Comparative functional characteristics of DMSP lyases extracted from polar and temperate Phaeocystis species

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ABSTRACT: Members of the marine phytoplankton genus Phaeocystis (Prymnesiophyceae) produce large amounts of the intracellular osmolyte DMSP and they are known to also produce lyase enzymes that cleave DMSP into the biogeochemically important trace gas DMS. The functional characteristics of DMSP lyase activity in Phaeocystis spp. are not well known. We characterized DMSP lyase activity in extracts from 2 ecologically important species from this genus, the mesophile P. globosa (strain CCMP629) and the psychrophile P. antarctica (strain CCMP1374). Results from whole cell extracts showed that both algal species were potent producers of DMSP lyase, with Michaelis-Menten constant (Km) and maximum reaction velocity (Vmax) values of 1.77 mM and 17.3 nmol DMS min⁻¹ mg protein⁻¹, respectively, for P. globosa, and 2.31 mM and 28.2 nmol DMS min⁻¹ mg protein⁻¹, respectively, for P. antarctica. The optimal DMSP lyase activity was recorded at pH 4 and 30°C for P. globosa, and at pH 5 and 20°C for P. antarctica. The half-life of the DMSP lyase of P. globosa was 210 min at 25°C, which was longer than that of the P. antarctica enzyme (61.9 min). First-order kinetic analysis of DMSP lyase thermal denaturation demonstrated that the activation energy, free energy, enthalpy and entropy of denaturation in P. antarctica extracts were lower than for P. globosa extracts, confirming that the P. antarctica DMSP lyase was more thermolabile than the lyase from the temperate strain. Inhibitor tests with metals, a chelator (EDTA) and a serine binding agent (PMSF) suggested that the DMSP lyases from both Phaeocystis species were metalloenzymes with serine and sulfhydryl groups at the active site. The acidic pH optima for the Phaeocystis strains are consistent with findings from other Prymnesiophyceae, and we speculate that this may reflect adaptation to an acidic sub-cellular location for the DMSP lyase.

KEY WORDS: Dimethylsulfide · Dimethylsulfoniopropionate · DMSP · DMSP lyase · Prymnesiophyte · Psychrophile

INTRODUCTION

DMSP is a secondary metabolite synthesized by a wide variety of marine algae (reviewed in Keller et al. 1989). DMSP has been linked to different physiological functions, such as osmoregulation (Dickson & Kirst 1987), cryoprotection (Kirst et al. 1991, Karsten et al. 1996), oxidative stress protection (Sunda et al. 2002), methyl donor (Ishida 1968) and overflow metabolite for excess reducing power during photosynthesis (Stefels 2000). DMSP degradation in marine environments occurs via 2 different biochemical pathways (reviewed in Curson et al. 2011, Moran et al. 2012). In the first pathway, the DMSP is cleaved
via the enzyme DMSP demethylase to 3- (methyl-
mercapto)propionic acid (MMPA). In a subsequent
reaction, MMPA either undergoes demethylation to
form 3-mercaptopropionic acid and/or demethyla-
tion to form methanethiol (Reisch et al. 2011). In the
second pathway, DMSP lyase (EC 4.4.1.3) catalyzes
the lysis of DMSP into dimethylsulfide (DMS), acry-
late and a proton. There is worldwide interest in
evaluating the biochemical and molecular mecha-
nisms involved in the regulation of enzymatic cleav-
age of DMSP to DMS because DMS is an important
component of the global sulfur cycle and a potential
cclimate-active trace gas. DMS is supersaturated in all
surface ocean waters and, once transferred to the
atmosphere, it rapidly oxidizes to dimethylsulfoxide,
methanesulfonate (MSA) or sulfate. Sulfur-contain-
ing, acidic aerosols derived from these compounds
can affect the Earth’s climate system by scattering
incoming sunlight and contributing to the population
of aerosol particles that act as cloud condensation
nuclei (Charlson et al. 1987, Simó 2001).

DMSP lyases are the key enzymes responsible for
the biogenic production of DMS in marine waters.
They have been found in marine algae, including
phytoplankton (Steinke et al. 1996, 1998), and in
a variety of different bacteria (de Souza & Yoch
1995a) in which 6 different DMSP lyase proteins
(DddD, Dddl, DddP, DddQ, DddW and DddY) have
been identified (Todd et al. 2007, 2009, 2011, 2012,
these proteins except DddD catalyze cleavage of
DMSP into acrylate and DMS. The DddD protein
produces 3-hydroxypropionate instead of acrylate.
Most of the genes encoding bacterial DMSP lyases
are prone to horizontal gene transfers, and in the
case of dddl, the gene has been subjected to inter-
domain horizontal gene transfer between bacteria
and Ascomycete fungi (Todd et al. 2009). While
DMSP lyases from bacteria have been relatively
well characterized at the genetic level, much less
is known about phytoplankton DMSP lyases. Pub-
lished studies of phytoplankton DMSP lyases have
shown that their occurrence and properties are
often species- and strain-specific (Steinke et al.
2009, Franklin et al. 2010), but few species have
been well characterized.

*Phaeocystis* (Prymnesiophyceae) is a cosmopolitan
group of marine phytoplankton that comprises 6 dif-
ferent species, including colony-forming *P. globosa*,
P. antarctica and *P. pouchetii*, and unicellular *P. cor-
data*, *P. jahnii* and *P. scrobiculata* (Zingone et al.
1999). Colonial *Phaeocystis* species form massive
blooms in both temperate and polar regions and sub-
stantially impact the yield of commercially important
marine products (Schoemann et al. 2005). *Phaeoce-
tis* blooms play a major role in the biogeochemical
cycle of carbon and sulfur by fixing a large amount of
carbon dioxide and by producing high concentra-
tions of DMSP and DMS (Stefels & van Leeuwe 1998,
2003), and by providing an important substrate for
bacterial carbon demand (Rellinger et al. 2009).

Despite the importance of *Phaeocystis* species in
global biogeochemical cycles, climate dynamics and
coastal fisheries, very few studies have been per-
formed to characterize the DMSP lyase activity of
bloom-forming *Phaeocystis* (Stefels & van Boeke1993,
Stefels et al. 1995, Stefels & Dijkhuizen 1996). The
objective of the present study was to compare the
functional (i.e. DMSP cleaving) properties of
DMSP lyase extracted from 2 different, but closely
related *Phaeocystis* species: the mesophile *P. globosa*
and the psychrophile *P. antarctica*. Our findings de-
fine the significant properties of DMSP lyase in these
ecologically important species that will be useful in
future investigations into the ecophysiology of DMSP
cleavage in temperate and polar regions of the world
oceans.

**MATERIALS AND METHODS**

*Phaeocystis* culture

*P. globosa* (CCMP 629), originally isolated from the
subtropical North Atlantic (−23° N, 75° W; typical tem-
perature range 20 to 30°C) and *P. antarctica* (CCMP
1374), isolated from the Ross Sea (77.8° S, 163° E; −1.8
to 3.0°C), were obtained from The Provasoli-Guillard
Center for the Cultivation of Marine Phytoplankton
(CCMP, Maine, USA; now the National Center for
Marine Algae and Microbiota [NCMA]). The batch
cultivation of the algal species was carried out in
duplicate in 600 ml polycarbonate tissue culture
flasks with 0.2 µm vent caps (Corning), containing
500 ml f/2 medium (Guillard 1975). The incubation
temperature was 20 and 1°C for

P. antarctica

and

P. globosa

500 ml f/2 medium (Guillard 1975). The incubation
temperature was 20 and 1°C for

P. antarctica

and

P. globosa

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0.56 ± 0.05 µg ml⁻¹ for *P. globosa* and 0.47 ± 0.03 µg ml⁻¹ for *P. antarctica*. The chl *a* concentration was determined fluorometrically as described previously (Parsons et al. 1984). Both phytoplankton species were predominantly in the colonial cell form at the time of harvest.

**DMSP lyase extraction**

The extraction of DMSP lyase was performed as reported previously with some modifications (Harada & Kiene 2012). Briefly, algal pellets from each flask were resuspended in 5 ml of ice-cold 200 mM Tris-HCl buffer (pH 7.5) containing 500 mM NaCl, 2 mM dithiothreitol (DTT) and 0.1% (v/v) Triton X-100, followed by vigorous vortex mixing and centrifugation at 3500 × *g* (4°C) for 15 min. The supernatant was collected and concentrated using an Amicon Ultra 10000 MWCO centrifugal filter device (Millipore). As the standard deviation of the DMSP lyase activities of the replicate cultures of each algal species were <10% of the mean values, the concentrated fractions of each algal species were pooled together. The pooled fractions were diluted with 10 ml of 25 mM Tris-HCl buffer (pH 7.5), divided into 0.5 ml aliquots and stored at −20°C. The aliquots were used for characterization studies. There was no loss in enzyme activity detected during the 4 wk period during which characterization studies were performed.

**DMSP lyase assay**

DMSP lyase activity was defined as the rate of conversion of DMSP to DMS. DMSP lyase activity was assessed at 30 and 20°C for *P. globosa* and *P. antarctica*, respectively, in sealed 10 ml serum vials containing a standard assay mixture of 146 µl assay buffer (200 mM phosphate-citrate buffer adjusted to pH 4 for *P. globosa* and pH 5 for *P. antarctica*) and 50 µl of enzyme (~120 µg protein ml⁻¹). The reaction was started by the addition of 4 µl of 250 mM DMSP-HCl aqueous solution (5 mM final concentration), and the resulting DMS in the headspace of the vial was quantified as described in the DMSP lyase assay section. The abiotic corrections of DMS were made in a similar fashion with sterile f/2 medium. Bacterial abundance in the GF/C filtrate was quantified using DAPI staining as described previously (Porter & Feig 1980).

**DMSP lyase: pH and temperature dependence**

To determine the optimum pH for DMSP lyase activity in *Phaeocystis globosa* and *P. antarctica* extracts, the enzyme assay was performed with buffers of different pH added to the assay mixture: 200 mM phosphate-citrate (pH 3 to 6), 200 mM Tris-HCl (pH 7, 7.5 and 8) or 200 mM borate (pH 9 and 10). The temperature dependence of lyase activity was assayed from 5 to 50°C at 5°C increments. We also carried out pH and thermal stability studies in which the enzyme was exposed to a non-optimal pH (or temperature in a separate experiment) and then returned to the standard condition (pH 4 and 30°C for *P. globosa*, and pH 5 and 20°C for *P. antarctica*). The temperature dependence of lyase activity was assayed from 5 to 50°C at 5°C increments. We also carried out pH and thermal stability studies in which the enzyme was exposed to a non-optimal pH (or temperature in a separate experiment) and then returned to the standard condition (pH 4 and 30°C for *P. globosa*, and pH 5 and 20°C for *P. antarctica*). The pH stability tests were carried out by pre-incubation of the DMSP lyase solution at 20°C for *P. antarctica* or 25°C for *P. globosa* in one of the above-mentioned buffers at different pHs ranging from 3 to 10 for
15 min. The reaction mixture was then returned to pH 4 and 5 for P. globosa and P. antarctica, respectively, and the DMSP lyase activity was assayed. For the temperature stability tests, lyase preparations were pre-incubated for 30 min at different temperatures between 5 and 45°C (with 5°C increments), before the residual enzyme activity was assayed at optimum temperatures, as described in the DMSP lyase assay section.

**Determination of kinetic constants**

To determine the Michaelis-Menten constant ($K_m$) and maximum reaction velocity ($V_{max}$) for DMSP lyase extracted from the 2 different Phaeocystis species, the enzyme activity was measured as described in the DMSP lyase assay section by using DMSP substrate at different concentrations (0.1, 0.5, 1, 2.5, 5, 10 mM DMSP, 2-chloro DMSP, dimethylsulfonioacetate, choline-o-sulfate and glycine betaine) were added to the assay buffer (200 mM phosphate-citrate pH 4 buffer for Phaeocystis globosa and pH 5 buffer for P. antarctica). Then 50 µl of enzyme extract and 4 µl of DMSP were added to 146 µl of the ionic-strength-adjusted buffer solution, and the enzyme activity assayed as described in the DMSP lyase assay section.

**Effect of NaCl**

The effect of NaCl on DMSP lyase activity for both algal species was examined by adding aqueous NaCl (final added concentrations of 0.086 to 0.86 M in 0.086 M increments) to the assay buffer (200 mM phosphate-citrate pH 4 buffer for Phaeocystis globosa and pH 5 buffer for P. antarctica). Then 50 µl of enzyme extract and 4 µl of DMSP were added to 146 µl of the ionic-strength-adjusted buffer solution, and the enzyme activity assayed as described in the DMSP lyase assay section.

**Effect of DMSP analogs, metal ions and organic chemicals**

The effect of different DMSP analogs (2-methyl DMSP, 2-chloro DMSP, dimethylsulfonioacetate, choline-o-sulfate and glycine betaine) was determined by incubating the enzyme solution with the individual analog (final concentration 20 mM) for 10 min at 20 or 25°C for Phaeocystis antarctica or P. globosa, respectively. After a 10 min pre-incubation with the DMSP analog, the lyase enzyme activity was determined for each sample through the addition of 5 mM DMSP (final concentration) to the assay mixture. The enzyme activity was determined as described in the DMSP lyase assay section and compared to the control activity in the assay mixture without the added DMSP analog. Similarly, metal ions (Ca$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, Cu$^{2+}$, Ni$^{2+}$) and several organic chemicals, EDTA, DTT (dithiothreitol), PMSF (phenylmethanesulfonylfluoride) and pCMB (parachloromercuribenzoate), were tested at 0.1 and 1.0 mM. All the metal ions were in the chloride form and analytical reagent (ACS) grade. DTT, PMSF and pCMB were molecular biology grade. Separate controls were also prepared containing either the individual metal ion or organic chemical but with no added DMSP. Assays were repeated twice (n = 2) and data are presented as the mean ± SD. Differences were determined by calculating p-values using a one-tailed t-test (Microsoft Excel 2010).

**Thermodynamics of DMSP lyase**

The kinetics of thermal denaturation of DMSP lyase in extracts from both algal species were studied by incubating the enzyme solution at different temperatures (20 to 35°C in 5°C increments) for 60 min. Samples were removed at 15 min intervals, rapidly cooled in ice for 10 min and assayed for DMSP lyase activity using standard assay conditions (DMSP lyase assay section). The value of the first-order denaturation constant ($K_d$) was determined at each temperature by measuring the slope of the plot of ln ($A/A_0$) versus incubation time ($t$), where $A_0$ = initial enzyme specific activity, and $A$ = measured enzyme specific activity after heat treatment. The half-life of the enzyme ($t_{1/2}$, min) was calculated using Eq. (1), assuming first-order kinetic decay:

$$t_{1/2} = \ln 2/K_d$$

The activation energy of denaturation ($E_d$) was estimated from the slope ($-E_d/R$) of an Arrhenius plot of ln($K_d$) versus the reciprocal of absolute temperature ($1/T$), where $R$ (gas constant) = 8.314 J mol$^{-1}$ K$^{-1}$. Values for changes in free energy ($\Delta G_d$), enthalpy ($\Delta H_d$) and entropy ($\Delta S_d$) of denaturation were determined using Eqs. (2), (3) and (4), respectively:

$$\Delta G_d = -RT \ln ([K_d]h / [K_b]T)$$

$$\Delta H_d = E_d - RT$$

$$\Delta S_d = (\Delta H_d - \Delta G_d)/T$$

All kinetics measurements were performed twice and the mean values are reported. In all cases, SDs of the mean values were <15%.
RESULTS AND DISCUSSION

The _Phaeocystis_ cultures we used contained bacteria, but at relatively low abundances; 2.36 ± 0.19 × 10^5 cells ml⁻¹ and 2.45 ± 0.22 × 10^4 cells ml⁻¹ in the <1.2 μm size fractions (GF/C filtrate) of the _P. globosa_ and _P. antarctica_ cultures, respectively. The DMSP lyase activities were compared between the particles retained on GF/C filters (>1.2 μm; phytoplankton fraction) and those passing through the GF/C and subsequently retained on a 0.22 μm filter (bacterial fraction). For the _P. globosa_ culture, the activity in the phytoplankton fraction was 17.3 ± 0.94 nmol min⁻¹ ml⁻¹ whereas it was 0.33 ± 0.02 nmol min⁻¹ ml⁻¹ in the bacterial fraction. For the _P. antarctica_ culture, the values were 19.7 ± 2.06 and 0.29 ± 0.014 nmol min⁻¹ ml⁻¹ in the phytoplankton fraction and the bacterial fraction, respectively. Thus, >98% of the DMSP lyase activity was associated with the phytoplankton cells. This finding is consistent with previous studies that have shown that the majority of DMSP lyase activity is associated with phytoplankton size classes in seawater and phytoplankton cultures (Steinke et al. 2002, Yost & Mitchelmore 2009, Harada & Kiene 2012). While some bacteria may have been attached to the _Phaeocystis_ cells, our preliminary studies on the de novo peptide sequencing of the partially purified _Phaeocystis_ DMSP lyases indicated lack of homology with the 6 known proteins of bacterial DMSP lyases (B. R. Mohapatra et al. unpubl. data). Thus, the extracted lyase activities that we report on below are likely to reflect those of the _Phaeocystis_ enzymes.

The effect of pH on the DMSP lyase activity in extracts from _Phaeocystis globosa_ and _P. antarctica_ indicated that the enzymes of both algal species retained >70% of the maximum enzyme activity over a broad pH range (4.0 to 7.5) (Fig. 1). The optimum pH of the lyase activity was clearly acidic and nearly the same for both species, with an optimum rate at pH 4 for _P. globosa_ (11.7 ± 1.66 U) and pH 5 for _P. antarctica_ (19 ± 2.7 U) at 30°C and 20°C, respectively. The optimum pHs observed here are much lower than the pH 10.5 optimum reported for _Phaeocystis_ sp. strain K (likely _P. globosa_) by Stiefels & Dijkhuizen (1996), but are within the range of that previously reported for _in vitro_ DMSP lyase activity in other prymnesiophyte extracts, including 3 different strains of _Gephyrocapsa oceanica_ (pH 5) (Franklin et al. 2010) and 4 strains of _Emiliania huxleyi_ (pH 4 to 6) (Steinke et al. 1998, 2000). The lyases from _P. globosa_ and _P. antarctica_ also had lower pH optima than found in the green macroalga _Ulva curvata_ (pH 8) (de Souza et al. 1996), or in the bacteria _Roseovarius nubinhibens_ (pH 6) (Kirkwood et al. 2010) and _Alcigenes_ sp. strain M3A (pH 8) (de Souza & Yoch 1995a). For the _Phaeocystis_ strains studied here, DMSP lyase activity declined substantially at assay pHs >7.5, and at pH 9 the activity was diminished by >80% from the optimum.

Results of the pH stability study, in which the DMSP lyase enzyme was exposed to a test pH for 15 min, then returned to its optimum pH, revealed that the enzymes of both _Phaeocystis globosa_ and _P. antarctica_ were relatively stable in the pH range between 4 and 7.5, returning to >70% of maximum activity after exposure to a sub-optimal pH. The stability test also showed that lyase activity was lost rapidly at pH 3 (>60% loss) and above pH 8 (>80% loss), and the loss in activity at these or other pHs was not recovered when the assay solution pH was returned to the optimal pH (Fig. 2).

Collectively, the results of the pH tests suggest that the DMSP lyase enzymes in _Phaeocystis globosa_ and _P. antarctica_ function optimally at the low pH characteristic of some intracellular environments (e.g. secretory vesicles, chloroplast lumen) rather than the cell surface environment exposed to seawater (pH ∼8). Relatively little is known about the cellular location of DMSP lyases in algae or their physiological functions. Recently it was found that acidification of seawater or culture samples containing colonial _Phaeocystis_ sp. to pH ∼1 caused rapid losses of intracellular DMSP through conversion to DMS (del Valle et al. 2011). The authors concluded that DMSP lyases remained...
active for several minutes following acidification before being inactivated by the low pH (~1). The high tolerance towards acidity of the DMSP lyase in *P. globosa* and *P. antarctica* that we observed here (25 to 50% of maximum activity at pH 3) is consistent with the findings of del Valle et al. (2011). Recently, Orelñana et al. (2011) speculated that DMSP lyases could be present in acidic secretory vesicles of *P. antarctica*, where both DMSP and DMS are associated with condensed polysaccharides that ultimately expand to form mucous polymers upon release to alkaline seawater. Another potential sub-cellular location with low pH is the chloroplast lumen. DMSP lyase has been proposed to be part of an antioxidant system in phytoplankton (Sunda et al. 2002) where cleavage of DMSP near the site of reactive oxygen species production (e.g. chloroplasts) could be beneficial to cells. It will be interesting in future work to test whether DMSP lyases are specifically adapted to function in such acidic sub-cellular locations.

DMSP lyase activity in extracts of *Phaeocystis globosa* and *P. antarctica* showed sharp and distinct temperature optima with maximum enzyme activities at 30 and 20°C, respectively (Fig. 3a). At 40°C, the DMSP lyase of *P. globosa* and *P. antarctica* exhibited only 38 and 18% of maximum enzyme activity. Nearly complete inactivation of the enzyme activity was observed above 45°C. The activation energy of catalysis (Ea) of DMSP lyases of *P. globosa* and *P. antarctica*, calculated from an Arrhenius plot, were 70.1 and 71.1 kJ mol⁻¹, respectively (Fig. 3b). Previous temperature dependence studies on the DMSP lyases of different algal and bacterial species documented variation in temperature optima. The temperature optimum was reported as 25, 27 and 35°C for the algal species *Enteromorpha clatharata* (Steinke & Kirst 1996), *Cryptothecodinium cohnii* (Kadoya & Ishida 1968) and *Emiliania huxleyi* CCMP379 (Steinke et al. 1998), respectively, and 60°C for the bacterium *Roseovarius*
nubinhbens (Kirkwood et al. 2010). The different temperature dependence of various bacterial and algal species might be attributed to differences in the phenotypic and/or genotypic traits of the organisms which are likely influenced by environmental conditions in their growth habitat. In this regard, it is not surprising that the DMSP lyase from the polar strain P. antarctica had a lower optimum temperature and higher activity at low temperature (3.2 ± 0.4 U at 5°C) than did the temperate strain P. globosa (0.8 ± 0.1 U at 5°C). The observed shift to a lower temperature optimum, but with higher specific lyase activity at the lowest temperature in P. antarctica (natural habitat <5°C), is typical of enzyme adaptation in psychrophiles (Struvay & Feller 2012). Thermal stability of the isolated DMSP lyase from both P. globosa and P. antarctica was also investigated by pre-incubation of the extracted enzyme preparations at 11 different temperatures ranging from 5 to 45°C in steps of 5°C, and measuring the residual activity at the optimum temperatures of 30°C for P. globosa and 20°C for P. antarctica (Fig. 4). This test revealed that the DMSP lyase of P. globosa was more thermostable than that of P. antarctica. At 30°C, the P. globosa lyase retained 89 ± 13% of its enzyme activity; however, only 57 ± 9% of the P. antarctica DMSP lyase activity was retained at the same temperature.

The thermal denaturation process of DMSP lyase of both Phaeocystis globosa and P. antarctica followed first-order kinetics. The enzyme of P. globosa exhibited a $t_{1/2}$ of 210 and 30.1 min at 25 and 35°C, respectively (Table 1). In contrast, the DMSP lyase of P. antarctica had a $t_{1/2}$ of 61.9 and 14.6 min, respectively, at the same temperatures. The $E_d$ of DMSP lyase was estimated from an Arrhenius plot as 135 kJ mol$^{-1}$ for P. globosa and 106 kJ mol$^{-1}$ for P. antarctica. These $t_{1/2}$ and $E_d$ values suggested that the DMSP lyase of P. globosa was more thermostable than the P. antarctica lyase, again not surprising considering the polar habitat of P. antarctica. The $E_a$ values were higher than the DMSP lyase $E_a$, indicating the requirement of higher energy to initiate the thermal denaturation compared to catalysis.

In order to gain more knowledge on the mechanism of thermal denaturation of the DMSP lyase, the thermodynamic parameters such as $\Delta G_d$, $\Delta H_d$ and $\Delta S_d$ of denaturation were determined (Table 1). The $\Delta G_d$ and $\Delta H_d$ values of thermal denaturation of DMSP lyase of both algal species declined with increases in temperature, suggesting a considerable change in enzyme conformation during the process of denaturation and a requirement of lesser energy to denature the enzyme at higher temperatures. Lower values of $\Delta G_d$ and $\Delta H_d$ for Phaeocystis antarctica lyase compared to P. globosa lyase indicated that the former was more thermostable. Moreover, the positive $\Delta H_d$ and $\Delta S_d$ values obtained for both lyases provided supportive evidence that thermal denaturation of the enzymes is initiated by disruption of non-covalent linkages, which increases the disorder or entropy during denaturation (reviewed in Nosoh & Sekiguchi 1990, Vieille & Zeikus 1996). To our knowledge, the present study is the first to estimate the thermodynamic properties of DMSP lyase, and therefore it is not possible to compare our values with other studies.

We used Eadie-Hofstee plots to determine $K_m$ and $V_{max}$ for DMSP lyase extracted from the 2 different Phaeocystis species (data not shown). Under the opti-

Table 1. Phaeocystis globosa and P. antarctica. Half-life ($t_{1/2}$) and thermodynamic parameters for denaturation of DMSP lyase at different temperatures. $\Delta G_d$: free energy; $\Delta H_d$: enthalpy; $\Delta S_d$: entropy of denaturation

<table>
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<th>Species</th>
<th>$t_{1/2}$ (min)</th>
<th>$\Delta G_d$ (kJ mol$^{-1}$)</th>
<th>$\Delta H_d$ (kJ mol$^{-1}$)</th>
<th>$\Delta S_d$ (J K$^{-1}$ mol$^{-1}$)</th>
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</table>
mal assay conditions with DMSP as the substrate, the lyase of *P. globosa* (pH 4 and 30°C) and *P. antarctica* (pH 5 and 20°C) followed Michaelis-Menten saturation kinetics with $K_m$ values of 1.77 and 2.31 mM and $V_{max}$ values of 17.3 and 28.2 U, for *P. globosa* and *P. antarctica*, respectively. $K_m$ values ranging from 0.072 to 14 mM were reported for the in vitro DMSP lyase from several algae including *Phaeocystis* strain K (2.25 mM) (Stefels & Dijkhuizen 1996), *Polysiphonia paniculata* (0.072 mM) (Nishiguchi & Goff 1995) and *Ulva curvata* (0.52 mM) (de Souza et al. 1996), and bacteria including *Alcaligenes* sp. (1.4 mM) (de Souza & Yoch 1995a) and *Roseovarius nubinhibens* (13.8 ± 5.5 mM) (Kirkwood et al. 2010). Intracellular DMSP concentrations in *Phaeocystis* sp. are typically in the 100 to 300 mM range (Stefels & van Leeuwe 1998). These concentrations are well above the $K_m$ values that we determined, suggesting that lyase activity in *Phaeocystis* sp. could operate close to saturation in vivo if the enzyme is exposed to those high intracellular concentrations. But evidence from several studies suggests that in vivo lyase activity operates well below maximal potential activity in healthy phytoplankton cells (Wolfe & Steinke 1996, Wolfe et al. 2002, Galí et al. 2011), indicating that lyase activity must be regulated within the cells. Unfortunately, the mechanism of DMSP lyase regulation remains largely unknown, although factors such as grazing-induced cell disruptions, high light exposure and oxidative stress have been suggested to up-regulate lyase activity (Wolfe & Steinke 1996, Sunda et al. 2002, Gali et al. 2011).

The presence of NaCl affected DMSP lyase activity similarly for both *Phaeocystis globosa* and *P. antarctica* (Fig. 5). Optimal catalytic activity was found at 0.34 and 0.43 M NaCl for *P. globosa* and *P. antarctica* DMSP lyases, respectively (Fig. 5). For comparison, seawater with a salinity of 35 ppt has a NaCl concentration of ~0.5 M. Above 0.6 M the lyase activity sharply declined and the enzyme lost >50% of the activity. The optimal NaCl concentrations for DMSP lyase in vitro activity in the 2 *Phaeocystis* species was similar to that of several different strains of the prymnesiophyte *Emiliania huxleyi* (0.5 to 1.0 M NaCl for CCMP 370, 379, 1516; Steinke et al. 1998), but much higher than found for the macroalgae *Enteromorpha clatharata* (0.075 M NaCl; Steinke & Kirst 1996) and *Ulva curvata* (0.075 M NaCl; de Souza et al. 1996).

The relative catalytic efficacy of DMSP lyase isolated from *Phaeocystis globosa* and *P. antarctica* was assessed against different DMSP analogs (Table 2). For both organisms, the DMSP lyase activity was not significantly inhibited by 20 mM 2-methyl-DMSP (80.9 ± 8.9 and 89.1 ± 10.1% of the control, with p = 0.114 and 0.210, for *P. globosa* and *P. antarctica*, respectively), but was partially inhibited by 2-chloro-DMSP (57.9 ± 8 and 78.2 ± 7.1% of the control, with p = 0.003 and 0.013, for *P. globosa* and *P. antarctica*, respectively). Choline-ο-sulfate was strongly inhibitory to lyase activity in both species (5.3 to 7.09% of controls, p < 0.029). Dimethylsulfinioacetate, one carbon shorter than DMSP, had no significant effect on lyase activity. Glycine betaine, a compound known to inhibit transmembrane transport of DMSP in marine bacteria (Kiene 1998), did not inhibit DMSP lyase.

![Fig. 5. Phaeocystis globosa and P. antarctica. Effect of NaCl concentration on DMSP lyase activity (mean ± SD, n = 2). Relative specific activity of 100% = 11.9 ± 1.48 and 22.6 ± 1.93 nmol DMS min⁻¹ mg protein⁻¹ for P. globosa and P. antarctica, respectively](Image)

| Table 2. Phaeocystis globosa and P. antarctica. Effect of various DMSP analogs on DMSP lyase activity expressed as a percentage of the activity retained when DMSP was added to the assay mixtures lacking the DMSP analog. DMSP analogs (20 mM) were pre-incubated in the DMSP lyase assay mixture from *P. globosa* or *P. antarctica* for 10 min at 25 and 20°C, respectively. The residual enzyme activity was subsequently assayed with 5 mM DMSP as the substrate |
|-----------------|----------|----------|----------|
| DMSP analog     | *P. globosa* (%) | p        | *P. antarctica* (%) | p        |
| 2-methyl-DMSP   | 80.9 ± 8.9 | 0.114    | 89.2 ± 10.1 | 0.210    |
| 2-chloro-DMSP   | 57.9 ± 8  | 0.035    | 78.2 ± 7.1  | 0.086    |
| Dimethylsulfinioacetate | 91.7 ± 12.4 | 0.284   | 93.5 ± 13.2  | 0.326    |
| Choline-ο-sulfate | 45.3 ± 6.6 | 0.026    | 51.2 ± 6  | 0.029    |
| Glycine betaine | 95.5 ± 10.7 | 0.366    | 94.3 ± 4  | 0.305    |
activity appreciably, a result consistent with previous findings in bacteria (de Souza & Yoch 1995b). Bacic & Yoch (1998) observed induction of in vivo DMSP lyase activity in the fungus Fusarium lateritium by choline, glycine betaine and dimethylglycine, but no induction by dimethylselenonium propionate (DMSeP). We did not test specifically for induction of DMSP lyase activity in the Phaeocystis species, but we observed lyase activity in all P. globosa and P. antarctica cultures tested suggesting it is present at relatively high levels under the growth conditions we used.

The effect of various metal ions and organic chemicals on the extracted DMSP lyases were studied for both algal species (Table 3). DMSP lyase activities from both Phaeocystis species were not affected by the major divalent cations present in seawater, Ca\(^{2+}\) and Mg\(^{2+}\), however, the activities were strongly inhibited by Cu\(^{2+}\), Mn\(^{2+}\) and Ni\(^{2+}\). The metal chelating agent, EDTA, also inhibited lyase activity, suggesting that the extracted DMSP lyases of both the algal species were metalloenzymes. Additionally, inhibition of the lyase activity by PMSF and pCMB points towards the participation of both serine and sulphydryl (−SH) groups, respectively, at the active site of the enzyme. The thiol binding reagent pCMB has been used previously to inhibit DMSP lyase activity in Phaeocystis sp. (Stefels & Dijkhuizen 1996, del Valle et al. 2011). The non-inhibition of enzyme activity by DTT provided additional evidence of the involvement of an −SH group at the active site as suggested by Stefels & Dijkhuizen (1996). Metal ion and inhibitor studies have also been performed for DMSP lyases isolated from other algal and bacterial species. The proteins of bacterial DMSP lyase, DddL, DddQ and DddW contain metal binding cupin pockets (Curson et al. 2008, Todd et al. 2011, 2012). However, the DddP protein of the bacterium Roseovarius nubinhibens was not affected by EDTA, indicating no requirement of a metal cofactor for DMSP lyase activity (Kirkwood et al. 2010). It was reported that the in vitro DMSP lyase activity in the alga Polysiphonia paniculata (Nishiguchi & Goff 1995) was partially inhibited by EDTA, PMSF and pCMB, and was not affected by Ca\(^{2+}\) and Mg\(^{2+}\), similar to what we found with the 2 Phaeocystis species.

In conclusion, the DMSP lyases extracted from mesophilic Phaeocystis globosa and psychrophilic P. antarctica showed very similar functional characteristics. The main differences were lower pH optimum in P. globosa (pH 4) compared to P. antarctica (pH 5) and higher temperature optimum (and thermal stability) in P. globosa compared to P. antarctica. Further structural and genetic characterization will be required to determine the homology of the DMSP lyase of these 2 algal species. The DMSP lyases from the 2 Phaeocystis species tested function optimally at acidic pH and this is similar to what has been shown previously for several other prymnesiophytes. This raises the possibility that DMSP lyase in these organisms is adapted to function in a locally-acidic environment, but further work will be needed to determine the in vivo location and physiological function of DMSP lyase in these organisms. Our study has added important new information about the functional characteristics of DMSP lyase in Phaeocystis species that are globally important contributors to DMSP and DMS production in temperate and polar oceans. Future studies of DMSP lyase will benefit from purification of the enzyme and identification of gene(s) encoding these proteins.

Table 3. Phaeocystis globosa and P. antarctica. Effect of different metal ions and organic chemicals on DMSP lyase activity expressed as % enzyme activity retained. Individual metal ions or organic compounds, at either 0.1 or 1.0 mM, were preincubated with the DMSP lyase assay mixture from P. globosa or P. antarctica for 10 min at 25 and 20°C, respectively. The residual enzyme activity was subsequently assayed with 5 mM DMSP as substrate. DTT: dithiothreitol; pCMB: para-chloromercuribenzoate; PMSF: phenylmethanesulfonylfluoride.

| Metal ions and chemicals | P. globosa | | P. antarctica | |
|-------------------------|-----------|----------------|----------------|
|                         | 0.1 mM    | 1 mM           | 0.1 mM         | 1 mM          |
| Ca\(^{2+}\)             | 98.2 ± 12.4 | 108 ± 11       | 104 ± 13       | 97.2 ± 11     |
| Cu\(^{2+}\)             | 56.4 ± 6.8 | 9.1 ± 0.9      | 89.7 ± 5.7     | 97.2 ± 11     |
| Mg\(^{2+}\)             | 100 ± 11  | 104 ± 13       | 100 ± 12       | 95.1 ± 4.1    |
| Mn\(^{2+}\)             | 42.3 ± 4  | 23.2 ± 2.7     | 58.2 ± 8.4     | 36.1 ± 5.2    |
| Ni\(^{2+}\)             | 54.1 ± 5.9 | 27.1 ± 7.5     | 72.2 ± 5.9     | 48.2 ± 7.4    |
| EDTA                    | 13.5 ± 1.1 | 10.5 ± 1.3     | 22.1 ± 2.8     | 16.3 ± 2.1    |
| DTT                     | 103 ± 10  | 97.6 ± 8.5     | 110 ± 9        | 107 ± 8      |
| pCMB                    | 18.2 ± 2.4 | 9.2 ± 1.4      | 26.1 ± 3.6     | 14.2 ± 1.2    |
| PMSF                    | 18.3 ± 2.4 | 10.3 ± 1.3     | 29.1 ± 3.8     | 16.3 ± 1.7    |

In conclusion, the DMSP lyases extracted from mesophilic Phaeocystis globosa and psychrophilic P. antarctica showed very similar functional characteristics. The main differences were lower pH optimum in P. globosa (pH 4) compared to P. antarctica (pH 5) and higher temperature optimum (and thermal stability) in P. globosa compared to P. antarctica. Further structural and genetic characterization will be required to determine the homology of the DMSP lyase of these 2 algal species. The DMSP lyases from the 2 Phaeocystis species tested function optimally at acidic pH and this is similar to what has been shown previously for several other prymnesiophytes. This raises the possibility that DMSP lyase in these organisms is adapted to function in a locally-acidic environment, but further work will be needed to determine the in vivo location and physiological function of DMSP lyase in these organisms. Our study has added important new information about the functional characteristics of DMSP lyase in Phaeocystis species that are globally important contributors to DMSP and DMS production in temperate and polar oceans. Future studies of DMSP lyase will benefit from purification of the enzyme and identification of gene(s) encoding these proteins.
Acknowledgements. This work was funded by grants from the National Science Foundation (OPP-09-44686 to D.J.K. and OPP-09-44659 to R.P.K.). We thank B. Taylor for the kind gift of 2-methyl-DMS and 2-chloro-DMS. We also thank A. Hanson for providing choline-β-sulfate. We thank L. Oswald for general assistance in the laboratory.

LITERATURE CITED


Editorial responsibility: Wajih A. Naqvi, Dona Paula, India

Submitted: July 23, 2012, Accepted: February 24, 2013

Proofs received from author(s): April 23, 2013