Composition and cycling of marine organic phosphorus

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Abstract
Using tangential-flow ultrafiltration and solid-state $^{31}$P nuclear magnetic resonance (NMR) spectroscopy, the dominant compound classes of marine high–molecular weight (1–100-nm size fraction) dissolved organic phosphorus (DOP) have been characterized in 16 samples from the Pacific Ocean, the Atlantic Ocean, and the North Sea. NMR spectra of ultrafiltered dissolved organic matter (UDOM) from all sites and depths reveal that P esters (75%) and phosphonates (25%) are the major components of ultrafiltered DOP (UDOP). P esters and phosphonates are present in unchanging proportions throughout the ocean. The homogeneity of UDOP from different oceanic regions suggests that processes leading to this chemical composition are ubiquitous. Ultrafiltered particulate organic matter (UPOM; 0.1–60-μm size fraction) samples from the Pacific Ocean and the North Sea were also analyzed using $^{31}$P NMR. In these samples, P esters are the only P compound class measured. Differences in the observed chemical compound classes of UDOP versus UPOM may result from (1) less-reactive phosphonates accumulating relative to P esters as particulate organic matter (POM) decomposes to DOM or (2) phosphonates originating from another source. C:N:P ratios of UDOP are significantly higher than Redfield ratios for POM. In general, C:P and N:P ratios of UDOP double between surface waters and the deep ocean. Increasing C:P and N:P ratios suggest that P is preferentially remineralized from UDOP relative to C and N throughout the water column.

Oceanic fertility is largely dependent on the availability of the vital nutrient phosphorus. Over geologic time scales, P is the ultimate limiting nutrient for sustaining oceanic primary productivity (Redfield 1958; Broecker and Peng 1982). In surface waters of the oligotrophic ocean, dissolved organic P (DOP) often comprises a significant fraction of the total dissolved P pool (Jackson and Williams 1985; Smith et al. 1986; Orrett and Karl 1987; Björkman and Karl 1994; Karl and Yanagi 1997); thus, regeneration of P from dissolved organic matter (DOM) is a potentially important source of bioavailable P. Within pools of marine dissolved and particulate P, turnover rates of organic P are rapid and seasonal, enabling low inorganic P concentrations to support relatively high amounts of primary production (Benitez-Nelson and Buesseler 1999). In the open ocean, hydrolysis of inorganic P from DOM by picoplankton may significantly increase primary production, which has significant implications for CO$_2$ uptake and particulate organic carbon export (Benitez-Nelson and Buesseler 1999). Over geologic time scales, availability of P may control atmospheric oxygen and carbon dioxide levels through a mechanism involving marine primary productivity (Van Cappellen and Ingall 1996). Thus, new insights into the composition and cycling of marine organic P are imperative for understanding not only the global P cycle, but also the coupled cycles of carbon, nitrogen, and oxygen.

Recently, the major compound classes of marine high–molecular weight ultrafiltered DOP (UDOP; 1–100-nm size fraction) were identified for one oligotrophic site in the Pacific Ocean (12°S, 134°W; Clark et al. 1998). At this site, P esters were the dominant compound class (75%), and phosphonates (25%) were a lesser (though significant) component of marine UDOP. Phosphonates, a chemically stable group of compounds containing a direct C–P bond, were previously unrecognized as a significant fraction of marine DOP. P ratios from the Pacific Ocean depth profile in Clark et al. (1998) indicate that DOP is preferentially remineralized from ultrafiltered (also called high–molecular weight) DOM (UDOM) relative to dissolved organic carbon (DOC) and dissolved organic nitrogen (DON). In the current paper, we examine whether the UDOP compound classes and trends of preferential remineralization of DOP from DOM observed

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in the Clark et al. (1998) Pacific Ocean site are representative of the world ocean. An oceanwide survey of the chemical composition of UDOP should offer clues to the origin of the compounds observed. If compositional differences are observed between oceans, then processes within certain oceanographic regions may significantly influence the composition of marine UDOP. If marine UDOP is homogeneous throughout the ocean, then marine UDOP must originate from processes that occur on a global scale. In the current paper, C:N:P ratios and organic P structure were analyzed in >20 UDOM and ultrafiltered particulate organic matter (UPOM) samples from the Pacific Ocean (five sites), the Atlantic Ocean (one site), and the North Sea (two sites). Marine UPOM and a marine heterotrophic bacterial culture were also analyzed by nuclear magnetic resonance (NMR) to evaluate potential sources of marine dissolved organic phosphonates.

Redfield ratios are a benchmark from which to evaluate marine organic matter production and diagenesis, as well as nutrient regeneration. Redfield ratios approximate the average relative amounts of C, N, and P in marine phytoplankton, with the atomic C:N:P ratio of marine particulate organic matter (POM) being 106:16:1 (Redfield 1958). A major implication of Redfield ratios is that marine organisms (i.e., phytoplankton) influence the chemical composition of dissolved inorganic and organic nutrients in surrounding seawater. In surface waters with low nutrient concentrations, the Redfield ratios are most closely approximated by the ratio of total dissolved nitrogen (TDN) and total dissolved P (TDP) (rather than dissolved inorganic N and P alone), which suggests that DON and DOP are important sources for N and P in oligotrophic surface waters (Jackson and Williams 1985; Karl et al. 1993). Establishing common ranges of C:N:P ratios of DOM is essential for understanding the cycling of nutrient components within bulk DOM.

Materials and methods

Tangential-flow ultrafiltration—Tangential-flow ultrafiltration separates and isolates molecules primarily on the basis of size rather than chemical composition or reactivity. Ultrafiltration reproducibly collects 20 to 40% of DOM from seawater, which is the largest fraction of marine DOM that can currently be isolated (Benner 1991; Benner et al. 1992, 1997). UDOM has compositional similarities to bulk DOM, including similar C:N ratios, stable isotopic ratios, and bulk chemical composition (Benner et al. 1992, 1997; McCarthy et al. 1993; Pakulski and Benner 1994; Guo et al. 1995; McCarthy et al. 1996). Given the ability to rapidly isolate relatively large quantities of compositionally representative DOM, ultrafiltration is an excellent method for the collection of colloidal and dissolved molecules for structural characterization using methods, such as solid-state NMR spectroscopy, that require concentrated and desalted samples. The isolation of POM by ultrafiltration has also been previously evaluated (Benner 1991; Benner et al. 1997). Optical and electron microscopy of particles isolated by ultrafiltration revealed that fragile materials are recovered intact, including phytoplankton and other organisms, amorphous aggregates, and colloidal matter (Benner et al. 1997).

For most samples, UDOM (1–100 nm) and UPOM (0.1–60 μm) fractions were isolated from approximately 1,000-L seawater samples using tangential-flow ultrafiltration (UF) procedures described below and in Benner et al. (1997). The North Sea UF-4 sample was an integrated surface sample collected along a transect from 58°10′N, 2°42′E to 53°22′N, 3°21′E. Seawater (1,800 L) was ultrafiltered, 200 L at a time, from points along the transect. For the North Sea UF-5 sample, 200 L of seawater was collected during a *Phaeocystis* bloom.

Water samples were collected using Niskin bottles equipped with Teflon- or epoxy-coated closure springs or a surface-water pumping system. Water samples were then passed through a 60-μm pore size Nitex sieve to remove large organisms. Generally, two size fractions were isolated using ultrafiltration: UPOM (0.1–60 μm) and UDOM (1–100 nm). To isolate the 0.1–60-μm size fraction (UPOM), an Amicon DC10L ultrafiltration system equipped with a polysulfone hollow fiber filter (HSMP01) was used. To isolate the 1–100-nm size fraction (UDOM), the filtrate from the DC10L ultrafiltration system was fed directly into an Amicon DC30 ultrafiltration system equipped with nine spiral-wound polysulfone filters (S10N1) with a molecular weight cutoff of 1,000 Daltons. The samples were concentrated to a volume of 10 L then transferred to the DC10L system equipped with two S10N1 filters and further concentrated to a final volume of 1 L. Following ultrafiltration, samples were diafiltered with 18 L of Milli-Q water to remove sea salts; thus, no free inorganic ions (e.g., inorganic P) are present in the isolates. Diafiltered concentrates (approximately 1 L) were reduced in volume by rotary evaporation and dried under vacuum in a SpeedVac concentrator to produce a solid sample. A mass balance of organic carbon was performed for each ultrafiltered sample to assess DOC recovery (Benner et al. 1997).

We are unable to directly assess P recovery for samples presented in this study because DOP and particulate organic P (POP) measurements were not made on whole seawater samples. Here we estimate the percent recovery of DOP by dividing the measured concentration of UDOP by previously published DOP concentrations in Pacific Ocean seawater (Smith et al. 1986; Orrett and Karl 1987; Ridal and Moore 1992; Karl and Tien 1997). Using available estimates from the literature, DOP concentrations at the surface and 100 m deep were approximately 0.20 μmol L⁻¹ and 0.16 μmol L⁻¹, respectively. Recovery estimates for sites deeper than 100 m are limited by the paucity of measurements made at these depths. Based on these DOP values, calculated recoveries were generally between 25 and 50%, but never below 20%, which is in the same range as that of DOC (20 to 40%) (Benner et al. 1997; Biddanda and Benner 1997). These calculations are further substantiated by consistency with previously reported C:N:P ratios of marine DOM (Williams 1986). Also, the fact that NMR spectra were obtained suggests that the percent DOP recovery is similar to that of DOC, otherwise P concentrations would be too low to produce a NMR signal. We also estimated the percent recovery of POP by dividing measured ultrafiltered POP (UPOP) concentrations by the average of recent surface POP measurements in the Pacific Ocean, which is 0.022 μmol L⁻¹ (Loh and Bauer 2000). Calculated recovery estimates for UPOP
ranged from 23 to 32%, slightly below the surface-water ultrafiltered particulate organic carbon recovery average of 48.5% (Benner et al. 1997).

The potential problems that can be encountered during ultrafiltration of P from seawater include (1) production of contaminant P due to leaching of P from surfaces within the ultrafiltration system, (2) scavenging of inorganic P by sorption during concentration of trace metals, and (3) potential artifactual production of colloidal organic P via the association of inorganic P with organic molecules (Bauer et al. 1996). There is no evidence that any of these processes significantly affected DOM isolation in this study. Leaching of DOC, and therefore DOP, does not occur with these ultrafiltration membranes and the procedures used in the present study (Benner et al. 1997). We did not test for leaching of polyphosphates, the only inorganic form of P that would be concentrated during ultrafiltration. However, we did not observe the presence of polyphosphates in the NMR spectra of any of the UDOM samples. It is extremely doubtful that possible scavenging of inorganic P into trace metals or colloidal organic molecules would result in the synthesis of C–P bonds. Additionally, if trace metal (i.e., paramagnetic) concentrations in DOM were significant, it would have been impossible to obtain a signal using NMR.

There is also no evidence that the prevalence of phosphonates in marine DOM may result from sample contamination or artifactual production during the process of ultrafiltration. The stable isotope and elemental characterizations (δ¹³C, δ¹⁵N, C:N ratios) presented in Benner et al. (1997) clearly demonstrate that the UDOM is of marine origin. Phosphonates are not observed in all samples collected using this ultrafiltration system and membranes. Dissolved and particulate organic matter were isolated from several cultures of marine phytoplankton using the same ultrafiltration systems used in this study (Biddanda and Benner 1997). These ultrafiltered culture isolates were analyzed by ³¹P solid-state NMR in a subsequent study (Clark et al. 1999), which did not reveal phosphonate structures in the UDOM or UPOM isolates. If phosphonates were an artifact of the ultrafiltration system and procedures, then phosphonates should be present in all ultrafiltered samples, which they are not.

³¹P NMR experiments—³¹P NMR spectroscopy is a powerful, nondestructive technique for the determination of P chemical bond structures in natural organic samples. ³¹P is a very sensitive nucleus to detect with NMR due to its isotopic abundance (100%) and high gyromagnetic ratio. Figure 1 shows the chemical shift ranges for different groups of P compounds, and Fig. 2 contains the chemical structures for these compound classes. Until recently, NMR studies of DOP were not possible due to the low natural abundance of DOP in natural waters. However, the adaptation of tangential-flow ultrafiltration for oceanographic studies provided a method for isolation of DOM in quantities sufficient for solid-state NMR spectroscopy. POM has rarely been analyzed by NMR because of the small quantities recovered with ultrafiltration (usually 100–250 mg), but with newer NMR probes, it is now possible to accommodate smaller samples with improved resolution.

Solid-state ³¹P cross-polarized magic angle spinning (CPMAS) NMR spectra were collected on a Chemagnetics CMX 300 spectrometer with a Varian/Chemagnetics 5.0-mm HX MAS NMR probe at a frequency of 121.3 mHz. For all samples, spectra were acquired at a spin rate of 10 kHz using a pulse width of 4.2 µs, a pulse delay of 1 s and a contact time of 1 ms. Line broadening of 50 Hz was applied to all samples. Data were collected over 5,000–210,000 scans. All chemical shifts are referenced to phosphoric acid. NMR
standardization experiments were performed to confirm that peak areas were proportional to P concentrations. Detection limits of P compound classes were determined experimentally; approximately 1 μmol P per g of each compound class was the lowest detectable amount of P. Interference from paramagnetics was not a problem with these samples. Spinning sidebands (an artifact of solid-state magic angle spinning) are not seen in the NMR spectra presented here because the sidebands are located beyond the region of interest (50 to −50 ppm). At spinning rates of 10K, sidebands are very small and represent only a few percent (<5%) of total peak areas. By decreasing the size of sidebands, higher spinning rates increase the intensity of the signals of interest and improve the spectra.

**Bulk measurements**—The carbon and nitrogen contents of samples were measured after vapor phase acidification using a Carlo Erba 1108 CHN analyzer (Benner et al. 1997). Total phosphorus content of dried UDOM and UPOM samples was determined by a modification of the combustion method of Aspila et al. (1976). Briefly, 5 mg of each sample wereashed at 550°C for 2 h and treated with 15 ml of 1 N HCl. HCl extracts were filtered through a 0.45-μm puradisc polypropylene filter and were measured using standard spectrophotometric techniques (Murphy and Riley 1962).

**Bacterial culture experiment**—Four replicate cultures of marine heterotrophic microorganisms were grown in 5-L erlenmeyer flasks. Synthetic seawater medium was used to ensure defined chemical composition and low organic matter content. The medium was made in Milli-Q water and contained Sigma’s seawater synthetic basal salt mixture (Sigma G1469), buffer (NaHCO₃), inorganic nutrients, glucose, glycine, trace metals (Sigma G1775), and vitamins (Sigma G1525). Seawater was collected from the Gulf of Mexico (26°55.79’N, 96°48.447’W) at 10 m deep on 19 June 1999.
and used to inoculate the culture media. The seawater was filtered through a 0.8-μm pore size polycarbonate cartridge filter to remove eukaryotes but leave bacteria and smaller plankton, and 250 ml of this filtered seawater was used to filter to remove eukaryotes but leave bacteria and smaller plankton, and 250 ml of this filtered seawater was used to inoculate the culture media. Flasks were placed on a shaker table in a dark environmental chamber for 5 d at 27°C. During the 5-d culture period, samples were collected daily to monitor bacterial abundance. Samples were analyzed for bacterial abundance within a week of collection using 4′6′diamidino-2-phenolindole (DAPI) staining techniques and epifluorescence microscopy (Porter and Feig 1980). At the end of the experiment, samples were centrifuged for 10 min at 10,000 rpm in a superspeed centrifuge. Following centrifugation, the supernatant was decanted and the remaining pellet was freeze-dried.

Results

**C:N:P ratios of marine UDOM**—Table 1 includes depth profiles of ultrafiltered DOC (UDOC), ultrafiltered dissolved organic N (UDON), and UDOP concentrations from two sites in the Pacific Ocean (12°S, 134°W and 2°S, 140°W) and the Bermuda Atlantic Time Series (BATS) station in the Atlantic Ocean (32°N, 64°W). C:N:P ratios are similar at the two Pacific Ocean profiles. Surface UDOM C:N:P ratios are 247:15:1 for the 12°S site and 261:16:1 for the 2°S site. Deep UDOM C:N:P ratios are 539:30:1 for the 12°S site and 535:29:1 for the 2°S site—roughly twice the values of the surface UDOM. At the BATS Station in the Atlantic Ocean, the surface UDOM C:P ratio was 390 and the deep (2,400 m) UDOM C:P ratio was 438, both of which are significantly higher than Redfield values. The N:P ratio of UDOM at BATS ranged from 23 in surface waters to 24 in deep waters, which are also higher than Redfield values.

Other UDOM samples were analyzed from surface waters in the Pacific Ocean (10°N, 140°W; 18°N, 134°W; 22°N, 130°W) and the North Sea (UF-4 and UF-5). In the Pacific Ocean, there was a slight trend of increasing UDOM: C:N:P ratios (307–340) and UDOP: C:N:P ratios (17–20) with increasing latitude. In the North Sea, the C:N:P ratio for surface UDOM at UF-4 was 283:20:1. At UF-5, the C:N:P ratio for the UDOM sample from the surface-water *Pseudocystis* bloom was 501:23:1, indicating a C-rich DOM, likely enriched in carbohydrates.

**C:N:P ratios of marine UPOM**—Surface-water UPOM was analyzed from three sites in the Pacific Ocean (10°N, 140°W; 18°N, 134°W; 22°N, 130°W) and two sites in the North Sea (UF-4 and UF-5) (Table 2). The 10°N Pacific Ocean UPOM sample had a C:N:P ratio of 90:1:1, which was slightly lower than the other two Pacific Ocean UPOM samples, which had similar C:N:P ratios of 132:16:1 (18°N) and 134:16:1 (22°N). At UF-4, the C:P value of UPOM was 58 and the N:P value was 7. At UF-5, the UPOM from the *Pseudocystis* bloom was characterized by a C:P ratio of 248 and a N:P ratio of 34.

**Chemical composition of marine UDOP**—31P solid-state NMR spectra of UDOP are presented in Fig. 3A–D. Depth profiles from two locations in the Pacific Ocean (12°S, 134°W and 2°S, 140°W) are shown in Fig. 3A,B, respectively. Figure 3C is a UDOP depth profile from the Atlantic Ocean at the BATS station (32°N, 64°W). Surface-ocean UDOP from the Pacific Ocean (10°N, 140°W; 18°N, 134°W; 22°N, 130°W) and the North Sea (UF-4) are presented in

### Table 1. Sample description, UDOM, UDON, and UDOP concentrations in ultrafiltered DOM (UDOM) and C:N:P ratios (UDOC and UDON data from Benner et al. 1997).

<table>
<thead>
<tr>
<th>Site</th>
<th>Depth (m)</th>
<th>Latitude</th>
<th>Longitude</th>
<th>UDOM (μM)</th>
<th>UDON (μM)</th>
<th>UDOP (μM)</th>
<th>C:N:P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacific Ocean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Surface</td>
<td>1°30'S</td>
<td>140°00'W</td>
<td>21.7</td>
<td>1.35</td>
<td>0.083</td>
<td>261:16:1</td>
</tr>
<tr>
<td>2</td>
<td>100 m</td>
<td>2°00'S</td>
<td>140°00'W</td>
<td>14.4</td>
<td>0.87</td>
<td>0.045</td>
<td>320:19:1</td>
</tr>
<tr>
<td>3</td>
<td>400 m</td>
<td>2°00'S</td>
<td>140°02'W</td>
<td>11.7</td>
<td>0.7</td>
<td>0.029</td>
<td>403:24:1</td>
</tr>
<tr>
<td>4</td>
<td>4000 m</td>
<td>1°57'S</td>
<td>140°03'W</td>
<td>8.02</td>
<td>0.44</td>
<td>0.015</td>
<td>535:29:1</td>
</tr>
<tr>
<td>5</td>
<td>Surface</td>
<td>12°12'S</td>
<td>134°40'W</td>
<td>22.2</td>
<td>1.33</td>
<td>0.090</td>
<td>247:15:1</td>
</tr>
<tr>
<td>6</td>
<td>100 m</td>
<td>11°58'S</td>
<td>135°07'W</td>
<td>22.2</td>
<td>1.42</td>
<td>0.083</td>
<td>267:17:1</td>
</tr>
<tr>
<td>7</td>
<td>200 m</td>
<td>12°06'S</td>
<td>134°55'W</td>
<td>12.6</td>
<td>0.78</td>
<td>0.061</td>
<td>207:13:1</td>
</tr>
<tr>
<td>8</td>
<td>375 m</td>
<td>12°19'S</td>
<td>134°26'W</td>
<td>10.6</td>
<td>0.63</td>
<td>0.033</td>
<td>321:19:1</td>
</tr>
<tr>
<td>9</td>
<td>4000 m</td>
<td>12°00'W</td>
<td>135°00'W</td>
<td>8.08</td>
<td>0.45</td>
<td>0.015</td>
<td>539:30:1</td>
</tr>
<tr>
<td>10</td>
<td>Surface</td>
<td>10°14'N</td>
<td>140°00'W</td>
<td>18.7</td>
<td>1.06</td>
<td>0.061</td>
<td>307:17:1</td>
</tr>
<tr>
<td>11</td>
<td>Surface</td>
<td>18°47'N</td>
<td>133°50'W</td>
<td>18.5</td>
<td>1.12</td>
<td>0.059</td>
<td>314:19:1</td>
</tr>
<tr>
<td>12</td>
<td>Surface</td>
<td>22°47'N</td>
<td>130°05'W</td>
<td>18.7</td>
<td>1.12</td>
<td>0.055</td>
<td>340:20:1</td>
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<td>Atlantic Ocean</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Surface</td>
<td>31°50'N</td>
<td>64°10'W</td>
<td>16.4</td>
<td>0.96</td>
<td>0.042</td>
<td>390:23:1</td>
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<tr>
<td>14</td>
<td>900 m</td>
<td>31°50'N</td>
<td>64°10'W</td>
<td>8.25</td>
<td>0.53</td>
<td>0.027</td>
<td>306:20:1</td>
</tr>
<tr>
<td>15</td>
<td>2400 m</td>
<td>31°50'N</td>
<td>64°10'W</td>
<td>9.20</td>
<td>0.51</td>
<td>0.021</td>
<td>438:24:1</td>
</tr>
<tr>
<td>North Sea</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>16 (UF-4)</td>
<td>Surface</td>
<td>Integrated</td>
<td></td>
<td>10.13</td>
<td>0.71</td>
<td>0.036</td>
<td>283:20:1</td>
</tr>
<tr>
<td>17 (UF-5)</td>
<td>Surface</td>
<td>51°30'N</td>
<td>2°50'E</td>
<td>29.38</td>
<td>1.36</td>
<td>0.059</td>
<td>501:23:1</td>
</tr>
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Table 2. Sample description, UPOC, UPON, and UPOP concentrations in ultrafiltered POM (UPOM) and C:N:P ratios (UPOC and UPON data from Benner et al. 1997).

<table>
<thead>
<tr>
<th>Site</th>
<th>Depth</th>
<th>Latitude</th>
<th>Longitude</th>
<th>UPOC (µM)</th>
<th>UPON (µM)</th>
<th>UPOP (µM)</th>
<th>C:N:P</th>
</tr>
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<tbody>
<tr>
<td>Pacific Ocean</td>
<td>Surface</td>
<td>10°14’N</td>
<td>140°00’W</td>
<td>0.63</td>
<td>0.08</td>
<td>0.007</td>
<td>90:11:1</td>
</tr>
<tr>
<td></td>
<td>Surface</td>
<td>18°47’N</td>
<td>133°50’W</td>
<td>0.66</td>
<td>0.08</td>
<td>0.005</td>
<td>132:16:1</td>
</tr>
<tr>
<td></td>
<td>Surface</td>
<td>22°47’N</td>
<td>130°05’W</td>
<td>0.67</td>
<td>0.08</td>
<td>0.005</td>
<td>134:16:1</td>
</tr>
<tr>
<td>North Sea</td>
<td>Surface</td>
<td>51°30’N</td>
<td>2°50’E</td>
<td>1.65</td>
<td>0.19</td>
<td>0.029</td>
<td>58:7:1</td>
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<tr>
<td></td>
<td>Integrated</td>
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<td></td>
<td>17.38</td>
<td>2.36</td>
<td>0.070</td>
<td>248:34:1</td>
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Fig. 3D. The outstanding features of spectra from all depths and locations are the dominant peak at 0 ppm and a lesser peak at 25 ppm. The peak centered at 0 ppm indicates the presence of P esters (C–O–P bond structure) and covers a chemical shift range that includes both monoester and diester phosphates (Figs. 1, 2). The lesser peak at 25 ppm indicates the presence of phosphonates (C–P bond structure; Figs. 1, 2). Peak area integrations indicate that P esters are three
times more abundant than phosphonates, with P esters and phosphonates comprising 75 and 25%, respectively, of the high–molecular weight DOP pool.

Chemical composition of marine UPOP—UPOM samples were analyzed from surface waters of the Pacific Ocean \((10^\circ N, 140^\circ W; 18^\circ N, 134^\circ W)\) and the North Sea (UF-5) using solid-state \(^{31}\)P NMR (Fig. 4). \(^{31}\)P NMR spectra of surface UPOM from sites in the oligotrophic Pacific Ocean and a Phaeocystis bloom in the North Sea revealed that P esters were the only measurable P compound class present (Fig. 4).

Marine bacterial culture experiment—Seawater was filtered through a 0.8-µm pore size filter and used as an inoculum for cultures of marine heterotrophic bacteria. The initial bacterial abundance in the culture flasks averaged \(1.2 \times 10^6\) cells ml\(^{-1}\). Bacterial abundance increased exponentially during the 5-d experiment, reaching a final cell abundance of \(1.5 \times 10^8\) cells ml\(^{-1}\). Solid-state \(^{31}\)P NMR analysis of the dried culture reveals that P esters (0 ppm) and polyphosphates (\(-20\) ppm) were the only compound classes detected (Fig. 5).

Discussion

\(C\!:\!N\!:\!P\) ratios of marine DOM—Elemental ratios of carbon, nitrogen, and phosphorus are commonly used to assess the relative remineralization of nutrients in marine organic matter. Redfield ratios estimate the average \(C\!:\!N\!:\!P\) ratio of marine POM as 106:16:1 (Redfield 1958). Although it is unknown whether DOM is produced in Redfield stoichiometry, our results show that \(C\!:\!N\!:\!P\) ratios of UDOM deviate substantially from Redfield ratios (Table 1). The Pacific Ocean depth profiles reveal increases in \(C\!:\!P\) and \(N\!:\!P\) with depth, which suggests preferential remineralization of P relative to C and N in UDOM. In the Atlantic Ocean depth profile, \(C\!:\!P\) increases with depth, but the \(N\!:\!P\) trends are less clear. Previous studies of \(C\!:\!N\!:\!P\) ratios in UDOM also indicate that P is preferentially remineralized from UDOM relative to C or N, suggesting that DOP is cycled more rapidly than either DOC or DON (Clark et al. 1998). In the Pacific and Atlantic Ocean profiles, low concentrations of UDOP (15–21 nmol L\(^{-1}\)) in deep waters relative to surface values (42–90 nmol L\(^{-1}\)) reveal (1) UDOP concentrations decrease three- to fourfold with depth, and (2) the bulk of measured UDOP (50–80%) is ultimately reactive and bioavailable to marine microorganisms. Ridal and Moore (1992) similarly concluded that most DOP is bioactive.

Recent work in the eastern North Pacific Ocean (three stations) and the Southern Ocean (one station) showed DOC:DOP and DON:DOP ratios increasing with depth, indicating preferential remineralization of P relative to C and N throughout the water column (Loh and Bauer 2000). Concentrations of DOP ranged from 0.01–0.23 µM in the eastern North Pacific and from 0.07–0.23 µM in the Southern Ocean (Loh and Bauer 2000). Near-surface \(C\!:\!P\) ratios in DOM from Georges Bank ranged from 400 to 800, and \(N\!:\!P\) ratios ranged from 24 to 55 (Hopkinson et al. 1997). \(C\!:\!N\!:\!P\) ratios of DOM indicated preferential utilization of N and P relative to C, resulting in deep water \(C\!:\!N\!:\!P\) values, which are significantly higher than Redfield values. In addition, DOM de-
composition experiments were performed to independently determine the relative reactivities of C, N, and P in DOM from the Georges Bank region. These DOM decomposition experiments also revealed preferential remineralization of P relative to C and N (Hopkinson et al. 1997).

**C:N:P ratios of marine UPOM**—In general, C:P and N:P values in Pacific Ocean UPOM were similar to Redfield stoichiometry. In the Pacific Ocean, C:P ratios of UPOM ranged from 90 to 134, and N:P ratios ranged from 11 to 16. The North Sea UPOM samples exhibited a wide variance in C:N:P ratios, with UF-4 UPOM having a low C:N:P ratio of 58:7:1 and UF-5 UPOM (Phaeocystis bloom) having a high C:N:P ratio of 248:34:1. It appears that the colonial form of *Phaeocystis* is relatively depleted in P. Previous studies have indicated preferential remineralization of P relative to C and/or N in suspended POM from deeper waters, due to the recycling of more reactive organic matter in the surface waters (Bishop et al. 1977; Williams 1986; Loh and Bauer 2000). At Station ALOHA’s 1,000-m reference depth, the estimated 9-yr average of C:N:P ratio for deep-water DOM is 2,600:129:1, and the averaged C:N:P ratio for deep-water POM is 190:26:1 (Thomson-Buldids and Karl 1998). These data suggest that P is preferentially remineralized as POM degrades and cycles through the DOM pool, resulting in the accumulation of less reactive fractions of DOM at depth, which are characterized by high C:P and N:P ratios (Thomson-Buldids and Karl 1998).

The reasons for the preferential regeneration of P from UDOM and UPOM are not clear. The rapid cycling of P relative to C and N may reflect the nutrient demands of marine microorganisms, which suggests that P is a limiting nutrient (Clark et al. 1998, 1999). If this is true, it is unclear why microorganisms would regenerate dissolved inorganic P (DIP) from DOP in the presence of the enormous DIP pool in the deep ocean. Alternatively, DOM may be utilized as a C or N source with P regenerated as DIP (Benitez-Nelson and Buesseler 1999).

**Chemical composition of UDOP**—In all ocean regions and depths surveyed in this study, UDOP is dominated by P esters and phosphonates. The relative proportions of the two compound classes are invariant with respect to depth and oceanographic regime; in other words, UDOP is compositionally similar throughout the world ocean. P esters (monoester and diester phosphates) are synthesized by all living organisms to form macromolecules such as membrane phospholipids and nucleic acids. The dominance of P esters in UDOP is undoubtedly related to the abundance of these compounds in all organisms, but the biological origin of marine phosphonates is less obvious. Previous studies indicate that phosphonate structures are incorporated into phosphoproteins (Quin 1967) and membrane phospholipids, an analog of phospholipids (Hori et al. 1984). Phospholipids in membranes may provide extra rigidity or protection from enzymatic degradation (Kennedy and Thompson 1970; Rosenburg 1973).

Previous work has documented that phosphonates comprise around 3% of total P in a mixed assemblage of plankton (Kittredge et al. 1969). Generally, P monoesters are thought to cycle very rapidly (Taft et al. 1977), whereas phosphonates are thought to cycle slowly (Kittredge and Roberts 1969; Hori et al. 1984). In the surface ocean, phosphonates may accumulate in UDOP relative to P esters due
to preferential utilization of more reactive P esters. This mechanism could reconcile the high proportion of phosphonates in surface-water UDOP with the low concentrations observed in living organisms.

The bulk composition of UDOP is similar throughout the water column with P esters and phosphonates remaining in constant relative proportions. This suggests that the components of DOP cycle differently in the surface ocean (described above) and the deep (i.e., below the surface) ocean (described below). Decreases in UDOP concentration with depth (Table 1), coupled with the constant relative proportions of UDOP compound classes throughout the water column (Fig. 3A–D), suggest that P esters and phosphonates are utilized at roughly equivalent rates below the surface. In other words, remineralization of UDOP compounds in the deep ocean is nonselective. P esters occur in numerous macromolecules of varying reactivities; certain P esters are more reactive than other P esters. This may explain apparent differences in P ester reactivity in surface versus deep waters. Reactive P esters are quickly utilized in the surface waters, and the less reactive P esters are transported out of surface waters. Below the surface waters, the less reactive P esters cycle at a rate similar to that of phosphonates, resulting in unchanging relative proportions of these compound classes with depth.

Several lines of evidence indicate the existence of distinct reservoirs within bulk marine DOP that differ in bioavailability and reactivity. Recent work on in situ P turnover rates has identified preferential remineralization of compounds with relatively high $^{32}$P and $^{33}$P activities, which suggests that these compounds are more bioavailable than the bulk DOP pool (Benitez-Nelson and Buesseler 1999). The high $^{32}$P and $^{33}$P activities imply that these compounds were very recently a part of an organism (Benitez-Nelson and Buesseler 1999).

In another study, Karl and Yanagi combined two methods for partial characterization of dissolved P pools in the North Pacific Ocean Hawaii Ocean Time Series (HOTS) station: continuous-flow ultraviolet (UV) photodecomposition and magnesium-induced coprecipitation (MAGIC) (Karl and Tien 1992; Karl and Yanagi 1997). DOP photodecomposition experiments suggested the existence of two distinct soluble nonreactive P (SNP, also called DOP) pools in the North Pacific Ocean. The upper water column (<100 m) was dominated by a UV-labile monophosphate ester-enriched pool, whereas a UV-stable pool increased at depths greater than 100 m and was attributed to nucleotides. At the Pacific Ocean HOTS station, a sustained 6-yr net accumulation of DOP has been observed and attributed to production of a soluble nonreactive P fraction during the El Niño Southern Oscillation period (Karl and Tien 1997).

Another study used stirred-cell ultrafiltration to isolate high–molecular weight DOP from the surface waters of Tokyo Bay (Suzumura et al. 1998). High–molecular weight DOP was characterized using two phosphohydrolytic enzymes, alkaline phosphatase and phosphodiesterase, which revealed three distinct pools of DOP: easily hydrolyzable monoester phosphates and diester phosphates and unhydrolyzable nonreactive DOP. The nonreactive DOP fraction constituted up to 67% of high–molecular weight DOP. The authors hypothesized that although esters in the nonreactive fraction may be intrinsically labile, they could be protected from decomposition by forming macromolecular complexes and/or submicron particles (Suzumura et al. 1998). These studies all propose the coexistence of a fraction of DOP that cycles rapidly and a fraction that is relatively unreactive. Our data suggest that phosphonates and certain P esters are likely components of less reactive DOP observed in these studies.

Previous solid-state NMR studies of UDOM have illuminated the major compound classes of C and N. $^{13}$C NMR and chemical analyses have revealed that the chemical composition of UDOM is dominated by polysaccharides in the surface ocean (Benner et al. 1992; McCarthy et al. 1993, 1996). Polysaccharides constitute a larger fraction of UDOM in surface waters (~50%) than deep waters (~20%), which suggests both production and consumption of these compounds in the upper ocean (Benner et al. 1992). $^{15}$N NMR of UDOM from the Pacific Ocean determined that amidates are the primary component of UDOM throughout the water column (McCarthy et al. 1997). Collectively, solid-state NMR studies of the bulk chemical composition of UDOM suggest that UDOM is largely composed of common biochemical structures that are probably produced in surface waters of the world ocean.

**Chemical composition of UPOM—Phytoplankton are thought to be the dominant source of marine organic matter, and they contribute to DOM through both direct and indirect mechanisms, such as decomposition, exudation, viral lysis, and release by grazers (Romankevich 1984). In surface waters, POM composition should reflect that of phytoplankton, as particles are mainly composed of phytoplankton, partially degraded phytoplankton cells, and, to a lesser extent, bacteria and zooplankton (Copin-Montegut and Copin-Montegut 1983). Figure 4 reveals that P esters are the only P compound class observed for UPOM from the Pacific Ocean and the North Sea. Although marine DOM is thought to originate primarily from biological production in the euphotic zone, the absence of measurable phosphonates in UPOM suggests that marine DOP is chemically distinct from POP. Results presented here are consistent with previous work showing P esters as the only measurable organic P compound class in four cultures of marine phytoplankton (Clark et al. 1999).

One mechanism that may explain the compositional differences between POM and DOM is selective preservation of phosphonates during POM decomposition. Phosphonates may initially be present in POM in quantities too low to be detected by NMR (i.e., <1 $\mu$mol P g$^{-1}$). As POM decomposes to DOM, less reactive phosphonates can accumulate relative to P esters, thus becoming selectively preserved in the DOM pool. Alternatively, the discrepancy may suggest that phosphonates in DOM originate from a source other than phytoplankton and POM.

**Phosphonates: characteristics, occurrences, and degradation—**In 1969, Kittredge and Roberts (p. 42) proposed that “the fraction of total P which becomes incorporated into C–P compounds may be only slowly returned to the cycle.” The C–P bond is highly resistant to chemical hydrolysis, thermal decomposition, and photolysis (Cook et al. 1978). A number of organisms have been documented as phospho-
nate synthesizers. The freshwater ciliated protozoan *Tetrahymena pyriformis* is a commonly studied organism because of its high phosphonate content (13% of total P) (Rosenberg and La Nauze 1967; Kittredge et al. 1969; Kittredge and Roberts 1969; Rosenberg 1973). Previous reports have suggested that the coccoliths *Emiliania huxleyi* and *Syracosphaera elongata* are phosphonate synthesizers (Kittredge et al. 1969), but our NMR data reveals no detectable C–P bond structures in *E. huxleyi* (Clark et al. 1999). Several species of dinoflagellates synthesize phosphonates: *Amphidinium carteri*, *Exuviola cassubica*, and *Peridinium trochoidum* (Kittredge et al. 1969). Phosphonate structures have also been observed in marine protozoans and metazoans (Hori et al. 1984; Nakhel et al. 1988; Matsubara et al. 1990; Sul and Erwin 1997). Previous work suggests that the bacterium *Bdellovibrio bacteriovorus*, as well as certain mycobacteria, contains phosphonates (Smith 1983). A common characteristic of phosphonate-synthesizing organisms is the lack of a protective outer coating, such as chitin or cellulose (Rosenberg 1973). The presence of C–P compounds in the outer membranes may provide increased strength and protection to the organism (Rosenberg 1973).

Bacteria are the only organisms with the documented ability to cleave C–P bonds (Cook et al. 1978; Schowanek and Verstraete 1990). Phosphonate degradation is inhibited in the presence of compounds containing orthophosphate, such as phosphate monoesters (Rosenberg and La Nauze 1967). Due to constraints inherent in quantitative measurement of phosphonates, measurable concentrations of phosphonates have not been reported for the marine environment (Cembella and Antia 1986; Nowack 1997). Interestingly, a recent coastal study of DOP measurement techniques revealed data suggesting that phosphonates were not a dominant component of the DOP reservoir in waters of the Eel River Shelf (Monaghan and Ruttenburg 1999).

**Source of phosphonates**—The compositional similarity among UDOP samples from vastly different regions of the ocean suggests that phosphonates are derived from organisms and processes with widespread distribution. Using ultrafiltration, Biddanda and Benner (1997) isolated UDOM and UPOM from cultures of several marine primary producers, including *Emiliania huxleyi*, *Synechococcus baiil- laris*, *Skeletonema costatum* and *Phaeocystis* sp. These UDOM and UPOM culture isolates were analyzed by $^{31}$P NMR in a subsequent study, which revealed that P esters were the only P compound detected (Clark et al. 1999). Because bacterial cell walls may contribute significantly to the marine DON pool (McCarthy et al. 1998), Clark et al. (1999) also examined particulate matter from cultures of bacteria (*Azotobacter vinelandii*, *Pseudomonas fluorescens*, and *Bacillus subtilis*). $^{31}$P NMR spectra of all bacteria revealed P esters, and the two heterotrophic Gram-negative species (*Azotobacter vinelandii* and *Pseudomonas fluorescens*) revealed phosphonate structures as well (Clark et al. 1999).

Based on (1) previous work showing that bacterial cell walls are an important source of marine DON (McCarthy et al. 1998) and (2) $^{31}$P NMR work revealing phosphonate synthesis by bacteria (Clark et al. 1999), marine bacteria are a potential source of marine phosphonates.

The marine bacterial culture experiment (Fig. 5) revealed synthesis of P esters and polyphosphates, but there was no indication of phosphonate structures. It is important to recognize that culture conditions select for certain organisms, not necessarily for the organisms that are most representative of the water used as an inoculum. Our results from this preliminary culture experiment did not reveal phosphonate synthesis by marine bacteria, but it would be premature to rule out bacteria as candidates for phosphonate synthesis in the ocean, especially in light of the fact that some, but not all, bacteria synthesize phosphonates (Clark et al. 1999). It is possible that phosphonates are present in the culture, but in quantities too low to be detected by NMR (i.e., <1 μmol P g$^{-1}$). An interesting outcome of the culture experiment was the synthesis of polyphosphates by marine heterotrophic microorganisms. Under well-oxygenated conditions and when nutrients are plentiful, some bacteria sequester inorganic P and store it as long-chain polyphosphates (Kulaev and Vagabov 1983). When oxygen is depleted, the stored P can be utilized by the microorganisms as an energy source. Polyphosphate metabolism has been observed in lake sediments (Hupfer and Gächter 1995) and has been proposed as a regulator of benthic fluxes of P in marine sediments (Ingall and Jahnke 1997).

**Conclusions**—In vast regions of the ocean, a significant fraction of the vital nutrient P in surface waters is associated with DOM. C : N : P ratios of UDOM suggest that DOP is preferentially remineralized relative to DOC and DON. C : N : P ratios of UDOM are significantly higher than Redfield ratios, whereas C : N : P ratios of UPOM are similar to Redfield ratios. Depth profiles of UDOP show loss of DOP with depth, indicating that the majority of the measured bulk DOP is ultimately reactive. $^{31}$P NMR analysis of >20 UDOP samples reveals that UDOP is compositionally similar throughout the world ocean: UDOP is dominated by P esters (75%) and phosphonates (25%), which occur in similar proportions with depth. The similarity of UDOP in terms of both structure and relative abundance in surface waters from different oceanic regions suggests that oceanwide processes influence the chemical composition of DOP. In contrast to UDOP, marine UPOP is composed predominantly of P esters. Future work that can pinpoint the origin of marine phosphonates will likely illuminate key mechanisms of DOP production and cycling.

**References**


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