximate concentrations. Typical solution strengths were 100 and 1 mM, and typical addition volumes were 6 to 600 μl into a 60-ml sample, for a final concentration range of 0.1 to 1,000 μM. Most compounds were tested at four concentrations: 0.1, 10, 500, and 1,000 μM, but several compounds (chlorinated methanes, dimethyl ether) were examined in greater detail. Each inhibitor concentration was a single bottle result, but some compounds (dimethyl ether) were tested on more than one occasion. Acetylene was prepared by the CaC_2-H_2O reaction. A gastight syringe was used to remove a portion from the acetylene gas stream generated during the reaction as it flowed through silicone tubing. Acetylene and dimethyl ether volumes were added in ranges from 0.1 to 10% of the headspace volume (about 10 ml), yielding additions of 10 to 1,000 μl and final water concentrations of approximately 7.4 to 740 μM at standard temperature and pressure.

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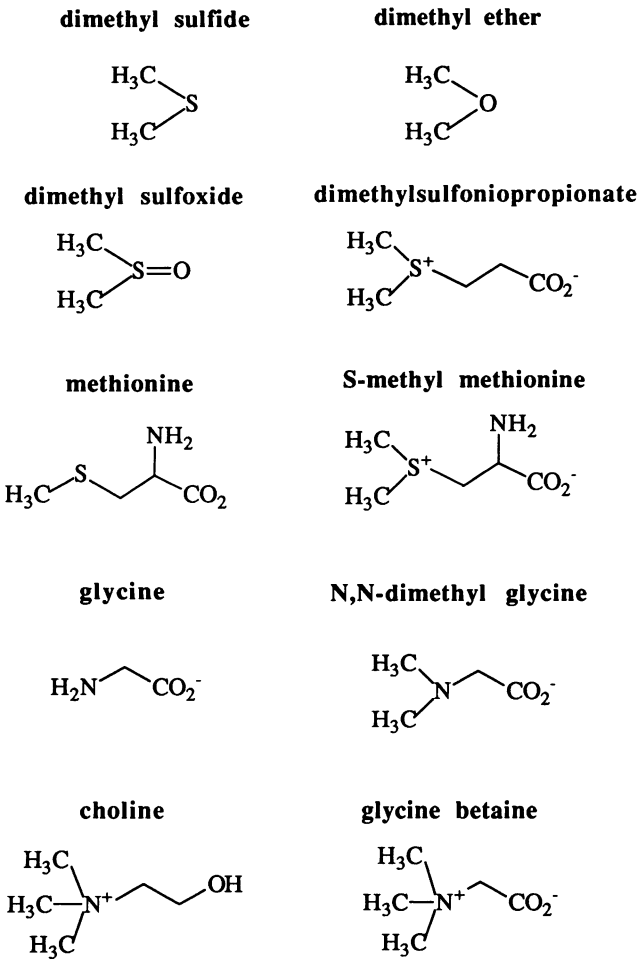

FIG. 1. Chemical structures of methylated substrates tested.

TABLE 1. Concentrations of compounds necessary to inhibit DMS consumption by 90% for all substrates tested

Substrate group	Compound	Concn to achieve 90% inhibition
Chlorinated methanes	CH_2Cl_2	50 μM
	$CHCl_3$	100 μM
	CCl_4	500 μM
Methane oxidation series	CH_3OH	>1 mM
	CH_2O	1 mM
	CH_2O_2	500 μM
Gases	C_2H_2	750 μM
	$(CH_3)_2O$	30 μM
Methylated amines	CH_3NH_2	≥1 mM
	$(CH_3)_2NH$	1 mM
	$(CH_3)_3N$	>1 mM
	$(CH_3)_3NO$	NS[a]
Other methylated organics	Dimethyl glycine	1 mM
	Methionine	≥1 mM
	DMSP	1 μM
	S-Methyl methionine	500 μM
	Choline	NS
	Glycine betaine	1 mM
S compounds	DMDS	0.1 μM
	Dimethyl sulfoxide	>1 mM
	CS_2	500 μM
Inorganic substrates	NO_3^-	NS
	S_2O_3	NS
	NH_4^+	>1 mM
Organic substrates	Glucose	NS
	Acetate	NS
	Glycine	NS

[a] NS, no significant inhibition at ≤1 mM.

Inhibition of the uptake of isotope label could be the result of several possible actions: (i) general metabolic poisoning, (ii) competition with DMS as substrates for consumption, (iii) conversion to other compounds which compete with DMS, (iv) conversion to other inhibitory products, and (v) production of unlabeled DMS, which would cause isotopic dilution of the ^{14}C pool. In these whole-water samples, it is also likely that multiple DMS consumers were present, possibly utilizing different pathways, so that a given compound might not have the same effect on all consumers. Pending multiple DMS concentration tests necessary to determine the enzyme kinetics, the hypothesis that reduced consumption was at least partially caused by competitive inhibition remains.

Table 1 summarizes the approximate concentrations at which all compounds caused 90% inhibition of DMS consumption. Each substrate group was a separate incubation run on different water samples over the course of about 2 weeks. However, physical, chemical, and DMS consumption patterns were very similar during this time (20), so the different results probably reflect inhibitor effects.

The survey showed that many methylated substrates in the micromolar range inhibited [^{14}C]DMS consumption, while nonmethylated organic and inorganic substrates did not. This is perhaps most clearly illustrated by the difference between glycine and *N*,*N*-dimethyl glycine (Table 1), which showed that the addition of the methyl groups led to inhibitory action. Methylated substrates that inhibited DMS consumption were not limited to sulfur compounds but also included methylated amines, chlorinated methanes, and other methylated organics, suggesting that DMS consumers were not sulfur specific and may potentially utilize methyl groups from other organics.

In general, compounds with dimethyl groups caused inhibition at lower concentrations than related compounds without methyl groups or with mono- or trimethyl groups, possibly reflecting a steric preference of the DMS-oxidizing enzyme(s) (for example, dimethyl glycine versus glycine, *S*-methyl methionine versus methionine, and the amine series in Table 1). All other mono- and trimethyl compounds were partially inhibitory below 1 mM, with the exceptions of trimethylamine oxide and choline, for which no inhibitory effects were noted. Interestingly, choline's breakdown product glycine betaine showed increasing inhibition with concentration, reaching 90% by 1 mM. It is not clear why choline was so ineffective. However, additions of glycine betaine have recently been shown to alter DMSP and DMS pools in marine waters (12). In that study, additions of 0.1 to 5 μM glycine betaine elevated DMS concentrations, possibly because of algal release of DMSP or inhibition of DMSP uptake by glycine betaine, but choline appeared to cause a

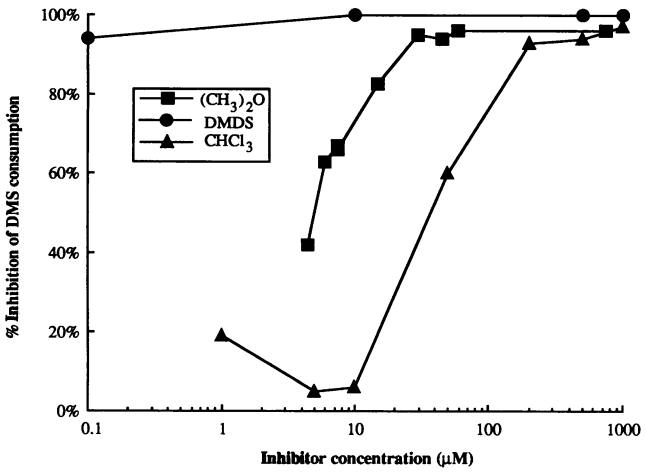

FIG. 2. Inhibition of DMS consumption versus concentration in estuarine waters for DMDS, dimethyl ether [$(CH_3)_2O$], and chloroform ($CHCl_3$).

much weaker response. It is therefore possible that addition of glycine betaine to our radioisotope incubations may have stimulated DMS production, diluting the isotope pool.

Carbon disulfide (CS_2) was an effective inhibitor of DMS consumption above 10 μM. This observation is important, because CS_2 has been observed to bleed out of rubber products such as punctured Teflon-coated rubber septa which are commonly used in bottle incubations (6), including those used in this study. We did observe CS_2 by gas chromatography in some of our unamended samples; however, the peaks were small and concentrations certainly did not approach the micromolar levels necessary to strongly inhibit DMS consumption.

Acetylene and dimethyl ether were both inhibitory at low concentrations (helium, used as a control gas, had no effect). Both have been shown to be cooxidation substrates for methanotrophs (1, 13), and dimethyl ether was recently reported to be a useful inhibitor for studies of methanotrophy (15). However, the other methanotrophic substrates in this study gave mixed results: methanol had little effect, and formaldehyde and formate were inhibitory only above 500 μM. It seems unlikely that methanotrophs in surface waters could be major DMS consumers since both methane concentrations and oxidation rates in this environment are low (7).

This study suggests several alternatives to chloroform as inhibitors of DMS consumption. In particular, DMDS and dimethyl ether were inhibitory at much lower concentrations than chloroform (Fig. 2), and preliminary results indicated that neither compound affected [^{3}H]leucine uptake in these whole-water samples (data not shown), suggesting that their inhibitory effects do not extend to an indicator of general bacterial growth. Furthermore, higher doses of chloroform (>500 μM) may increase DMS production rates by causing the release of DMSP from cells, leading to overestimates of DMS consumption (20). However, previous work (8) found no major changes in DMSP pools with 500 μM chloroform, so this effect is most likely variable and difficult to predict. The best inhibitor is that which is most selective for DMS consumption and which has minimal overall disruption of other biological processes; further investigation is necessary to determine whether one inhibitor is most effective in all situations.

Additional natural marine compounds which would be useful to test include other organic sulfides and mercaptans, simple alkanes and alcohols, other methylated halides, and methylated metals or metalloids such as arsenic and mercury, as well as other methylated organic substrates which are likely present in seawater, including sarcosine, choline sulfate, 3-methiol propionate, arsenobetaine, and methane sulfonate. Little is known of the abundance of most of these compounds, which are common biosynthetics (9) and are likely to occur in nanomolar-to-micromolar concentrations in the ocean. More data on the abundance and activity of other C_1 compounds in the marine environment are needed in order to establish which, if any, may affect DMS consumption.

This work was supported by the NASA Earth Science and Applications Division, Interdisciplinary Research Program, and National Science Foundation grants OCE-88-17442 and OCE-92-03728.

The University of Georgia Marine Institute very kindly provided housing at Sapelo Island for Gordon Wolfe.

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