

Marine phosphorus is selectively remineralized

Phosphorus is a vital nutrient of the world's oceans^{1,2}, where in vast regions it is associated with dissolved organic matter (DOM) in surface waters^{3,4}. We have characterized the major compound classes of high-molecular-weight marine dissolved organic phosphorus, phosphorus esters and phosphonates, by using tangential-flow ultrafiltration and phosphorus-31 nuclear magnetic resonance (³¹P NMR). We find that the composition and abundance of organic phosphorus in DOM differ significantly from the values in fresh organic matter, indicating that dissolved organic phosphorus (DOP) is preferentially remineralized from DOM.

It has become feasible, with the advent of tangential-flow ultrafiltration, to concentrate and isolate sufficient dissolved and particulate organic matter from natural waters for NMR spectroscopic analysis^{5,6}. Tangential-flow ultrafiltration of sea water reproducibly isolates a greater fraction of DOM than other methods, so ultrafiltered isolates represent the largest fractions of marine DOM ever characterized⁵⁻⁷.

In this study, 19–38% of total DOM was

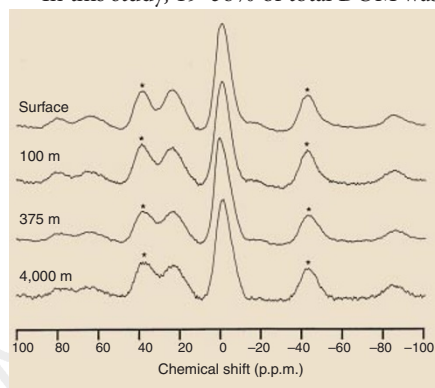


Figure 1 Phosphorus-31 cross-polarized magic-angle spinning NMR spectra of organic matter in sea water. Dried high-molecular-weight DOM isolated from ~1,000 litres of sea water from the surface, 100 m (chlorophyll maximum), 375 m (oxygen minimum) and 4,000 m in the Pacific Ocean (12° S, 134° W)⁶. Spectra were collected on a Chemagnetics CMX300 spectrometer at a frequency of 121.3 MHz and a spin rate of 5 kHz. Chemical shifts are referenced to phosphoric acid. Spectra were acquired using a pulse width of 4.4 μs and a pulse delay of 500 ms. Contact time was 1 ms. Data for surface and 100-m samples were collected over 20,000 transients, and data for 375- and 4,000-m samples were collected over 100,000 transients. Line-broadening of 50 Hz was applied to all samples. Asterisks denote spinning sidebands. NMR experiments with organic P standard compounds were performed to ensure that changes in peak area are linearly related to changes in abundance of the P compounds.

Table 1 Sample depth and concentration of DOC, DON and DOP, and C/N/P ratios

Sample	DOC	DON	DOP	C/N/P
Surface	22.2	1.33	0.090	247/15/1
100 m	22.2	1.42	0.083	267/17/1
375 m	10.6	0.63	0.033	321/19/1
4,000 m	8.08	0.45	0.015	539/30/1

Concentrations of ultrafiltered dissolved organic carbon (DOC), nitrogen (DON) and phosphorus (DOP) are expressed as micromolar; C/N/P values are the calculated atom ratios.

recovered by ultrafiltration⁶. All the ultrafiltered DOM isolates characterized here are in the high-molecular-weight or colloidal fraction (1–100-nm size range)⁶, which is a significant source of carbon and energy for marine bacteria⁸.

The ³¹P NMR spectrum of surface-water DOM indicates that DOP is dominated by two major classes of compound, phosphorus esters and phosphonates (Fig. 1). The most prominent feature of all spectra is the large peak centred at a chemical shift of 0 p.p.m., attributed to phosphorus esters. In marine plankton, phosphorus esters are present as monoester and diester phosphates in macromolecules such as nucleic acids and membrane phospholipids⁹. The smaller peak at 25 p.p.m. indicates the presence of phosphonates, a group of compounds containing a C–P bond. Phosphonates are often associated with phosphoproteins¹⁰ and membrane phosphonolipids⁹. Phosphonolipids in outer-membrane structures may protect cells from enzymatic degradation or provide extra rigidity^{11,12}.

About 3% of total phosphorus in a natural assemblage of plankton was attributed to phosphonates¹³. Although phosphonates comprise only a few per cent of phosphorus in plankton, integration of NMR peak areas reveals a surprisingly high abundance of phosphonates in high-molecular-weight DOP (about 25%). The relatively high proportion of phosphonates in surface DOP indicates preferential use of phosphorus esters relative to phosphonates during organic matter decomposition.

In sea water, some dissolved phosphorus esters are a rapidly used source of phosphorus whereas phosphonates are considered to be chemically stable compounds, more resistant to hydrolysis and bacterial degradation⁹. Given the higher proportion of phosphonates in surface DOP relative to marine plankton, regeneration must initially be a selective process, with reactive phosphorus esters as a preferred substrate for microorganisms.

Although the absolute concentration of high-molecular-weight DOP decreases from 90 nM in surface waters to 15 nM in deep waters (Table 1), ³¹P NMR spectra of DOP show phosphorus esters and phosphonates in unchanging proportions throughout the water column (Fig. 1), indicating that phosphorus esters and phosphonates are used at equivalent rates in deeper waters. Apparent differences in the reactivity of phosphorus esters in surface compared with deeper

waters may result from phosphorus esters occurring in a variety of biochemical classes of differing reactivities. Reactive phosphorus esters are used rapidly in surface waters, resulting in enrichment of deep waters in less reactive DOP compounds. The persistence of phosphorus esters and phosphonates from surface to deep waters suggests that less reactive fractions of DOP consist of common biochemical structures that are probably produced in surface waters.

The regeneration of nutrients in sea water is often compared to the Redfield ratio¹, which approximates the average composition of marine planktonic organisms (ratio of carbon/nitrogen/phosphorus (C/N/P) atoms = 106/16/1). Although it is largely unknown whether DOM is produced in Redfield stoichiometry, our data show that DOM is depleted in phosphorus throughout the entire water column relative to Redfield values (Table 1). The dramatic increase in C/P and N/P ratios with depth indicates that phosphorus is preferentially regenerated from DOM, which implies that DOP must ultimately cycle more efficiently than either DOC or DON.

Selective removal of phosphorus from DOM presumably reflects the nutrient demands of marine microorganisms, suggesting that phosphorus is a limiting nutrient in surface waters of oligotrophic regions.

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- Redfield, A. C. *Am. Sci.* **46**, 205–222 (1958).
- Broecker, W. S. & Peng, T. S. *Tracers in the Sea* (Eldigio, Palisades, NY, 1982).
- Smith, S. V., Kimmerer, J. & Walsh, T. W. *Limnol. Oceanogr.* **31**, 161–167 (1986).
- Karl, D. M. & Yanagi, K. *Limnol. Oceanogr.* **42**, 1398–1405 (1997).
- Benner, R., Pakulski, J. D., McCarthy, M., Hedges, J. I. & Hatcher, P. G. *Science* **255**, 1561–1564 (1992).
- Benner, R., Biddanda, B., Black, B. & McCarthy, M. *Mar. Chem.* **57**, 243–263 (1997).
- McCarthy, M. D., Hedges, J. I. & Benner, R. *Chem. Geol.* **107**, 503–507 (1993).
- Amon, R. M. W. & Benner, R. *Nature* **369**, 549–552 (1994).
- Hori, T., Horiguchi, M. & Hayashi, A. *Biochemistry of Natural C–P Compounds* 1–200 (Maruzen, Shiga, 1984).
- Quin, L. D. in *Topics in Phosphorus Chemistry* (eds Grayson, M. & Griffith, E. J.) 23–48 (Wiley, New York, 1967).
- Kennedy, K. E. & Thompson, G. A. Jr *Science* **168**, 989–991 (1970).
- Rosenberg, H. in *Form and Function of Phospholipids* (eds Ansell, G. B., Hawthorne, J. N. & Dawson, R. M. C.) 333–344 (Elsevier, New York, 1973).
- Kittredge, J. S., Horiguchi, M. & Williams, P. M. *Comp. Biochem. Physiol.* **29**, 859–863 (1969).