Cryptic carbon and sulfur cycling between surface ocean plankton

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About half the carbon fixed by phytoplankton in the ocean is taken up and metabolized by marine bacteria, a transfer that is mediated through the seawater dissolved organic carbon (DOC) pool. The chemical complexity of marine DOC, along with a poor understanding of which compounds form the basis of trophic interactions between bacteria and phytoplankton, have impeded efforts to identify key currencies of this carbon cycle link. Here, we used transcriptional patterns in a bacterial-diatom model system based on vitamin B12 auxotrophy as a sensitive assay for metabolite exchange between marine plankton. The most highly up-regulated genes (up to 347-fold) for a marine Roseobacter clade bacterium when cocultured with the diatom Thalassiosira pseudonana were those encoding the transport and catabolism of 2,3-dihydroxypropane-1-sulfonate (DHPS). This compound has not currently recognized role in the marine microbial food web. As the genes for DHPS catabolism have limited distribution among bacterial taxa, T. pseudonana may use this sulfonate for targeted feeding of beneficial associates. Indeed, DHPS was both a major component of the T. pseudonana cytosol and an abundant microbial metabolite in a diatom bloom in the eastern North Pacific Ocean. Moreover, transcript analysis of the North Pacific samples provided evidence of DHPS catabolism by Roseobacter populations. Other such biogeochemically important metabolites may be common in the ocean but difficult to discriminate against the complex chemical background of seawater. Bacterial transformation of this diatom-derived sulfonate represents a previously unidentified and likely sizeable link in both the marine carbon and sulfur cycles.

Carbon-cycle-relevant interactions between heterotrophic marine bacteria and phytoplankton are mediated through compounds excreted from living phytoplankton cells or released when the cells are grazed or lysed (1). Current views of these interactions are rooted in the idea of passive trophic transfer to heterotrophic bacteria, although recent findings of pathogen-like genes in bacterial genomes (2) and predictable associations between microbial taxa (3, 4) suggest that more strategic interactions link autotrophic and heterotrophic plankton. Compounds serving as intermediates in bacterial-phytoplankton interactions are likely to be present in low concentration or to reach high concentration only within the cells’ diffusive boundary layers (5, 6) and in either case are difficult to discriminate over the complex chemical background of seawater. To the extent that these compounds remain unrecognized as currencies of trophic interactions, particularly when abundant plankton groups are involved, they represent gaps in our understanding of marine elemental cycles.

We established a model microbial system for exploring bacterial-phytoplankton exchanges in which the marine bacterium Ruegeria pomeroyi DSS-3 had an obligate dependency on the diatom Thalassiosira pseudonana CCMP1335 for fixed carbon while the diatom obtained an essential vitamin from the bacterium. As is common among marine eukaryotic phytoplankton, T. pseudonana harbors the B12-requiring version of the methionine synthase gene (metH) yet cannot synthesize B12 (7) and must obtain it from an exogenous source. The >50 sequenced members of the Roseobacter lineage all carry genes for B12 biosynthesis (ref. 8; www.roseobase.org). Both groups of organisms are important in the ocean, with diatoms responsible for up to 40% of global marine primary productivity (9) and roseobacters ubiquitously distributed, metabolically active (10), and commonly associated with diatoms (3).

In coculture, R. pomeroyi DSS-3 recovered B12-limited T. pseudonana, restoring growth rates comparable to those of axenic cultures supplemented with exogenous B12 (Fig. 1). When bacterial transcriptomes collected after 8 h of co-growth with diatoms (n = 4) were compared with control incubations (n = 2), ten of the most highly up-regulated genes (up-regulated 56- to 374-fold) encoded proteins for the transport and catabolism of the C3-sulfonate 2,3-dihydroxypropane-1-sulfonate (DHPS; Table S1). Eight of these genes were colocated on the R. pomeroyi DSS-3 chromosome and included DHPS TRAP transporter components hpsKLM (11), dehydrogenases hpsNOP for convertible DHPS to (R)-sulfolactate, and slcD for converting sulfolactate to sulfopyruvate (Fig. 2). Two genes were colocated on the bacterium’s megaplasmid, including cuyA, which mediates conversion of cysteate to pyruvate and

Significance

In the surface ocean, organic matter released by phytoplankton and degraded by heterotrophic bacteria is a key step in the carbon cycle. Compounds important in this trophic link are poorly known, in part because of the thousands of chemicals making up marine dissolved organic matter. We cocultured a Roseobacter clade bacterium with the diatom Thalassiosira pseudonana and used gene expression changes to assay for compounds passed to the bacterium. A C3-sulfonate with no previously known role in the microbial food web was identified and subsequently shown to be an abundant diatom metabolite and actively cycling compound in seawater. This work identifies a missing component of the marine carbon and sulfur cycles.


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Data deposition: The nucleic acid sequences reported in this paper have been deposited in the NCBI BioProject database, www.ncbi.nlm.nih.gov/nuccoreproject (accession no. PRJNA261079).

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ammonium, and the bisulfite exporter cayZ. Quantitative RT-PCR (RT-qPCR) of hpsN, the gene encoding dihydroxypropanesulfonate-3-dehydrogenase, confirmed that providing DHPS as a sole carbon source to R. pomeroyi DSS-3 resulted in the same pattern of gene regulation as growth with the diatom (Fig. 2). Although the transcriptional response was strongest to DHPS, six colocated genes involved in transport and catabolism of C₂-sulfonate N-acetyltaurine were also up-regulated by the bacterium. (Fig. S1). The enzymatic function of these bacterial genes was established previously (11, 12), but their ecological role had remained unknown. Identification of both sulfonates as currencies in this model system suggests a diatom-derived oxidized organic sulfur pool that may be important in fueling the marine microbial food web.

Coincident changes in cocultured T. pseudonana CCMP1335 were assessed with poly(A)-selected transcriptomes comparing cells cultured with R. pomeroyi (n = 2) to those that were axenic but B₁₂-replete (n = 2) during exponential growth (Table S2). As anticipated, the gene encoding the B₁₂-binding cobalamin acquisition protein 1 (CBA1) (13) was one of the most highly up-regulated diatom genes (11-fold) when bacteria served as the source of B₁₂ compared with when the vitamin was added to the culture in excess. No other genes reported previously to be induced by B₁₂ limitation (13) were differentially regulated in the coculture. However, several sulfur-related genes were down-regulated in the diatom when cocultured with the bacterium, including sqdB, which mediates the first committed step in biosynthesis of the sulfolipid sulfocinovosyl diacylglycerol (SQDG) (Tp269393; sixfold; Fig. S2), two genes involved in the synthesis of diacylglycerol precursors for SQDG biosynthesis (Tp7093 and Tp263660; fourfold), and a gene annotated as a sulfate transporter (Tp261147; fourfold). These changes in gene expression suggest shifts in sulfur metabolism by the diatom in the presence of R. pomeroyi.
Some bacteria are capable of producing DHPS by degrading sulfoquinovosine, the headgroup of SQDG (14). In our model system, however, the up-regulation of a dedicated DHPS transporter by R. pomeroyi and the previous identification of this compound in the cytosol of a freshwater diatom (15) argues that direct release of DHPS by the diatom formed the basis of this interaction. Diatoms might produce DHPS by a reductive deamination of cysteinolic acid, a potential cytosolic reservoir of reduced sulfur (16). Despite significant changes in evolutionary patterns, indicators of the physiological state of T. pseudonana (i.e., growth rate, photosynthetic efficiency $F_v/F_m$) showed no differences between the axenic and cocultured conditions.

The three known routes of bacterial DHPS degradation share an initial oxidation step mediated by HpsN, but carry out desulfuration via different enzymes (CuyA, SuyAB, or Xsc; Fig. 2 and Table S3). Over half of R. pomeroyi’s sequenced relatives in the Roseobacter clade (26 of 41 genomes) harbor at least one of the DHPS degradation pathways (Table S3), as do some marine relatives in the Rhodobacteriales (4 of 17 genomes) and Rhizobiaceae (4 of 15 genomes) (Fig. 3 and Table S3). Members of the alphaproteobacterial SAR11 clade also have a possible HpsN protein, but the sequences have an unresolved evolutionary relationship with experimentally verified sequences even when accounting for the substantial compositional heterogeneity due to differences in G+C content of the corresponding genes (Fig. 3, Fig. S3, and Table S4); further, the SAR11 genomes lack orthologs to metabolically upstream genes $hpsO$ and $hpsP$ (Table S3). All sequenced members of the Roseobacter clade and most of the marine Rhizobiaceae and the other marine Rhodobacterales that harbor a DHPS degradation pathway also have a B12 biosynthetic pathway (8, 17) (Table S3), whereas B12 biosynthesis is not nearly as widespread in the candidate DHPS-degrading SAR116 and Gammaproteobacteria taxa (<20%) and is absent in the SAR11 clade. Several taxa of soil bacteria, including strains associated with root nodules in plants, have DHPS pathway orthologs (Table S3), suggestive of similar sulfonate-based interactions between heterotrophic bacteria and terrestrial primary producers.

Whether the release of unique organic metabolites by T. pseudonana and other phytoplankton is used to enable mutualisms with vitamin-supplying or otherwise beneficial bacteria, or is simply excretion of excess materials that are subsequently scavenged, is a matter of debate (18, 19). In the case of DHPS, we asked whether the diatom might use a chemical signal based on the presence of a beneficial bacterium as a cue to initiate a mutualism (20). The concentration of DHPS in the T. pseudonana cytosol was high (3.3 mM) and similar to concentrations of compounds known to be abundant in microbial cells (21, 22), including the nitrogen homeostasis metabolite glutamate and compatible solute proline (Fig. 4). Importantly, DHPS was present in similar concentrations in the axenic and cocultured diatom cells, and present but slightly lower in B12-limited diatom cells (Fig. 4). By comparison, concentrations of another ecologically important organic sulfur compound, cosmylate dimethylsulfonopropionate (DMSP), ranged from 5 to 12 mM in cells of T. pseudonana, and also did not differ in the presence of bacteria. As for the bacterial side of the association, there was also no evidence of differential regulation of B12 pathway genes in R. pomeroyi DSS-3 during growth with the diatom. These findings suggest that neither of the cocultured organisms regulates biosynthesis of the currencies exchanged based on a cue linked to the physical presence of the other. They do not rule out, however, the possibility of species-specific recognition systems in naturally occurring roseobacter-diatom associations. Alternatively, it is conceivable that these compounds do not serve as the basis of an explicit, taxonomically narrow symbiosis (23), but rather represent general metabolic linkages between broad taxonomic groups. The ubiquity of bacteria associated with the diatom phycosphere (the region surrounding the cell that is enriched in extracellular products; ref. 24) under natural conditions assures that roseobacters or other DHPS-utilizing, B12-releasing bacteria will be in close proximity in the ocean.

To determine whether DHPS metabolism occurs in marine surface waters, metatranscriptomic and metabolomic data were collected from four stations along a coastal-to-open-ocean transect in the eastern North Pacific during an active diatom bloom. Counts of bacterial $hpsN$ transcripts (those mapping to the Roseobacter clade with experimentally verified function; Fig. 3) reached 2.5 x 10^4 L⁻¹ in coastal regions of the transect.

Fig. 3. A Bayesian phylogenetic tree derived from reference HpsN sequences including experimentally verified sequences (bold) and orthologs in marine genomes available through the Integrated Microbial Genomes database (img.jgi.doe.gov; see Table S3 for additional information). The tree was constructed using the P4 software following a χ² test on posterior distribution samples showing that a Bayesian composition-homogeneous model was adequate. Outgroups included a variety of histidinol dehydrogenase orthologs from marine genomes available through the Integrated Microbial Genomes database (Table S3) and orthologs in experimentally verified sequences (bold) and orthologs in the SAR11 clade. Some bacterial DHPS-degrading genes are not necessarily present in all members of the SAR11 clade, as indicated by their presence only in the starred sequences in Table S3. The tree was rooted using the BphS ortholog from Pelagibacter ubique (DSS-3). Branch length estimates were obtained from the maximum likelihood analysis using RAxML under the GTR+I+G model. The scale bar indicates the number of substitutions per site.
(Station P1), decreasing 25-fold to 1.0 \times 10^6 \text{ L}^{-1} in the open ocean (Station P8; Fig. 5A) and tracking well with diatom cell counts. DHPS was identified in the metabolome of the eukaryotic plankton size fraction (cells > 1.6 \mu m), where it accounted for up to 27% of a suite of major metabolites (Fig. 5B). Maximum concentrations of DHPS were similar to those of DMSP (Fig. 5C), currently considered the central metabolite of the surface ocean sulfur cycle (25) and produced primarily by dinoflagellates and haptophytes. DHPS concentration in the seawater dissolved organic matter pool was estimated to be 0.7 ± 0.5 nM (n = 4), consistent with efficient bacterial consumption. Given the importance of diatoms to carbon fixation in the global ocean, DHPS likely plays a major role in the flux of sulfur and carbon through marine food webs.

Biogeochemically important microbial metabolites may be cryptic because they have not yet been identified in the complex chemical milieu of seawater, or they are not readily targeted with existing analytical methods, or their concentrations are vanishingly low outside the phyosphere of phytoplankton. In waters of the eastern North Pacific, for example, chemical analysis of the organic carbon pools resolved >7,000 unique compounds. Unrecognized currencies in plankton interactions are highly likely to be represented among them. In this study, transcriptional responses in a two-member model organism system offered a sensitive methodology for identifying candidate metabolites of biogeochemical relevance. For DHPS, detection of this previously unrecognized diatom metabolite suggests significant new links in both the carbon and sulfur cycles that are mediated by the specific bacterial lineages capable of sulfonate catabolism.

**Methods**

*Trinema pseudonana* CCMP1335 was cultured in f/2 (+Si) medium at 18 °C on a 16:8 h light:dark cycle at ~160 μmol photons m\(^{-2}\) s\(^{-1}\). *R. pomeroyi* DSS-3 cells were harvested from 1/2 YTSS medium in exponential phase and washed in f/2 medium. Four treatments were established (n = 2 or 4) in f/2 (+Si) medium: *T. pseudonana* +B\(_{12}\), *T. pseudonana* −B\(_{12}\), *T. pseudonana* +*R. pomeroyi*, and *R. pomeroyi* alone. For RT-qPCR, *R. pomeroyi* DSS-3 was also grown in f/2 (+Si) −B\(_{12}\) with either acetate or DHPS. Diatom and bacterial growth was tracked by chlorophyll fluorescence and/or flow cytometry.

Cells were collected after 8 h (bacterial transcriptomics) or during exponential phase (bacterial RT-qPCR, diatom transcriptomics/metabolomics) and flash frozen in liquid N\(_2\). RNA was extracted and processed as described (26), with slight modifications, and sequenced on either Illumina MiSeq (Illumina) or SOLID version 4 (Life Technologies) platforms. Reads mapped using BWA (v0.5.9) (27) were analyzed for differential expression using the baySeq package in R, with 0.2–4.4 \times 10^6 mapped reads per sample for *R. pomeroyi* and ~8 \times 10^6 mapped reads per sample for *T. pseudonana*. RT-qPCR analysis of hpsN transcripts was performed as described (28). *T. pseudonana* metabolites were measured using ultra-performance liquid chromatography-high resolution mass spectrometry (UPLC-HRMS) on an Exactive Plus Orbitrap (29) using external calibration curves generated with pure metabolite standards. These data were converted to cytosolic concentrations based on an experimentally determined fluorescence:cell number relationship and assuming a cytosolic cell volume of 30 μm\(^3\).

Experimentally verified HpsN sequences (11, 12) were used as queries in BLASTP analysis to identify orthologs in marine bacterial genomes. Protein sequences were aligned and trimmed, and phylogenetic trees were created assuming three different evolutionary models using the P4 and PhyloBayes software packages (30).

Marine plankton consisting primarily of eukaryotic cells (retained on a 1.6 μm or 2.0 μm pore-size filter) and seawater dissolved organic matter concentrated on solid phase extraction columns were collected in May 2012 along the North Pacific Line P transect. Analysis of cellular metabolites was carried out by liquid chromatography triple-stage quadrupole mass spectrometry (LC/TOQMS). Metatranscriptomic datasets were obtained as above, with some modifications, including addition of an internal standard to all samples immediately before cell lysis (26, 31). Reads representing hpsN transcripts were identified using a BLASTX search (bit score cutoff ≥40), and hits were confirmed by analysis of the positive reads against the RefSeq protein database. Further details of all methods are given in SI Methods.
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