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Evidence for Intracellular and Extracellular Dimethylsulfoniopropionate (DMSP) Lyases and DMSP Uptake Sites in Two Species of Marine Bacteria

DUANE C. YOCH,* JOHN H. ANSEDE, AND KRISTINA S. RABINOWITZ

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

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The kinetics of dimethylsulfoniopropionate (DMSP) uptake and dimethylsulfide (DMS) production from DMSP in two bacterial species, *Alcaligenes* sp. strain M3A, an isolate from estuarine surface sediments, and *Pseudomonas doudoroffii*, from seawater, were investigated. In *Alcaligenes* cells induced for DMSP lyase (DL) activity, DMS production occurred without DMSP uptake. In DL-induced suspensions of *P. doudoroffii*, uptake of DMSP preceded the production of DMS, indicating an intracellular location of DL; intracellular DMSP levels reached ca. 7 mM. DMSP uptake rates in noninduced cells showed saturation at three concentrations (K_t [transport] values, 3.4, 127, and 500 μM). In DL-induced cells of *P. doudoroffii*, DMSP uptake rates increased ca. threefold (V_{max}, 0.022 versus 0.065 μmol of DMSP taken up min^{-1} mg of cell $protein^{-1}$), suggesting that the uptake binding proteins were inducible. DMSP uptake and DL activity in *P. doudoroffii* were both inhibited by CN^-, 2,4-dinitrophenol, and membrane-impermeable thiol-binding reagents, further indicating active uptake of DMSP by cell surface components. The respiratory inhibitors had limited or no effect on DL activity by the *Alcaligenes* sp. Of the structural analogs of DMSP tested for their effect on DMSP metabolism, glycine betaine (GBT), but not *methyl*-3-mercaptopropionic acid (MMPA), inhibited DMSP uptake by *P. doudoroffii*, suggesting that GBT shares a binding protein with DMSP and that MMPA is taken up at a separate site. Two models of DMSP uptake, induction, and DL location found in marine bacteria are presented.

The high concentration of dimethylsulfoniopropionate (DMSP) found in various marine phytoplankton species (18) appears to serve as an osmoprotectant (14) or cryoprotectant (21) in these organisms. DMSP is released to the water column following zooplankton grazing on phytoplankton (7, 39) and lysis which occurs in the senescing bloom (26, 27). DMSP may therefore be an important source of carbon and energy for free-living and epiphytic marine bacteria (3, 5, 19). Since bacterial numbers increase as blooms senesce (4, 5, 31), it seems probable that DMSP released into the water column would be available to those bacteria having the genes for synthesizing either DMSP lyase (DL) or DMSP demethylase. Large numbers of bacteria have indeed been shown to grow on either acrylate or *methyl*-3-mercaptopropionic acid (MMPA), the respective products of these two enzymes (35). One species having DL activity, *Alcaligenes* sp. strain M3A, has in fact been shown to have a strong chemotactic response to levels of DMSP found in natural environments (41). Bacteria having DMSP demethylase (29, 33, 37) or DL (9, 10, 22) activities have also been isolated and extensively characterized, as have several fungi with DL activity (2). Other marine isolates have been shown to take up and use DMSP as an osmoprotectant (12, 13, 38).

Of the two DMSP-utilizing enzymes from bacteria, only DL has been purified and characterized (9, 11, 32). It was isolated from two aerobes (one a salt marsh isolate, *Alcaligenes* sp. strain M3A, and the other, *Pseudomonas doudoroffii*, isolated from the water column) and an anaerobe (*Desulfovibrio acrylicus*) (32). The kinetic characteristics of the purified enzymes showed all to have relatively low affinities for DMSP cleavage (K_m values of 0.5 to 2 mM), which would seem to preclude these strains from utilizing the nanomolar levels of DMSP in seawater. However, preliminary data for *P. doudoroffii* indicated that DL was intracellular and DMSP was actively transported (11), which suggested that this organism could compensate for the low K_m by concentrating DMSP intracellularly, a conclusion reached previously with another dimethylsulfide (DMS)-producing organism (22). On the basis of limited supporting data (10), a preliminary model showing the location of the lyase enzymes and characteristics of the DMSP uptake system (or lack thereof) in *Alcaligenes* and *P. doudoroffii* was published in the proceedings of a symposium (11). Confirmation and a more-detailed comparative models are presented here.

MATERIALS AND METHODS

Culture methods. Both *Alcaligenes* sp. strain M3A and *P. doudoroffii* (ATCC 27123) were cultured overnight on a rotary shaker (100 rpm) at 30°C in tryptic soy broth (TSB) (Difco), which was supplemented with 3% NaCl for the pseudomonad. These cultures (50 ml), which had Klett readings of ≥400 and routinely yielded 0.1 (±10%) mg of cell protein ml^{-1}, were harvested by a 10-s centrifugation at 14,500 × *g* and resuspended in an equal volume of 50 mM phosphate buffer (pH 7.2) (the *Alcaligenes* sp.) or seawater (*P. doudoroffii*).

DL induction. DL was induced by the addition of 1 mM DMSP to cell suspensions incubated at 22°C on a rotary shaker at 100 rpm for 2 to 2.5 h. The lyase-induced cells were collected as described above and resuspended in fresh buffer or seawater for use in the experiments described below. The effects of inhibitory compounds on induction were measured by first washing the compounds from the cell suspension and then measuring the DL activity. This activity is not to be confused with the constitutive DL activity measured in the presence of these inhibitors. The two experiments were carried out with parallel cell suspensions.

DMS and DMSP analyses. The DMSP used in this study was synthesized from DMS and acrylate (6). It was quantified by alkaline hydrolysis (1 ml of 4.25 M NaOH added to a 1-ml sample) to yield DMS, which was then compared to a standard curve constructed by using a pure commercial standard of DMSP obtained from Research Plus Inc., Bayonne, N.J. DMS was analyzed by gas chromatography after 18 h (10). Total DMS concentrations, the sum of the

* Corresponding author. Phone: (803) 777-2322. Fax: (803) 777-4002. E-mail: yoch@biol.sc.edu.

concentrations in the gas and liquid phases produced by cell suspensions, were determined by quantitating the DMS in the gas phase and then calculating the DMS concentration in the liquid phase by using the Henry's Law constants of Dacey et al. (8).

DMSP uptake kinetics. The rate of uptake of DMSP in *P. doudoroffii* was measured by monitoring either the accumulation of intracellular DMSP or the loss of DMSP from the dissolved phase or both. The latter method was required to monitor consumption by *Alcaligenes* because it does not accumulate intracellular DMSP. After the cells were removed from the TSB medium and resuspended in buffer or seawater, they were incubated on the shaker for 2 h before use, to allow them to equilibrate after the downshift in metabolism. Uptake experiments were carried out with 1-ml aliquots of cells placed in 15-ml serum bottles (silylated and capped with Teflon-faced rubber septa) to which DMSP (100 μM, final concentration) had been added. After the appropriate time of incubation with DMSP, the cells were transferred to microcentrifuge tubes and centrifuged for 25 s to separate the dissolved from the cell-associated DMSP. The interstitial DMSP was removed by washing the pellet with 1 ml of seawater (*Pseudomonas*) or buffer (*Alcaligenes*). The intracellular concentration of DMSP at a given time was quantitated by resuspending the pellet in seawater (or buffer) and adding NaOH to convert the DMSP to DMS. The cell volume used for calculating the intracellular DMSP concentration was determined by estimating the volume of water in the cells (25). This was done by subtracting the dry weight from the wet weight of a cell pellet (from 1 ml); this value, after correction for the interstitial volume, was taken as the cytoplasmic (water) volume and used to calculate the intracellular (molar) concentration of DMSP.

To calculate the rate of DMSP uptake by its rate of removal from solution, aliquots of cells were rapidly removed from the medium by centrifugation for 25 s in a microcentrifuge, and 1 ml of NaOH (4.25 M) was added to the supernatant to cleave DMSP to DMS. In experiments using DL-induced *P. doudoroffii*, the DMS (i.e., DMSP) values had to be corrected by subtracting the DMS (in solution) resulting from DL activity. By using the Henry's law constant, that value could be calculated from the DMS concentration in the gas phase. Finally, K_t values for DMSP uptake by *P. doudoroffii* were determined by adding DMSP (0.5 to 1,500 μM) to 1-ml cell suspensions at 2-min intervals. After 15 min, the intracellular concentration of DMSP was determined as described above. K_t is defined as the concentration of DMSP that results in half the maximum rate of DMSP uptake.

Use of inhibitors. In the experiments involving the effects of inhibitors or DMSP analogs on DMSP uptake, induction, or DMSP cleavage, the cells were preincubated with the inhibitor at the indicated concentrations for 20 min before DMSP was added to initiate the reaction. The cells were washed free of the inhibitor (or analog) and residual DMSP or DMS after the induction period. The effect of inhibitors on the rate of DMSP uptake is presented as percent inhibition and was calculated as follows: (1 − rate of inhibitor-treated sample/rate of sample with DMSP only) × 100. $[I]_{0.5}$ is defined as the concentration of inhibitor that reduces the velocity of DL to half its V_{max}.

Because of the number of concentrations of each inhibitor or analog tested and the time required for sample analysis, except for the no-addition controls, duplicate assays were not performed. Instead, each experiment was replicated at least twice and usually three or more times, and results of a representative experiment are presented here.

RESULTS

Kinetics of DMSP uptake and cleavage. The time courses of DMSP uptake, intracellular accumulation, and DMSP cleavage (DMS production) by cell suspensions of *Alcaligenes* and *P. doudoroffii* are shown in Fig. 1 and 2. Figure 1 shows that suspensions of DL-induced *Alcaligenes* cells removed DMSP (100 μM) from solution at a rate comparable to the appearance of DMS (0.15 μmol min^{-1} mg of cell $protein^{-1}$), but no DMSP was observed to accumulate in these cells. No DMSP was observed to accumulate in noninduced cells of this organism, either (data not shown). In contrast, both DL-induced and -noninduced suspensions of *P. doudoroffii* accumulated DMSP intracellularly (Fig. 2). The pool size reached a maximum of ca. 7 mM in DL-induced cells and then declined rapidly as DMSP was depleted from the seawater medium due to concomitant DMS production (Fig. 2A). The initial rate of DMSP removal from solution paralleled the rate of cellular uptake and was considerably higher than the rate of DMSP cleavage, i.e., DMS production. Figure 2B shows that noninduced *P. doudoroffii* cells also accumulated DMSP but at a threefold-lower rate than DL-induced cells (ca. 0.012 versus 0.730 μmol min^{-1} mg of cell $protein^{-1}$). The production of DMS by this cell suspension was just beginning at 30 to 40 min, as DL was being induced. The observed differences in DMSP metabolism between these two species are consistent with *Alcaligenes* cleaving DMSP at the cell surface (no uptake required) and *P. doudoroffii* transporting DMSP across the cell membrane and being cleaved by a cytosolic lyase. Formalin-treated *P. doudoroffii* cells (negative controls) were incapable of taking up DMSP.

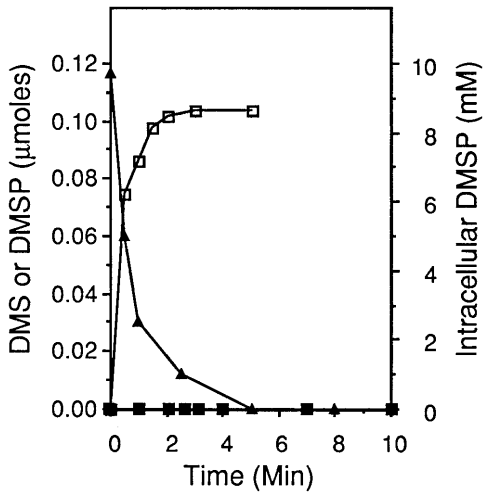

FIG. 1. DMSP uptake and DMS production by DL-induced *Alcaligenes* sp. strain M3A. DMSP removal from solution (▲), intracellular accumulation (■), and DMS production (□) in 1-ml cell suspensions (240 μg of cell protein ml^{-1}) are shown. DMSP was added at a concentration of 100 μM. Ambient temperature (20 to 22°C) was used.

Saturation curves of DMSP uptake by non-DL-induced *P. doudoroffii* cells were produced by using DMSP concentrations between 0.5 and 1,500 μM (Fig. 3). Changes in the rate of intracellular concentration of DMSP (millimolar per minute) were recorded. Analyses of the rates of uptake as a function of DMSP concentration indicated inflection (saturation) points near 150 μM and at ca. 1,000 μM (K_t, ca. 127 ± 40 [mean ± standard deviation] and 500 μM, respectively). The inflection point was observed in four independent experiments. The cell suspensions were diluted 10-fold in order to determine the existence of other saturation points in the rate of DMSP uptake; indeed, one was apparent at 15 μM (K_t, ca. 3.4 μM).

Effect of energy production inhibitors. Evidence of an active uptake system in bacteria can often be seen by the effect of inhibitors of energy production on this process. The effects of two inhibitors on DMSP metabolism were examined: cyanide (CN^-), an inhibitor of electron transport, and 2,4-dinitrophenol (2,4-DNP), an ionophore uncoupler of ATP synthesis. Control experiments showed that these inhibitors (used in the experiment whose results are shown in Fig. 4) had no effect on in vitro DL activity in either organism. In vivo, cyanide had a small (<25%) inhibitory effect, while 2,4-DNP had no effect on *Alcaligenes* DL activity (indicating no energy requirement) (Fig. 4A and C). However, both reagents strongly inhibited induction of DL activity in this organism. It must be noted that the effects of these inhibitors on DMSP uptake and DL activity were measured in the same cell suspensions but that DL induction was measured in a parallel cell suspension (see Materials and Methods). While DL activity (DMSP cleavage) by cell suspensions of *Alcaligenes* does not appear to require energy, consistent with a cell surface location, the energy requirement for DL induction presumably represents its requirement for protein synthesis.

In contrast to the results for *Alcaligenes*, both CN^- and

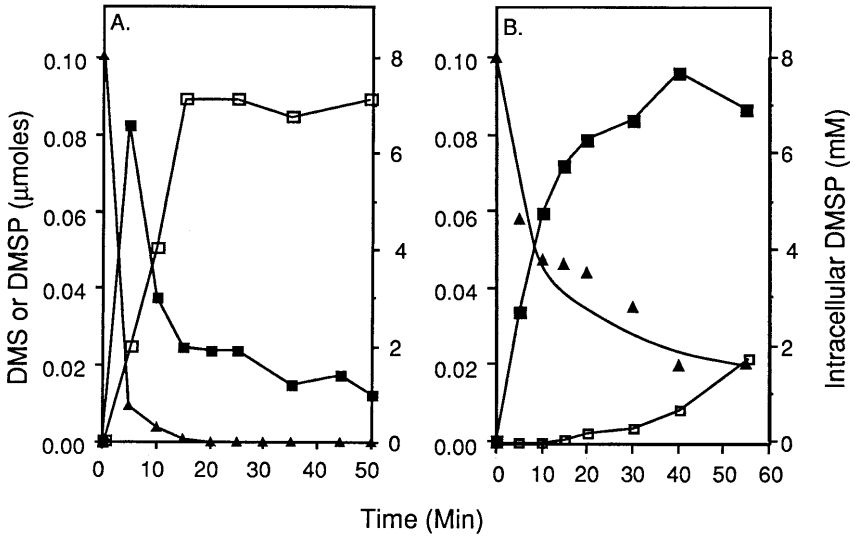

FIG. 2. DMSP uptake and accumulation and DMS evolution by DL-induced (A) and non-DL-induced (B) suspensions of *P. doudoroffii*. DMSP removal from solution (▲), intracellular accumulation (■), and DMS production (□) in 1-ml suspensions (270 μg of cell protein ml^{-1}) are shown. DMSP was added at a concentration of 100 μM. Of four replica experiments of DMSP accumulation by noninduced cells, the rates were highest in the one whose results are shown here.

2,4-DNP completely shut down DMSP metabolism in *P. doudoroffii*. DMSP uptake, DL induction, and DL activity were all inhibited by both compounds (Fig. 4B and D). While CN^- inhibited all three activities at approximately the same concentration ($[I]_{0.5}$ = 0.5 mM), 2,4-DNP inhibited DMSP accumulation and DL induction at different concentrations, with $[I]_{0.5}$s of 0.01 and 0.5 mM, respectively. The significance of this large difference is not clear, but it is reproducible. Although DL induction was inhibited by CN^- and 2,4-DNP, we cannot distinguish between the energy needs of DMSP uptake and protein synthesis. The effect of these inhibitors is consistent with a model in which DMSP is actively taken up by the pseudomonad, meaning that DMSP metabolism in vivo is also energy dependent due to the cytosolic location of DL.

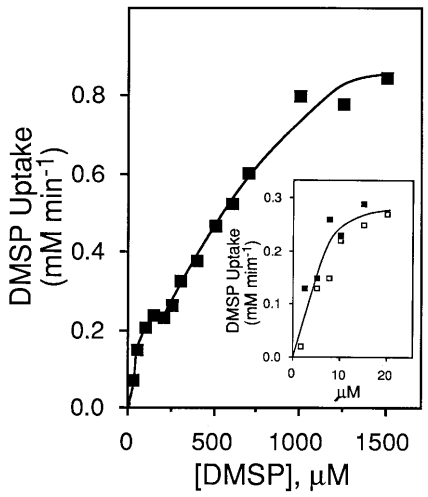

FIG. 3. Saturation curves of DMSP uptake rates by *P. doudoroffii*, as measured by changes in intracellular DMSP concentration. Experiments were conducted with suspensions of noninduced cells. (Inset) DMSP accumulation by cell suspensions diluted 10-fold. Data from two separate experiments (open and closed symbols) are shown. The same amount of cell protein as for Fig. 2 was used.

Effect of thiol reagents. To further probe the physical differences between the *Alcaligenes* sp. and *P. doudoroffii* regarding the location of their DL enzymes and putative DMSP-binding proteins, reagents which bind to reactive SH groups in proteins were employed. *p*-Chloromercuriphenylsulfonate (*p*-CMBS), a nonpermeable SH reagent which acts only on the outside of the cell, and *p*-chloromercuribenzoate (*p*-CMB), which is membrane permeable (34), were equally effective as inhibitors of *Alcaligenes* DL activity (Fig. 5). As inhibition of activity by *p*-CMB was not strong until a rather high level (1 mM) was used, these results suggest minor surface exposure of the *Alcaligenes* DL enzyme. Since DL does not require energy, the inhibition is presumably unrelated to any damage done to the cytoplasmic membrane. In vitro controls showed DL activity to display about the same sensitivity to *p*-CMB as it does in vivo, or less (40).

In *P. doudoroffii*, all three functions, DMSP uptake (Fig. 6A), DL induction (40), and DL activity (Fig. 6B), were inhibited by *p*-CMB and *p*-CMBS. Both DMSP uptake and DL activity were inhibited more strongly by the membrane-permeable reagent, *p*-CMB, suggesting that the uptake protein (5) is not fully exposed to the cell surface.

Effect of DMSP analogs. MMPA ($CH_3SCH_2CH_2COO^-$), which is a structural analog of DMSP, inhibits DL induction by DMSP and DL activity (measured in parallel cell suspensions) in both *Alcaligenes* (Fig. 7A) and *P. doudoroffii* (Fig. 7B). The inhibition of *P. doudoroffii* DL by MMPA indicates that this analog is taken up by the cell, since it inhibits the cytosolic enzyme (Fig. 7B). The fact that MMPA did not, however, inhibit DMSP uptake is consistent with DMSP and MMPA being taken up at different sites on the cell surface. Inhibition of DL activity in vivo by MMPA is consistent with its inhibition of in vitro activity (11). We recently learned that in *Alcaligenes* acrylate (not DMSP) is the inducer of DL (1) and that MMPA also inhibits acrylate-dependent DL induction (40).

Glycine betaine (GBT) [$(CH_3)_3N^+CH_2COO^-$], another analog of DMSP, inhibited both DMSP uptake and DL induction in cell suspensions of *P. doudoroffii*, with a similar $[I]_{0.5}$ of 5×10^{-4} M (Fig. 8). The low level of inhibition of DL activity by

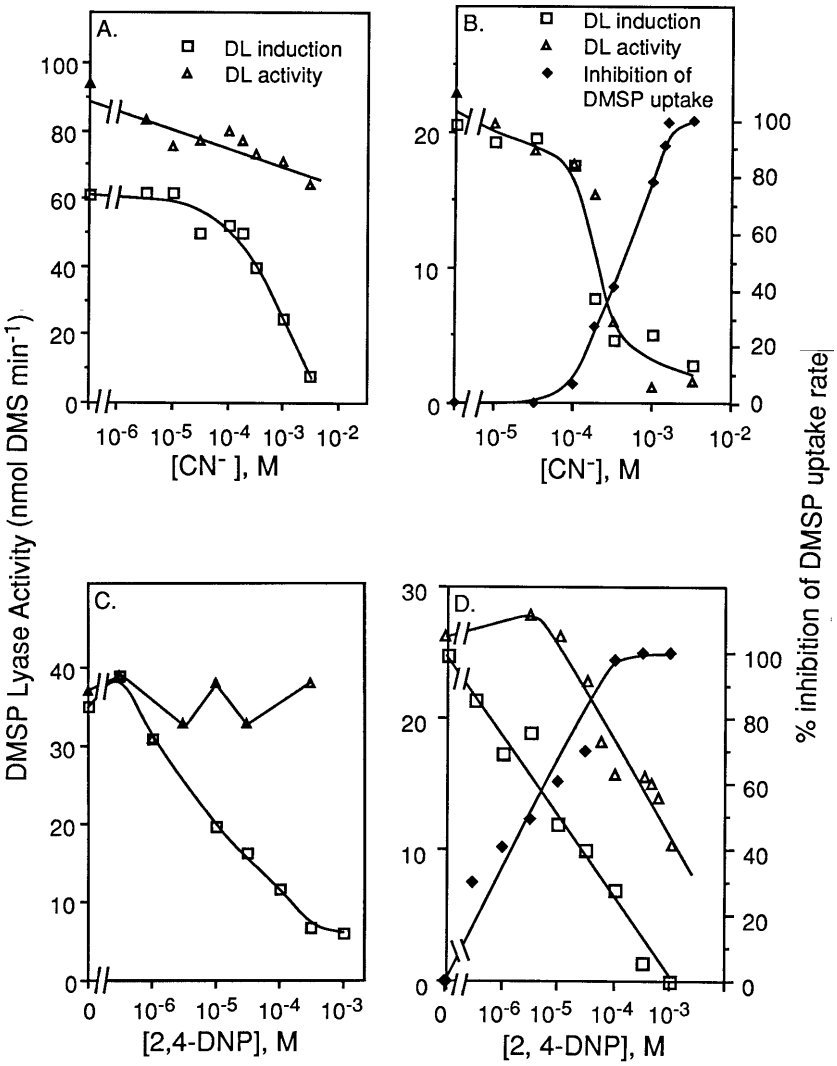

FIG. 4. Effects of cyanide and 2,4-dinitrophenol on DMSP accumulation, DL induction, and DL activity by *Alcaligenes* (A and C) and *P. doudoroffii* (B and D). Induction was measured as DL activity of cell suspensions after 3 h of exposure to 1 mM DMSP. Other details are found in Materials and Methods, and the amount of protein is as for Fig. 1 and 2. The 100% rates of DMSP intracellular accumulation in panels B and D were 650 and 810 μM min^{-1}, respectively.

GBT (ca. 15%) appears to be inconsistent with its strong inhibition of DMSP uptake; it does, however, confirm our earlier observation (10) that GBT is recognized as an analog of DMSP by the DMSP-binding protein(s) and the DL induction machinery of *P. doudoroffii* but not by its lyase.

DISCUSSION

To obtain a better understanding of the mechanism of DMS emission from marine bacteria, we have examined DMSP metabolism in two gram-negative isolates, *Alcaligenes* sp. strain M3A and *P. doudoroffii*. From these and other data (13, 22, 23), two models of bacterial structure and function relating to DMSP uptake, induction of DL, and cleavage of DMSP to DMS have emerged. In one model, represented by the *Alcaligenes* sp., DMSP is cleaved directly on the surface of DL-induced cells, with no uptake required (Fig. 9A). In the other model, represented by *P. doudoroffii*, DMSP is actively taken up by an inducible uptake system and concentrated intracellularly, where it is cleaved to DMS and acrylate (Fig. 9B). These conclusions and the supporting data are discussed below.

Localization of DL. Evidence suggesting a surface location for *Alcaligenes* DL is summarized as follows: (i) no DMSP accumulated in the cytosol; (ii) rates of DMSP removal from the medium and DMS production were nearly identical; (iii) DMS production was not inhibited by CN^- or 2,4-DNP, consistent with the proposition that active uptake of DMSP was not required for DL activity (DMSP cleavage); and (iv) DL activity was inhibited by the nonpermeable thiol reagent (*p*-CMBS). It was previously reported that *p*-CMB did not inhibit DL activity in this organism (10), but that report was incorrect; the reagent used to make the solutions was subsequently found to be nearly inactive, probably due to its age.

In *P. doudoroffii*, the intracellular location of DL was indicated from the following observations: (i) DMSP was taken up at a rate that exceeded the rate of DMS formation, and (ii) DL activity (DMS production) was strongly inhibited by CN^- and 2,4-DNP, consistent with DMSP being actively accumulated prior to its catabolism by the lyase (Fig. 9B).

DMSP uptake proteins. The prevention of DMS production in *P. doudoroffii* by inhibitors of respiration (CN^- and 2,4-

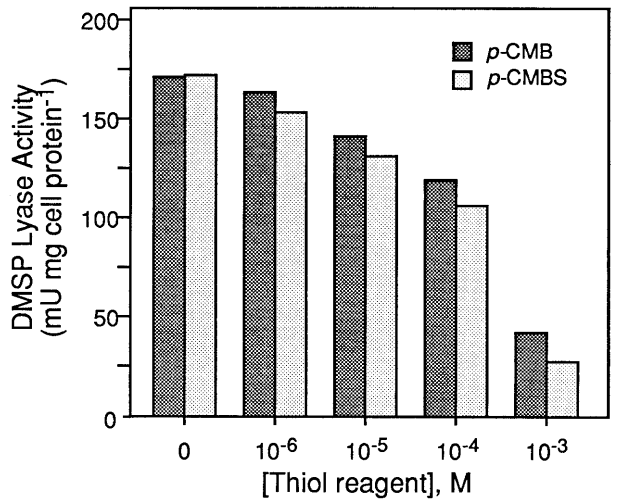

FIG. 5. Effects of *p*-CMB and *p*-CMBS on *Alcaligenes* DL activity. Cell protein concentrations were similar to those reported for Fig. 1 and 2. Data are the means of two experiments.

DNP) and thiol reagents strongly suggests the presence of a binding protein(s) on the cell surface needed for transport of DMSP into the cell (Fig. 9B). The differences in thiol sensitivity (*p*-CMB inhibition was stronger than that by *p*-CMBS) (Fig. 6) are consistent with the DMSP transport protein being partially accessible to the surface, since *p*-CMBS does not readily penetrate cell membranes (34).

The kinetics of DMSP uptake by noninduced *P. doudoroffii* cells suggest at least three constitutive uptake systems, with K_ts of ca. 3.4, 127 ± 40, and 500 μM. No attempt was made to measure uptake sites of higher affinity for DMSP (i.e., between our DMS detection limits of 80 nM and 1 μM), and therefore such sites cannot be ruled out. The threefold (minimum)-greater rate of DMSP accumulation by DL-induced cells (compared to that by noninduced cells) suggests that the DMSP-binding protein(s) is inducible in *P. doudoroffii*. For strain LFR (22), DMSP uptake rates and K_m values for DMSP uptake were reported to be similar for DL-induced and -noninduced cells. It is not known if strain LFR has other binding sites for DMSP with affinities lower than the 105 to 190 nM values reported, since the effect of higher concentrations was apparently not examined (22). The presence of an active DMSP uptake system in *P. doudoroffii* and strain LFR is consistent with observations that in natural populations of microbes in Monterey Bay seawater the half-saturation values for DMSP uptake were higher than the range of DMSP concentrations found in these waters (23). These data suggested to those workers that "we may not fully understand the microscale distribution of DMSP(d) in seawater."

Uptake sites for DMSP analogs. MMPA inhibited DL activity in *P. doudoroffii* cells (as it does in vitro), but DMSP uptake was unaffected by this analog (Fig. 7). Taken together, these data suggest that MMPA and DMSP are taken up at different sites on the cell surface. MMPA also inhibited DL induction by DMSP in both organisms. The explanation for the inhibition of DL induction in *P. doudoroffii* is mainly speculative at this time, but in *Alcaligenes*, MMPA prevents the small amount of constitutive DL from cleaving DMSP to DMS and acrylate (the inducer) (11). The constitutive DL in the model is depicted as DL* in Fig. 9A.

GBT is widely distributed in marine environments, being found as an osmoprotectant in *Spartina alterniflora* and other halophytes in salt marshes (28) and marine algae (15). The similar structures and cellular functions of GBT and DMSP make the effect of GBT on DMSP metabolism a matter of special interest. Observations that GBT inhibited DMSP uptake in *P. doudoroffii* (Fig. 8) and strain ML-G (12) suggest that these two analogs are taken up at the same site (Fig. 9B). Kiene and Gerard (20) drew a similar conclusion from GBT inhibition of DMSP degradation by microbes in seawater.

Proline and dimethyldisulfide, unlike GBT, did not inhibit DMSP uptake (or DL induction) by *P. doudoroffii* (20). This differs from the case for *Escherichia coli*, in which DMSP and GBT uptake have been linked to ProP and ProU (for a review, see reference 24), and suggests that in *P. doudoroffii*, DMSP and proline are transported by different binding proteins. That proline is transported by *P. doudoroffii* is seen by the fact that it was used as a carbon source (data not shown).

Evidence for an acrylate uptake site on *Alcaligenes* cells (Fig. 9A), although indirect, is as follows: (i) these cells grow on

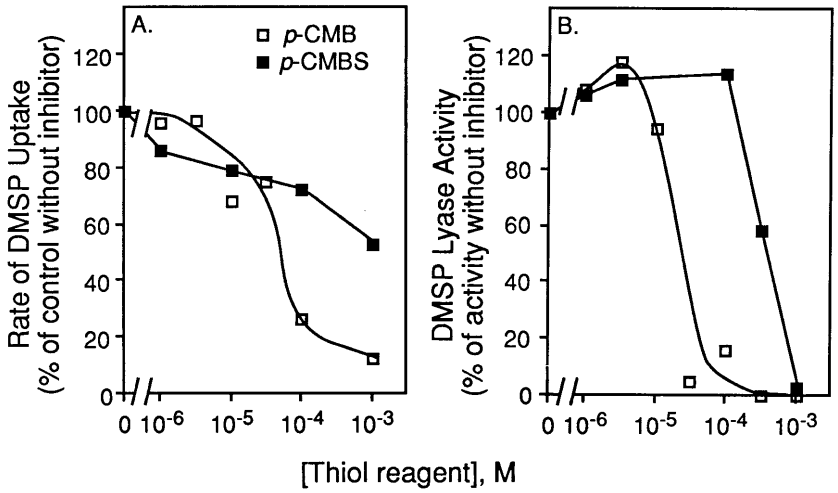

FIG. 6. Effects of *p*-CMB and *p*-CMBS on uptake of DMSP (A) and DL activity (B) in *P. doudoroffii*. The 100% initial rate of 100 μM DMSP uptake (in the absence of *p*-CMB) was 650 μM min^{-1}. All determinations were made with 1-ml cell suspensions. The protein concentration was 0.17 mg ml^{-1}.

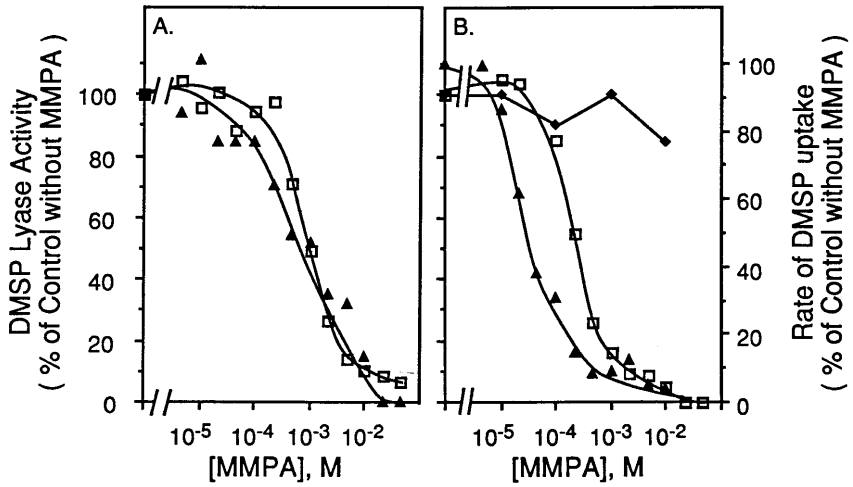

FIG. 7. Effect of MMPA on DL induction, DL activity, and the rate of DMSP uptake by *Alcaligenes* (A) and *P. doudoroffii* (B). The 100% rate of DL induction represents the DL activity after 3 h of exposure to DMSP in the absence of MMPA (0.063 [A] and 0.037 [B] μmol of DMS min^{-1} mg of cell $protein^{-1}$). The 100% rates of DL activity were 0.07 (A) and 0.05 (B) μmol of DMS min^{-1} mg of cell $protein^{-1}$. The 100% rate of *P. doudoroffii* DMSP uptake was ca. 490 μM intracellular accumulation min^{-1}. Note that *Alcaligenes* does not take up DMSP, so no attempt was made to measure it.

acrylate; (ii) acrylate induces DL activity, so it is probably taken up; and (iii) *p*-CMBS (the nonpermeable thiol reagent) partially inhibits DL induction by acrylate (40).

DMSP pools. *P. doudoroffii* has the ability to actively transport and concentrate into its cytoplasm high levels (3 to 40 mM) of DMSP, depending on the external concentration and the time allowed for accumulation (Fig. 2) (40). This finding appears to answer one of the persistent questions concerning the mechanism of DMS emissions, namely, how microorganisms with K_m values for DMSP cleavage in the micromolar (or higher) range can effectively utilize the ambient levels of DMSP (<10 nM) usually found in seawater (30). Our data suggest that the cells may accumulate DMSP via active transport systems, but how they manage to do this in seawater having such low concentrations is not understood. Unlike *E. coli* (16, 24) and the marine isolates which appeared to store DMSP (12, 13, 38), *P. doudoroffii* either cleaves DMSP immediately or induces DL so it can be used; only in the presence of MMPA, a DL inhibitor, does the intracellular pool level of DMSP remain elevated (40).

Alcaligenes, which does not accumulate DMSP intracellularly, would presumably be able to induce DL and utilize DMSP only in environments where DMSP levels are high, since the K_m values for induction and DL activity are 1 to 2 mM (11). We surmise that environments having such levels of DMSP might well be found near senescing phytoplankton populations that are leaking DMSP, as various species of these microorganisms are known to contain DMSP in concentrations

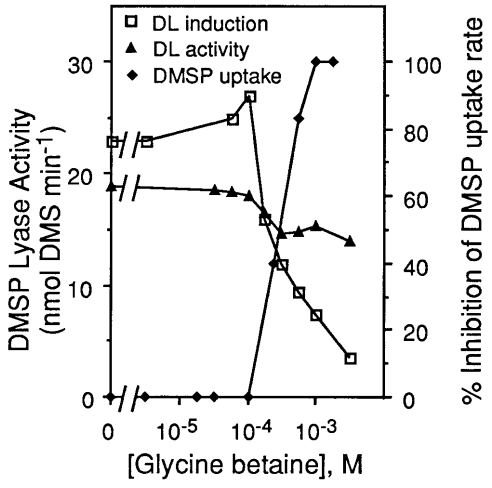

FIG. 8. Effect of GBT on DMSP uptake, DL induction, and DL activity by *P. doudoroffii*. Details are found in Materials and Methods.

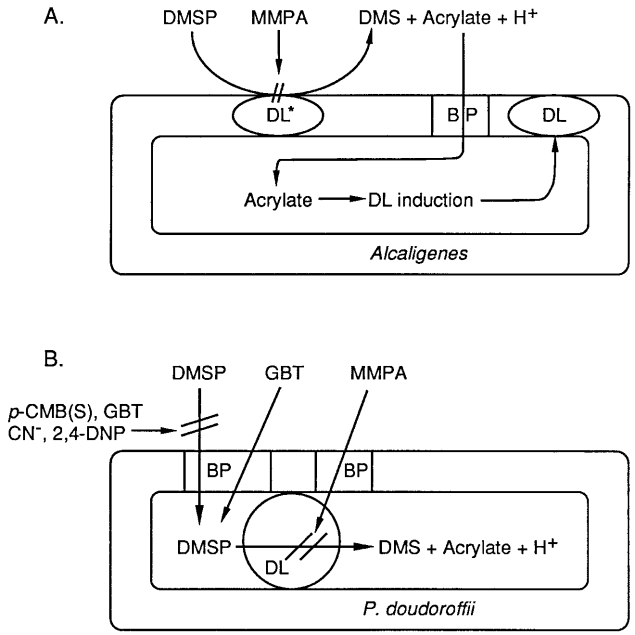

FIG. 9. Model of DMSP lyase location and uptake sites in *Alcaligenes* sp. strain M3A (A) and *P. doudoroffii* (B). BP, binding protein; DL*, the constitutive level of DL in the cell prior to induction. The existence of an acrylate BP is circumstantial; it is equally feasible that acrylate binding (uptake) is a functional domain of DL* itself.

in the millimolar range of tens to hundreds (17, 18, 31). Other environments with high DMSP levels are probably as numerous; for example, Visscher et al. (36) measured DMSP excreted from dead brine shrimp in shoreline lake sediments of Mono Lake which can be calculated to be ca. 20 mM in the porewater. In conclusion, on the basis of kinetic and inhibitor studies, a working model of DMS production which includes a DMSP-binding protein(s) and an intracellular DL in *P. doudoroffii* and a cell surface (extracellular) DL in *Alcaligenes* has been presented.

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