Vol. 507: 31–38, 2014
doi: 10.3354/meps10842

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published July 17

Dimethylsulfoxide reduction activity is linked to nutrient stress in *Thalassiosira pseudonana* NCMA 1335

Christopher E. Spiese*, Elvira A. Tatarkov

Department of Chemistry & Biochemistry, Ohio Northern University, 525 South Main St., Ada, OH 45810, USA

ABSTRACT: The response of dimethylsulfoxide (DMSO) reduction activity (DRA) was examined in the marine diatom *Thalassiosira pseudonana* under nutrient limitation (nitrogen- or vitamin B_{12}-limitation). DRA was higher in nutrient-limited cultures (160 ± 42% of control for N-limited and 168 ± 52% for B_{12}-limited) than controls. The increased DRA is thought to be due to up-regulation of defense/repair enzymes as opposed to increased substrate availability. By supplying a chiral sulfoxide (methylphenylsulfoxide, MPSO), DRA could be tentatively assigned to a methionine sulfoxide reductase B (MsrB) enzyme due to a decrease in the amount of (*R*)-MPSO present in limited cultures versus control, in a similar fashion to other marine organisms. Cellular dimethylsulfoniopropionate (DMSP) and DMSO contents were also significantly higher under nutrient stress versus control, indicating physiological stress. This is the first time DRA and cellular DMSO concentrations have been shown to increase during stress conditions in a marine alga. Together, these results suggest a role in nutrient-stress management for DRA, and may provide a link between stress at the cellular level and the biogeochemical cycling of sulfur in the marine environment.

KEY WORDS: Sulfur · Nitrogen · Cobalamin · Dimethylsulfide · Diatom · Methionine sulfoxide reductase

INTRODUCTION

Dimethylsulfide (DMS) is a trace sulfur gas with large emissions from the ocean to the atmosphere (Bates et al. 1992). DMS emissions to the atmosphere are important in global biogeochemistry because they redistribute sulfur around the globe and potentially contribute to the formation and growth of aerosol particles that can influence climate (Charlson et al. 1987). DMS is also important in the marine ecosystem as a source of carbon and energy for microbes (De Bont et al. 1981, Simó et al. 2009) and as a potential signaling agent (Pohnert et al. 2007). While many marine phytoplankton produce its biological precursor dimethylsulfoniopropionate (DMSP) in relatively high concentrations (2 to 300 mmol l^{-1} cell volume; Keller et al. 1989, Matrai & Keller 1994), fewer are known to enzymatically cleave DMSP to form DMS via DMSP lyase (DLA; Steinke et al. 1996). Until recently, this cleavage was the only known pathway for DMS formation in marine algal cells. However, reduction of the related compound dimethylsulfoxide (DMSO) was found to be a source of DMS, particularly for phytoplankton lacking the ability to cleave DMSP (Fuse et al. 1995, Spiese et al. 2009). DMSO is formed naturally by the oxidation of DMS and DMSP (Spiese 2010).

DMSO reduction activity (DRA) has been reported to be ubiquitous in marine phytoplankton, regardless of DMSP production or concentration, and is thought to be mediated by a methionine sulfoxide reductase (Msr; Spiese et al. 2009). This activity was hypothesized to play a role in oxidative stress mitigation because the Msr enzyme family plays a key role in protein repair, but this has not been shown in marine phytoplankton. In *Arabidopsis thaliana*, up-regulation of (*S*)-methionine sulfoxide reductase (MsrA)

*Corresponding author: c-spiese.1@onu.edu

has been shown to occur under high ultraviolet light intensity or increased ozone (Romero et al. 2004). *Ulva fasciata*, a marine macroalga, has been shown to up-regulate the expression of an (*R*)-methionine sulfoxide reductases (MsrB) in response to similar stressors (Hsu & Lee 2010). Coupling of DRA to oxidative stress via either MsrA or MsrB would be in keeping with the idea presented by Sunda et al. (2002) of an antioxidant function for DMSP and its degradation products, including DMSO.

DMSP, along with DMS and DMSO are thought to form an antioxidant system in marine algae (Sunda et al. 2002, 2007), based on the intracellular concentrations of each species (2 to 300 mmol l^{-1} cell volume for DMSP, 0.01 to 10 mmol l^{-1} cell volume for DMSO, and <10 mmol l^{-1} cell volume for DMS; Keller et al. 1999, Sunda et al. 2002, Spiese et al. 2009) and their respective rate constants for reaction with various reactive oxygen species (ROS; Bardouki et al. 2002, Sunda et al. 2002, Barnes et al. 2006). DRA is thought to fit into this antioxidant system by allowing marine phytoplankton that lack DLA to generate DMS, and may account for essentially all of the DMS produced during normal growth (Spiese et al. 2009). However, there has been no clear demonstration that DRA is increased under oxidative stress, which would support a role in oxidative stress management.

This study examined the response of DRA in the marine diatom *Thalassiosira pseudonana* under nutrient-limited treatments, determined the class of Msr enzyme likely responsible for such activity, and measured the cellular concentrations of DMSP and its oxidation products. This study also directly investigated for the first time the response of cellular DMSO during nutrient stress. All of these factors are crucial to understanding the response of DMSP-producing algae to stress and the effect this may have on the biogeochemical cycling of sulfur from the ecosystem to the global scale.

MATERIALS AND METHODS

Chemicals. All chemicals were reagent grade and were purchased from commercial sources and used without further purification. Solutions were prepared using distilled water.

Algal culturing. Axenic cultures of *Thalassiosira pseudonana* NCMA 1335 were obtained from the National Center for Marine Algae and Microbiota (Boothbay Harbor, ME) and maintained in batch culture in f/2 medium (Guillard & Ryther 1962) based on artificial seawater (Parsons 1984), with slight modifications (see next paragraph). Cultures were manipulated under sterile conditions and were periodically tested for heterotrophic bacterial growth using a sterile yeast extract-tryptone broth (0.4 g l^{-1} yeast extract and 0.25 g l^{-1} tryptone). Further detection of live bacteria was performed using fluorescein diacetate staining and fluorescence microscopy (FDA, Agustí & Sánchez 2002). Cultures (10 to 30 ml) were grown in triplicate 25 cm^2 canted neck, vented (0.2 µm membrane) culture flasks (Corning) at 21 ± 2°C under incandescent lighting (65 µmol photons m^{-2} s^{-1}, 14 h light:10 h dark cycle). Absolute culture density and cell volume was measured using a Beckman Coulter Z2 Particle Analyzer, while relative culture density for growth rate measurements was determined using a 96-well plate reader (BioTek Synergy H1) at 660 nm.

Nutrient limitation was induced by reducing to one-tenth either the concentration of nitrate (88.3 µM for N-limited cultures, 883 µM for all other cultures) or vitamin B_{12} (10 pM for B_{12}-limited cultures, 100 pM for all others). All other macro- and micronutrients were supplied as described in the media recipe (Guillard & Ryther 1962) and were sufficient for growth. Cultures were sampled after 14 d of growth, corresponding to late exponential or early stationary phase.

Chemical analyses. DMSP was determined as DMS released after addition of 200 µl 2 M NaOH to 1 ml of sample in a 14 ml flask sealed with a Teflon-faced butyl rubber septum. Samples were allowed to react at room temperature in the dark for >24 h. Samples were analyzed by a headspace gas chromatograph (Shimadzu GC2010-Plus) equipped with a flame photometric detector (GC-FPD) with sulfur-selective filters. The column used was a Supelco SPB-5 (30 m × 0.25 mm × 0.25 µm). The injector and detector were held at 250°C, and the column at 45°C. Ultra-high purity He was the carrier gas (2 ml min^{-1}), and a split ratio of 2:1 was employed. Samples were analyzed using a fixed 500 µl injection of headspace. Standards were prepared from solid DMSP·HCl, synthesized according to the procedures of Chambers et al. (1987), recrystallized from hot ethanol, and prepared identically to samples.

DMSO was analyzed on the same GC-FPD system as DMSP, according to the method of Kiene & Gerard (1994). Cellular DMSO was calculated as the difference between total DMSO (unfiltered) and dissolved DMSO (DMSO passing through a GF/F), normalized to cell volume. Culture aliquots were gravity filtered, according to Kiene & Slezak (2006). All filters and vials for DMSO analysis were pre-combusted at 450°C overnight and stored at 120°C until use.

DRA was measured according to the method of Spiese et al. (2009), without modification. In brief, 1 ml aliquots of culture were placed in 14 ml glass vials with Teflon-faced butyl rubber septa. DMSO was added to a final concentration of 10 mM, and the vial sealed. Vials were incubated in the same conditions as for growth for 24 h, at which time DMS in the headspace was measured as described at the start of this section. DMS accumulation in the headspace after DMSO addition was corrected for DMS production without additional DMSO. DMS accumulation was normalized with respect to cell volume, in keeping with the protocol of Spiese et al. (2009).

In order to confirm that the DRA in *T. pseudonana* was due to up-regulation of oxidative stress defense and repair enzymes (e.g. MsrA or MsrB), a racemic sulfoxide was supplied and the change in enantiomeric excess (ee) of the (*R*)-(+) enantiomer was determined. The absolute stereochemistry of methylphenylsulfoxide (MPSO) is identical to that of methionine sulfoxide (MetSO), and therefore the stereospecificity of enzymes utilizing MPSO as a substrate is expected to be the same as for MetSO. Higher ee-values indicate up-regulation of MsrA, while lower ee values indicate up-regulation of MsrB. Racemic MPSO was added at a concentration of 10 mM to cultures in triplicate 25 cm^2 canted neck, vented culture flasks and cultured as described in 'Algal culturing'. After 7 d incubation, MPSO was removed from cultures by extraction into hexanes followed by evaporation under a gentle air stream. Samples were re-dissolved into 90:10 hexanes:isopropanol. The ee of MPSO was measured using HPLC (Chiracel OD-H 25 cm × 4.5 mm i.d. × 5 µm) with elution under isochratic conditions (0.75 ml min^{-1} 90:10 hexanes: isopropanol). Compounds were detected at 210 nm, and ee was determined from the ratio of the integrated peak areas. Under these conditions, the (*R*)-(+) enantiomer eluted slightly before the (*S*)-(–) enantiomer (Le Maux & Simonneaux 2011).

Statistical analyses. Statistical analyses were performed using 2-tailed *t*-tests with $\alpha = 0.05$. All treatments were compared to the control (nutrient-replete) values. Results are reported at mean ± standard deviation unless otherwise noted.

RESULTS AND DISCUSSION

Culture growth, density, and cell volume

Overall growth in N-limited and B_{12}-limited cultures was reduced versus control over the middle 10 d of growth (Culture Days 5 to 16). Nitrogen-limited cultures were found to have a specific growth rate of 0.14 ± 0.02 d^{-1} (78 ± 12% of control, p = 0.0062, Fig. 1). A similar reduction in growth rate was observed for B_{12}-limited cultures (0.09 ± 0.02 d^{-1}, 50 ± 11% of control, p = 0.0001). Control cultures grew at a rate of 0.18 ± 0.01 d^{-1} (Culture Days 5 to 16). Control cultures likely left a pure exponential phase around Day 10 due to self-shading. All N-limited and B_{12}-limited cultures also had lower cell culture biomass relative to controls. N-limited cultures had the lowest cell culture biomass (28 ± 5% of control, p < 0.0001, Fig. 2). Low-B_{12} cultures had a similar low overall biomass accumulation (58 ± 13% of control, p = 0.0005), although not as small as N-limited cultures. The lower culture density for N-limited cultures versus B_{12}-limited cultures despite a higher growth rate is likely due to the presence of non-viable cells in the culture. In the limited cultures, the mean cell size was lower (52 ± 3 fl for

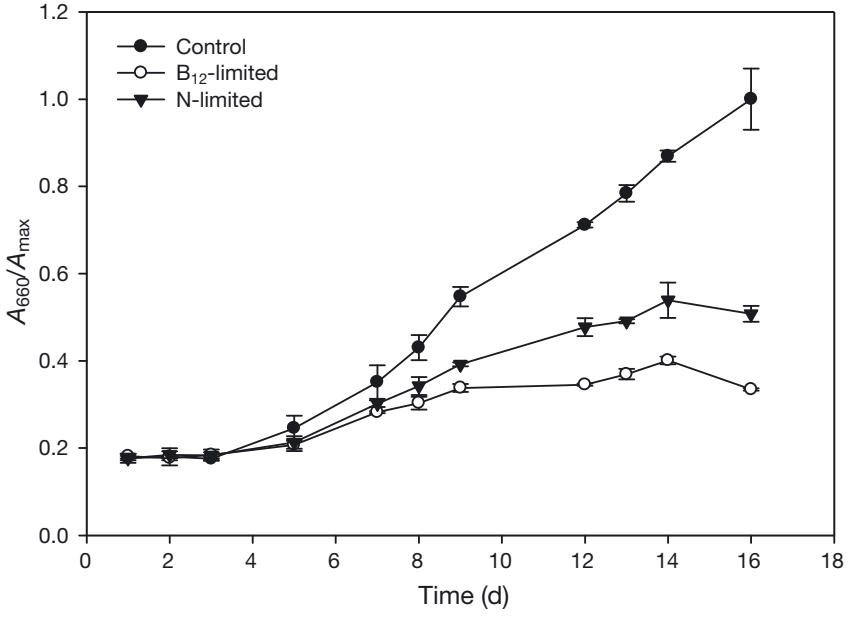

Fig. 1. Cell culture growth curves. *Thalassiosira pseudonana* culture density as measured by absorbance at 660 nm in triplicate culture flasks for each treatment. (●) Control cultures, (○) cultures with restricted B_{12}, and (▼) low N cultures. Points denote the mean absorbance for triplicate flasks and error bars represent 1 SD. Day 0 corresponds to inoculation of the culture. Cultures were sampled for all other assays and analyses on Day 14

N-limited, p = 0.0001, and 55 ± 2 fl for B_{12}-limited, p = 0.0002) compared to the controls (74 ± 3 fl). These results indicate that growth was limited by either nitrogen or vitamin B_{12}, depending on experimental treatment.

DMS, DMSO and DMSP

The cellular concentrations of both DMSO and DMSP were measured, as these have both been noted to change when phytoplankton are subjected to stress conditions (Keller et al. 1999, Sunda et al. 2002, 2007). Under N-limitation, DMSP increased substantially to 194 ± 34% of the control (p = 0.0077, Fig. 3). This result supports the findings of Bucciarelli & Sunda (2003) and Keller et al. (1999), who observed increased DMSP under N-limitation conditions. Increased DMSP during N-limitation suggests a number of roles, including a possible antioxidant function for DMSP (Sunda et al. 2002), recycling of fixed N (Andreae 1986, Turner et al. 1988), a system for metabolic overflow (Stefels 2000), or a replacement for N-containing osmolytes such as glycine betaine (Sunda et al. 2007). These results do not support one role over the others, and it is possible that more than one of these responses is contributing to the increase in DMSP.

DMSP concentrations were significantly decreased in cultures under low-B_{12} conditions (45 ± 9% of control, p = 0.0006, Fig. 3). Vitamin B_{12} plays a central role in the biosynthesis of methionine (Met, Banerjee & Matthews 1990), and thus lower Met concentrations would logically lead to lower DMSP concentrations, as DMSP is derived from Met (Summers et al. 1998). Decreased cellular DMSP has also been found in B_{12}-limited natural assemblages versus B_{12}-replete treatments (Bertrand et al. 2007). Control over DMSP concentrations by restricting B_{12} has been hypothesized before by Gröne & Kirst (1992), who suggested that control over the synthesis of Met is a key control over DMSP concentrations in some algae.

DMSO is thought to play a role in oxidative stress management in DMSP-producing algae (Sunda et al. 2002); however, this hypothesis has never been tested directly. Under nutrient limitation, *Thalassiosira pseudonana* was found to have significantly increased cellular DMSO ($DMSO_c$) content (Fig. 4a). N-limited cultures were found to have $DMSO_c$ concentrations 52-fold higher than controls (p < 0.0001). B_{12}-limited cultures were not found to have significantly higher $DMSO_c$ concentrations (353 ± 235% of control, p = 0.4334).

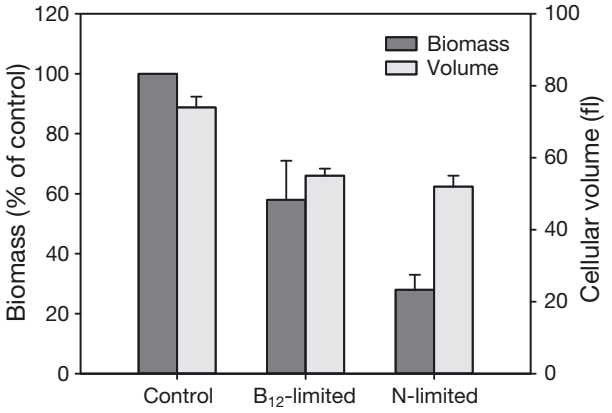

Fig. 2. *Thalassiosira pseudonana* culture biomass and mean cell volume under different nutrient treatments. Dark bars represent mean of triplicate measurements of culture biomass normalized to control cultures, with error bars denoting 1 SE. Light bars denote the mean cell volume (fl). Cell volumes are the mean of triplicate measurements in 3 separate experiments, and error bars represent 1 SD

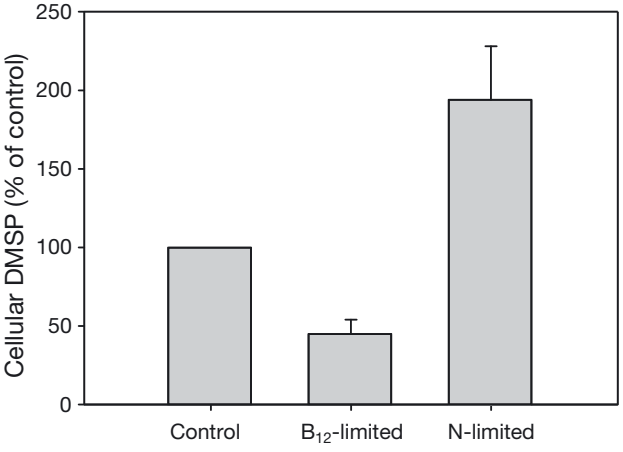

Fig. 3. Intracellular DMSP concentrations. DMSP was analyzed in 1 ml samples of *Thalassiosira pseudonana* cultures under different nutrient treatments. Cellular DMSP concentrations were normalized to control concentrations and bars denote mean of all samples. Error bars represent 1 SD. Control DMSP concentrations were in the order of 7.0 ± 1.0 mmol l^{-1} cell volume

Two prior studies have shown increases in the DMSO:DMSP ratio under stress in the DMSP-producing plant *Spartina alterniflora* (Husband & Kiene 2007, Husband et al. 2012). A higher DMSO:DMSP ratio is interpreted as the organism experiencing oxidative stress. *T. pseudonana* was observed to have a DMSO:DMSP ratio of 0.005 ± 0.006 in nutrient-replete (control) conditions, rising to 0.05 ± 0.03 under B_{12}-limitation (p = 0.0136) and 0.16 ± 0.02 under N-limitation (p = 0.0031) (Fig. 4b). These results suggest that *T. pseudonana* cultures were experiencing

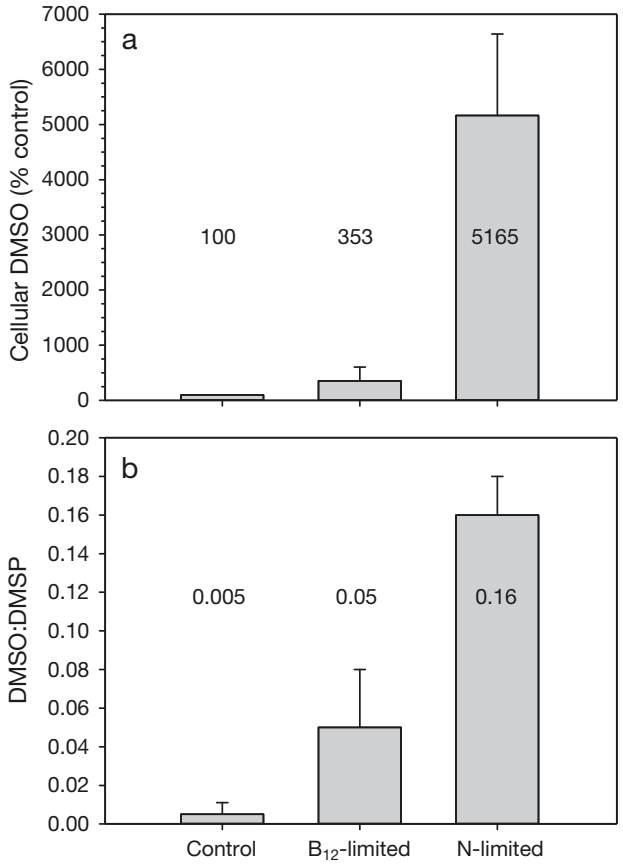

Fig. 4. (a) Intracellular DMSO concentrations and (b) DMSO:DMSP ratios for *Thalassiosira pseudonana* cultures. Cellular DMSO was determined as the difference between total (unfiltered) DMSO and dissolved (filtered) DMSO, normalized to cell volume and control values in 3 separate experiments. Values for each bar are denoted next to the corresponding bar, expressed as percent of control value (DMSO) or as the DMSO:DMSP value. Error bars represent 1 SE. Control DMSO concentrations ranged from 10 to 55 mmol l^{-1} cell volume

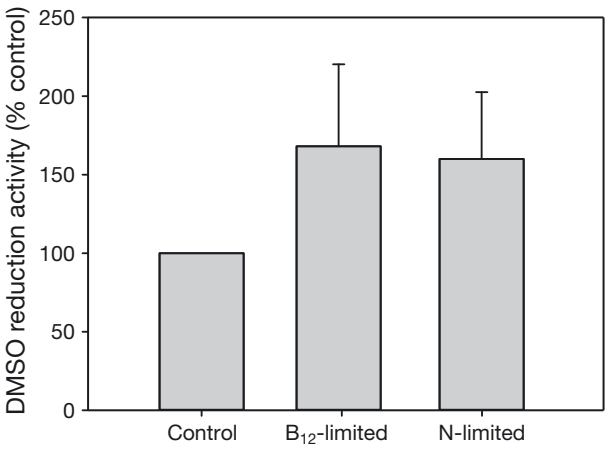

Fig. 5. Cellular DMSO reduction activity (DRA). DRA was analyzed in 1 ml samples of unfiltered *Thalassiosira pseudonana* cultures under different nutrient treatments and determined as the difference between cultures with and without added DMSO (10 mM). DRA was normalized to control activity and bars denote mean of all experiments. Error bars represent 1 SE

oxidative stress during nutrient limitation. Given the hypothesized role in oxidative stress for DMSP and its degradation products, it is not surprising that an increase in the more oxidized product (DMSO) is observed under these conditions. Simó et al. (1998) found that DMSO was produced primarily in the late exponential phase, leading to higher DMSO: DMSP ratios as nutrients became scarce. Simó & Vila-Costa (2006) observed increasing particulate DMSO (DMSOp):DMSPp ratios in natural assemblages across a latitudinal gradient. They interpreted these results as an indication of increased solar irradiance, and therefore increased oxidative stress in these communities. Whether these compounds constitute an actively controlled system or are a by-product of increased ROS in the cell, however, is an open question. A putative link between DMSO reduction — and therefore, DMS production — and the antioxidant enzymes MsrA and MsrB seems to indicate that DMS production may be actively controlled by the cell as a defense against ROS.

DMSO reduction activity

Spiese et al. (2009) suggested that the enzyme responsible for the reduction of DMSO in marine phytoplankton and specifically in *T. pseudonana* is MsrA. This enzyme has been shown to be up-regulated in response to various stressors in *Arabidopsis thaliana* (Romero et al. 2004). To test whether a similar increase in DRA occurs in *T. pseudonana*, the activity was assayed in all cultures. Both N-limited and B_{12}-limited treatments showed increased DRA over controls (Fig. 5). Cultures under N-limitation had a DRA of 160 ± 42 % of the control ($p = 0.0022$ for volume-normalized DRA) and B_{12}-limited cultures had a DRA of 168 ± 52 % of control ($p = 0.0044$ for volume-normalized DRA). All cultures were assayed at saturating DMSO concentrations (Spiese et al. 2009), and therefore it is likely that higher enzyme expression is the underlying cause for higher DRA.

To further support up-regulation of DRA activity, a chiral sulfoxide (MPSO) was added to *T. pseudonana* cultures. A change in the ee of this sulfoxide indicates that the activity of either MsrA (higher *R* ee) or MsrB (higher *S* ee) is increased over controls, in-

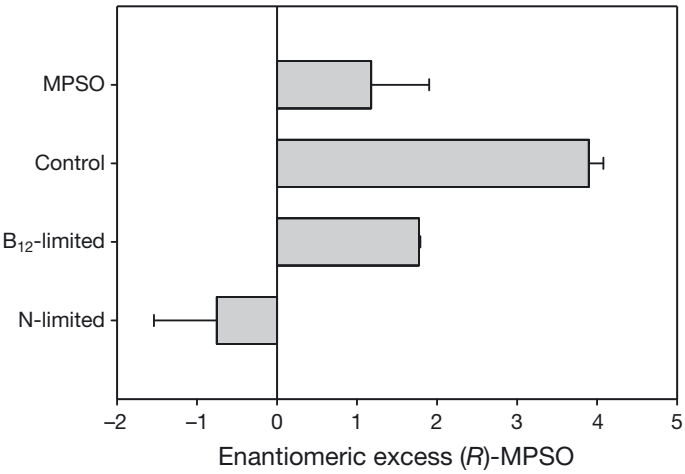

Fig. 6. Enantiomeric excess of (*R*)-methylphenylsulfoxide (MPSO) after incubation with *Thalassiosira pseudonana* cultures. Cultures were exposed to MPSO for 7 d, and then the relative amount of each enantiomer was measured. Negative values indicate more (*S*)-MPSO than (*R*)-MPSO. Bars denote the mean of triplicate cultures, and error bars represent 1 SD. MPSO corresponds to the original standard, without incubation

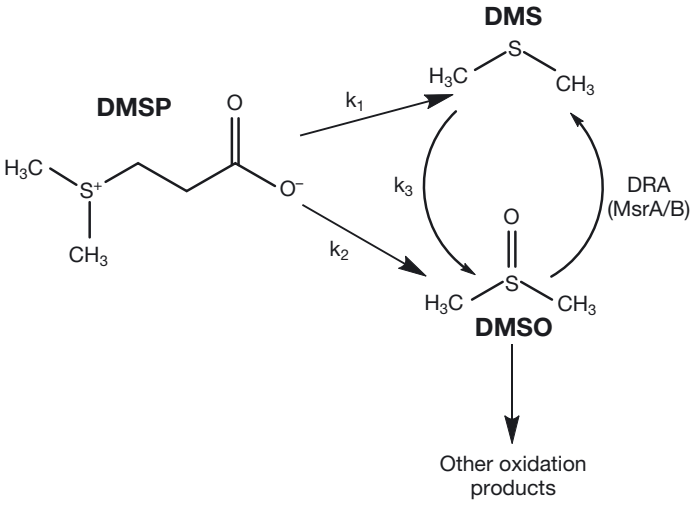

Fig. 7. Schematic of putative DMSP-based antioxidant system in *Thalassiosira pseudonana*. Pseudo-first order rate constants are based on published values for reactive oxygen species (ROS, Boveris & Cadenas 1997) and second-order rate constants (Sunda et al. 2002 and references therein, Spiese 2010). For *T. pseudonana*, $k_1 = 1.1 \times 10^{-10}$ s^{-1}, $k_2 = 1.3 \times 10^{-10}$ s^{-1}, and $k_3 = 1.92 \times 10^{-8}$ s^{-1} (for details, see Spiese et al. 2009, Spiese 2010 and references therein). Cofactors have been removed for clarity

creasing the amount of *R* or *S* enantiomer, respectively. Cultures of *T. pseudonana* were supplied with 10 mM racemic MPSO (ee = 1.2 ± 0.7% *R*, Fig. 6) for 7 d. In the absence of applied stress, the ee was found to increase to 3.9 ± 0.2% *R* ($p = 0.0104$ versus initial mixture, $n = 4$), indicating that MsrA (the *S*-selective isoform) is constitutively expressed. For B_{12}- and N-limited cultures, the ee was found to decrease significantly versus control cultures (1.78 ± 0.02% *R* for B_{12}-limited cultures, $p < 0.0005$, $n = 5$; 0.8 ± 0.8% *S* for N-limited cultures, $p = 0.0062$, $n = 5$ for both). Therefore, at least 1 MsrB isoform is up-regulated in response to stress, increasing the relative amount of the *S* enantiomer by selectively reducing the *R* enantiomer. This result is similar to that of Hsu & Lee (2010), who demonstrated up-regulation of MsrB in response to stress. While our results indicate that MsrB activity is increased, it remains to be determined which isoform of this enzyme is responsible for DMSO reduction, or even if DMSO is the primary substrate.

Oxidative stress and DMSO reduction

Under oxidative stress, organisms will typically increase expression of enzymatic antioxidant defenses, such as superoxide dismutase, catalase, and MsrA/B. Increased DRA fits this general trend, as DMS produced by this activity is significantly more active in scavenging ROS than other known (e.g. glutathione, ascorbate) or suspected (e.g. DMSO, DMSP) antioxidants (Sunda et al. 2002). A redox cascade comprising DMSP and its oxidation products, cycled by MsrA/B, would thus comprise a component of the antioxidant defenses in DMSP-producing organisms like *T. pseudonana* (Fig. 7). However, the reduction of DMSO by MsrA/B may also be a result of increased expression of MsrA/B due to increased MetSO concentrations, and not a specific response to increased DMSO concentrations. If this is the case, then DRA would not be part of an antioxidant system per se, although the increase in DMS production due to DRA would be indicative of oxidative stress.

A further complication arises when the repair of oxidized Met residues is considered. Kwak et al. (2009) observed that several MsrB isoforms were unable to reduce DMSO, the addition of which led to lower MetSO reduction rates and inhibition of activity of the affected enzymes. Endogenous DMSO production may prevent repair of protein Met residues oxidized by ROS due to competitive inhibition by DMSO (Kwak et al. 2009). The extent to which this occurs would depend on a number of factors, including the subcellular localization of MsrA/B and the relative rates of oxidation between DMS(P) and protein Met.

Direct production of DMSO from DMSP reacting with OH radicals can be measured (Spiese 2010) and a pseudo-first order rate constant determined. For an intracellular DMSP concentration of 10 mM (midd-

ling between N-replete and N-limited), the pseudo-first order rate constant for reaction with the OH radical is expected to be in the order of 1.3×10^6 s^{-1} (assuming $k = 1.3 \times 10^8$ M^{-1} s^{-1}, Spiese 2010). By comparison, the oxidation rate for protein-bound Met (1.5 µmol l^{-1} cell volume based on 0.3 µmol l^{-1} cell volume total protein, 5 Met per protein; Keller et al. 1999) is approximately 11 100 s^{-1}, some 100-fold less. Although these values are approximate, it appears that DMSO would be produced significantly faster than MetSO. In such a case, DMSP may be acting as a general antioxidant, while Met is specific to an individual enzyme (Levine et al. 1996). This is further supported by the fact that DMSP is expected to be free in the cytosol or internal matrices of organelles, unlike Met, which is bound to the surfaces of protein.

For an alga such as *T. pseudonana*, DRA is very likely the primary source of DMS (Spiese et al. 2009), given that *T. pseudonana* lacks DLA (Harada 2007). The increased DMS production in *T. pseudonana* observed by Sunda et al. (2002) during CO_2 or Fe limitation may be accounted for by DRA. Other hypotheses regarding the role of DMSP and its degradation product have been proposed, however. Stefels (2000) suggested that DMS may act as a way for the cell to eliminate excess carbon and sulfur during high protein turnover. DRA supports this, as the cell would be able to regulate DMS production via MsrA/B rather than DLA. Although the exact role of DRA in the overall physiology of DMSP and related compounds cannot be ascertained in this study, the linkage between stress and DRA strongly points to an antioxidant function for this activity and for DMSP and DMSO in general.

Given the key role of DMS in the global sulfur cycle, its potential role as a cellular antioxidant, and its importance via DMSP as a carbon source in the marine ecosystem, understanding the various cellular processes leading to its formation is critical. This study demonstrates clearly that DMSO reduction is a response to nutrient limitation and suggests a potential mechanism for DMS formation in marine algae lacking DLA activity.

Acknowledgements. This work was supported by the Signature Program in Chemistry and Biochemistry at Ohio Northern University (ONU). The authors thank Dr. Amy Aulthouse and Dr. Dennis de Luca, both in the ONU Department of Biological and Allied Health Sciences, for their material assistance. We also thank Dr. Jake Zimmerman (ONU Department of Chemistry & Biochemistry) for assistance with chiral HPLC and Dr. Ronald P. Kiene (Dauphin Island Sea Lab) for editorial assistance. Lastly, we thank 4 anonymous reviewers for their constructive criticism which strengthened this paper.

LITERATURE CITED

Agustí S, Sánchez MC (2002) Cell viability in natural phytoplankton communities quantified by a membrane permeability probe. Limnol Oceanogr 47:818–828

Andreae MO (1986) The ocean as a source of atmospheric sulfur compounds. NATO Adv Sci Inst C 185:331–362

Banerjee RV, Matthews RG (1990) Cobalamin-dependent methionine sythase. FASEB J 4:1450–1459

Bardouki H, da Rosa MB, Mihalopoulos N, Palm WU, Zetzsch C (2002) Kinetics and mechanism of the oxidation of dimethylsulfoxide (DMSO) and methanesulfinate (MSI^-) by OH radicals in aqueous medium. Atmos Environ 36:4627–4634

Barnes I, Hjorth J, Mihalopoulos N (2006) Dimethyl sulfide and dimethyl sulfoxide and their oxidation in the atmosphere. Chem Rev 106:940–975

Bates TS, Lamb BK, Guenther A, Dignon J, Stoiber RE (1992) Sulfur emissions to the atmosphere from natural sources. J Atmos Chem 14:315–337

Bertrand EM, Saito MA, Rose JM, Riesselman CR and others (2007) Vitamin B_{12} and iron colimitation of phytoplankton growth in the Ross Sea. Limnol Oceanogr 52: 1079–1093

Boveris A, Cadenas E (1997) Cellular sources and steady-state levels of reactive oxygen species. In: Clerch LB, Massaro DJ (eds) Oxygen, gene expression, and cellular function. Marcel Dekker, New York, NY, p 1–88

Bucciarelli E, Sunda WG (2003) Influence of CO_2, nitrate, phosphate, and silicate limitation on intracellular dimethylsulfoniopropionate in batch cultures of the coastal diatom *Thalassiosira pseudonana.* Limnol Oceanogr 48:2256–2265

Chambers ST, Kunin CM, Miller D, Hamada A (1987) Dimethylthetin can substitute for glycine betaine as an osmoprotectant for *Escherichia coli.* J Bacteriol 169: 4845–4847

Charlson RJ, Lovelock JE, Andreae MO, Warren SG (1987) Oceanic plankton, atmospheric sulfur, cloud albedo, and climate. Nature 326:655–661

De Bont JAM, Van Dijken JP, Harder W (1981) Dimethyl sulfoxide and dimethyl sulfide as a carbon, sulfur, and energy source for growth of *Hyphomicrobium* S. J Gen Microbiol 127:315–323

Fuse H, Takimura O, Kamimura K, Murakami K, Yamaoka Y, Murooka Y (1995) Transformation of dimethylsulfide and related compounds by cultures and cell extracts of marine phytoplankton. Biosci Biotechnol Biochem 59: 1773–1775

Gröne T, Kirst GO (1992) The effect of nitrogen deficiency, methionine and inhibitors of methionine metabolism on the DMSP contents of *Tetraselmis subcordiformis* (Stein). Mar Biol 112:497–503

Guillard RR, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229–239

Harada H (2007) Physiological roles of dimethylsulfoniopropionate (DMSP), DMSP lyase, dimethylsulfide (DMS) and dimethylsulfoxide (DMSO) in phytoplankton. PhD thesis, University of South Alabama, Mobile, AL

Hsu YT, Lee TM (2010) Photosynthetic electron transport mediates the light-controlled up-regulation of methionine sulfoxide reductase A and B from marine macroalga *Ulva fasciata.* J Phycol 46:112–122

Husband JD, Kiene RP (2007) Occurrence of dimethylsulfox-

ide in leaves, stems, and roots of *Spartina alterniflora*. Wetlands 27:224–229

Husband JD, Kiene RP, Sherman TD (2012) Oxidation of dimethylsulfoniopropionate (DMSP) in response to oxidative stress in *Spartina alterniflora* and protection of a non-DMSP producing grass by exogenous DMSP + acrylate. Environ Exp Bot 79:44–48

Keller MD, Bellows WK, Guillard RRL (1989) Dimethyl sulfide production in marine phytoplankton. In: Saltzman ES, Cooper WJ (eds) Biogenic sulfur in the environment. American Chemical Society, New York, NY, p 167–182

Keller MD, Kiene RP, Matrai PA, Bellows WK (1999) Production of glycine betaine and dimethylsulfoniopropionate in marine phytoplankton. II. N-limited chemostat cultures. Mar Biol 135:249–257

Kiene RP, Gerard G (1994) Determination of trace levels of dimethylsulfoxide (DMSO) in sea water and rainwater. Mar Chem 47:1–12

Kiene RP, Slezak D (2006) Low dissolved DMSP concentrations in seawater revealed by small-volume gravity filtration and dialysis sampling. Limnol Oceanogr Methods 4:80–95

Kwak GH, Choi SH, Kim JR, Kim HY (2009) Inhibition of methionine sulfoxide reduction by dimethyl sulfoxide. BMB Rep 42:580–585

Le Maux P, Simonneaux G (2011) First enantioselective iron-porphyrin-catalyzed sulfide oxidation with aqueous hydrogen peroxide. Chem Commun (Camb) 47:6957–6959

Levine RL, Mosoni L, Berlett BS, Stadtman ER (1996) Methionine residues as endogenous antioxidants in proteins. Proc Natl Acad Sci USA 93:15036–15040

Matrai PA, Keller MD (1994) Total organic sulfur and dimethylsulfoniopropionate (DMSP) in marine phytoplankton: intracellular variations. Mar Biol 119:61–68

Parsons TR (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York, NY

Pohnert G, Steinke M, Tollrian R (2007) Chemical cues, defence metabolites and the shaping of pelagic interspecific interactions. Trends Ecol Evol 22:198–204

Romero HM, Berlett BS, Jensen PJ, Pell EJ, Tien M (2004) Investigations into the role of the plastidial peptide methionine sulfoxide reductase in response to oxidative stress in *Arabidopsis*. Plant Physiol 136:3784–3794

Simó R, Vila-Costa M (2006) Ubiquity of algal dimethylsulfoxide in the surface ocean: Geographic and temporal distribution patterns. Mar Chem 100:136–146

Simó R, Hatton AD, Malin G, Liss PS (1998) Particulate dimethyl sulphoxide in seawater: production by microplankton. Mar Ecol Prog Ser 167:291–296

Simó R, Vila-Costa M, Alonso-Sáez L, Cardelús C, Guadayol Ò, Vázquez-Domínguez E, Gasol JM (2009) Annual DMSP contribution to S and C fluxes through phytoplankton and bacterioplankton in a NW Mediterranean coastal site. Aquat Microb Ecol 57:43–55

Spiese CE (2010) Cellular production and losses of dimethylsulfide in marine phytoplankton. PhD thesis, State University of New York College of Environmental Science and Forestry, Syracuse, NY

Spiese CE, Nomura CT, Kiene RP, Kieber DJ (2009) Reduction of dimethyl sulfoxide to dimethyl sulfide by marine phytoplankton. Limnol Oceanogr 54:560–570

Stefels J (2000) Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. J Sea Res 43:183–197

Steinke M, Daniel C, Kirst GO (1996) DMSP lyase in marine macro- and microalgae. In: Kiene RP, Visscher PT, Keller MD, Kirst GO (eds) Biological and environmental chemistry of DMSP and related sulfonium compounds. Plenum, New York, NY, p 317–324

Summers PS, Nolte KD, Cooper AJL, Borgeas H, Leustek T, Rhodes D, Hanson AD (1998) Identification and stereospecificity of the first three enzymes of 3-dimethylsulfoniopropionate biosynthesis in a chlorophyte alga. Plant Physiol 116:369–378

Sunda W, Kieber DJ, Kiene RP, Huntsman S (2002) An antioxidant function for DMSP and DMS in marine algae. Nature 418:317–320

Sunda W, Hardison R, Kiene RP, Bucciarelli E, Harada H (2007) The effect of nitrogen limitation on cellular DMSP and DMS release in marine phytoplankton: climate feedback implications. Aquat Sci 69:341–351

Turner S, Malin G, Liss PS, Harbour DS, Holligan PM (1988) The seasonal variation of dimethyl sulfide and dimethylsulfoniopropionate concentrations in nearshore waters. Limnol Oceanogr 33:364–375

Editorial responsibility: Steven Lohrenz, New Bedford, Massachusetts, USA

Submitted: May 15, 2013; Accepted: May 3, 2014
Proofs received from author(s): June 30, 2014