

A microbial source of phosphonates in oligotrophic marine systems

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Phosphonates, compounds with a carbon-phosphorus bond, are a key component of the marine dissolved organic phosphorus pool¹. These compounds serve as a phosphorus source for primary producers, including the nitrogen-fixing cyanobacteria *Trichodesmium*². Phosphonates can therefore support marine primary production, as well as climate-driven increases in marine nitrogen fixation³, carbon sequestration⁴ and possibly methane production, through the breakdown of methylphosphonate⁵. Despite their importance, the source of phosphonates to the open ocean has remained uncertain. Here, we use solid-state nuclear magnetic resonance spectroscopy to screen for the presence of phosphonates in cultured strains of *Trichodesmium erythraeum*. We show that phosphonates comprise an average of 10% of the cellular particulate phosphorus pool in this species. We therefore suggest that these cyanobacteria produce phosphonates, and might be a significant source of these compounds in the ocean, particularly in nutrient-poor regions, where *Trichodesmium* blooms occur. Given that *Trichodesmium* also thrives in a warm, carbon-dioxide-rich environment³, phosphonate production may increase in the future. This, in turn, might select for a microbial community that can use phosphonate, and could have implications for nitrogen fixation, carbon sequestration and greenhouse-gas production.

Phosphorus (P) has a key role in constraining the growth of marine primary producers over both modern and geologic timescales^{6,7}. In many regions of the ocean, standing stocks of dissolved inorganic phosphorus are so low that organically bound P dominates the dissolved P reservoir. With future P limitation scenarios predicted from natural (for example, increased N₂ fixation) and anthropogenic (for example, increased atmospheric nitrogen deposition) responses to climate change^{3,4,7}, microbial community structure, oceanic carbon export, and hence the oceanic uptake of atmospheric CO₂, may be controlled by dissolved organic phosphorus (DOP) concentration and composition^{5,8}. Furthermore, the presence and microbial degradation of methylphosphonate in the upper water column has been suggested to result in methane release to the atmosphere⁵. However, these hypotheses, and the ability to model climate-driven changes in oceanic biogeochemical cycles, are limited by the lack of information regarding the chemical composition, production and degradation of the present-day DOP pool.

DOP exists as two main bond classes, phosphoester (P–O–C bond) and phosphonate (P–C bond). ³¹P NMR analysis of high-molecular-weight (HMW) DOP (the only fraction concentrated enough for study) has shown that both bond classes are a significant and constant percentage (75% phosphoester and 25% phosphonate) of marine DOP (ref. 1). The phosphoester pool

comprises nucleotides, sugars and other biomolecules that are thought to be rapidly produced and consumed by marine microbes. The ubiquitous distribution of phosphonates within marine systems¹ also reflects a dynamic balance between sources and sinks, but the processes controlling the consumption and production of phosphonates are largely unknown. It is only with the sequencing of marine microbial genomes and environmental samples that this bond class was recognized as bioavailable to marine cyanobacteria^{2,5,9}. These studies suggest that the ability to degrade phosphonates is relatively widespread, and that phosphonate is consumed in the upper water column to support both carbon and N₂ fixation².

The maintenance of a relatively high standing stock of phosphonates in the presence of utilization suggests that there is a significant, yet unidentified, phosphonate source that contributes to upper-water-column DOP in the open ocean. Phosphonate compounds are known to be present in benthic marine invertebrates^{10,11}, and have been detected at low levels (<3.0%) in a planktonic amphipod¹². Phosphonates are also present in some organic pesticides and xenobiotics¹³. Although it may be possible that invertebrates or terrestrial runoff could influence dissolved phosphonate concentrations in certain cases, such as near-shore marine environments, neither of these potential sources adequately explains the dominance of the phosphonate bond class in the DOP of the ocean gyres and in about 2,000-year-old deep waters^{14,15}. It is widely accepted that microbe-derived phosphoesters (for example, sugars) and organic nitrogen compounds (for example, amino acids) contribute to the DOP and dissolved organic nitrogen pools, respectively^{16,17}. Indeed, the phosphonate bond has been detected in marine particulate organic matter^{12,18,19} and microbes could be a source of phosphonates to the ocean gyres. However to the best of our knowledge, this bond class has never been found to comprise a substantial proportion of the total P in any marine microbe examined until this study^{12,15} (Table 1).

Using solid-state ³¹P NMR spectroscopy, a non-destructive method for identifying the dominant classes of P bonds, the marine N₂-fixing cyanobacteria *T. erythraeum*, *T. theibautii*, *T. tenue* and *Crocospaera watsonii* were screened for the presence of phosphonates. Eukaryotic phytoplankton species, including a diatom and a coccolithophore were also tested (Table 1). Solid-state ³¹P NMR spectroscopy is advantageous in that it avoids extraction issues (for example, compound breakdown and alteration) associated with other methods¹⁹. No phosphonates were detectable in the N₂-fixing cyanobacteria *T. theibautii*, *T. tenue* or *C. watsonii*, or in the eukaryotes, consistent with previous work¹⁵ (Table 1, Fig. 1). In contrast, the phosphonate

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Table 1 | The average percentage of total phosphorus as the phosphonate bond class in cultures of marine phytoplankton.

Organism	Strain	P (μM)*	Phosphonate (% of total particulate phosphorus) [†]	Reference
<i>Amphidinium carteri</i>	—	2	0.01%	Ref. 12
<i>Coccolithus huxleyi</i>	—	2	<0.01%	Ref. 12
<i>Crocospaera watsonii</i>	WH8501	45	N.D.	This study
<i>Crocospaera watsonii</i>	WH8501	0	N.D.	This study
<i>Emiliana huxleyi</i>	CCMP 374	36	N.D.	This study
<i>Emiliana huxleyi</i>	CCMP 374	1	N.D.	This study
<i>Emiliana huxleyi</i>	CCMP 372	3.6	N.D.	Ref. 15
<i>Peridinium trochoidem</i>	—	2	<0.01%	Ref. 12
<i>Phaeocystis</i> sp.	CCMP 627	3.6	N.D.	Ref. 15
<i>Skeletonema costatum</i>	CCMP 775	3.6	N.D.	Ref. 15
<i>Synechococcus bacillaris</i>	CCMP 1333	3.6	N.D.	Ref. 15
<i>Syracosphaera elongata</i>	—	2	0.004%	Ref. 12
<i>Thalassiosira weissflogii</i>	CCMP 1336	36	N.D.	This study
<i>Thalassiosira weissflogii</i>	CCMP 1336	0	N.D.	This study
<i>Trichodesmium erythraeum</i>	IMS101	15	10%	This study
<i>Trichodesmium erythraeum</i>	IMS101	0	11%	This study
<i>Trichodesmium erythraeum</i>	ST6-5	15	8%	This study
<i>Trichodesmium erythraeum</i>	ST6-5	0	17%	This study
<i>Trichodesmium tenue</i>	Tenue	15	N.D.	This study
<i>Trichodesmium tenue</i>	Tenue	0	N.D.	This study
<i>Trichodesmium theibautii</i>	II-3	15	N.D.	This study
<i>Trichodesmium theibautii</i>	II-3	0	N.D.	This study

*Phosphate concentration estimated based on culture media at the start of the experiment.

[†] Average per cent phosphonate of cultures sampled at single time points; averages are from the ³¹P NMR data for two or more independent experiments, with the exception of the ST6-5 0 μM P treatment, which was not replicated. A previous ³¹P NMR study¹⁵ used a similar approach. Error on the ³¹P NMR integrations of phosphonate is roughly $\pm 3\%$. The presence of trace (<0.01%) phosphonate in undisclosed strains of coccolithophores and dinoflagellates was reported using an alternative to the ³¹P NMR method¹². N.D.: not detectable.

bond class was detected in cultures of *T. erythraeum*, with average concentrations from replicate single-time-point experiments ranging from 10–17% (Table 1, Fig. 1), depending on the strain and its physiology. The phylogenetic relationship deduced from the internal transcribed spacer region of the ribosomal RNA gene suggests that *T. erythraeum* occupies a clade that is unique from the other *Trichodesmium* species²⁰, and the genetic machinery for phosphonate biosynthesis may be associated only with this clade. To our knowledge, this is the first report of the presence of the phosphonate bond class in cultures of an abundant species of marine cyanobacteria.

Phosphonates were detected in two strains of *T. erythraeum*, isolated from the North Carolina coast (IMS101) and the oligotrophic northern Sargasso Sea (ST6-5) (Table 1). This suggests that the presence of phosphonates in these strains is not an artefact of the regime from which they were isolated. Although *T. erythraeum* has genes for the transport and metabolism of phosphonate compounds², mere surface adsorption or accumulation of phosphonate compounds does not explain the high percentages of the phosphonate bond class detected in the cultures. First, the genes for phosphonate transport are upregulated under low-P conditions² and here similar percentages of phosphonate compounds were detected in both low-P and P-replete cultures (Table 1). Second, mass balance calculations confirm that the phosphonate concentration in the sea water used in the culture studies is over four orders of magnitude too low to explain the measured concentrations of *T. erythraeum* phosphonate if accumulation or surface adsorption were the only mechanisms of incorporation (see Supplementary Information). *Trichodesmium* is difficult to maintain axenically, but an examination of the heterotrophic bacteria in the *T. erythraeum* cultures indicated that heterotrophic contamination was minimal (~ 100 – $1,000$ cells ml^{-1}). Heterotrophic bacteria were routinely separated from the *Trichodesmium* cultures by

collecting the *Trichodesmium* cells onto 5 μm filters, and collecting heterotrophic bacteria in the filtrate onto 0.2- μm filters. Biomass on the 0.2- μm filters never had detectable phosphonates (data not shown). Although this procedure does not control for the presence of heterotrophic bacteria tightly attached to the *Trichodesmium* trichomes, the total particulate P on the 5- μm filters used for this study is heavily dominated by *Trichodesmium*. Given these results, it seems that *T. erythraeum* is the source of the phosphonates detected herein.

The total phosphonate content in *T. erythraeum* IMS101 cultures increases as a function of increasing total particulate P ($R^2 = 0.60$, $p < 0.0007$), with an average slope resulting in a 10% allocation of total particulate P to phosphonate (Fig. 2). That phosphonates are a constant percentage of the total particulate P in culture is consistent with previous findings that these compounds consistently comprise at least 6% of total DOP (see Supplementary Information). The phosphonate bond can be present in a diverse set of biomolecules, including lipids, proteins and antibiotics¹³. Recent work with *T. erythraeum* IMS101 has shown that it will decrease the production of P-containing lipids under P stress, when the particulate P concentration is low²¹. Although phosphonate-containing lipids were not explicitly examined in this study, the decline in P allocation to phospholipids during P stress²¹ suggests that the phosphonates detected herein are not primarily associated with lipids. A lack of a relationship between phosphonates and lipids was previously found for marine HMW dissolved organic matter¹⁴. The chemical composition of marine phosphonates in DOP is unknown, because the compounds are difficult to isolate from marine organic matter at a concentration appropriate for characterization. As such, the finding that *T. erythraeum* produces roughly 10% of its cellular particulate P as phosphonate provides an important model system for characterizing the chemical composition of marine phosphonates produced *in situ*.

