Purification and Characterization of Dimethylsulfoniopropionate Lyase from an *Alcaligenes*-Like Dimethyl Sulfide-Producing Marine Isolate

MARK P. DE SOUZA AND DUANE C. YOCH*

Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208

Received 23 May 1994/Accepted 19 October 1994

Dimethyl sulfide (DMS) is quantitatively the most important biogenic sulfur compound emitted from oceans and salt marshes. It is formed primarily by the action of dimethylsulfoniopropionate (DMSP) lyase which cleaves DMSP, an algal osmolyte, to equimolar amounts of DMS and acrylate. This report is the first to describe the isolation and purification of DMSP lyase. The soluble enzyme was purified to electrophoretic homogeneity from a facultatively anaerobic gram-negative rod-shaped marine bacterium identified as an *Alcaligenes* species by the Vitek gram-negative identification method. The key to successful purification of the enzyme was its binding to, and hydrophobic chromatography on, a phenyl-Sepharose CL-4B column. DMSP lyase biosynthesis was induced by its substrate, DMSP; its product, acrylate; and also by acrylamide. The relative effectivenesses of the inducers were 100, 90, and 204%, respectively. DMSP lyase is a 48-kDa monomer with a Michaelis-Menten constant (K_m) for DMSP of 1.4 mM and a V_{max} of 408 $\mu\text{mol/min/mg}$ of protein. It converted DMSP to DMS and acrylate stoichiometrically. The similar K_m values measured for pure DMSP lyase and the axenic culture, seawater, and surface marsh sediment suggest that the microbes in these ecosystems must have enzymes similar to the one purified from our marine isolate. Anoxic sediment populations, however, have a 40-fold-lower K_m for this enzyme (30 μM), possibly giving them the capability to metabolize much lower levels of DMSP than the aerobes.

Dimethyl sulfide (DMS) is quantitatively the most important volatile sulfur compound (approximately 90%) emitted from oceans (1) and may have a role in the acidity of precipitation (3, 34), cloud formation, and climate regulation (2, 10, 32, 49). Dimethylsulfoniopropionate (DMSP), the major precursor of DMS, is the principal sulfonium compound in marine environments and is produced by marine phytoplankton (20, 35, 48), microbial mats (44), many species of macroalgae (8, 21, 35), and *Spartina alterniflora*, the dominant salt marsh halophyte (12). DMSP, which is an organosulfur compound analogous to glycine betaine in structure, is thought to function as an osmolyte in algae (35, 42) and *S. alterniflora* (12). DMSP is ubiquitous in both oceanic and nearshore surface waters (18, 25, 41, 47) and has also been detected in salt marsh sediments (23); it is rapidly degraded in these marine environments by the resident microbiota (24, 25, 29). DMSP is degraded enzymatically to produce equimolar amounts of DMS and acrylate (7, 46): $(\text{CH}_3)_2\text{S}^+\text{CH}_2\text{CH}_2\text{COO}^- \rightarrow \text{H}_3\text{C}-\text{S}-\text{CH}_3 + \text{H}_2\text{C}=\text{CH}-\text{COO}^- + \text{H}^+$. The enzyme that carries out this reaction has been called DMSP lyase.

DMS is emitted from *S. alterniflora* marshes (12, 38, 39) and is released (along with dissolved DMSP) during senescence of DMSP-containing algae (33, 37); furthermore, grazing by copepods on a DMSP-containing dinoflagellate greatly enhanced the rate of DMS release (13). DMSP can also be demethylated to 3-mercaptopropionate in marine sediments (27). DMS production from trimethylsulfonium chloride (45) and methylated sulfur compounds other than DMSP (22, 27, 29) has also been described. It is believed that, in marine environments, DMS production from sources other than DMSP is negligible.

DMSP lyase activity has been demonstrated in axenic cell cultures of bacteria (11, 15, 24, 31, 46) and marine phytoplank-

ton (19, 37), and there are several reports that describe the activity of DMSP lyase in crude extracts of algae (7, 19) and a bacterium (46). Despite the extensive literature on DMS emissions and DMSP production by algae, this report is the first to describe the purification and characterization of DMSP lyase from a marine bacterium.

MATERIALS AND METHODS

Media. Enrichment, isolation, cultivation, and experiments with cell suspensions were carried out with the following medium. The basal medium contained the following (per liter): 10 ml of basal salts solution ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 20 g liter⁻¹; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 7.5 g liter⁻¹; Fe-EDTA [Sigma], 2.95 g liter⁻¹; 10 ml of trace elements [MnSO_4 , 15.99 g liter⁻¹; H_3BO_3 , 28 g liter⁻¹; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.79 g liter⁻¹; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 2.4 g liter⁻¹; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 12.6 g liter⁻¹]; $(\text{NH}_4)_2\text{SO}_4$, 0.66 g; NaCl, 1.17 g; yeast extract, 0.05 g; acrylic acid, 0.34 ml of a 100% solution or 5 mM final concentration. This medium was supplemented with 1 ml of a vitamin mixture containing vitamin B₁₂ (2 mg liter⁻¹) and the following at 10 mg liter⁻¹: *p*-aminobenzoic acid, riboflavin, vitamin B₆, niacin, vitamin C, thiamine, and D-(+)-biotin. The vitamin solution was autoclaved and stored refrigerated. The pH of the medium was adjusted to 6.4 prior to autoclaving. Phosphate buffer (1 M; pH 6.8; KH_2PO_4 [60 g liter⁻¹] plus K_2HPO_4 [90 g liter⁻¹]) at 10 ml liter⁻¹ was autoclaved separately and added after the medium had cooled; the final pH of the medium was 7.0.

Isolation of strain M3A. Salt marsh sediment (30 g) from the bank of Shem Creek in Charleston Harbor was vortexed with an equal volume of filtered (pore size, 0.22 μm) seawater and then allowed to settle. The supernatant was used to inoculate a minimal medium containing acrylate as a carbon-energy source. After 72 h, the culture was streaked onto plates containing the acrylate medium described above. Numerous colonies which appeared after a week at 30°C were restreaked onto separate plates to obtain pure cultures. These cultures were washed off the plates with 50 mM phosphate buffer (pH 7.0) and assayed for DMSP lyase activity. One strain, designated M3A, was chosen since it showed the highest rates of DMSP lyase activity.

Growth experiments. Growth experiments were carried out in 50 ml of medium in 125-ml Erlenmeyer flasks covered with aluminum foil and placed in a shaker incubator (100 rpm) at 30°C. Maximum growth on 5 mM acrylate occurred in approximately 24 h. The mass culturing of strain M3A for enzyme preparations was done in 9-liter carboys containing the liquid medium described above. The cultures were given a 10% inoculum and aerated with magnetic stirring bars. After a 4-day incubation at room temperature, the cultures attained an A_{420} of 0.6 to 0.8 and were then harvested by centrifugation at $8,600 \times g$ for 10 min. Cell yields were approximately 0.25 g (wet weight) liter⁻¹. After harvesting, the cells were immediately frozen (unwashed) at -70°C.

* Corresponding author. Phone: (803) 777-2322. Fax: (803) 777-4002.

Induction of DMSP lyase. Strain M3A was grown on medium containing either acetate, propionate, DMSP, acrylate, tryptone plus yeast extract, glucose, or acrylamide at 5 mM concentrations prior to determining DMSP lyase activity. To test the effect of DMS, dimethylsulfonioacetate, dimethylglycine, methacrylate, acrylonitrile, methacrylonitrile, dimethylsulfoxide, dimethyldisulfide, 3-mercaptopropionate, glycine betaine, and methionine on induction of DMSP lyase, 5 mM acetate was added to the growth medium along with the test compound (also at 5 mM). The acetate was used to support growth since the organism did not grow on these putative inducers. To determine the concentration of acrylate necessary for induction, acetate-grown cells were harvested and inoculated into medium with acrylate at concentrations of 1 μ M to 10 mM. A parallel experiment was set up with 50 μ g of chloramphenicol per ml. The cells were incubated for 18 h with the different concentrations of acrylate, after which DMSP lyase activity was measured.

Purification of DMSP lyase. The buffer used in all procedures and assays consisted of a 50 mM phosphate buffer (pH 8.0) containing 5% glycerol and the proteinase inhibitors 1 mM benzamide and 0.1 mM phenylmethylsulfonyl fluoride; it will simply be referred to as the buffer. Frozen cells of strain M3A (30 g) were suspended in 300 ml of the buffer and sonicated four times (at 120 W) for 1 min, making sure that the temperature of the suspension was below 10°C at all times. The suspension was then centrifuged at $39,200 \times g$ for 20 min. Reagent-grade ammonium sulfate was added slowly to the supernatant to a final concentration of 55% saturation (16). The mixture was stirred with a magnetic stirrer at 6°C for 1 h and then centrifuged at $27,200 \times g$ for 10 min. The supernatant, which contained the activity, was loaded onto a phenyl-Sepharose CL-4B column (1.5 by 20 cm) which had been equilibrated with buffer containing $(\text{NH}_4)_2\text{SO}_4$ at 55% saturation. The column was eluted with buffer containing $(\text{NH}_4)_2\text{SO}_4$ at 25% saturation, after which a gradient from 25 to 0% $(\text{NH}_4)_2\text{SO}_4$ was used. Fractions were collected and assayed for activity, and those containing activity were concentrated with Centricon 10 filters (Amicon Corp., Danvers, Mass.). The pH of the concentrated enzyme was adjusted to 8.4 and loaded onto a DEAE-cellulose DE-52 column (1.5 by 64.5 cm) which had been equilibrated with the buffer at pH 8.4. The buffer at pH 8.4 was also used for elution with a flow rate of 3.7 ml/20 min. Fractions were concentrated to 0.6 ml and loaded onto a Sepharose 6B-100 gel filtration column (1.5 by 65 cm) which was equilibrated with the buffer (pH 7). The first two fractions were pure, and the rest of the fractions were pooled, concentrated, loaded onto a Sephadex G-100 column (2.5 by 85 cm), and eluted with the buffer (pH 7). Nondissociating discontinuous electrophoresis was performed on 10% polyacrylamide gels by the method of Hames (17). The pH values of the gel buffer and the reservoir buffer were 8.8 and 8.3, respectively.

Molecular weight determinations. To determine the molecular weight of DMSP lyase by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), a Laemmli-type discontinuous system buffer (pH 8.6) was employed (17). Gels were stained for protein with 0.1% Coomassie blue R-250. A G-100 Sephadex column (2.5 by 85 cm) was equilibrated and eluted with the buffer (containing 300 mM NaCl) and used to determine the molecular weight of native DMSP lyase by gel filtration chromatography. Two individual runs of a mixture of molecular weight standards (Bio-Rad) were performed to create a standard curve. The elution volume of DMSP lyase was determined by measuring the elution of its activity peak.

The enzyme assay. The reaction mixture (in 1 ml) contained buffer (the same buffer described above for the purification step) and the enzyme preparation in glass serum vials (total volume, 14.5 ml). DMSP (at a final concentration of 2.5 mM) was added from a neutralized stock solution (150 mM), and the bottles were immediately capped with gray, Teflon-lined, butyl rubber stoppers, sealed with aluminum crimp caps, and placed on an orbit shaker at 100 rpm until analysis. Unless otherwise mentioned, all assays were carried out at room temperature by gas chromatographic analysis of 50- μ l samples of headspace gas. DMS was analyzed on a Shimadzu GC-8A gas chromatograph with a flame ionization detector by use of a Poropak R column maintained at 190°C and a carrier gas flow rate of 36 ml/min; peaks were integrated on an HP 3390A integrator. The Henry's law constant used for the calculation of liquid-phase DMS was 2.16 atm [ca. 219 kPa] liter mol^{-1} (14).

Determination of the optimum pH and temperature. DMSP lyase activity was assayed in both cell suspensions and crude extracts following preincubation for 10 min in the buffer at temperatures ranging between 10 and 60°C or a pH range of 4 through 9.

Kinetics. The initial rate of DMS production from DMSP was used to calculate the Michaelis-Menten constant (substrate concentration at half-maximum velocity, i.e., K_m) and maximum velocity (V_{max}) for the pure enzyme and cell culture. To determine the kinetic values for microbial populations in aerobic and anaerobic sediments, the top 2 mm and sediment from a depth of approximately 10 cm were used, respectively, in 14.5-ml serum vials. Sediment-seawater slurries (1:1 ratio) in bottles with an air atmosphere were used to measure DMSP lyase activity in aerobic sediments. For anaerobic sediment slurries, deoxygenated seawater was used to prepare the slurry and anoxia was maintained in the reaction vessels by subjecting the vials to a vacuum for 5 min and then replacing the headspace with argon. For measuring activity in seawater, 100 ml was used in 120-ml bottles. The Henry's law constant used for calculating DMS in seawater and in slurries was 2.54 atm [ca. 257 kPa] liter mol^{-1} (14).

FIG. 1. Growth curve of strain M3A on a basal salts medium with DMSP or acrylate as a sole carbon source. The substrate concentration at the time of inoculation was 5 mM. After approximately 6 h of growth on DMSP, the pH of the medium was adjusted to 7.

Protein determinations. Cell suspensions of M3A were boiled in 3.5 M NaOH for 2 min in Eppendorf tubes and centrifuged for 3 min; the supernatant was used for protein estimations. Total protein was determined by using Bradford reagent (Bio-Rad) and bovine serum albumin as a standard.

Materials. DMSP was synthesized by the method of Chambers et al. (9). Dimethylglycine, acrylic acid, DMS, methacrylate, and acrylonitrile were obtained from Aldrich Chemical Corp., Inc., Milwaukee, Wis. 3-Mercaptopropionic acid and S-methylmethionine were obtained from Sigma Chemical Co., St. Louis, Mo. Dimethylsulfonioacetate was obtained from Jaques Minet, and an initial supply of dimethylglycine was from Ronald Kiene.

RESULTS

Characterization of the isolate. A facultatively anaerobic gram-negative rod-shaped bacterium, designated M3A, was chosen from among the several pure salt marsh isolates because it grew rapidly on DMSP or acrylate under aerobic conditions and attained a cell density of 0.6 to 0.8 (A_{420}) in a 9-liter batch culture within 72 h. Strain M3A grew equally well on DMSP or acrylate (Fig. 1). This strain also exhibited high rates of DMSP lyase activity compared with the other isolates.

The most obvious characteristic of strain M3A, which was also seen in a number of DMSP lyase-containing isolates, was the diffusible yellow, nonfluorescent pigment from cells grown on Trypticase soy agar. Strain M3A showed a number of characteristics in common with the genus *Alcaligenes*. Indeed, strain M3A was identified with 95% certainty as an *Alcaligenes* species by the Vitek gram-negative identification card method. However, strain M3A differed from *Alcaligenes* species in several key characteristics, such as catalase, Simmons citrate, and xylose utilization (6). We would prefer to call M3A an *Alcaligenes*-like species. The characteristics of strain M3A are shown in Table 1.

Induction of DMSP lyase. DMSP lyase in strain M3A is an inducible enzyme. It was induced by both DMSP and acrylate (Table 2); these observations are consistent with those of other marine isolates (24, 30). It was also shown that millimolar levels of acrylate were necessary for DMSP lyase induction and that induction was inhibited by 50 μ g of chloramphenicol per ml (Fig. 2), suggesting that enzyme synthesis is induced by acrylate rather than merely being activated by it. Interestingly, acrylamide consistently induced much higher levels of this en-

TABLE 1. Morphological and biochemical characteristics of strain M3A^a

Characteristic	Value or description
Morphology.....	Short rods in pairs
Cell length (μm).....	1
Cell width (μm).....	0.6
Gram reaction.....	Negative
Motility.....	+
Colony type.....	Dimorphic—punctate and diffuse
Soluble pigment production.....	+
Oxidase.....	+
Aerobic acid production from:	
Maltose.....	+
Mannitol.....	+
Xylose.....	+
L-Arabinose (fermentative).....	+
Growth on acetate.....	+
NO ₃ ⁻ as an N source.....	+
Arginine dihydrolase.....	Variable
Action on blood.....	Green to brown
Oxygen requirement.....	Facultative anaerobe

^a The following tests were negative: lysine and ornithine decarboxylase, tryptophan; indole; malonate; DP300 (glucose fermentation in the presence of trichloro-2'-hydroxy-diphenyl ether); *p*-coumaric acid; acetamide; urea; citrate; catalase; nitrogenase; gelatinase; amylase; polymyxin B resistance; H₂S production; fermentation of raffinose, sorbitol, sucrose, inositol, adonitol, rhamnose, *o*-nitrophenyl-β-D-galactopyranoside, and esculin; aerobic acid from glucose and lactose.

zyme. The significance of this observation is not yet apparent. Compounds with a double bond between carbons 2 and 3 seem to be necessary for induction of activity since propionate (no double bond) and acrylonitrile (a C₄ compound) induced only low rates of DMSP lyase activity. Addition of a methyl group to the unsaturated C₂ carbon reduced the activity by 87% as demonstrated by the activity induced by methacrylate. Acetate and propionate, which are products of acrylate metabolism in a clostridial species and in anaerobic sediments (28, 46), did not induce DMSP lyase nor did DMS, the other product of this enzyme. Several other compounds were also tested as inducers and showed no effect (Table 2).

Purification of DMSP lyase. After sonication and centrifugation, the membrane fraction showed less than 1% of the activity of the soluble fraction. Very little DMSP lyase activity was also measured in the 0 to 55% (NH₄)₂SO₄ precipitate. Therefore, both of these fractions which showed low activity

TABLE 2. Inducibility of DMSP lyase in strain M3A^a

Inducer	% of DMSP-induced activity ^b
Acrylate.....	90
Acrylamide.....	204
Methacrylate.....	11
Acrylonitrile.....	5
Acetate.....	2
Propionate.....	0
DMS.....	0
Dimethylsulfonylacetate.....	0

^a Tryptone plus yeast extract, glucose, dimethyl sulfoxide, dimethyl disulfide, glycine betaine, methionine, dimethylglycine, S-methylmethionine, and 3-mercaptopropionate did not induce DMSP lyase activity.

^b Maximum rates of DMSP lyase activity were normalized to protein mass and are expressed as a percentage of enzyme activity shown by cells grown on DMSP. These data are representative of at least two separate experiments.

FIG. 2. Induction of DMSP lyase activity in strain M3A. Acrylate was added as the inducer to the cell suspensions (A_{420} , 0.6) at the concentrations indicated for a period of 12 to 18 h. Chloramphenicol (CAP; 50 μg/ml) was added in a parallel experiment to inhibit protein synthesis.

were discarded. The activity in the 55% (NH₄)₂SO₄ supernatant bound tightly to phenyl-Sepharose CL-4B, which effected a 12-fold purification after a step elution with buffer containing (NH₄)₂SO₄ at 25% saturation, which was followed by a gradient elution from 25 to 0% (NH₄)₂SO₄ and then buffer only until the activity eluted (Fig. 3). A very small protein peak coincided with the DMSP lyase activity peak. The enzyme bound weakly to a DEAE-cellulose DE-52 column at pH 8.4 and chromatographed without the aid of an added anion. This was followed by gel filtration on Sepharose 6B-100; several fractions that resulted showed one band on a native polyacrylamide gel (Fig. 4A). This procedure purified the enzyme 130-fold, with the specific activity increasing to 390 U/mg of protein (Table 3). The remaining enzyme in the Sepharose fractions was pooled and purified by chromatography on a Sephadex

FIG. 3. Hydrophobic chromatography of DMSP lyase showing its elution from a phenyl-Sepharose CL-4B column (1.5 by 20 cm). The solid line shows the concentration of (NH₄)₂SO₄ in the buffer, and the open circles represent the A_{280} .

FIG. 4. PAGE of purified DMSP lyase. (A) Native gel (10%). Lanes: 1, BSA (10 µg); 2, DMSP lyase (1.8 µg). (B) SDS-polyacrylamide gel (10%). Lanes: 1, molecular weight markers (phosphorylase *b*, 94 kDa; BSA, 66 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa); 2, DMSP lyase (1.8 µg). Gels were stained for protein with 0.1% Coomassie blue R-250.

G-100 column. The enzyme is stable indefinitely at -70°C in the glycerol-containing buffer.

Molecular weight characteristics. Gel filtration with molecular weight standards showed that DMSP lyase is a polypeptide with a molecular weight of 48,000 (data not shown). Its migration on an SDS-polyacrylamide gel as a peptide of 48 kDa indicates that it exists as a monomer (Fig. 4B). In spite of its small size, DMSP lyase comigrated with the dimer of bovine serum albumin (BSA; molecular size, 132 kDa) on a native polyacrylamide gel (Fig. 4A).

Stoichiometry. The characteristics of DMSP lyase are summarized in Table 4. Whole cells and the pure enzyme completely degraded DMSP to DMS in a stoichiometric conversion within minutes. An alternative pathway for DMSP degradation, by two successive demethylation steps (27), has been described for microbes in marine sediment, but this pathway was not found in strain M3A since all the DMSP was accounted for as DMS. Acrylate was produced concomitantly with DMS in an equimolar ratio (data not shown).

Kinetics. The Michaelis constants (K_m) for DMSP were determined for pure enzyme and whole cells to be 1.41 and 2.02 mM, respectively (Fig. 5; Table 5). An analysis of the K_m values for microbes in aerobic sediment (top 2 mm) and aerobic seawater showed similar results, suggesting that the microbial

TABLE 3. Purification of DMSP lyase from strain M3A

Purification step	Total protein (mg)	Total activity (U) ^a	Yield (%)	Sp act (U/mg) ^a	Purification (fold)
Crude extract	693.4	2,108	100	3.0	1.0
55% (NH ₄) ₂ SO ₄ supernatant	167.7	1,140	54.0	6.8	2.3
Phenyl-Sepharose CL-4B	15.6	1,144	54.0	73.4	24.5
DEAE-cellulose DE-52	5.9	868	41.2	146.0	48.7
Sepharose 6B-100	0.4	156	6.4	390.1	130.0

^a A unit of DMSP lyase activity is defined as the amount of enzyme required to produce 1 µmol of DMS (in the liquid plus the gas phase) in 1 min at ambient temperature.

TABLE 4. Some physical and biochemical properties of DMSP lyase

Parameter measured	Value
Molecular mass ^a (kDa)	48
No. of subunits ^b	1
Sp act ^c (U/mg)	390
Purification ^d (fold)	130
Reaction stoichiometry (DMS/acrylate)	1:1
Optimum pH	8
Optimum temp (°C)	37–40

^a Determined by gel filtration.

^b Determined by SDS-PAGE and gel filtration chromatography.

^c For the definition of a unit of DMSP lyase activity at ambient temperature (27°C), see Table 3, footnote *a*. At 37°C, the specific activity increases by a factor of 1.5.

^d Determined by comparing the specific activity of the pure enzyme with that of the crude cell extract.

populations in these natural samples may have DMSP lyase enzymes with kinetic constants analogous to the one described for strain M3A (Table 5). The K_m of microbial DMSP lyase in anoxic sediments, however, was more than an order of magnitude lower than that of the enzyme assayed in the isolate (in vivo) or the pure enzyme; the ecological significance of this observation is discussed below.

Effect of alternative substrates and inhibitors on DMSP lyase activity. Dimethyl sulfoxide, dimethylsulfonyacetate, and *S*-methylmethionine (tested at 1 to 6 mM) did not serve as alternative substrates nor did they inhibit DMS production (after a 10-min preincubation) from DMSP (data not shown). Methionine, dimethylglycine, β-mercaptoethanol, glycine betaine, acrylate, and 3-mercaptopropionate (tested at concentrations of 0.25 to 6 mM) also did not inhibit or promote DMS evolution from DMSP (data not shown). These data indicate that DMSP lyase is quite specific for its substrate.

DISCUSSION

There have been several reports of microbial DMSP lyase activity in aerobic and anaerobic salt marsh sediments and in

FIG. 5. Effect of DMSP concentration on DMSP lyase activity of the pure enzyme and cell culture. Velocity equals units of activity per 0.83 µg of protein or per milliliter of cell suspension (A_{420} , 0.5). The inset is the accompanying Lineweaver-Burk plot for cell culture ($R^2 = 0.966$) and DMSP lyase ($R^2 = 0.991$). K_m and V_{max} values are shown in Table 5.

TABLE 5. Kinetic characteristics of DMSP lyase from various marine environmental sources^a

Source ^b	K_m (mM)	V_{max}	
		$\mu\text{mol/min/mg}$ of protein	$\mu\text{mol/min/liter}$
Pure enzyme	1.41	408	
Cell culture	2.02	1.09	
Aerobic seawater	1.99		1.15
Aerobic sediment slurries	0.33		10.31
Anaerobic sediment slurries	0.034		4.98

^a The K_m and V_{max} of DMSP lyase were determined by the Lineweaver-Burk method. Velocity equals units of activity per 0.83 μg of pure enzyme or per 1 ml of cell suspension (A_{420} , 0.5). The density of both surface and anaerobic sediments was assumed to be 0.8 g/ml. Values represent the means of two experiments.

^b The preparation of sediment slurries and seawater samples is described in Materials and Methods.

oceanic and nearshore waters. It was not surprising, therefore, that bacteria from salt marsh sediment could be isolated and cultured on a medium containing DMSP as a sole carbon source. Strain M3A is similar to other aerobic strains isolated in that it rapidly degrades DMSP to DMS and acrylate in vitro and then takes up the acrylate (data not shown), using it as an energy source. The relative impermeability of the cell membrane to zwitterions such as DMSP (11) and the rapid appearance (1.1 $\mu\text{mol/min/mg}$ of cell protein) of both DMS and acrylate in the reaction mixture suggest that DMSP lyase may be located in the periplasmic space of strain M3A or is loosely associated with the outer cell membrane. DMSP lyase (48 kDa) comigrated with the dimer of BSA (132 kDa) on native polyacrylamide gels (Fig. 4A); the atypically slow migration of DMSP lyase could be a function of either its hydrophobic nature or a lack of negatively charged amino acids on the surface of the protein.

Enzyme synthesis is induced by both the substrate, DMSP, and the product, acrylate; product induction has also been reported in aromatic hydrocarbon metabolism, where this phenomenon was assumed to confer substrate specificity on the enzyme (36). In fact, our data show that structural analogs of DMSP (*S*-methylmethionine, dimethylsulfonioacetate, glycine betaine, and dimethylglycine) did not function as inducers (Table 2) or alternative substrates for DMSP lyase (data not shown). It has been suggested that DMSP lyase activity is constitutive in some bacteria isolated from coastal waters (24). The kinetics of DMSP lyase induction in strain M3A was slow (12 to 18 h), similar to that of the clostridial enzyme (46) and unlike that in strain LFR (30) and *Pseudomonas doudoroffii* (unpublished data), where induction was complete in 2 to 3 h.

DMSP lyase-containing bacteria that populate algal mats and blooms and salt marsh sediments probably catabolize the DMSP produced by algae and *S. alterniflora*, using it as an energy source. DMSP degradation may provide a readily utilizable energy source for bacteria, which may help explain the peak in bacterioplankton biomass following crashes of algal blooms (33, 37, 43). Purification of DMSP lyase from several bacterioplanktonic and phytoplanktonic algal species and a subsequent comparison of these enzymes may help determine the relative importance of the procaryotic and eucaryotic components in DMS production by the planktonic community. There is a preliminary report of a DMSP dethiomethylase-specific antibody being produced from an algal enzyme, but no details about the protein were given (33a).

The K_m values for DMSP lyase measured under aerobic

conditions are all in the millimolar range, even though the concentrations of DMSP in seawater and sediment are 10^3 to 10^6 lower than these K_m values. Microbes may, however, routinely encounter microenvironments with high levels of DMSP in seawater or surface sediment during senescence and autolysis of the *Spartina* plant cells or algae (4, 5), which contain millimolar concentrations of DMSP (4, 12, 37). The low K_m of the anaerobic sediment population may provide them with the means of metabolizing much lower levels of DMSP than those available to microbes which reside in seawater or the surface sediments.

The rates of DMS production from surface sediments were linear over a DMSP concentration range of 25 to 100 μM , suggesting that DMSP lyase does not have a second binding site of higher affinity (data not shown). It may be argued that K_m measurements of 1 to 2 mM for DMSP lyase in bacteria in estuarine waters samples are artefactual. Our data do not preclude a second substrate-binding site existing in the low micromolar or nanomolar range; enzymes with both high and low substrate affinity forms are in fact well known (40). However, DMS production from DMSP at endogenous levels (3 to 30 nM) did not saturate the enzyme in seawater samples (25).

For the demethylation pathway (26) to predominate in anoxic sediments, it would seem that the K_m for the demethylating enzyme must be lower than that of DMSP lyase of microbes in these sediments, which is 34 μM . A comparison of the K_m s for DMSP for the demethylating enzyme and DMSP lyase in marine environments might indicate the relative importance of the two pathways.

DMSP lyase activity has been demonstrated in axenic cell cultures (11, 15, 24, 31, 37, 46) and in crude extracts (7, 14a, 19, 46) of algae and bacteria. The K_m of the DMSP lyase from strain M3A reported here of approximately 1.4 mM is comparable to that found for the unicellular alga *Gyrodinium cohnii* (1.5 mM) (19) but 2 orders of magnitude higher than the K_m of a *Phaeocystis* species (11.7 μM) (37). Future comparisons of the kinetic parameters of algal and bacterial enzymes may help to determine the relative importance of algae and bacteria in the release of DMS from the marine environment to the atmosphere.

ACKNOWLEDGMENTS

We thank Mei-Jing Shen, Kristina Schmidt, and Ashton Estridge for excellent technical assistance and Y. P. Chen for helpful discussions.

REFERENCES

- Andreae, M. O. 1985. The emission of sulfur to the remote atmosphere, p. 5–25. In J. N. Galway, R. J. Charlson, M. O. Andreae, and H. Rodhe (ed.), The biogeochemical cycling of sulfur and nitrogen in the remote atmosphere. D. Reidel Publishing Co., Boston.
- Bates, T. S., R. J. Charlson, and R. H. Gammon. 1987. Evidence for the climatic role of marine biogenic sulphur. *Nature* (London) **329**:319–321.
- Bates, T. S., and J. D. Cline. 1985. The role of the ocean in a regional sulfur cycle. *J. Geophys. Res.* **90**:9168–9172.
- Belviso, S., S. K. Kim, B. Krajka, B. C. Nguyen, N. Mihalopoulos, and P. Buat-Ménard. 1990. Production of dimethylsulfoniumpropionate (DMSP) and dimethylsulfide (DMS) by a microbial food web. *Limnol. Oceanogr.* **35**: 1810–1821.
- Billen, G., C. Joiris, L. Meyer-Reil, and H. Lindeboom. 1990. Role of bacteria in the North Sea ecosystem. *Neth. J. Sea Res.* **26**:265–293.
- Busse, H.-J., and G. Auling. 1992. The genus *Alcaligenes*, p. 2544–2555. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes. Springer-Verlag, New York.
- Cantoni, G. L., and D. G. Anderson. 1956. Enzymatic cleavage of dimethylpropiothetin by *Polysiphonia lanosa*. *J. Biol. Chem.* **222**:171–177.
- Challenger, F., and M. I. Simpson. 1948. Studies on biological methylation. Part XII. A precursor of the dimethyl sulfide evolved by *Polysiphonia fastigata*. Dimethyl-2-carboxyethylsulfonium hydroxide and its salts. *J. Chem. Soc.* **3**:1591–1597.
- Chambers, S. T., C. M. Kunin, D. Miller, and A. Hamada. 1987. Dimethyltin

- can substitute for glycine betaine as an osmoprotectant molecule for *Escherichia coli*. J. Bacteriol. **169**:4845–4847.
10. Charlson, R. J., J. E. Lovelock, M. O. Andreae, and S. G. Warren. 1987. Oceanic phytoplankton, atmospheric sulfur, cloud albedo and climate. Nature (London) **326**:655–661.
 11. Dacey, J. W. H., and N. V. Blough. 1987. Hydroxide decomposition of DMSP to form DMS. Geophys. Res. Lett. **14**:1246–1249.
 12. Dacey, J. W. H., G. M. King, and S. G. Wakeham. 1987. Factors controlling emission of dimethylsulfide from salt marshes. Nature (London) **330**:643–645.
 13. Dacey, J. W. H., and S. G. Wakeham. 1986. Oceanic dimethyl sulfide: production during zooplankton grazing on phytoplankton. Science **233**:1314–1316.
 14. Dacey, J. W. H., S. G. Wakeham, and B. L. Howes. 1984. Henry's law constants for dimethylsulfide in freshwater and seawater. Geophys. Res. Lett. **11**:991–994.
 - 14a. Diaz, M. R., and B. F. Taylor. 1994. Comparison of dimethylsulfoniopropionate (DMSP) lyase activity in a prokaryote and a eukaryote, abstr. N-18, p. 319. In Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Microbiology, Washington, D.C.
 15. Diaz, M. R., P. T. Visscher, and B. F. Taylor. 1992. Metabolism of dimethylsulfoniopropionate and glycine betaine by a marine bacterium. FEMS Microbiol. Lett. **96**:61–66.
 16. Green, A. A., and W. L. Hughes. 1955. Protein fractionation on the basis of solubility in aqueous solutions of salts and organic solvents. Methods Enzymol. **1**:67–90.
 17. Hames, B. D. 1981. An introduction to polyacrylamide gel electrophoresis, p. 1–91. In B. D. Hames and D. Rickwood (ed.), Gel electrophoresis of proteins: a practical approach. IRL Press, Inc., Oxford.
 18. Iverson, R. L., F. L. Nearhoof, and M. O. Andreae. 1989. Production of DMSP and DMS by phytoplankton in estuarine and coastal waters. Limnol. Oceanogr. **34**:53–67.
 19. Kadota, H., and Y. Ishida. 1968. Effect of salts on enzymatical production of dimethyl sulfide from *Gyrodinium cohnii*. Bull. Jpn. Soc. Sci. Fish. **34**:512–518.
 20. Kadota, H., and Y. Ishida. 1972. Production of volatile sulfur compounds by microorganisms. Annu. Rev. Microbiol. **26**:127–138.
 21. Karsten, U., C. Wiencke, and G. O. Kirst. 1990. The β -dimethylsulfoniopropionate (DMSP) content of macroalgae from Antarctica and southern Chile. Bot. Mar. **33**:143–146.
 22. Kelly, D. P., and N. A. Smith. 1990. Organic sulfur compounds in the environment—biogeochemistry, microbiology and ecological aspects. Adv. Microbiol. Ecol. **11**:345–385.
 23. Kiene, R. P. 1988. Dimethylsulfide metabolism in salt marsh sediments. FEMS Microbiol. Ecol. **53**:71–78.
 24. Kiene, R. P. 1990. DMS production from DMSP in coastal seawater samples and bacterial cultures. Appl. Environ. Microbiol. **56**:3292–3297.
 25. Kiene, R. P. 1992. Dynamics of DMS and DMSP in oceanic water samples. Mar. Chem. **37**:29–52.
 26. Kiene, R. P., and S. K. Service. 1991. Decomposition of dissolved DMSP and DMS in estuarine waters: dependence on temperature and substrate concentration. Mar. Ecol. Prog. Ser. **76**:1–11.
 27. Kiene, R. P., and B. F. Taylor. 1988. Demethylation of dimethylsulfoniopropionate and production of thiols in anoxic marine sediments. Appl. Environ. Microbiol. **54**:2208–2212.
 28. Kiene, R. P., and B. F. Taylor. 1989. Metabolism of acrylate and 3-mercaptopropionate, p. 222–230. In E. S. Saltzman and W. J. Cooper (ed.), Biogenic sulfur in the environment. American Chemical Society Symposium Series No. 393. American Chemical Society, Washington, D.C.
 29. Kiene, R. P., and P. T. Visscher. 1987. Production and fate of methylated sulfur compounds from methionine and dimethylsulfoniopropionate in anoxic salt marsh sediments. Appl. Environ. Microbiol. **53**:2426–2434.
 30. Ledyard, K. M., and J. W. H. Dacey. 1994. Dimethylsulfide production from dimethylsulfoniopropionate by a marine bacterium. Mar. Ecol. Prog. Ser. **110**:95–103.
 31. Ledyard, K. M., E. F. DeLong, and J. W. H. Dacey. 1993. Characterization of a DMSP-degrading bacterial isolate from the Sargasso sea. Arch. Microbiol. **160**:312–318.
 32. Legrand, M. R., R. J. Delmas, and R. J. Charlson. 1988. Climate forcing implications from Vostok ice-core sulphate data. Nature (London) **334**:418–420.
 33. Matrai, P., and M. D. Keller. 1993. DMS in a large scale coccolithophore bloom in the Gulf of Maine. Cont. Shelf. Res. **13**:831–843.
 - 33a. Nishiguchi, M. K. 1994. Identification and detection of dimethylpropiothetin dethiomethylase (4.4.1.3) from macro and micro algae using biomolecular techniques, abstr. 022L-13, p. 98. In Abstracts of the ASLO Ocean Science Meeting 1994. Eos, Transactions, American Geophysical Union, Washington, D.C.
 34. Nriagu, J. O., D. A. Holdway, and R. D. Coker. 1987. Biogenic sulfur and the acidity of rainfall in remote areas of Canada. Science **237**:1189–1192.
 35. Reed, R. H. 1983. Measurement and osmotic significance of β -dimethylsulfoniopropionate in marine macroalgae. Mar. Biol. **4**:173–181.
 36. Stanier, R. Y., and L. N. Ornston. 1973. The β -ketoadipate pathway, p. 89–151. In A. H. Rose and D. W. Templest (ed.), Advances in microbial physiology. Academic Press, Inc., New York.
 37. Stefels, J., and W. H. M. van Boekel. 1993. Production of DMS from dissolved DMSP in axenic cultures of the marine phytoplankton species *Phaeocystis* sp. Mar. Ecol. Prog. Ser. **97**:11–18.
 38. Steudler, P. A., and B. J. Peterson. 1984. Contribution of gaseous sulphur from salt marshes to the global sulphur cycle. Nature (London) **311**:455–457.
 39. Steudler, P. A., and B. J. Peterson. 1985. Annual cycle of gaseous sulfur emissions from a New England *Spartina alterniflora* marsh. Atmos. Environ. **19**:1411–1416.
 40. Stryer, L. 1988. Biochemistry, 3rd ed., p. 233–259. W. H. Freeman & Co., New York.
 41. Turner, S. M., G. Malin, and P. S. Liss. 1988. The seasonal variation of dimethyl sulfide and dimethylsulfoniopropionate concentrations in near-shore waters. Limnol. Oceanogr. **33**:364–375.
 42. Vairavamurthy, A., M. O. Andreae, and R. L. Iversen. 1985. Biosynthesis of dimethyl sulfide and dimethyl propiothetin by *Hymenomonas carterae* in relation to sulfur source and salinity variations. Limnol. Oceanogr. **30**:59–70.
 43. van Boekel, W. H. M., F. C. Hansen, R. Riegman, and R. P. M. Bak. 1992. Lysis-induced decline of a *Phaeocystis* spring bloom and coupling with the microbial foodweb. Mar. Ecol. Prog. Ser. **81**:269–276.
 44. Visscher, P. T., and H. van Gernerden. 1991. Production and consumption of dimethylsulfoniopropionate in marine microbial mats. Appl. Environ. Microbiol. **57**:3237–3242.
 45. Wagner, C., S. M. Lusty, Jr., H. F. Kung, and N. L. Rogers. 1966. Trimethylsulfonium-tetrahydrofolate methyltransferase, a novel enzyme in the utilization of 1 carbon units. J. Biol. Chem. **241**:1923–1925.
 46. Wagner, C., and E. R. Stadtman. 1962. Bacterial fermentation of dimethyl- β -propiothetin. Arch. Biochem. Biophys. **98**:331–336.
 47. Wakeham, S. G., and J. W. H. Dacey. 1989. Biogeochemical cycling of dimethyl sulfide in marine environments, p. 152–166. In E. S. Saltzman and W. J. Cooper (ed.), Biogenic sulfur in the environment. American Chemical Society Symposium Series No. 393. American Chemical Society, Washington, D.C.
 48. White, R. H. 1982. Analysis of dimethylsulfonium compounds in marine algae. J. Mar. Res. **40**:529–536.
 49. Wigley, T. M. L. 1989. Possible climate change due to SO₂ derived cloud condensation nuclei. Nature (London) **339**:365–367.