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NOTE

Characteristics of DMSP-lyase in *Phaeocystis* sp. (Prymnesiophyceae)

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ABSTRACT: The marine phytoplankton species *Phaeocystis* sp. is one of the few microalgae known to be able to convert dimethylsulfoniopropionate (DMSP) enzymatically into dimethyl sulfide (DMS) and acrylic acid. The function of this enzymatic process for the organism is not known. From experiments with crude extracts and whole cells of axenic cultures of *Phaeocystis* it was concluded that DMSP-lyase is membrane-bound and located extracellularly because: (1) the enzyme activity in extracts and in whole cells varied in a similar manner with pH; (2) between 50 and 80% of the DMSP-lyase activity was associated with the membrane fraction; (3) lyase activity in whole cells was inhibited by the non-permeable thiol-reagent *p*-chloromercuribenzenesulfonic acid (*p*CMBS). The pH optimum was 10.5 or higher, which is in contrast with available data for the enzyme from other organisms. The pH profile, the requirement for reduced thiol groups in extracts and the inhibition by *p*CMBS suggest the involvement of cysteine residues at the active site. Production of DMSP as well as its cleavage by DMSP-lyase are apparently not involved in the short term regulation of the osmotic potential of cells upon changes in salinity.

KEY WORDS: DMSP-lyase · DMSP · DMS · *Phaeocystis* Phytoplankton

The production of the most important volatile organo sulfur compound in the marine environment, dimethyl sulfide (DMS), occurs mainly through enzymatic cleavage of its precursor, dimethylsulfoniopropionate (DMSP). DMSP is produced by several groups of macro- and microalgae (Keller et al. 1989, Blunden et al. 1992). Since research began on the production of DMS in the marine environment, many suggestions have been made on the physiological role of DMSP. Most research has focused on the possible contribution of DMSP to the regulation of the cellular osmotic potential. Indeed, the compatibility of this solute with algal metabolism was confirmed in studies on the impact of DMSP on enzyme activities in extracts of the marine phytoplankter *Tetraselmis subcordiformis* (Gröne & Kirst 1991). Several authors have reported an increased cellular DMSP concentration at higher salinity levels (Vairavamurthy et al. 1985, Dickson & Kirst 1986, 1987a, b), but rapid changes in the intracellular concentrations of DMSP upon salinity changes were not always observed (van Diggelen et al. 1986, Edwards et al. 1987, Stefels et al. in press). Others suggested a function as cryoprotectant (Kirst et al. 1991, Nishiguchi & Somero 1992), methyl donor (Ishida 1968) or sulfur storage (van Diggelen et al. 1986). It has also been hypothesized that DMSP can substitute for other compatible solutes, e.g. those containing nitrogen under N-deficient conditions (Turner et al. 1988, Gröne & Kirst 1992).

With the function of DMSP within the algal cell still unclear, mechanisms involved in the regulation of intracellular DMSP levels are also under discussion. Only a few reports on the partial purification of the DMSP-lyase enzyme responsible for the cleavage of DMSP have appeared. The enzyme has been found in the macroalga *Polysiphonia lanosa* (Cantoni & Anderson 1956) and the heterotrophic dinoflagellate *Gyrodinium cohnii* (Ishida 1968). More reports have focused on the ability of microorganisms to produce DMS from DMSP (Kadota & Ishida 1972). Several aerobic as well as anaerobic bacterial strains able to cleave DMSP have been isolated from tidal flat areas (Quist & van der Maarel pers. comm.). A bacterium has been isolated from the Sargasso Sea which cleaves DMSP into DMS and acrylate (Ledyard & Dacey 1994). It in fact grows on acrylate, which the bacterium uses for its carbon source, and does not further metabolize DMS. The first homogeneous DMSP-lyase protein preparation

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has recently been obtained from a marine *Alcaligenes*-like bacterium (de Souza & Yoch 1995). The many field data suggest that bacteria may play an important role in the conversion of DMSP to DMS. Evidence for a contribution to the conversion of DMSP by algae has remained scarce, notwithstanding the fact that DMSP can account for 50 to 70% of total organic sulfur in some algal species (Matrai & Keller 1994), and that the suggested functions of DMSP imply the presence of a mechanism to remove it from the cell. Recently, Stefels & van Boekel (1993) reported the presence of DMSP-lyase activity in axenic cultures of the Prymnesiophyte *Phaeocystis* sp. Moreover, in natural seawater samples taken during a spring bloom off the Dutch coast, DMSP-lyase activity was found to be highly correlated with *Phaeocystis* numbers (Stefels et al. 1995).

In the present study, we report on some of the characteristic properties of DMSP-lyase in crude extracts and whole cells of *Phaeocystis* sp. and discuss the possible role of this enzyme in cellular physiology.

Materials and methods. ***Algal strain and culturing conditions:*** An axenic strain of *Phaeocystis* sp. (strain K) was used and regularly checked for bacterial contamination using Hoechst dye no. 33258 and fluorescence microscopy (Paul 1982). The organism was grown in filtered, sterilized (by autoclaving) North Sea water to which minor salts, vitamins and nutrients were added as described by Veldhuis & Admiraal (1987), with the exception that nitrate was the only nitrogen source. Cultures were grown in serum bottles placed on a rolling device, and incubated at 11°C and a light intensity of 85 $\mu E\ m^{-2}\ s^{-1}$ in a 14 h light:10 h dark cycle. Experiments with whole cells as well as with crude extracts were always performed with samples taken from the end-exponential growth phase to ensure physiologically comparable conditions.

Extract preparation: To 15 ml culture aliquots, 5 ml of a 50 mM Tris buffer (prepared in seawater, pH 8.3) with dithiothreitol (DTT, minimal end concentration 1 mM) was added. Cells were harvested by centrifugation at 800 × *g* for 20 min. Pellets were resuspended in the same Tris/DTT buffer, and disrupted using a French Pressure cell at 130 MPa. Buffer conditions during the experiments are given below.

Assay for DMSP-lyase activity: DMSP.HCl was used as substrate in the enzyme assay. Primary DMSP standards of approximately 10 mM were prepared in seawater and stored frozen. All assays were performed in 14 or 60 ml vials stoppered with a teflon Mininert valve. Vials were placed in the dark in a constant-temperature waterbath, usually at a temperature of 20°C unless stated otherwise. The standard assay mixture for crude extracts (5 ml) contained 50 mM Tris buffer (pH 8.3) with 3 to 3.7 mM DTT and an extract concentration of approximately 0.5 µg protein ml^{-1}. In whole cell assays, the mixture (5 ml) contained culture aliquots (2 to 4 × 10^5 cells ml^{-1}, equivalent to approximately 2 to 4 µg protein ml^{-1}) and 20 mM Bis-Tris Propane (BTP) buffer (pH 8.6). Deviations from the standard procedure in specific experiments are indicated. Reactions were started by addition of 100 µM DMSP. Under regular shaking of the vials, DMS evolution was measured in the headspace over 60 min, at 15 min intervals. A 100 µl gas sample from the headspace of the vial was injected into a Varian 3600 gas chromatograph equipped with a sulfur-specific Hall Electrolytic Conductivity Detector (Stefels & van Boekel 1993). For practical reasons, the substrate concentrations used in the experiments did not saturate the enzyme. During the 1 h assay, the amount of substrate used was typically 2 to 6% of the initial concentration and never exceeded 12%, resulting in activities linear in time (with typical r^2 values of 0.996) and proportional to enzyme concentrations. After each experiment, the pH of the assay mixture was checked. A calibration curve was prepared from DMSP standards in seawater to which NaOH was added (final concentration 1 M), resulting in an equimolar conversion of DMSP into DMS and acrylic acid (White 1982, Dacey & Blough 1987). Working standards were stored under the same conditions as assay samples. To obtain absolute values, DMS concentrations calculated from the working standards were multiplied by 1.4, to correct for the difference in gas/liquid equilibrium of DMS in samples with (the standards) or without (the assay samples) 1 M NaOH.

Protein measurements: Whole cell samples (harvested by centrifugation at 800 × *g* for 20 min) and 200 to 400 µl aliquots of crude extracts were treated with boiling NaOH (0.5 M) for 15 min and centrifuged subsequently. Supernatants were analysed with the Bio-Rad protein assay. Bovine serum albumin (BSA) was used as a standard.

Experiments with crude cell extracts: Extracts prepared from large cell numbers were first tested in dilution experiments to ascertain that DMSP-lyase activity was proportional to the amount of extract used.

Effect of DTT on enzyme activity: Crude extracts were prepared in 25 mM Tris buffer with 1 mM DTT (pH 8.0); 100 µl samples were then added to 20 ml 25 mM Tris buffer with increasing DTT concentrations (5 µM to 5 mM DTT). Following addition of 145 µM DMSP, DMS production over time was measured.

Effect of pH on enzyme activity: Crude extracts were prepared in 50 mM Tris buffer (pH 8.5, in 30‰ NaCl) with 5 mM DTT. A pH range from 4.5 to 10.5 was prepared with a buffer complex. For the pH range 4.5 to 8, a combination was made of 200 mM acetic acid + 200 mM $NaH_2PO_4.H_2O$ + 200 mM Tris in 30‰ NaCl;

for the pH range 8.5 to 10.5, 200 mM $NaHCO_3$ was added to the former complex. The pH was set by adding HCl or NaOH. In the assay, the final concentration of the buffer components was 50 mM and of DTT 3.7 mM. Assays were started by addition of 110 µM DMSP. Control samples for abiotic conversion of DMSP were treated in exactly the same way but without addition of the extract.

Effect of salinity and NaCl on enzyme activity: Crude extracts were prepared in 50 mM Tris buffer (pH 8.5, prepared in NaCl) with 5 mM DTT. A salinity range from 0 to 50‰ S was prepared by dilution or evaporation of a natural seawater sample; 0‰ S was equivalent to 100% demineralized water. Also, an NaCl range with comparable ionic strength was prepared (500 mM NaCl = 31‰ S). All samples were Tris/DTT buffered (pH 8.7). Assays were started by addition of 110 µM DMSP. Abiotic conversion of DMSP was checked for the highest and lowest salinity values only. Salinity was measured with a WTW Microprocessor Conductivity Meter LF 196.

Determination of kinetic properties: For practical purposes, the apparent K_m and V_{max} values were determined in crude extracts under non-optimal conditions (pH 8.3), using the initial rate of DMS production over a range of 0 to 15 mM DMSP. The assays were performed in 100 mM Tris/DTT buffer. After addition of DMSP from a neutralized stock solution, the rate of abiotic conversion in the buffer was measured first, then the assay was started with the addition of 50 µl crude extract.

Experiments with whole cells: Proportionality between enzyme activity and cell concentration was confirmed in dilution experiments.

Effect of temperature on enzyme activity: Culture aliquots of 20 ml were incubated at 5 different temperatures for 30 min, along with their own DMSP standard range for calibration. Abiotic conversion of DMSP was measured using GF/F-filtered culture medium, treated in the same way as whole cell samples. Assays were started by addition of 50 µM DMSP.

Effect of pH on enzyme activity: A pH range from 7.5 to 9.5 was prepared in 2 different buffers: 200 mM Tris (pKa = 8.3) and 200 mM BTP (pKa_1 = 6.8, pKa_2 = 9.0), both in seawater. The assay mixture contained 4.5 ml of culture and 0.5 ml buffer. Assays were started by addition of 50 µM DMSP. As a check for a possible deleterious effect of the buffers on living cells, a culture sample was analyzed in which the buffer was replaced with seawater. Abiotic conversion of DMSP was measured in a Tris buffer series, in which the culture was replaced with seawater.

Effect of salinity on enzyme activity: A salinity range from 6 to 56‰ S was prepared by dilution or evaporation of a natural seawater sample. Each sample was buffered with 20 mM BTP buffer (pH 9.4; comparable with the pH of late exponential phase cultures). Prior to the assay, aliquots of a culture (34‰ S) were mixed 1:1 with one of the prepared salinity samples and incubated for 30 min at 16°C. Final salinities ranged from 20 to 45‰ S. Assays were started by addition of 100 µM DMSP. Abiotic conversion was measured by replacing the culture medium with autoclaved seawater which was then subjected to the same salinity range. The physiological condition of the cells was checked by following the DMS evolution in a parallel series of shocked cultures to which no DMSP was added. Increased DMS evolution from intracellular DMSP in axenic cultures of *Phaeocystis* can be regarded as an indicator for lysis (Stefels & van Boekel 1993).

Localization of DMSP-lyase: Two experiments were performed: a membrane isolation from cell free extracts and an inhibition experiment with whole cells using the thiol-reagent *p*-chloromercuribenzenesulfonic acid (*p*CMBS) which acts only on the outside of cell membranes as a consequence of its large size.

Membrane fractions were isolated as follows: crude extract preparations were centrifuged at 2000 × *g* for 10 min to sediment cell debris. Aliquots of the supernatants were used for estimation of total DMSP-lyase activity; the remainder was carefully suspended on a 50% sucrose layer and centrifuged for 1 h at 100000 × *g*. The supernatant was used for estimation of the enzyme activity in the dissolved fraction; the membrane fraction was resuspended with the sucrose and subsequently assayed. The assay was performed in Tris/DTT buffer (pH 8.4). After addition of DMSP, the rate of abiotic conversion in the buffer was measured first, then assays were started by addition of 50 µl of the fractions.

Inhibition experiments with whole cells were performed: 1 series without a buffer and 2 series with BTP buffer (20 and 10 mM BTP at pH 8.6 and 9.4, respectively). Cultures were incubated with different concentrations of *p*CMBS (0.5, 1 and 2 mM) for 1 or 2 h. Because preparations of *p*CMBS from its sodium salt may be contaminated with small quantities of mercury chloride ($HgCl_2$) — also a thiol-reagent that acts in the same way as *p*CMBS, but it is able to penetrate the cell — simultaneous incubation of culture aliquots with a range of $HgCl_2$ concentrations was carried out, assuming a maximum dissociation of *p*CMBS of 10%. Thus, $HgCl_2$ concentrations of 50, 100 and 200 µM were used. As an abiotic control, GF/F-filtered culture medium was used and treated in the same way as culture samples. Assays were started by addition of 100 µM DMSP.

Chemicals: DMSP.HCl was prepared according to Chambers et al. (1987). *p*CMBS and DTT were obtained from Fluka BioChemika; $HgCl_2$ from Merck.

Results. In contrast to whole cells, crude extract preparations required addition of DTT to maintain DMSP-lyase activity. A maximum activity was reached at DTT concentrations of 1 mM (Fig. 1). At the minimum DTT concentration used in the assay (5 µM), DMSP-lyase activity had dropped to 20% of the maximum. No inhibition by DTT was measured at higher concentrations (assays were performed up to 20 mM DTT; data not shown). A DTT concentration of approximately 3 mM was used in subsequent experiments.

The DMSP-lyase activity in whole cells decreased with decreasing temperature (Fig. 2). Because the temperature optimum for growth of the *Phaeocystis* strain used lies between 12 and 21°C (van Boekel 1992), incubation temperatures for further experiments were chosen between 16 and 20°C.

Effect of pH on enzyme activity: In both extracts and whole cells, DMSP-lyase exhibited maximum activity at alkaline pH (Fig. 3). As abiotic decomposition of DMSP increases with increasing pH, it became problematic to measure enzyme activity above pH 10.5. At this pH, abiotic conversion of DMSP contributed over 30% of total DMS production in the extract preparations. At pH 7, enzyme activity had dropped to ca 25% of the activity at pH 10.5. In experiments with whole cells, abiotic decomposition of DMSP contributed 4 to 8% of total DMS production at the maximum pH of 9.5. DMSP-lyase activities in whole cells and extracts displayed comparable pH profiles. Buffers in the whole cell experiments were chosen for their non-permeability through the cell membrane. Although it is known that Tris sometimes interferes with enzyme reactions, whereas BTP is thought to be inactive, samples without buffer more closely followed the Tris profile.

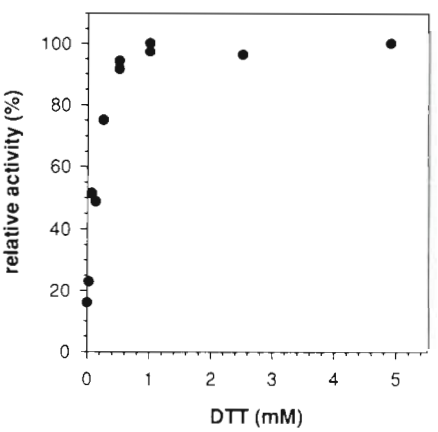

Fig. 1. Effect of dithiothreitol concentrations on DMSP-lyase activity in crude extracts of axenic *Phaeocystis* sp. cultures, expressed as percentage of the maximum activity; data are corrected for abiotic DMSP conversion

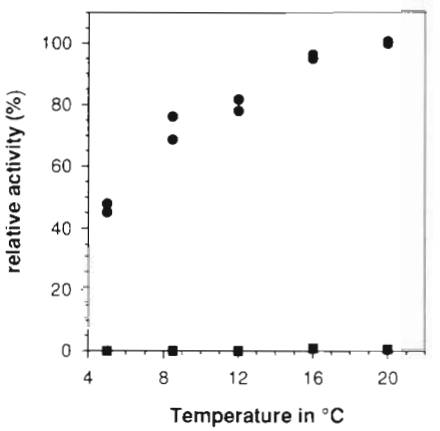

Fig. 2. Effect of temperature on DMSP-lyase activity in whole cells of axenic *Phaeocystis* sp., expressed as percentage of maximum activity; data are corrected for abiotic DMSP conversion; (●) activity in whole cells, (■) abiotic DMSP conversion

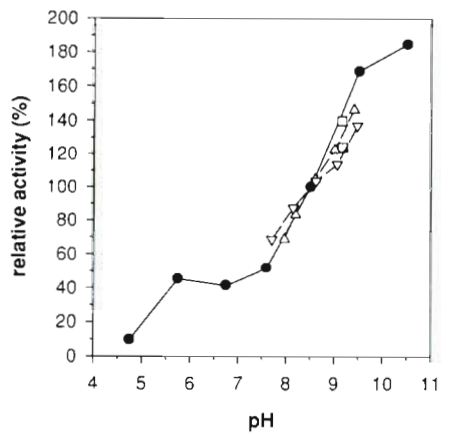

Fig. 3. Effect of pH on DMSP-lyase activity in crude extracts and whole cells of axenic *Phaeocystis* sp. cultures, expressed as percentage of the activity at pH 8.5; data are corrected for abiotic DMSP conversion; (—●—) activity in extracts; activity in whole cells: (--△--) (Tris-buffered), (--▽--) (BTP-buffered), (□) (without buffer)

Effect of salinity and NaCl on enzyme activity: The DMSP-lyase activity profiles of extracts and whole cells in response to salinity were also comparable (Fig. 4). A gradual decrease of activity was observed with increasing ionic strength (expressed as salinity). Abiotic decomposition of DMSP increased gradually with salinity. At 50‰ S abiotic conversion had increased by a factor of 1.5 when compared with activities at 0‰ S. Growth experiments at different salinities (Stefels unpubl.) had set limits on the salinity range used in the experiments with whole cells. The minor DMS release from intracellular DMSP during a 6 h period in a parallel series of shocked cultures indicated no severe damage to cells. In cultures which had experienced no or only slight salinity changes, 1.7% of total particulate DMSP

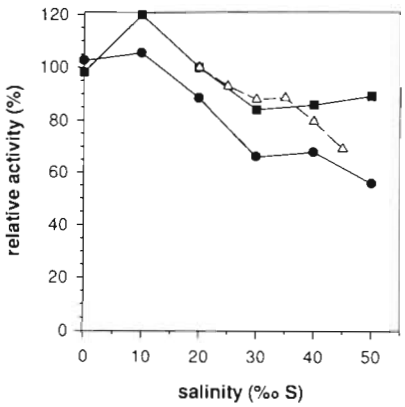

Fig. 4. Effect of salinity on DMSP-lyase activity in crude extracts and whole cell samples of axenic *Phaeocystis* sp. cultures, expressed as percentage of the activity at 20‰ S in seawater; data are corrected for abiotic DMSP conversion; activity in extracts: (—■—) in seawater, (—●—) in NaCl; (--△--) activity in whole cells

($DMSP_p$) was released as DMS during this 6 h period; cultures with largest salinity changes (up as well as down) released 2.5% of $DMSP_p$ as DMS. Total DMSP had increased slightly in all cultures during these 6 h, indicating stable physiological conditions (Stefels et al. in press). Extracts were also incubated in NaCl solutions which inhibited DMSP-lyase activity more than seawater. In Fig. 4, activities are related to total ionic strength of the incubation medium. It should be noted that the NaCl content of seawater accounts for approximately 50% of the total salt. Except for deviations in the higher salinity regions, this might indicate that lyase inhibition is almost exclusively the result of NaCl inhibition. Maximum activity was found at 10‰ S, equivalent to 160 mM NaCl in the NaCl series.

Kinetic properties: Because of the high abiotic conversion of DMSP in alkaline media, a suboptimal pH was chosen for kinetic experiments; at pH 8.3, DMSP-lyase activity is approximately 50% of the pH 10.5 activity. The Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) in crude extracts were calculated from repeated experiments using Lineweaver-Burk plots. The apparent V_{max} varied between 0.35 and 1.23 µmol min^{-1} mg^{-1} protein; the apparent K_m was determined as 2.25 mM DMSP. Crude extracts retained almost 90% of their activity even after 24 h on ice. The interpretation of the V_{max} values should be approached with caution, because only crude extracts were used and not pure enzyme.

Localization of DMSP-lyase: Cell fractionation experiments revealed that between 50 and 80% of total DMSP-lyase activity was associated with the membrane fraction, resulting in an increase of specific activity in this fraction by a factor of 3.5 compared with cell free extracts.

The experiments with the thiol-reagent *p*CMBS on whole cells demonstrated a clear reduction of DMSP-lyase activity (Table 1). Inhibition was strongest at high pH values. Considering the nature of the chemical reaction — the formation of mercaptide ions that associate with the organo mercurial — this was to be expected. At a *p*CMBS concentration of 2 mM, less than 6% of the activity was left when the pH was 9.35. It was assumed that at most 10% of *p*CMBS in solution is dissociated into $HgCl_2$, also a thiol-reagent. Although both inhibitors react with SH-groups, direct comparison of their reactivity can not be made. It is known that Hg^{2+} can react with either 1 or 2 SH-groups, whereas organic mercurials react with only 1. The difference in molecular structure may allow the inhibitors to react with different thiol groups within the enzyme. In general, however, $HgCl_2$ is more reactive than *p*CMBS. In our experiments, samples with 200 µM $HgCl_2$ exhibited a specific activity twice as high as samples with 2 mM *p*CMBS, indicating that *p*CMBS indeed did inhibit DMSP-lyase.

Discussion. The aim of this work was to carry out an initial characterization of the DMSP-lyase enzyme in *Phaeocystis* cells. By identifying the location of DMSP-lyase and analyzing its activity under different experimental conditions, it was expected that this information would provide a better insight in the physiological role of DMSP-lyase.

Although unequivocal evidence for the localization of an enzyme can only be obtained in specific labeling

Table 1. Inhibitory effects of the thiol reagents *p*-chloromercuribenzenesulfonic acid (*p*CMBS) and mercury chloride ($HgCl_2$) on DMSP-lyase activity in whole cells of *Phaeocystis* sp. In each series, a non-inhibited sample was measured simultaneously as a reference

Assay conditions	pH	*p*CMBS (mM)	$HgCl_2$ (µM)	Percentage of maximum activity
Non-buffered	9.06	0.5		58
1 h incubation	9.02	1		30
	8.86	2		17
Non-buffered	9.06	0.5		44
2 h incubation	9.02	1		20
	8.86	2		10
20 mM BTP buffer	8.61	0.5		57
2 h incubation	8.61	1		47
	8.59	2		34
	8.61		50	63
	8.62		100	57
	8.62		200	53
10 mM BTP buffer	9.35	2		6
2 h incubation	9.43		50	14
	9.41		100	13
	9.42		200	12

experiments, our data provide strong evidence for an extracellular location of DMSP-lyase in *Phaeocystis* cells. Firstly, DMSP-lyase activities in extracts and whole cells clearly varied with buffer pH values. It has to be expected that under the conditions used the intracellular pH will be influenced only slightly by changes in extracellular pH. The comparable responses of DMSP-lyase activity in whole cells and extracts thus provide a strong argument for an extracellular location of the lyase. Secondly, we found that DMSP-lyase in *Phaeocystis* is membrane-bound: 50 to 80% of total activity was present in the membrane fraction following cell fractionation. The pH response and this association with the membrane fraction suggest that the enzyme is bound to the outer surface of the cell membrane. An association with an intracellular membrane — e.g. vacuolar membranes that are involved in osmotic regulation — is not likely to be the case, considering the enzyme kinetics found in whole cells: instantaneous conversion of extracellular DMSP was observed in the low micromolar ranges, whereas intracellular DMSP concentrations, present at 150 to 200 mM (Stefels & van Boekel 1993), remained unaffected. Thirdly, the inhibition with *p*CMBS — a non-permeable thiol-reagent — strongly suggests an extracellular location.

The characteristics of the *Phaeocystis* DMSP-lyase (this study) allow some interesting comparisons with other known DMSP-lyases. Ishida (1968) and Cantoni & Anderson (1956) studied crude enzyme preparations of the heterotrophic dinoflagellate *Gyrodinium cohnii* and the macroalga *Polysiphonia lanosa*, respectively, and also observed a requirement of DMSP-lyase for reduced thiol groups. In contrast, these authors observed an acidic pH optimum of the DMSP-lyase enzymes, whereas the pH optimum of this enzyme in crude extracts of *Phaeocystis* sp. was clearly alkaline. In *G. cohnii*, a pH optimum was found between 6 and 6.5; in *P. lanosa*, a pH optimum around 5.1 was observed that declined gradually on the alkaline side of this pH. Recently, de Souza & Yoch (1995) purified DMSP-lyase from a facultatively anaerobic marine bacterium. In their study, no requirement for reduced thiol groups was mentioned; the optimal pH was found to be 8.

The reactivity of an enzyme with pH gives an indication of the identity of the amino acid residues involved in catalysis at the active site. Although the *pKa* of this residue may vary considerably according to the nature of its environment within the enzyme, and therefore should be used with caution, the *pKa* of approximately 8.5 in *Phaeocystis* DMSP-lyase is, however, indicative of a cysteine residue. Also, the need for a reducing environment in extracts (DTT) and inhibition by *p*CMBS is consistent with an active role of cysteine residues at the active site of the enzyme. Under alkaline conditions, the deprotonated form of the thiol group of cysteine may act as proton acceptor when DMSP is cleaved into DMS and acrylate:

$$(CH_3)_2S^+-CH_2-CH_2-COO^- \rightarrow (CH_3)_2S + CH_2=CH-COO^- + H^+$$

What does this tell us about the physiological role of DMSP-lyase in *Phaeocystis*? The main function of DMSP in marine micro- and macroalgae may be the regulation of the osmotic potential of the cell. If we assume this to be the case in *Phaeocystis*, there are 2 ways of regulating intracellular DMSP concentrations upon changes of the water potential of the environment. One or both of the following mechanisms can be triggered: the production of DMSP can be affected, or a change in activity of the DMSP-lyase is induced. In up- and down-shock experiments with whole cells, however, we could not detect significant changes in intracellular DMSP content within 6 h after the shock (Stefels et al. in press). This is in contrast to other observations, e.g. of *Hymenomonas carterae* (Vairavamurthy et al. 1985). Also, the effect of salinity changes upon DMSP-lyase activity (Fig. 4) can not be regarded as an accurate mechanism in the regulation of intracellular DMSP concentrations, especially when considering the salinity range in which *Phaeocystis* can grow effectively: 25 to 40‰ S (Stefels unpubl.). Although intracellular DMSP concentrations do change when cultures are grown in different salinity media, these concentrations change only gradually rather than being regulated actively. We conclude from these observations that, in the case of *Phaeocystis*, DMSP can not be regarded as an osmoticum in the sense of an active regulator, although its high intracellular concentration of course contributes to the osmotic potential of the cell.

An active role for the extracellularly located DMSP-lyase in the cleavage of intracellularly produced DMSP would necessitate the need to transport DMSP across the cell membrane. In experiments with *Phaeocystis* cultures, a small but constant release of DMS could be observed during the exponential growth phase, amounting to 1-2% of intracellular DMSP per day (Stefels unpubl.). The presence of DMSP-lyase suggests that this release is beneficial to the alga and not merely the result of leakage. Further study into the physiological function of this catalytic process will be undertaken.

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