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Dimethylsulfide production from dimethylsulfoniopropionate by a marine bacterium

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ABSTRACT: The processes of dimethylsulfoniopropionate (DMSP) uptake and dimethylsulfide (DMS) production from DMSP were investigated using a marine bacterium isolated from Sargasso Sea water. Uptake of DMSP into the cell preceded cleavage to form DMS, indicating an intracellular location for the DMSP lyase. The rate of DMS production accelerated rapidly during the timecourse of DMSP cleavage, independent of cell growth; this increase in rate was suppressed in the presence of 500 µM chloramphenicol, consistent with induction of the DMSP lyase. Additionally, preincubation with acrylic acid, the putative byproduct of the cleavage, as well as with DMSP, stimulated the initial rate of DMS production. Two determinations of the K_s, V_{max} values for DMSP uptake in induced cells yielded 190 nM, 1.3 fmol cell^{-1} h^{-1} and 105 nM, 3.2 fmol cell^{-1} h^{-1} A single determination of these parameters in uninduced cells yielded 280 nM and 1.9 fmol cell^{-1} h^{-1}. The rate of DMS production from DMSP exhibited a sigmoidal dependence on extracellular DMSP concentration.

KEY WORDS: Dimethylsulfide · Dimethylsulfoniopropionate · DMSP lyase

INTRODUCTION

Although present at trace levels, dimethylsulfide (DMS) is the major volatile organosulfur compound in seawater, and its transfer across the sea-air interface may account for as much as 90% of the marine biogenic sulfur flux to the atmosphere (Andreae 1990). A number of recent studies have implicated DMS as a quantitatively significant precursor of non-sea-salt sulfate aerosol and, by extension, of cloud droplet nuclei in the marine atmosphere (Bates et al. 1987, Charlson et al. 1987, Wigley 1989). These developments have spurred interest in the controls on DMS production in surface seawater.

The tertiary sulfonium compound dimethylsulfoniopropionate (DMSP), an abundant osmoticum in many taxa of marine algae, is considered the main precursor of DMS in marine environments. The structural similarity of DMSP to known osmoregulants, and evidence that its intracellular levels are adjusted in response to salinity stress (Vairavamurthy et al. 1985, Dickson & Kirst 1986, 1987a, b), support a role for this compound in algal osmoregulation. A DMSP lyase catalyzing the cleavage of DMSP to DMS and acrylic acid has been partially isolated from a red macroalga (Cantoni & Anderson 1956) and a heterotrophic dinoflagellate (Ishida 1968), although the metabolic role of this cleavage is unclear. A number of studies have additionally documented direct production of DMS by DMSP-containing phytoplankton (Andreae 1980, Vairavamurthy et al. 1985, Dacey & Wakeham 1986).

Microbial formation of DMS from dissolved, extracellular DMSP has recently begun to receive attention as an alternative mode of DMS formation in the marine environment. Cleavage of dissolved DMSP to form DMS has been observed in marine bacterial cultures and aerobic seawater incubations (Dacey & Blough 1987, Wakeham et al. 1987, Kiene 1990, Kiene & Service 1991, Taylor & Gilchrist 1991, Diaz et al. 1992, Ledyard et al. 1993) and has recently been documented in an axenic *Phaeocystis* sp. as well (Stefels & van Boekel 1993). Growth on DMSP as the sole source of carbon and energy has been observed in several of the bacterial studies, which suggests that in some cases procurement of the non-DMS cleavage product

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(presumably acrylic acid) for growth is the basis for the cleavage. Diaz et al. (1992) observed that a marine bacterium which ordinarily catabolizes DMSP accumulates it at elevated salinities, suggesting that bacteria may also utilize DMSP as an osmoprotectant. Although bacterial metabolism of dissolved DMSP is an accepted source of DMS in seawater, no information is available on the kinetics of this process in marine bacteria. In this study we report the concentration dependence of DMSP uptake and cleavage in a bacterium isolated from Sargasso Sea water.

MATERIALS AND METHODS

Isolation and stock culture maintenance. The bacterium, designated strain LFR, was isolated from Sargasso Sea surface (1 m) water. Strain LFR is a strictly aerobic, carotenoid-containing bacterium which requires Na^+ for growth, and has been placed in the α-subgroup of the *Proteobacteria* by analysis of its small-subunit rRNA. The bacterium was maintained on marine agar 2216 (Difco) or on a seawater mineral medium (f/2; Guillard 1975) containing 36 µM sodium phosphate, 0.88 mM sodium nitrate, and vitamin and trace metal mixes, amended with 0.4 mM ammonium chloride. The pH of the medium is 7.5 after autoclaving. 500 µM DMSP was used as the carbon source. This medium, lacking DMSP, is referred to below as the basal maintenance medium. Stocks were also stored frozen at −80 °C and in liquid nitrogen. A full description of the isolation, physiological and phylogenetic characterization and maintenance of this bacterium is given elsewhere (Ledyard et al. 1993).

Analysis. Cells were counted by acridine orange epifluorescence microscopy (Hobbie et al. 1977). DMS was quantified by sulfur-specific gas chromatography using a Varian 3700 gas chromatograph equipped with a Sievers 350A sulfur chemiluminescence detector (SCD). Volatile sulfur compounds were separated on Chromosil 330 (Supelco) at a column temperature of 74 °C, with high-purity nitrogen as the carrier gas at a flow rate of 35 ml min^{-1}. Most determinations of DMS were by direct injection of 0.25 to 1 ml headspace gas. When DMS levels < 20 nM were anticipated, DMS was preconcentrated from solution as follows. Unfiltered liquid sample (1 to 3 ml) was stripped in a fritted glass tube by a stream of nitrogen (100 ml min^{-1}) for 4 to 6 min. DMS was cryofocused in a 3.2 mm FEP (fluorinated ethene propene) loop packed with the solid adsorbent Tenax-GC (35/60 mesh size; Alltech Associates) submerged in a 50/50 water/methanol circulating bath held at −20 °C. Water vapor was removed from the sample stream during trapping by a glass cold-finger trap submerged in the same −20 °C bath. The sample was injected by diverting carrier, by means of a 6-port switching valve, to backflush the contents of the loop onto the chromatographic column. The sample loop was then raised and DMS thermally desorbed from the Tenax by passing current through a stainless steel sheath encasing the loop. DMS sampling and analysis were automated (J. W. H. Dacey unpubl.), which allowed unattended operation of the system and minimized potential error due to sample handling and variability in timing the phases of the trapping procedure. Quantitative recovery of DMS was confirmed by comparing on-column injection of headspace gas with identical injections into the sparger. The detection limit of the SCD is about 0.5 pmol. DMSP was determined as DMS following reaction with 1 N KOH for a minimum of 2 h (White 1982).

Induction of DMSP lyase. Early in the timecourse of DMS production from DMSP by LFR, a rapid increase in the rate of DMSP cleavage, independent of cell growth, was observed. To explore the possibility that this increase reflected induction of the DMSP lyase or one of the enzymes involved in DMSP-lyase activity, the effect of chloramphenicol, an inhibitor of protein synthesis (Hahn 1983), on DMSP-lyase activity was examined. Cells harvested in the exponential phase of growth on mannitol were suspended in basal maintenance medium and preincubated for 2 h with or without 500 µM chloramphenicol. DMSP was then added to a concentration of 1 µM, and headspace gas monitored for DMS accumulation.

We tested the effect of preincubation with DMSP and the presumed products of its cleavage, DMS and acrylic acid, on DMSP-lyase activity. Cells were grown to mid-logarithmic phase on mannitol, washed twice in sterile seawater and resuspended in medium containing either 20 µM DMSP, DMS, acrylic acid, or mannitol as the sole carbon source. Following a 4 h incubation, cells from each treatment were harvested, washed 3 times in sterile seawater, and resuspended in medium containing 1 µM DMSP in serum bottles. Headspace gas was then monitored for DMS accumulation. Incubation with mannitol, which did not stimulate the initial rate of DMSP cleavage, served as a negative control. DMS was not produced from 1 µM DMSP in sterile controls.

Assay of DMSP uptake. Based on results described elsewhere in this paper, cells grown on DMSP are referred to as 'induced' and cells grown on mannitol or marine medium 2216 as 'uninduced'. The kinetics of DMSP uptake were examined in both induced and uninduced cells by monitoring loss of DMSP from the dissolved phase. Cells were grown to exponential phase on 1 mM DMSP or mannitol, washed 3 times in sterile seawater, and harvested by centrifugation at 7700 × *g* for 8 min at 15 °C. A suspension of these cells

in sterile seawater served as the inoculum for simultaneously conducted assays. Cells from the same original culture were freshly harvested for each set of simultaneous assays to minimize potential differences among inocula due to storage. Assays were carried out in silylated, sterile serum bottles capped with Teflon-faced butyl rubber septa, each containing different concentrations of DMSP in the basal maintenance medium. DMSP uptake was assayed at DMSP concentrations from 5 nM to 1.5 µM. Following sample bottle inoculation, 4 ml aliquots of culture medium were periodically withdrawn into a 10 ml B-D Plastipak syringe (Becton-Dickinson), expelled into a 25 mm glass filtration unit (Millipore) and filtered by vacuum through a 0.2 µm Gelman Supor-200 filter directly into a 10 ml serum bottle. Filtration separated dissolved and cell-associated DMSP and instantly terminated DMSP cleavage in the medium. Filtered aliquots were stripped of DMS with nitrogen, and KOH added to a final concentration of 1 N. Aliquots reacted overnight at room temperature, and the resulting DMS was analyzed as described above. Following each timecourse, a 1 ml aliquot was withdrawn from the sample bottle and fixed by addition of glutaraldehyde to a level of 0.5% for later cell counts. Cell density in the sample bottles was approximately 10^5 cells ml^{-1}.

Assay of DMS production from DMSP. Preparation of inocula and sample bottles was as above. However, because filtration was not used to terminate activity, sampling proceeded more rapidly and higher cell densities were used (approximately 10^6 ml^{-1}) than for assays of DMSP uptake. DMS production was assayed using 10 nM to 4 µM DMSP. To avoid the effect of induction on rate estimation, cells were grown on DMSP prior to assays, with the exception of a single experiment assaying DMS production from DMSP between 10 and 80 nM. Here cells were grown on marine medium 2216 (Difco) rather than DMSP, and were therefore uninduced by our definition. In this case, rates were approximated from the maximum, rather than initial, rate of DMS accumulation. The 3 rates obtained in this way were consistent with those measured in DMSP-grown cells in this concentration range. Intracellular DMSP blanks were assessed in controls consisting of unamended, inoculated medium, and combined with the DMSP added to calculate the total substrate available to DMSP-grown cells. DMS production and DMSP uptake were usually assayed in separate experiments due to time constraints of the sampling process. However, in one experiment the 2 processes were monitored simultaneously in induced cells.

The time required for equilibration of DMS between the solution and gas phases in a sample bottle introduces a complicating factor into timecourses of DMS production measured by real-time headspace analysis. However, preconcentration of DMS from solution imposes serious constraints on sampling frequency. To address this problem, a preservation scheme was devised that permitted sampling at half-minute intervals and accurate quantification of solution-phase DMS. 4 ml aliquots were removed by syringe from the sample bottle at each timepoint and transferred to 10 ml serum bottles containing 40 µl 10% sodium dodecyl sulfate (SDS), which terminated DMS production by LFR instantly and did not interfere with DMS analysis. The increase in sample bottle headspace over a typical timecourse would be expected to cause a maximum decrease in solution-phase DMS concentrations of about 3%, according to the Henry's Law constant for DMS in seawater (Dacey et al. 1984), and probably less on the short timescale (10 to 30 min) of these assays. DMS in the dispensed aliquots was allowed to equilibrate completely with the gas phase and then analyzed in the headspace. DMS standards were prepared identically to aliquot bottles with respect to dispensing and SDS concentration. Where analysis required preconcentration of DMS from SDS-preserved aliquots, 30 µl half-strength Antifoam A (Sigma) was added immediately prior to sparging to reduce foaming of the sample.

Assay of simultaneous DMSP uptake, accumulation and cleavage. An experiment that simultaneously monitored loss of dissolved DMSP from the medium, increase in intracellular DMSP and production of DMS in mannitol-grown cells was carried out in a 1 l amber Qorpak bottle (All-Pak) containing 950 ml of the basal maintenance medium. DMSP was added to a final concentration of 65 nM and cells added to a density of $\sim 5 \times 10^5$ cells ml^{-1}. Aliquots of unfiltered culture medium were automatically withdrawn and analyzed for DMS. Simultaneously, 15 ml aliquots of culture medium were periodically withdrawn by hand for measurement of dissolved and particulate DMSP. Measurement of dissolved DMSP was as described above, except that a GF/F filter (Whatman, nominal pore size 0.7 µm) was used and the filtrate collected in 30 ml serum bottles. For measurement of intracellular DMSP, the filter was rinsed with 3 ml sterile seawater, suspended in 16 ml 1 N KOH, allowed to react overnight at room temperature, and DMS analyzed as above.

Rate estimation and kinetic analysis. Rates were calculated from the initial linear portion of the timecourse of DMS accumulation (in the case of DMS production assays) or of dissolved DMSP disappearance (in the case of DMSP uptake assays) in the culture medium. A brief lag of 2 to 4 min was often discernible at the start of DMS production timecourses, possibly reflecting the time required for DMSP uptake, after which the rate of

DMS accumulation was constant. No such lag was observed in timecourses of DMSP uptake. Duplicate assays were carried out for some substrate levels in DMS production experiments, but were not carried out in DMSP uptake assays, due to the time required for sampling and the need to cover the concentration range adequately. The range of rates calculated from duplicate assays of the same DMSP concentration was between 0.3 and 39%, and was usually better than 9%. Replication between experiments was provided by 6 separate analyses of DMSP cleavage kinetics and 2 analyses of DMSP uptake in induced cells. A single experiment was carried out to determine the kinetics of DMSP uptake in uninduced cells. Kinetic parameters were estimated using the Eadie-Hofstee (single reciprocal) transformation of the Michaelis-Menten equation (Fersht 1985).

Reagents and chemicals. DMS was obtained from Fluka Chemical Corp., Ronkonkoma, NY, USA. The HBr salt of DMSP was synthesized (Challenger & Simpson 1948), and its purity checked by melting point determination and by comparison to DMS standards. Chloramphenicol was purchased from Sigma. Prepared media and media components were from Becton Dickinson Microbiology Systems, Cockeysville, MD, USA.

RESULTS

Aspects of DMSP utilization and DMS production

A low level of constitutive DMSP-lyase activity was present in strain LFR, as evidenced by the modest (relative to untreated cells) but constant rate of DMS production from DMSP by uninduced cells treated with 500 µM chloramphenicol (Fig. 1a). Some curvature in the DMS production timecourse was apparent even in treated cells, indicating that induction may not be completely suppressed at this level of chloramphenicol. In contrast, the rate of DMS production by untreated cells increased rapidly in the early part of the timecourse. The maximum rate of DMS production in untreated cells was approximately 6 times that observed in chloramphenicol-treated cells.

In testing the capacity of various compounds to stimulate production of DMSP lyase, enhancement of the initial rate of DMS production from DMSP was considered a measure of induction. Preincubation with both DMSP and acrylate greatly increased the initial rate of DMS production from DMSP relative to that of cells grown on mannitol. Cells preincubated with DMS produced DMS from DMSP at a rate similar to that of mannitol-incubated cells (Fig. 1b).

The timecourse of DMSP uptake and accumulation and DMS production in uninduced cells (Fig. 2) shows

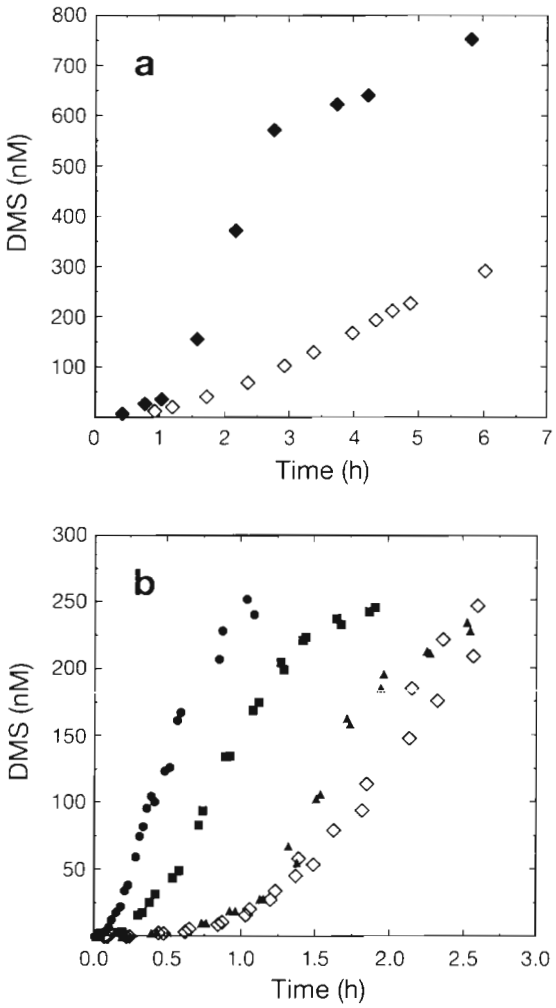

Fig. 1. (a) Effect of chloramphenicol on DMS production from 1 µM DMSP by strain LFR. (◆) Untreated; (◇) 500 µM chloramphenicol added. Cell density was 4.3×10^6 cells ml^{-1} (b) Timecourse of DMS production from 1 µM DMSP following preincubation of cells with 20 µM DMSP (●), acrylic acid (■), DMS (▲), or mannitol (◇). Duplicate treatments shown. Cell density was 1.3×10^6 cells ml^{-1}, except for the mannitol-preincubated cells, which were used at a density of 6.6×10^5 cells ml^{-1}

that loss of dissolved DMSP from the medium, and concomitant accumulation in the cells, occurred before production of DMS was initiated (subsequent DMS production was not monitored). This is consistent with a transport system for DMSP and an intracellular location for the DMSP lyase in strain LFR.

Kinetics of DMSP uptake and cleavage

Based on saturation curves and Eadie-Hofstee linearizations of the DMSP uptake data (Fig. 3a, b), K_s values of 190 ± 14 and 105 ± 15 nM were obtained

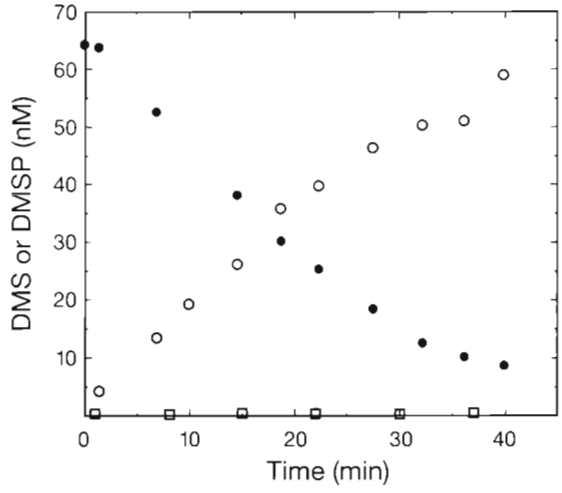

Fig. 2. DMSP uptake and accumulation, and DMS production, in response to a 65 nM DMSP addition to mannitol-grown LFR. (●) Dissolved DMSP; (○) intracellular DMSP; (□) DMS. Uptake rate is equal to the accumulation rate, 0.22 fmol cell^{-1} h^{-1}. Cell density was 5.05×10^5 cells ml^{-1}

for induced cells, with corresponding maximum velocities of 1.34 ± 0.08 and 3.2 ± 0.2 fmol cell^{-1} h^{-1} (errors represent the standard error of the regression). K_s and V_{max} for uninduced cells were 275 ± 36 nM and 1.91 ± 0.11 fmol cell^{-1} h^{-1}, respectively. Physiological variability between strains used in separate experiments may account for the difference between the 2 sets of kinetic parameters determined for induced cells. The single set of DMSP uptake kinetic parameters determined for uninduced cells was not strikingly different from those of induced cells. However, more estimates of the kinetic parameters of DMSP uptake in both induced and uninduced cells are needed to statistically support this observation. The general similarity between kinetic parameters of DMSP uptake in induced and uninduced cells, and the lack of a lag in timecourses of DMSP uptake, may imply that it is the cleavage, rather than the uptake, system which is inducible.

The concentration dependence of DMS production from DMSP by induced cells is shown in Fig. 4. The overall appearance of the curve suggests saturation-type kinetics (Fig. 4a), and for dissolved DMSP concentrations greater than about 350 nM, the DMS production data can in fact be fit with a saturation kinetics model. At lower DMSP levels, however, a sigmoidal curvature is apparent (Fig. 4b). If the DMSP lyase is intracellular, meaningful kinetic parameters cannot be obtained by modelling rates of DMS production as a function of extracellular DMSP concentration, especially when uptake and catalysis are closely coupled, as appears to be the case in strain LFR. In the interest of comparing the apparent kinetic parameters for DMSP cleavage obtained in this way with those of DMSP uptake, both processes were monitored simultaneously in induced cells (Fig. 5). The apparent V_{max} of DMSP cleavage (3.2 ± 0.2 fmol cell^{-1} h^{-1}) was the same within the error of the regression as the V_{max} of uptake (3.24 ± 0.31 fmol cell^{-1} h^{-1}), while the apparent half-saturation value calculated for DMSP cleavage was 640 ± 279 nM.

DISCUSSION

Although evidence has mounted in favor of a microbial pathway for DMS production in the marine environment, both field and laboratory work on the concentration dependence of dissolved DMSP uptake and DMS production has been scanty. The only field data of this kind have been provided by Kiene & Service

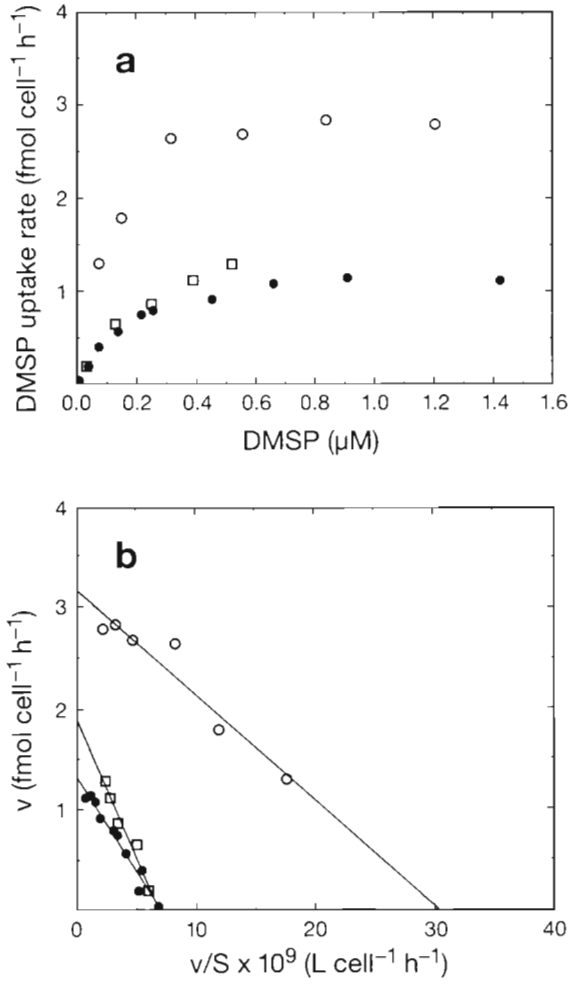

Fig. 3. (a) Saturation curves and (b) Eadie-Hofstee plots of DMSP uptake by induced (2 separate experiments; ●, ○) and uninduced (mannitol-grown; □) LFR

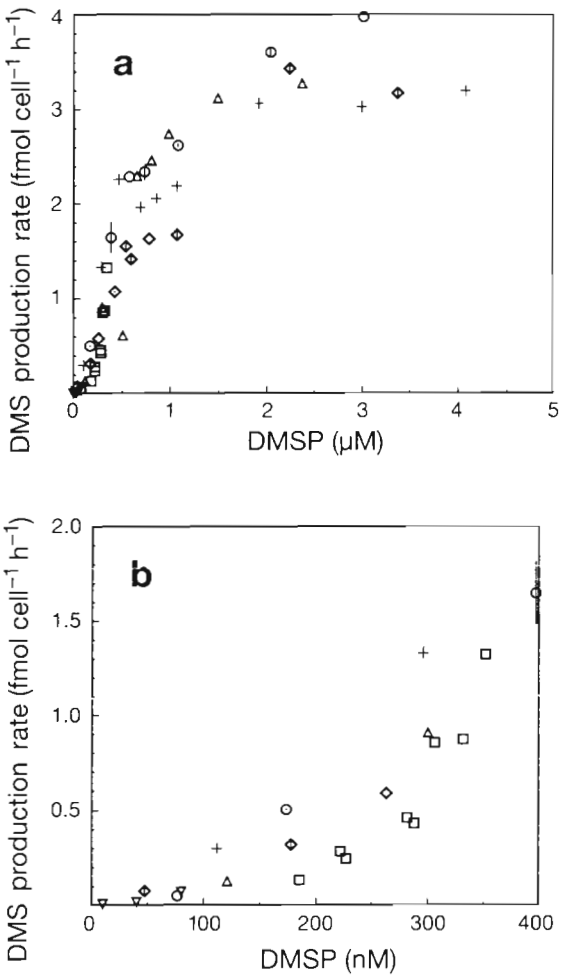

Fig. 4. Concentration dependence of rate of DMS production from DMSP by induced LFR. Rate data are shown for (a) entire DMSP concentration range assayed and (b) expanded range for DMSP < 400 nM. (○, □, △, ◇, ▽, +) 6 separate experiments. Bars represent range of duplicate measurements

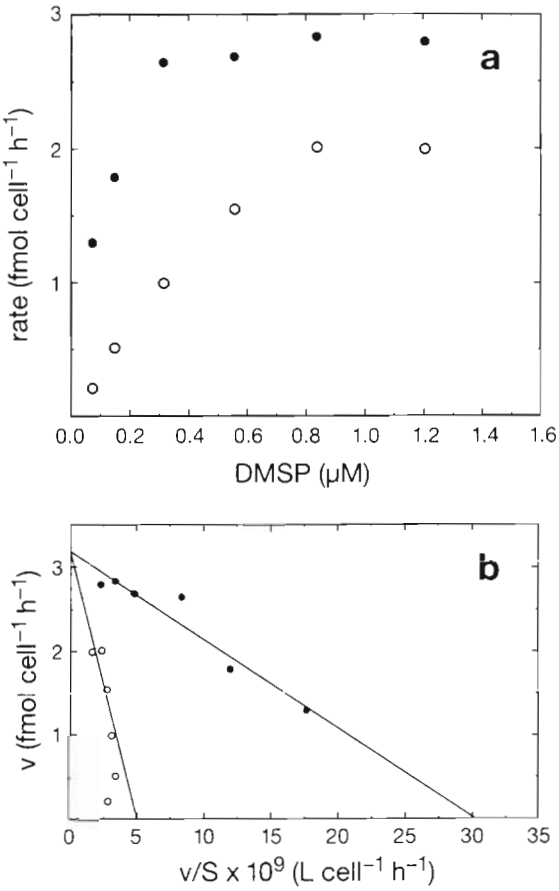

Fig. 5. (a) Saturation curves and (b) Eadie-Hofstee plots of DMSP uptake (●) and cleavage (○) simultaneously monitored in induced LFR. In the case of DMSP cleavage, the linearization is based only on rate data for DMSP > 350 nM

(1991), although kinetic parameters could not be estimated for either process because saturation of activity within the experimental range of DMSP concentrations was not observed. Stefels & van Boekel (1993) reported kinetic parameters for a *Phaeocystis* sp., broadening the focus on production of DMS from dissolved DMSP to include phytoplankton, and emphasizing the need for further examination of the ubiquity and function of this activity in algae. In contrast, although isolation of bacteria able to produce DMS from DMSP has been reported (Kiene 1990, Taylor & Gilchrist 1991, Diaz et al. 1992, Ledyard et al. 1993), no published studies of the kinetics of DMSP uptake and cleavage in pure cultures of marine bacteria exist. Here we have undertaken such a study with the aim of providing a suitable model for further examination of the kinetics of these processes in the marine environment.

Although dissolved DMSP levels as high as hundreds of nanomolar have been documented in seawater (Turner et al. 1988, Gibson et al. 1990), these concentrations are not typical of the Sargasso Sea water from which strain LFR was isolated. In this regard, it is interesting that half-saturation values for DMSP uptake measured in this study were in the low hundreds of nanomolar. It is possible that strain LFR cannot effectively utilize ambient DMSP levels, although this is difficult to reconcile with possession of a specific system for transport and catabolism of DMSP. Alternatively, this bacterium may occupy a niche in which dissolved DMSP is elevated relative to bulk seawater, for instance in close association with particulate matter or DMSP-containing organisms. While dissolved DMSP is usually present in oligotrophic seawater at nanomolar levels, intracellular DMSP in cultured algae can reach concentrations of several hundred mmol per liter of cell volume (Keller et al. 1989). It has been postulated that microzones containing high concentrations of algal intracellular compounds exist in the immediate vicinity

of phytoplankton cells (Bell & Mitchell 1972, Sjoblad & Mitchell 1979, Mitchell et al. 1985), and consideration of the effect of turbulence on diffusive flux suggests that these zones may be more stable than previously estimated (Lazier & Mann 1989). The apparent inducibility of the bacterial DMSP lyase in this study is also consistent with microscale patchiness in DMSP concentrations. Under low-concentration conditions, little energy is expended on synthesis of DMSP lyase, but when high DMSP levels are encountered, enzyme induction may confer rapid enhancement of processing capabilities.

The K_m and V_{max} of DMSP cleavage recently reported for whole cells of a *Phaeocystis* sp. are 11.7 µM and 0.2 pmol cell^{-1} h^{-1}, respectively (Stefels & van Boekel 1993). The fact that simple kinetics models cannot be applied to the decomposition of DMSP by strain LFR precludes direct comparison of the bacterial and phytoplankton cleavage processes. However, rates of DMS production by this *Phaeocystis* sp. can be compared to rates of DMSP uptake by strain LFR. This sort of analysis indicates that, although the algal lyase has a relatively low affinity for DMSP, *Phaeocystis* could compete well with a bacterium such as LFR, on a per cell basis, for environmental concentrations of DMSP. For instance, 5 nM DMSP would be cleaved by *Phaeocystis* at an initial rate of 85 amol cell^{-1} h^{-1}, while strain LFR could take up the same level of DMSP at an initial rate in the range of 33 to 145 amol cell^{-1} h^{-1}. These results are difficult to extrapolate to the environment in a quantitative way, as *Phaeocystis* blooms appear to be limited to high-latitude and coastal waters. While the geographic range of strain LFR is unknown, it may be more representative of DMSP-decomposing bacteria in oligotrophic regions of the ocean. Our ability to generalize about the outcome of competition between algae and bacteria for this substrate is limited, as kinetic data are available for only 1 algal and 1 bacterial species at this point. Moreover, our assessment of the ubiquity of dissolved DMSP utilization in natural microbial assemblages is only as good as our ability to culture these organisms; molecular phylogenetic approaches have indicated that natural bacterial populations in the oligotrophic ocean may be dominated by species not previously isolated by standard techniques (Britschgi & Giovannoni 1991, Schmidt et al. 1991).

Since the non-DMS product(s) of DMSP cleavage in strain LFR provides carbon for growth, identification of this product will require *in vitro* study of the bacterial enzyme. Cantoni & Anderson (1956) demonstrated that the products of DMSP cleavage catalyzed by partially isolated lyase from *Polysiphonia lanosa* are DMS and acrylic acid. While we do not have direct evidence that acrylic acid is the other product of the bacterial cleavage, several observations strongly favor this possibility. Not only does LFR grow on acrylate as the sole source of carbon and energy (Ledyard et al. 1993), but preincubation with acrylate stimulated initial rates of DMSP cleavage (Fig. 1b). Additionally, Kiene (1990) observed that bacteria able to cleave DMSP to DMS can be isolated from acrylate-enriched coastal seawater.

Two possible scenarios can be envisioned for DMSP utilization by the cleavage pathway. Either DMSP is cleaved at the cell surface, and the resulting acrylate taken up, or DMSP is transported and cleaved intracellularly. Since DMS, the other product of the cleavage reaction, is not utilized, and a surface-associated lyase would obviate the need to transport DMSP across the cell membrane, the former model would appear energetically advantageous. However, although strain LFR can take up acrylic acid, consistent with this model, our results indicate that uptake of DMSP precedes cleavage in this isolate, in accord with the latter mechanism. Both anaerobiosis and treatment with dinitrophenol, an uncoupler of respiration, have been observed to suppress DMS production from DMSP, suggestive of a requirement for active uptake of DMSP (J. W. H. Dacey unpubl. data).

An intracellular location for the DMSP lyase precludes obtaining meaningful kinetic parameters for the lyase in a whole-cell system, as the enzyme will respond to physiological rather than extracellular DMSP concentrations. At DMSP levels below 350 nM, where uptake rates increased most rapidly with concentration (Fig. 3a), the concentration dependence of DMS production rate appears sigmoidal, and it is unclear whether this phenomenon reflects actual properties of the cleavage catalysis or is a consequence of close coupling between uptake and cleavage kinetics. Presumably, uptake kinetics determine the maximum amount of DMSP cells can accumulate before DMSP cleavage causes a net decline in intracellular concentration, therefore imposing an artificial cap on the V_{max} of cleavage. Consistent with this hypothesis, when the kinetics of both processes were assessed simultaneously, the maximal velocities obtained were identical.

While it is clear that a cell-free system is required to elucidate lyase kinetics, the observational data presented here on the concentration dependence of DMS production may provide insight into the results of similar kinetic experiments carried out in natural seawater. Kiene & Service (1991) observed a disparity between the rates of dissolved DMSP uptake and DMS production at an estuarine site, and attributed this to an alternative pathway for DMSP degradation, possibly demethylation. Although there is other evidence that a demethylation pathway is indeed operative in seawater (Taylor & Gilchrist 1991), a simple mass balance approach must be used with caution. This is especially relevant in natural seawater incubations, where DMSP decomposition via

different pathways, some of which do not result directly in volatile sulfur production, and DMS production and consumption, occur concomitantly. The results of this study demonstrate that, due to kinetic considerations, rates of DMSP uptake and DMS production in response to a single DMSP concentration are not identical even in a pure bacterial culture, especially at DMSP concentrations at which uptake enzyme is not saturated. Measurements of dissolved DMSP loss and DMS production alone may not suffice to support inferences about the proportion of DMSP metabolism mediated by various pathways.

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