Dimethylsulfoniopropionate Uptake by Marine Phytoplankton

Maria Vila-Costa, *et al.*

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sequences are available at NCBI under accession numbers AAV95190 (*S. pomeroyi*), ABF64177 (*Silicibacter* sp. TM1040), ABD55296 (*Jannaschia* sp. CCS1), EAP76657 (*Roseovarius nubinhibens* ISM), AAZ21068 (*P. ubique* HTCC1062), EAS85076 (*P. ubique* HTCC1002), EAS69357 (*P. torquis* genome sequence contaminant), DU750654 and DU737812 (Station Aloha metagenome), and DQ874604-DQ874613 (Sapelo Island metagenome).

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Dimethylsulfoniopropionate Uptake by Marine Phytoplankton

Maria Vila-Costa,[1]* Rafel Simó,[1]* Hyakubun Harada,[2] Josep M. Gasol,[1] Doris Slezak,[2] Ronald P. Kiene[2]

Dimethylsulfoniopropionate (DMSP) accounts for most of the organic sulfur fluxes from primary to secondary producers in marine microbial food webs. Incubations of natural communities and axenic cultures with radio-labeled DMSP showed that dominant phytoplankton groups of the ocean, the unicellular cyanobacteria *Prochlorococcus* and *Synechococcus* and diatoms, as well as heterotrophic bacteria take up and assimilate DMSP sulfur, thus diverting a proportion of plankton-produced organic sulfur from emission into the atmosphere.

Dimethylsulfoniopropionate (DMSP) is synthesized by ubiquitous phytoplankton taxa as a solute, probably for osmoregulatory and antioxidant purposes (*1*–*4*). DMSP is the precursor of the climate-active gas dimethylsulfide (DMS), the main natural source of sulfur to the global atmosphere and a major aerosol and cloud droplet precursor over the ocean (*5*–*7*). Enzymatic cleavage of DMSP into volatile DMS is the fate of only a fraction (generally <50%) of all DMSP produced (*8*). Recent research has revealed that algal DMSP plays an important role in food-web processes supplying sulfur and carbon to heterotrophic bacteria and, to a lesser extent, to microzooplankton herbivores (*9*–*12*). Thus, the biogeochemical fate and function of DMSP is largely determined by a switch between conversion into DMS and sulfur assimilation by microorganisms, which in turn depends on the composition, structure, and dynamics of the planktonic food web. The ability to assimilate DMSP sulfur seems to be widespread among taxa of heterotrophic bacterioplankton (*13*, *14*) and has also been observed in the cyanobacterium *Synechococcus* (*15*). Our work aimed to find out whether major non-DMSP–producing phytoplankton also assimilate DMSP sulfur.

To investigate the distribution of DMSP sulfur uptake and assimilation among picoplankton, we used flow cytometry cell sorting and measured assimilation by picophototrophs and heterotrophic bacteria by using radio-labeled DMSP. Surface seawater samples were collected from the coasts of the Gulf of Mexico, the northwest Mediterranean, and Gran Canaria Island and from the Sargasso Sea. After light and dark incubations with [^{35}S]DMSP, sample aliquots were passed through the flow cytometer and sorted into four major groups: heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*, and autofluorescent picoeukaryotes. All groups showed some capability for assimilating DMSP sulfur (Fig. 1). The most notable DMSP sulfur assimilators were heterotrophic bacteria, followed by *Prochlorococcus*, *Synechococcus*, and picoeukaryotes. Incubation of samples in the light stimulated DMSP sulfur assimilation by picophototrophs by as much as a factor of 2.2. The phototrophs accounted for 10 to 34% of picoplanktonic DMSP consumption in the light, with the remaining 66 to 90% being carried out by heterotrophic bacteria.

Until now the only phototrophs for which DMSP use had been observed were *Synechococcus*. It seems that, in a similar way to that of heterotrophic bacteria (*10*) and *Synechococcus* (*15*), *Prochlorococcus* may also benefit from using a reduced sulfur source such as DMSP, probably by saving the energy required to reduce sulfate. Studies with cultured and natural assemblages of heterotrophic bacteria have provided evidence for a common membrane transporter for DMSP and glycine betaine (GBT) (*16*, *17*), and, interestingly, putative GBT transporter genes have been found in the genome of *P. marinus* MIT9313 (*18*).

In one of the samples (Gran Canaria Island), eukaryotic picophytoplankton also showed significant incorporation of ^{35}S from [^{35}S]DMSP (Fig. 1). It is possible, however, that some of these eukaryotes were mixotrophic bacterivores that had fed on ^{35}S-radio-labeled bacteria.

We used microautoradiography with [^{35}S]DMSP to follow DMSP sulfur assimilation by organisms larger than 5 μm collected during an annual time series in the coastal Mediterranean. Consistent with our flow-cytometric observations of picoeukaryotes, many phytoplankton cells, including dinoflagellates, cryptophytes, and diatoms, became radio-labeled (Fig. 2). Mixotrophy by bacterivory has been described for dinophytes, cryptophytes, and haptophytes (*19*), but not for diatoms, which consequently must have directly taken up ^{35}S from dissolved radio-labeled DMSP.

The DMSP-to-chlorophyll (DMSP:chl-*a*) ratio is a good indicator of how strong a DMSP producer a phytoplankton assemblage is and how much of the available carbon and sulfur are accounted for by DMSP (*9*, *11*). We found that the proportion of diatoms that had assimilated [^{35}S]DMSP sulfur followed a pattern very similar to that of independently measured DMSP:chl-*a* ratios from parallel samples (Fig. 3) through the annual course of sampling, with highest values observed in June and August. In other words, higher numbers of DMSP sulfur–assimilating diatoms did not occur when these phytoplankters were more abundant (late winter) but when DMSP was more abundant with respect to total sulfur and carbon fluxes (summer).

DMSP is a zwitterion that cannot cross cell membranes without a specific transporter (*17*). DMSP sulfur assimilation by diatoms implies, therefore, that either these algae have a DMSP transport system or they were taking up by-

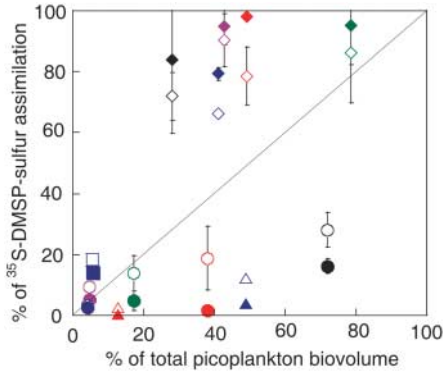

Fig. 1. Contribution of different groups of picoplankton to total picoplankton [^{35}S]DMSP assimilation versus their contribution to the total picoplankton biovolume. Solid and open symbols correspond to dark and light incubations, respectively (diamonds, heterotrophic bacteria; circles, *Synechococcus*; squares, *Prochlorococcus*; and triangles, picoeukaryotes). Green, Blanes Bay (northwest Mediterranean); black, off Dauphin Island (Gulf of Mexico); purple, Sargasso Sea; red, Pensacola beach (Gulf of Mexico); and blue, Gran Canaria Island. Error bars represent standard deviation of the mean (*n* values from 2 to 6). The 1:1 line is included as a reference.

[1]Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar (CSIC), Pg Marítim de la Barceloneta 37-49, 08003 Barcelona, Catalonia, Spain. [2]Department of Marine Sciences, University of South Alabama, Mobile, AL 36688, USA.

*To whom correspondence should be addressed. E-mail: mariavila@icm.csic.es (M.V.-C.); rsimo@icm.csic.es (R.S.)

products of [^{35}S]DMSP degradation by bacteria, such as [^{35}S]methanethiol. To check for the capability of diatoms to take up DMSP, we grew two axenic strains of the centric diatoms *Thalassiosira pseudonana* (CCMP1335) and *T. oceanica* (CCMP1005). We chose these two species for their low cellular DMSP content (1.3 and 0.9 mM, respectively) and their small size [circa (ca.) 5 and 8 μm diameter, respectively].

After 12 hours of incubation with trace concentrations of [^{35}S]DMSP, both species had taken up most of it. Contrasting with what was observed with picophototrophs, light only stimulated uptake by ~10% in diatoms (Fig. 4). When nonlabeled DMSP was added at a concentration of 10 μM, the uptake of [^{35}S]DMSP was almost completely suppressed. Addition of 10 μM of nonlabeled GBT produced the same effect (Fig. 4, top). Trace amounts of [^{14}C]GBT were taken up by both species, and, likewise, when 10 μM of nonlabeled GBT was added, [^{14}C]GBT uptake was suppressed. Addition of 10 μM of DMSP inhibited ^{14}C-GBT uptake by half in *T. pseudonana* and by a third in *T. oceanica* (Fig. 4, bottom). The two species of *Thalassiosira* seemed to use the same transport system for both compounds, in a way similar to that of heterotrophic bacteria (*16*, *17*). Genes encoding for a putative GBT membrane transporter have been found in the genome of this same *T. pseudonana* CCMP1335 strain (*20, 21*).

Our results provide evidence that diatoms and the two major groups of pelagic nonfilamentous cyanobacteria can take up and use DMSP. Production of DMSP, although ubiquitous in the ocean, is taxon dependent and, to some extent, size dependent too: Small haptophytes and dinoflagellates are generally high producers, whereas diatoms (except for those that grow in sea ice) and cyanobacteria are low or nonproducers (*22–24*). Tests with two axenic strains of the haptophyte *Emiliana huxleyi* (CCMP373 and CCMP374) and the dinoflagellate *Karenia brevis* (CCMP 2281), strong and moderate DMSP producers, respectively, revealed no uptake of [^{35}S]DMSP (table S2).

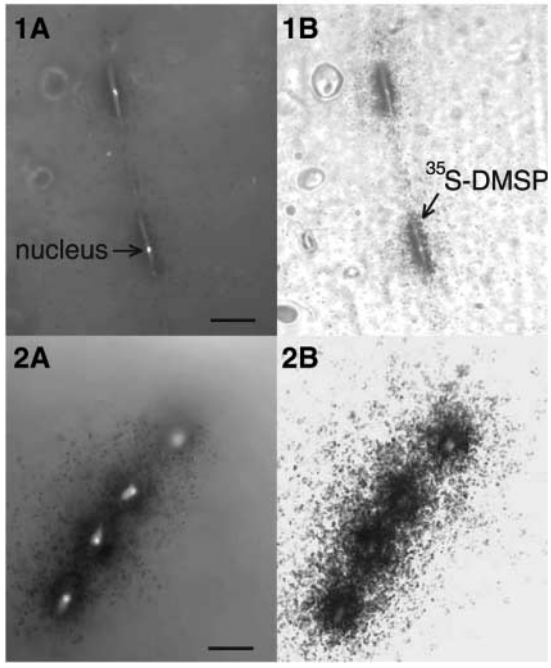

Fig. 2. Photomicrographs of two species of diatoms, (1) *Pseudo-nitzschia* sp. and (2) *Chaetoceros* sp. occurring in a natural phytoplankton assemblage from Blanes Bay (northwest Mediterranean), after being processed by microautoradiography. (**A**) Epifluorescence micrographs under UV light, showing 4′,6′-diamidino-2-phenylindole–stained nuclei. (**B**) Same cells observed under transmitted light. Black dots surrounding cells indicate assimilation of [^{35}S]DMSP by diatoms. Scale bars indicate 10 μm.

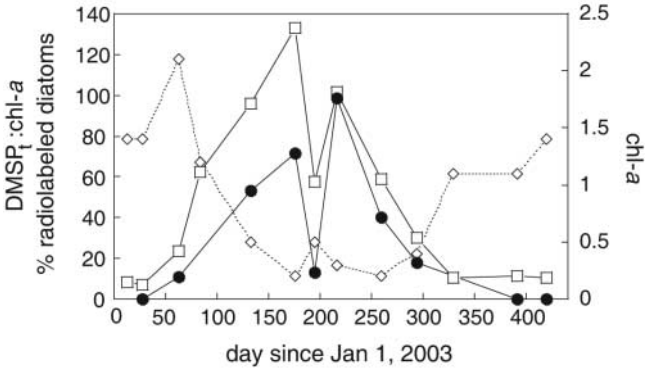

Fig. 3. Annual variation of the percentage of DMSP sulfur–assimilating diatoms (circles) and the in situ DMSP:chl-*a* ratio in nmol•μg^{-1} (squares) in surface waters of Blanes Bay (northwest Mediterranean). Chl-*a* concentration (μg•l^{-1}) is also shown (diamonds).

Our results thus suggest that low- or non–DMSP-producing diatoms and cyanobacteria consume DMSP released by high-producing phytoplankton partners.

But what is the quantitative relevance of this process in nature? *Prochlorococcus* are numerically the dominant phytoplankters in the oligotrophic central oceanic gyres and tend to be replaced by *Synechococcus* in productive tropical waters and in the transitional zones to temperate waters (*25*). Both co-occur in the euphotic zone with small high-DMSP–producing eukaryotic algae. In upwelling regions and in waters that receive pulses of nutrients from continental discharges or from episodic or seasonal mixing, diatoms grow among a background of small algae and tend to dominate primary production (*26*). We have shown that picophototrophs contributed 10 to 34% of DMSP sulfur assimilation in the light-exposed waters studied. The contribution of total phytoplankton (including diatoms) is harder to quantify. Size-fractionated DMSP-sulfur assimilation experiments conducted in the surface Sargasso Sea in April 2002 and July 2004 revealed that, in the dark, 100% of the DMSP sulfur assimilation was carried out by microorganisms smaller than 0.6 μm (i.e., mostly heterotrophic bacteria), whereas, in the light, assimilation was stimulated by two- to threefold; 50 to 70% of the total assimilation was by organisms larger than 0.6 μm (i.e., mostly phototrophic prokaryotes and all eukaryotes).

All of our incubations were conducted in the absence of ultraviolet (UV) radiation; hence, it is likely that we are underestimating the relative contribution of phytoplankton (UV-protected by pigments) versus heterotrophic bacterioplankton as DMSP sulfur sinks. In any case, phytoplankton DMSP utilization confirms a major role of DMSP as a carrier for sulfur and carbon through multiple levels of marine microbial food webs (*9*, *11*). Ours results show that, in the illuminated conditions of the surface ocean, phytoplankton assimilate DMSP sulfur in similar proportions to heterotrophic bacterioplankton, both together assimilating ca. 20% of total DMSP consumption. If we also include the assimilation by microzooplankton (ca. 20%), then the total assimilative consumption by microbial food web components is of similar magnitude to DMS production (ca. 10 to 50% of total DMSP turnover) and much higher than eventual DMS ventilation to the atmosphere (ca. 3%) (fig. S1). Assimilation of DMSP, therefore acts to regulate sulfur emissions into the atmosphere, with potential important consequences to the global biogeochemical sulfur cycle and climate (*5*, *7*).

Another broad implication of our results refers to the use of organic substrates by phytoplankton. Our data add to previous observations (*15*, *27–29*) to demonstrate that widespread and numerically dominant phytoplankton groups are capable of taking up essential elements in reduced organic forms. This, together with the phagotrophic bacterivory described in many algal

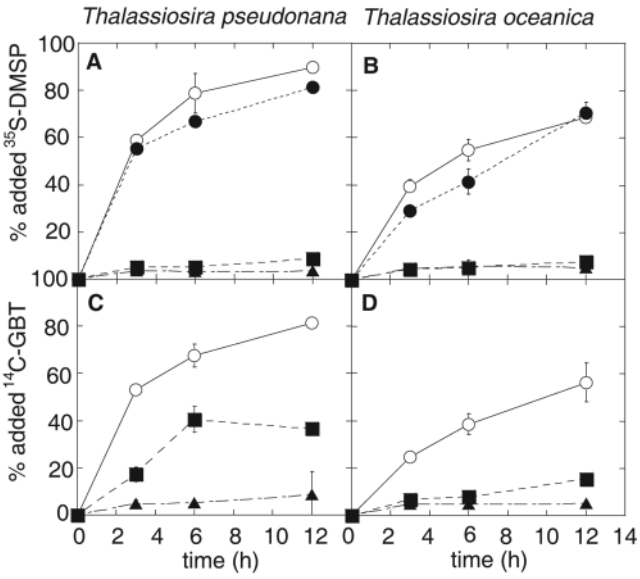

Fig. 4. (**A** to **D**) Uptake of [^{35}S]DMSP (top, circles) and [^{14}C]GBT (bottom, circles) by axenic cultures of *T. pseudonana* (left) and *T. oceanica* (right). Solid and open symbols correspond to dark and light incubations, respectively. Time series of isotope uptake in the presence of potential competitive inhibitors, 10 μM of non–radio-labeled DMSP (squares), and 10 μM GBT (triangles) are also shown. Error bars correspond to standard deviation from triplicate measurements.

taxa (*19*, *30*), further reveals how metabolically versatile phytoplankton are as a fundamental ecological player in the ocean and how challenging it becomes to implement their dynamics in oceanic biogeochemical models.

References and Notes

1. G. Malin, G. O. Kirst, *J. Phycol.* **33**, 889 (1997).
2. J. Stefels, *J. Sea Res.* **43**, 183 (2000).
3. D. C. Yoch, *Appl. Environ. Microbiol.* **68**, 5804 (2002).
4. W. Sunda, D. J. Kieber, R. P. Kiene, S. Huntsman, *Nature* **418**, 317 (2002).
5. R. J. Charlson, J. E. Lovelock, M. O. Andreae, S. G. Warren, *Nature* **326**, 655 (1987).
6. R. Simó, *Trends Ecol. Evol.* **16**, 287 (2001).
7. M. O. Andreae, P. J. Crutzen, *Science* **276**, 1052 (1997).
8. R. Simó, C. Pedrós-Alió, *Nature* **402**, 396 (1999).
9. R. P. Kiene, L. J. Linn, J. A. Bruton, *J. Sea Res.* **43**, 209 (2000).
10. R. P. Kiene, L. J. Linn, J. M. González, M. A. Moran, J. A. Bruton, *Appl. Environ. Microbiol.* **65**, 4549 (1999).
11. R. Simó, S. D. Archer, C. Pedrós-Alió, L. Gilpin, C. E. Stelfox-Widdicombe, *Limnol. Oceanogr.* **47**, 53 (2002).
12. R. Simó, *Can. J. Fish. Aquat. Sci.* **5**, 673 (2004).
13. R. R. Malmstrom, R. P. Kiene, D. L. Kirchman, *Limnol. Oceanogr.* **49**, 597 (2004).
14. M. Vila *et al.*, *Appl. Environ. Microbiol.* **70**, 4648 (2004).
15. R. R. Malmstrom, R. P. Kiene, M. Vila, D. L. Kirchman, *Limnol. Oceanogr.* **50**, 1924 (2005).
16. B. Kempf, E. Bremer, *Arch. Microbiol.* **170**, 319 (1998).
17. R. P. Kiene, L. P. Hoffmann Williams, J. E. Walker, *Aquat. Microb. Ecol.* **15**, 39 (1998).
18. G. Rocap *et al.*, *Nature* **424**, 1042 (2003).
19. J. A. Raven, *Limnol. Oceanogr.* **42**, 198 (1997).
20. E. V. Armbrust *et al.*, *Science* **306**, 79 (2004).
21. Transporter Protein Analysis Database, www.membranetransport.org.
22. M. D. Keller, W. K. Bellows, R. R. L. Guillard, in *Biogenic Sulfur in the Environment*, E. Saltzman, W. J. Cooper, Eds. (American Chemical Society, Washington, DC, 1989), pp. 167–182.
23. M. Corn *et al.*, in *Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds*, R. P. Kiene, P. T. Visscher, M. D. Keller, G. O. Kirst, Eds. (Plenum, New York, 1996).
24. S. Belviso, H. Claustre, J. C. Marty, *Limnol. Oceanogr.* **46**, 989 (2001).
25. F. Partensky, J. Blanchot, D. Vaulot, *Bull. Inst. Oceanogr. (Monaco)* **19**, 457 (1999).
26. V. Smetacek, R. Scharek, E. M. Nöthig, in *Antarctic Ecosystems Ecological Change and Conservation*, K. R. Kerry, G. Hempel, Eds. (Springer, Berlin, 1990), pp. 103–114.
27. H. W. Paerl, *Appl. Environ. Microbiol.* **57**, 473 (1991).
28. L. R. Moore, A. F. Post, G. Rocap, S. W. Chisholm, *Limnol. Oceanogr.* **47**, 989 (2002).
29. M. V. Zubkov, B. M. Fuchs, G. A. Tarran, P. H. Burkill, R. Amann, *Appl. Environ. Microbiol.* **69**, 1299 (2003).
30. R. I. Jones, *Mar. Microb. Food Webs* **8**, 87 (1994).

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A Centrosome-Independent Role for γ-TuRC Proteins in the Spindle Assembly Checkpoint

Hannah Müller, Marie-Laure Fogeron, Verena Lehmann, Hans Lehrach, Bodo M. H. Lange*

The spindle assembly checkpoint guards the fidelity of chromosome segregation. It requires the close cooperation of cell cycle regulatory proteins and cytoskeletal elements to sense spindle integrity. The role of the centrosome, the organizing center of the microtubule cytoskeleton, in the spindle checkpoint is unclear. We found that the molecular requirements for a functional spindle checkpoint included components of the large γ-tubulin ring complex (γ-TuRC). However, their localization at the centrosome and centrosome integrity were not essential for this function. Thus, the spindle checkpoint can be activated at the level of microtubule nucleation.

The classical function of the centrosome is the organization of microtubules in higher eukaryotic cells. Its duplication and function are tightly integrated into cell cycle regulatory processes (*1*, *2*). A role for the centrosome in the spindle assembly checkpoint, as an essential guardian of cell cycle progression, has been suggested but not established on the molecular level (*3*). γ-tubulin is a highly conserved component of the microtubule-organizing center (MTOC) in most animal cells and is involved in the initiation of microtubule nucleation (*4*, *5*). γ-tubulin is mainly found in two complexes: the large γ-tubulin ring complex (γ-TuRC) (comprising Grip71, Grip75, Grip84, Grip91, Grip128, Grip163, and γ-tubulin in *Drosophila*) and its subunit, called the γ-tubulin small complex (γ-TuSC) (comprising Grip84, Grip91, and γ-tubulin) (*6*, *7*). γ-TuRC promotes the nucleation of a microtubule filament (*4*, *6*, *8*). In addition, γ-tubulin is thought to be required for a G_1-related checkpoint pathway and spindle formation (*9*–*12*). Finally, the centrosome-associated fraction of γ-tubulin ring proteins is essential for coordinating mitotic events (*13*–*15*).

We investigated the role of core centrosomal proteins such as γ-TuRC proteins and centrosomin (cnn) (*16*) in spindle checkpoint activation in *Drosophila* cells (supporting online material text) by depleting target proteins using RNA interference (RNAi) (*17*) (Fig. 1). We focused on the analysis of the γ-TuSC components γ-tubulin and Grip84, the γ-TuRC component Grip71, and the

Department of Vertebrate Genomics, Max-Planck Institute for Molecular Genetics, Ihnestrasse 73, D-14195 Berlin, Germany.

*To whom correspondence should be addressed. E-mail: lange_b@molgen.mpg.de