

Nature and dynamics of phosphorus-containing components of marine dissolved and particulate organic matter

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Abstract

The molecular sources, dynamics and analytical characterizations of the phosphorus (P) containing components of marine dissolved and particulate organic matter (OM) are reviewed. Using a combination of ¹³C and ³¹P nuclear magnetic resonance spectroscopy on samples collected from a depth profile (20–4000 m) at Station Aloha in the North Pacific subtropical gyre, the biomolecular associations of P functional groups in marine OM are identified. Compositional differences between ultrafiltered dissolved organic matter (UDOM; 1–100 nm size fraction) and ultrafiltered particulate organic matter (UPOM; 0.1–60 μm size fraction) as reflected by NMR and elemental analyses indicate that UDOM does not originate from simple solubilization of UPOM, and the aggregation of UDOM is not the primary source of UPOM. Regression analyses indicated a large fraction of the P in UDOM is associated with carbohydrates and amino acids, but not with lipids. Similar analyses for UPOM indicated that P is associated with carbohydrates, amino acids and lipids. The P functional groups also appear to be balanced in their distribution among molecular classes, because they remain in relatively constant proportion throughout the ocean.

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1. Introduction

The oceans play a major role in the biogeochemical cycling of several key elements including carbon (C), nitrogen (N) and phosphorus (P). Both particulate and dissolved organic matter (OM) are continuously produced and remineralized in the marine water column, making them dynamic components of the biogeochemical cycles of C, N and P. Dissolved OM (DOM) is the dominant form of organic matter in the oceans and comprises approximately 700 Gt of C, making it one of the largest dynamic reservoirs of organic C on Earth (Hedges and Oades, 1997). Though a much smaller reservoir in size than DOM, marine particulate organic matter (POM) is also important as the sinking of POM from the surface to deep ocean waters is a major pathway for transporting C and other biological-

ly associated elements within the ocean (Lee et al., 2004). Burial of C in deep oceans and marine sediments is an effective long-term sink in the global C cycle. Phosphorus is an essential nutrient required by all living organisms and biomolecules containing P are integral to various cellular components including membranes (phospholipids), genetic material (DNA and RNA) and energy storage (ATP and ADP). In the marine water column and sediments, P occurs in both organic and inorganic compounds. The DOM and POM pools in the ocean include many compounds that contain both C and P and thus the cycling of P in DOM and POM should be considered in conjunction with that of C. The crucial balance between carbon dioxide and oxygen in the atmosphere is largely controlled by marine primary productivity, which is in turn linked to the availability of limiting nutrients such as N and P to marine phytoplankton. Therefore, an understanding of the coupled marine C and P cycles is also central to determining the long-term controls and variability of marine primary productivity.

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Recent studies have suggested that P may play a significant role in limiting primary productivity and nitrogen fixation in oligotrophic ocean regions (for example, Sañudo-Wilhelmy et al., 2001). In one such oligotrophic region, the North Pacific subtropical gyre (NPSG), the concentration of dissolved inorganic P (DIP), which is considered the most readily available form of P for microorganisms, has been decreasing in the upper water column for at least the last several years (Karl et al., 2001a,b). However, levels of primary productivity are being maintained and may even be increasing in this region, indicating that either DIP recycling and utilization has greatly increased in efficiency or, other sources of P are being exploited. In oligotrophic regions such as the NPSG and Sargasso Sea, dissolved organic P (DOP) often comprises a major fraction of the total dissolved P (TDP) pool especially in surface waters (Jackson and Williams, 1985; Björkman and Karl, 1994; Karl and Yanagi, 1997; Dyhrman et al., 2006). The DOP pool at Station Aloha located in the NPSG, constitutes ~75–80% of the TDP pool and is frequently 5–10 times larger than the measured DIP pool (Karl and Björkman, 2002). Thus, DOP is an important potential P source for the ecosystem at this site and obtaining new insights into its composition is an important step for understanding the dynamics of P cycling.

Recently, results of DOP bioavailability, hydrophobic P distribution, oxygen isotopic ratios of DIP and multi-year OM inventory studies, have provided new insights into P cycling at Station Aloha (Church et al., 2002; Björkman and Karl, 2003; Suzumura and Ingall, 2004; Colman et al., 2005). Some of these studies have indicated possible changes in the P cycle dynamics in this region. Results from Church et al. (2002) indicate that over a 10-year time period from 1989 to 1999, the dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) inventories at Station Aloha increased without a corresponding increase in the DOP pool. These changes resulted in increases in C:P and N:P ratios on the order of 17%, which indicate changes in the OM cycling in this ecosystem resulting from reorganization of the phytoplankton community. In particular, the phytoplankton community once dominated by eukaryotes, appears to have shifted to a phototrophic community dominated by smaller prokaryotic cells such as *Prochlorococcus* and *Synechococcus* (Karl, 1999). As a result of this ecological reorganization, a doubling of the chlorophyll *a* inventory and rate of primary production has also been reported (Karl, 1999; Karl et al., 2001a).

Given the significance of phosphorus in the marine environment, several excellent and extensive reviews on various aspects of the P cycle have appeared in the recent literature (e.g., Benitez-Nelson, 2000; Karl and Björkman, 2002; Suzumura, 2005). Benitez-Nelson (2000) presented a comprehensive review of the biogeochemical cycling of P in the marine system. This review summarized the processes that affect the distribution and cycling of P within the upper ocean and also gave an overview of the sources and sinks in the marine P cycle. One of the unique aspects

of this article was its emphasis on the techniques for estimation of marine P turnover times using artificial as well as natural tracers. Suzumura (2005) reviewed the analytical techniques for the extraction and measurement of phospholipids in marine samples and the dynamics and role of P-lipids in the biogeochemical cycling of P. The most comprehensive review on the composition and cycling of marine dissolved organic P has been provided by Karl and Björkman (2002). This review covered nearly every facet including compositional information, details of P-containing biomolecules in DOM and analytical techniques for measuring and quantifying DOP and P-biomolecules. In this paper, we draw upon various aspects of P cycling as reviewed in previous studies and focus mainly on the nature and dynamics of the coupled C-P cycling in DOM and POM. In order to further understand the association of P functional groups in DOM and POM with biomolecules, a case study of samples taken from a Station Aloha depth profile is presented.

2. Background

In this section, we briefly review the analytical techniques used for the measurement and characterization of inorganic and organic P in marine OM. The composition of marine DOP and POP can be characterized using various techniques such as direct analyses of certain biomolecules/compound classes in seawater, enzymatic assays, partial photochemical degradation, chromatographic methods and bulk compositional measurements using tools such as nuclear magnetic resonance (NMR) and X-ray spectroscopy. Many of these techniques have been discussed thoroughly in some of the previous reviews (Karl and Björkman, 2002; Suzumura, 2005) and are only broadly reviewed here. NMR spectroscopy, a powerful and versatile technique for determining the bulk composition of marine OM is reviewed in greater detail here than in earlier reviews and we also introduce a new technique, X-ray spectromicroscopy which has just begun to be applied to study marine OM.

2.1. Measurement and characterization of dissolved and particulate organic P

Measurements of inorganic phosphate play a key role in quantifying dissolved organic phosphate in the marine environment. In most cases, DOP is estimated as the difference between total dissolved phosphorus (TDP) and dissolved inorganic phosphate or what is operationally termed as soluble reactive phosphorus (SRP). Thus the precision of the DOP pool estimation is tied directly to that of the inorganic phosphate analyses. Quantitative analysis of inorganic phosphate (or SRP) is usually performed using some form of the phosphomolybdate blue method in which SRP is measured spectrophotometrically (Murphy and Riley, 1962). Over the years, numerous modifications have been made to the basic method and research has been car-

ried out on the optimum conditions for color development and the effects and treatment of potentially interfering compounds. One of the problems with the most commonly used protocol, which employs a reagent containing sulfuric acid, is that of the potential acid hydrolysis of DOP compounds leading to an overestimation of the SRP pool. In order to circumvent the problem of acid hydrolysis and to improve detection limits, Karl and Tien (1992) developed the Magnesium induced co-precipitation (MAGIC) method, which uses an alkaline solution for the pre-concentration of phosphate. This reduces the potential interference from the hydrolysis of acid-labile DOP compounds and since phosphate is concentrated from seawater prior to adding the reagents, the signal-to-noise ratio is improved (Karl and Björkman, 2002). MAGIC has also been utilized to separate phosphate from DOP in samples with high phosphate/DOP ratios (Thomson-Bulldis and Karl, 1998). In case of organic matter concentrated and isolated from seawater using tangential flow ultrafiltration, the measurement uncertainties are somewhat reduced as DOP can be directly measured using a single total phosphorus measurement. This is discussed further in Section 5, which describes results from the Station Aloha case study.

Quantitative measurements of TDP require an effective conversion of all inorganic and organic non-reactive P to SRP. Some methods employed to facilitate the complete oxidation and hydrolysis of all combined P include: (1) dry combustion, which is sometimes followed by acid hydrolysis of polyphosphate, (2) wet combustion with permanganate, persulfate or perchloric acid as the oxidant, (3) UV photo-oxidation, and, (4) alkali fusion with sodium carbonate or sodium nitrate followed by acid digestion.

2.1.1. Chemical techniques

Several organic P biomolecules such as nucleic acids, ATP, vitamins and lipids have been measured directly in seawater. A fairly detailed and historical account of these techniques and examples of their applications to marine samples has been presented by Karl and Björkman (2002) and is not repeated here. Here, we give a broad overview of the analytical protocols used to isolate and measure organic P biomolecules in seawater.

Nucleic acids are isolated from seawater by adsorption on to barium sulfate (Pillai and Ganguly, 1972) or hydroxyapatite (Hicks and Riley, 1980) or by precipitation using ethanol (DeFlaun et al., 1986), cetyltrimethylammonium bromide (Karl and Bailiff, 1989) or polyethylene glycol (Maruyama et al., 1993). The isolated nucleic acids are measured using colorimetric or fluorometric dye detection. Dissolved nucleic acids can also be measured indirectly by high performance liquid chromatography (HPLC) analyses of free nucleic acid bases released after hydrolysis (Breter et al., 1977). The quantitative determination of ATP involves isolation and partial purification (using a variety of techniques e.g., sulfuric acid extraction, adsorption on to activated charcoal and desalting) prior to detection by

the firefly luciferin–luciferase bioluminescence assay (Hodson et al., 1976). This method has a low recovery of ATP from seawater. Björkman and Karl (2001) developed a MAGIC based technique followed by firefly bioluminescence assay for ATP analysis, which has >90% recovery. Vitamins are measured using bioassay procedures based on the incorporation of ^{14}C bicarbonate during the growth of certain vitamin requiring phytoplankton (Carlucci and Silbernagel, 1966).

Dissolved P-lipids are extracted from seawater using liquid–liquid extraction techniques with chloroform or dichloromethane as solvents. Suzumura and Ingall (2001) examined the extraction efficiency of a commercially available phospholipid added to seawater, using chloroform, and concluded that phospholipid concentrations in seawater are underestimated due to low extraction efficiency (~60%). Extraction at lower pH may improve the extraction efficiencies of P-lipids by reducing the charge of the molecules, but these effects have not yet been quantitatively evaluated. Following extraction of the P-lipids from seawater, the simplest way to determine their concentration is by directly measuring the total P (using the standard phosphomolybdenum blue method) in the extracted fraction. This method does not require further separation of P-lipids from other kinds of lipids in the extracted material. Other methods used for hydrolysis of lipid-P extracted from marine samples include perchloric acid digestion for liquid samples and high temperature dry combustion after adding magnesium nitrate (to hydrolyze phosphonolipids). P lipids can also be separated and detected by HPLC or by thin-layer chromatography coupled with flame ionization detection (Parrish and Ackman, 1985). Phospholipids can be separated from phosphonolipids using a stepwise treatment with HCl to cleave phosphodiester, followed by APase to cleave monophosphate esters (Snyder and Law, 1970). For a more detailed discussion on the analytical techniques for P-lipid separation and quantification, the reader is referred to the review by Suzumura (2005).

2.1.2. Enzyme-based techniques

Marine microorganisms can utilize three forms of P, namely, orthophosphate or inorganic phosphate, phosphate esters and phosphonates. Most microorganisms synthesize one or more phosphohydrolytic enzymes in order to be able to degrade selected P compounds. Inorganic P is their preferred substrate, which is hydrolyzed using phosphatase enzymes. Given the use of different enzymes to breakdown P bonds or biomolecules, enzyme assay based techniques have been utilized to measure the concentrations of different P biomolecules in seawater. Addition of specific enzymes to whole, filtered or partially purified seawater can be used to characterize DOP and to determine its bioavailability. One technique that has been employed involves monitoring the appearance of SRP during timed incubations with the addition of alkaline phosphatase (APase) relative to controls without the enzyme addition. This technique assays the phosphomonoester associated

P, but in reality can also include polyphosphate and pyrophosphate derived P (Strickland and Solorzano, 1966). The contributions of the non P-monoester derived P have not been ascertained. Other components of the DOP pool have been similarly quantified using the enzymes DNase and RNase added to incubations (DeFlaun et al., 1987). Most of the extracellular nucleic acid pool was found to be nucleic acid hydrolysable indicating its bioavailability.

Phosphohydrolytic enzymes have also been used to compare the relative reactivity of different DOM pools (Suzumura et al., 1998). These authors studied the HMW and LMW DOP fractions (collected using tangential flow ultrafiltration) using two phosphohydrolytic enzymes, APase and phosphodiesterase. Suzumura et al. (1998) used stirred-cell ultrafiltration to isolate high molecular weight DOP from the surface waters of Tokyo Bay, which was then characterized using two phosphohydrolytic enzymes, alkaline phosphatase and phosphodiesterase. Their analyses revealed three distinct pools of DOP: easily hydrolyzable monoester phosphates, diester phosphates and unhydrolyzable non-reactive DOP. The non-reactive fraction was found to constitute up to 67% of the high molecular weight DOP (Suzumura et al., 1998). In a recent study (Ruttenberg and Dyhrman, 2005), APase activity was used as a measure of DOP bioavailability to phytoplankton off the Oregon coast. The identification of APase activity in several species indicates that DOP may contribute to phytoplankton P nutrition in this region (Ruttenberg and Dyhrman, 2005).

2.1.3. Nuclear magnetic resonance (NMR) spectroscopy

NMR spectroscopy is a technique based on the magnetic properties of atomic nuclei. It is used to distinguish between different functional groups/compound classes present in organic compounds and can be applied to solution as well as solid-state samples. The successful application of NMR spectroscopy to natural samples is often hampered by low concentrations of the nuclei of interest, resulting in poor spectral resolution. Despite these problems, a wealth of information on the composition of natural organic material has been obtained using this technique. In case of marine OM, concentration techniques such as tangential-flow ultrafiltration have been applied to obtain suitable samples for NMR analyses. The coupling of ultrafiltration and NMR has been applied to solid-state ^{13}C (Benner et al., 1992; McCarthy et al., 1993; Sannigrahi et al., 2005), ^{31}P (Clark et al., 1998, 1999; Kolowith et al., 2001) and ^{15}N (McCarthy et al., 1997). Solution and solid-state NMR have also been applied to marine sediments and particle/sediment trap material (Ingall et al., 1990; Carman et al., 2000; Paytan et al., 2003; Benitez-Nelson et al., 2004; Cade-Menun et al., 2005; Sannigrahi and Ingall, 2005).

The high natural isotopic abundance of ^{31}P (100%) makes it an ideal nucleus for NMR spectroscopy. ^{31}P nuclear magnetic resonance (NMR) spectroscopy has been shown to be an excellent tool for determining P composi-

tion of a wide variety of natural samples. Recently, Cade-Menun (2005) reviewed the principles and applications of ^{31}P NMR to environmental and agricultural samples. The major P compound classes present in marine organic matter (OM) have been identified for several sites in the world oceans using ^{31}P NMR (Clark et al., 1998, 1999; Kolowith et al., 2001; Paytan et al., 2003; Benitez-Nelson et al., 2004). These NMR studies have shown that P esters and phosphonates are the dominant forms of organic P observed in marine organic matter (Clark et al., 1998, 1999; Kolowith et al., 2001; Paytan et al., 2003; Benitez-Nelson et al., 2004; Cade-Menun et al., 2005). Other studies have indicated that compounds such as polyphosphates and pyrophosphates are present as minor constituents in marine sediments and sinking particulate matter (Sundareswar et al., 2001; Paytan et al., 2003; Sannigrahi and Ingall, 2005). Identification of functional groups by ^{31}P NMR is based on their chemical shift relative to an external phosphoric acid standard. Chemical shift values are used to represent differences in resonance frequency of different P compound classes. These values are dimensionless and are expressed in parts per million (ppm) relative to the phosphoric acid standard set at 0 ppm. The chemical shift ranges (in ppm) and structures of some P functional groups are given in Fig. 1.

One of the primary advantages of solid-state NMR spectroscopy is that it is non-destructive and requires almost no sample processing except drying. However, there may be problems associated with chemical shift anisotropy (which can be overcome to a large extent by using magic angle spinning), low spectral resolution and broad peaks that may overlap in certain cases. In case of natural OM, another concern is the inability of solid-state ^{31}P NMR to distinguish between P mono and di-esters. Solid-state ^{31}P NMR spectroscopy has been used to study marine high-molecular weight (HMW) ultrafiltered dissolved OM (UDOM; 1–100 nm), ultrafiltered particulate OM (UPOM; 0.1–60 μm), sinking particles and sediments (Ingall et al., 1990; Clark et al., 1998, 1999; Kolowith et al., 2001; Benitez-Nelson et al., 2004; Cade-Menun et al., 2005; Sannigrahi and Ingall, 2005). This technique has also been employed in the Station Aloha case study presented later in this paper.

Solution ^{31}P NMR has also been applied to elucidate the composition of marine sinking particles from various locations (Paytan et al., 2003; Cade-Menun et al., 2005). Solution ^{31}P NMR has higher spectral resolution than solid-state NMR and can distinguish between the two types of P-ester linkages, however in case of most natural samples and especially for marine samples an extraction step is necessary. One of the earliest sequential extraction methods termed SEDEX was developed by Ruttenberg (1992), which separates P into five operationally defined pools: loosely sorbed or exchangeable P (extracted with MgCl_2 , pH 8); Fe-oxide bound P (dithionite in citrate-bicarbonate, pH 7.6); authigenic carbonate fluorapatite, biogenic apatite and CaCO_3 -associated P (Na-acetate, pH 4); detrital P,

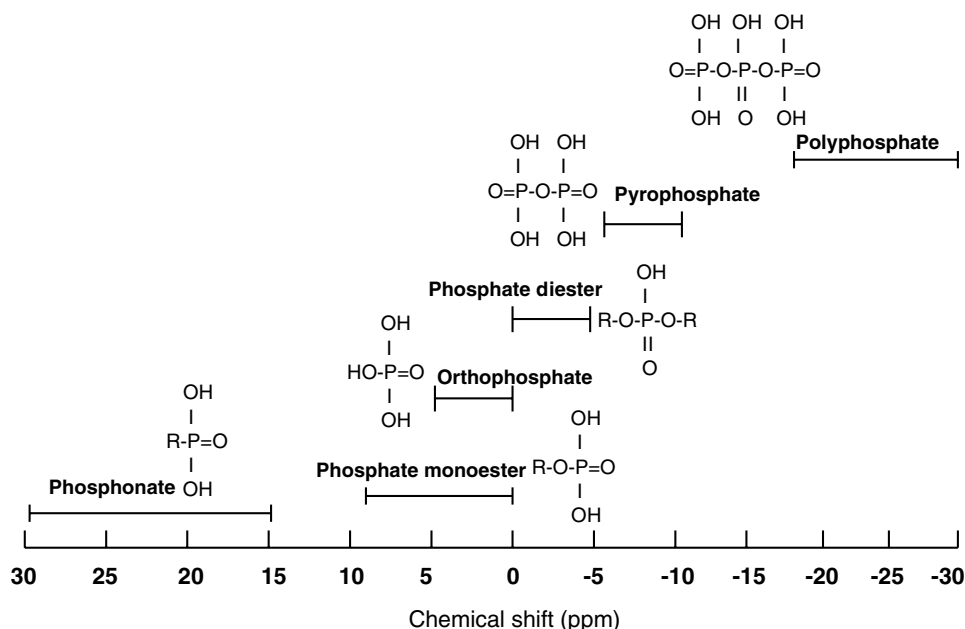


Fig. 1. ^{31}P NMR chemical shift ranges (on the ppm scale) and chemical structures of P functional groups observed in marine organic matter samples (Nanny and Minear, 1997; Turner et al., 2003). Chemical shifts are measured relative to an external phosphoric acid standard.

including apatite (HCl); and organic P (ashing and HCl extraction). An alternative procedure for extracting organic P, developed originally for soil samples utilizes a mixture of NaOH and EDTA (Cade-Menun and Preston, 1996). The NaOH-EDTA extraction protocol has been modified for use on small (≤ 1 g) samples and applied to marine sediment trap material (Paytan et al., 2003). The harsh conditions in these extractions often lead to hydrolysis of P esters (producing a large orthophosphate peak in most solution ^{31}P NMR spectra) or cause certain P species to be preferentially extracted over others. For example, the NaOH-EDTA extraction procedure is not as quantitatively effective for phosphonates as it is for P-esters (Cade-Menun et al., 2005).

Cade-Menun et al. (2005) compared solid-state versus solution ^{31}P NMR in terms of their application to sinking particulate organic matter. These authors also evaluated the effects of sample storage and processing conditions for sinking particulate OM collected in traps. In marine particulate OM, the extracted P concentrations were reported to increase in samples that were stored (by refrigerating or freezing) as compared to freshly collected samples. The relative abundances of P functional groups however were not affected. Hydrolysis of organic compounds occurs with storage and even more so with oven-drying. Cade-Menun et al. (2005) recommended that a combination of solid-state and solution ^{31}P NMR is necessary for a more complete characterization of marine OM.

2.1.4. Emerging techniques: X-ray spectromicroscopy

X-ray spectromicroscopic techniques have recently been shown to have great potential for the analysis of P in marine samples. With these techniques organic, mineral, and

polymeric P phases can be distinguished and mapped at a resolution of $0.1\ \mu\text{m}$ with minimal sample handling. The ability to map and identify P containing phases at submicron scales is particularly relevant for examining the microbially-mediated storage and degradation mechanisms often proposed for P cycling (Hirschler et al., 1990; Konhauser et al., 1994; Roden and Edmonds, 1997; Sannigrahi and Inggall, 2005; Schulz and Schulz, 2005).

Synchrotron-based soft X-ray microscopes have been used to examine P distributions within phytoplankton cells (Twining et al., 2003, 2004). Such instruments rely upon the interaction of X-rays in the <10 keV range with inner shell electrons in selected elements. The position and shape of absorption and fluorescence spectra collected from samples provide insights into the bonding environment of P within a particular phase. Differences in bonding environments in phosphate containing mineral, organic and inorganic phases result in a unique spectral “fingerprints” for each phase (Myneni, 2002; Brandes et al., in press). P X-ray absorption spectroscopy has the potential to identify organic P speciation (i.e., phosphonates and phosphates) and also to separate and identify inorganic phases, such as apatite and iron phosphate minerals, from organics. Additionally, X-ray microscopy techniques can be used to produce fluorescence maps of a sample, which are useful in identifying the association of other elements with P in a sample (Brandes et al., in press). In a recent X-ray microscopy study of P in marine sediments, fluorescence mapping of a natural marine sediment sample revealed that phosphorus was distributed heterogeneously throughout the sample (Brandes et al., in press). High P concentrations were observed in domains ranging from $<1\ \mu\text{m}$ to $\sim 6\ \mu\text{m}$ in diameter. Several of these high P abundance regions examined using nanoscale X-ray

fluorescence spectroscopy were found to consist of either apatite minerals or polyphosphates.

2.2. C:N:P stoichiometry of marine organic matter

Depending on various factors such as ambient nutrient concentrations, energy availability and growth rate, marine microorganisms can produce organic matter with elemental C:N:P ratios that deviate significantly from the Redfield value of 106:16:1. Despite the increasing number of studies dealing with DOM, simultaneous measurements of DOC, DON and DOP are not common in the literature. Loh and Bauer (2000) analyzed and compiled an extensive biogeochemical dataset of elemental C:P and N:P ratios of marine dissolved and particulate organic matter. They analyzed samples from an upper continental slope to abyssal plain transect in the eastern North Pacific, and a single station in the Southern Ocean. DOM and suspended POM had unique C:N:P ratios with POM concentrations generally 1 or 2 orders of magnitude less than the corresponding DOM concentrations. For all the OM pools measured in this study, the elemental ratios reveal that organic P is preferentially remineralized over organic C and N at these sites. The data also suggest that DOP is preferentially degraded over C and N as a function of depth. Cavender-Bares et al. (2001) reported results from near-surface dissolved organic and inorganic N and P measurements along a >2500 km long transect in the North Atlantic Ocean. In the subtropical portion of this transect, the total dissolved N and P pools were dominated by organic N and P, respectively. The concentrations of the DON and DOP pools were relatively invariant over a wide geographical range with mean concentrations of 6.3 μM N and 0.12 μM P. The mean molar N:P ratio of surface DOM was greater than 25:1 throughout this transect and had a highest value of 50:1, around 3-fold higher than the Redfield ratio of 16:1.

Recently, Aminot and Kerouel (2004) compiled an extensive inventory of dissolved organic C, N and P values measured in deep profiles in the N-E. Atlantic and N-W. Mediterranean. These samples were collected and analyzed during an 18-year period from 1984 to 2002 and the older analyses were re-examined in light of the results of inter-laboratory DOC and DON comparisons published in the early 1990s (e.g., Sharp et al., 1995; Peltzer et al., 1996). At both sites studied by these authors, C:P ratios increased by about a factor of 3 in vertical profiles from surface to deep waters due to the more drastic decrease in DOP as compared to DOC. The average C:P value of the deep water masses (1500–4000 m) from both sites was 1900 ± 600 . N:P ratios showed the same depth trends as the C:P ratios and averaged 130 ± 40 in the deep waters. By coupling non-refractory DOM stoichiometry and relationships between the main DOM elements in the water column, the relative mineralization of C, N and P from DOM was studied. Aminot and Kerouel (2004) also used their extensive dataset to test whether the increasing C:P ratios with depth were a result of the preferential degrada-

tion of P relative to C, or simply due to a larger proportion of “refractory” DOM with higher C:P ratios in the deeper DOM. Considering the samples from 1500 to 2000 m as refractory DOM, the average C:N:P for this fraction was calculated to be 1570:100:1. The non-refractory fraction had much lower C:N:P ratios. The preferential remineralization of P relative to C in the semi-labile DOM fraction was confirmed below the thermocline, but that of N was not. Preferential C removal appeared to be more frequent in the surface ocean, where various processes contribute to the production and removal of DOM.

Details of a case study involving elemental C, N, P analyses and a combination of ^{31}P and ^{13}C NMR on two different size fractions of marine OM collected from a depth profile at Station Aloha are presented below. These results provide new insights in to the differences in C and P cycling and the biomolecular associations of P functional groups in DOM and POM.

3. Methods: Station Aloha case study

3.1. Site location and sample collection

Water samples were collected from various depths (20–4000 m) at Station Aloha in the Pacific Ocean. Station Aloha (22.75°N, 158°W), located approximately 100 km north of Oahu, Hawaii, has been the subject of intensive study for many years as part of the Hawaii Ocean Time Series program. The water depth at the sampling site is 4800 m. Further information on Station Aloha and annual time series data reports can be accessed at http://hahana.soest.hawaii.edu/hot/hot_jgofs.html.

Samples were collected during a cruise to Station Aloha in October 1999 aboard the R/V *Ka'imikai-o-Kanaloa*. Organic matter was isolated from seawater using tangential flow ultrafiltration (Benner et al., 1992, 1997). At each depth, ~700 L of seawater was collected with four separate casts of a Niskin bottle rosette. From 600 L of this composite, the ultrafiltered POM (UPOM, 0.1–60 μm) fraction was isolated aboard ship with an Amicon DC10L ultrafiltration system with a polysulfone hollow fiber filter. The filtrate from the DC10L system was fed directly into an Amicon DC30 ultrafiltration system with polysulfone membranes (S10N1; 1000 Da cutoff) to isolate the ultrafiltered DOM (UDOM, 1–100 nm), which is also referred to as high-molecular weight (HMW) DOM. The UDOM and UPOM concentrates were frozen for transportation to the laboratory, where they were dried under vacuum and stored for chemical analyses. A mass balance of organic C was performed for each ultrafiltered sample, details of which can be found in Benner et al. (1997) and Hernes and Benner (2002). The results indicate that appreciable amounts of C were not gained or lost during ultrafiltration.

The percentage recovery of DOP was determined by dividing the measured P concentration of UDOM (Table 1) by the DOP concentration of seawater at that depth. The seawater DOP concentrations used were the average values for the week immediately preceding and following our sampling cruise, obtained from the Hawaii Ocean Time series data set. These calculations indicate that on average 32% of the total dissolved organic phosphorus present was recovered during ultrafiltration. This recovery is comparable to that of DOC (Benner et al., 1997). Potential problems that may be encountered during ultrafiltration include contamination by P leaching from surfaces within the ultrafiltration system; scavenging of inorganic P during concentration of trace metals and; artifactual production of colloidal organic P via the association of inorganic P with organic molecules (Bauer et al., 1996). Evidence presented in Kolowith et al. (2001) shows that using our sampling protocol, these processes are not significant factors affecting the isolation of ultrafiltered organic matter.

Table 1
P contents and atomic C:P, N:P and C:N ratios of UDOM and UPOM from Station Aloha

Depth (m)	P (nm/l)	C:P atom	N:P atom	C:N atom
<i>UDOM</i>				
20	98.2	169	10.7	15.9
80	83.6	213	13.3	15.9
110	73.1	241	15.8	15.3
150	64.5	210	14.1	14.9
200	55.0	172	11.6	14.8
250	36.2	200	15	13.3
300	39.3	213	14.7	14.5
500	33.5	220	14.5	15.2
750	28.9	268	18	14.9
2500	27.0	219	12.9	16.9
4000	29.3	198	12.2	16.3
Average		211.2	13.9	15.3
SD		28.1	2.1	1.0
<i>UPOM</i>				
20	6.7	142	17.5	8.1
80	10.0	109	13	8.4
110	18.5	129	13.1	9.9
150	5.3	134	15.7	8.5
200	3.3			8.1
250	3.5	110	11	10
300	2.8	149	15.7	9.5
500				9.4
750				9.9
2500	3.0	92	8.9	10.4
4000	6.7	98	8.6	11.5
Average		120.4	12.9	9.4
SD		21.0	3.3	1.1

The C:N ratios are from Sannigrahi et al. (2005).

3.2. Elemental C, N, P measurements

C, N and P contents were measured on dried UDOM and UPOM samples. C and N contents were measured after vapor phase acidification using a Carlo Erba CHN analyzer (Hedges and Stern, 1984). Total P contents were determined by a modification of the combustion method of Aspila et al. (1976). Five milligram of each sample were ashed at 550 °C for 2 h and treated with 15 ml of 1 N HCl overnight. The HCl extracts were filtered through a 0.45 µm puradisc polypropylene filter and the P content measured using standard spectrophotometric techniques (Murphy and Riley, 1962).

3.3. ³¹P NMR experiments

Solid-state ³¹P NMR analyses of the UDOM and UPOM samples were carried out at the NMR center in the School of Chemistry and Biochemistry at the Georgia Institute of Technology. The ³¹P NMR spectra were acquired on a Bruker DSX 400 spectrometer using Cross Polarization-Magic Angle Spinning (CP-MAS) at a ³¹P frequency of 161 MHz. Approximately 90 mg of powdered sample was packed into a 4 mm-diameter cylindrical zirconia rotor fitted with a Kel-F cap and spun at 10,000 ± 10 Hz in a Bruker magic-angle spinning probe. The presence of spinning sidebands is an artifact of MAS, but at fast enough spinning rates such as the one used here, they do not interfere with the peak resonances. For all samples, a cross polarization sequence, optimized to obtain semi-quantitative data, was used with a 1.0 ms contact time and a pulse delay of 4 s. In order to obtain semi-quantitative data using CP-MAS techniques, the time delay between two consecutive NMR pulses (referred to as the

pulse delay), used should be much greater than the spin-lattice relaxation times ($T_{1\rho}$'s) of all the different functional groups present (Wilson, 1987). In complex material such as natural organic matter, where a range of $T_{1\rho}$'s are present, NMR optimization experiments have to be performed to determine an optimal value of pulse delay that satisfies the above condition. In a series of experiments performed on marine dissolved organic matter samples, Clark (2000) found that varying the pulse delay from 1 to 20 s did not appear to alter the relative peak areas of the P-ester and phosphonate peaks, suggesting that 4 s was a sufficient pulse delay for these peaks to relax in the natural organic matter sample matrix. A contact time of 1 ms, commonly used for natural organic matter samples, was found to be optimal in this case as well. A total of 8000 transients were collected for each UDOM sample and 32,000 transients for each UPOM sample. UDOM and UPOM spectra were processed with 30 Hz and 100 Hz exponential line broadening, respectively. Data processing was carried out off-line using the Mestre-C software package (Mestrelab Research, Santiago de Compostela, Spain).

4. Results: Station Aloha case study

4.1. C:P and N:P ratios of UDOM and UPOM

P contents and C:P, C:N and N:P atom ratios of UDOM and UPOM from Station Aloha are presented in Table 1. The C:N ratios presented in Table 1 have been previously presented in Hernes and Benner (2002) and Sannigrahi et al. (2005). P contents of UDOM showed a decreasing trend with depth and ranged from 98.2 nM in the surface waters to 27 nM in the deepest samples. The P contents of UPOM did not exhibit any uniform trends with depth and varied between 2.8 and 18.5 nM. The C:N ratios for UDOM ranged from 13.3 to 16.9 (average 15.3 ± 1) and those for UPOM varied between 8.1 and 11.5 (average 9.4 ± 1.1). The C:P ratio of UDOM ranged from 169 to 268 (average 211 ± 28). The corresponding values for UPOM ranged from 92 to 149 (average 120.9 ± 21). The average N:P ratio for UDOM was 13.9 ± 2.1 , whereas it was 12.9 ± 3.3 for UPOM.

4.2. ³¹P NMR spectroscopy

Fig. 2 shows a water column profile of UDOM ³¹P spectra. Spectra from all depths show a prominent peak at -3 ppm and a smaller peak at ~20 ppm. The peak at -3 ppm can be attributed to phosphate monoesters and diesters. Phosphonates, a group of compounds containing a direct C-P bond, are also present in UDOM as revealed by the peak at approximately 20 ppm in the UDOM spectra (Fig. 2). P-esters and phosphonates comprise 75% and 25%, respectively, of the organic P in terms of relative proportions. This ratio remains constant for UDOM throughout the water column. Essentially identical trends with depth have been observed in other oceanographic regions (Clark et al., 1999; Kolowith et al., 2001).

Results from ³¹P NMR spectroscopy of UPOM from Station Aloha are presented in Fig. 3. These spectra exhibit only a single peak at -3 ppm, attributable to P-esters. This peak is located in the same region as the P-ester peak observed in UDOM and does not change in intensity with

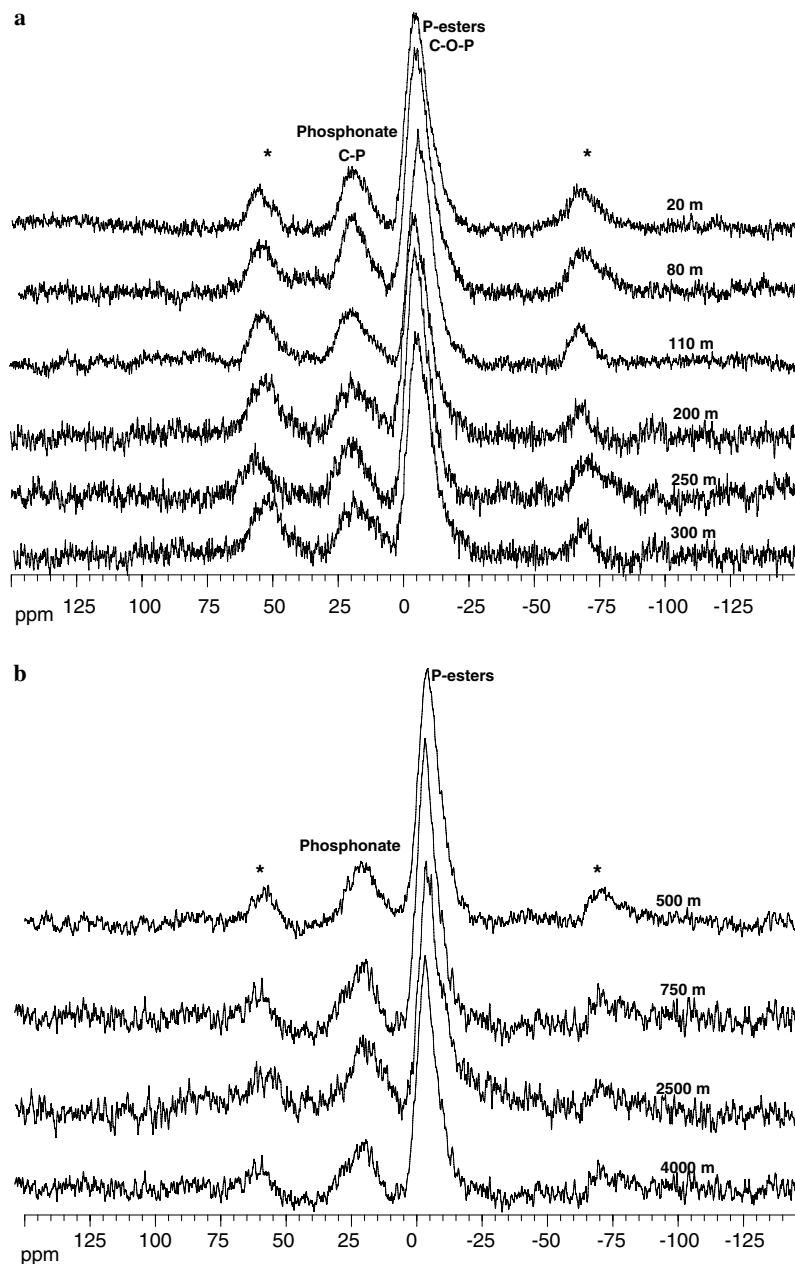


Fig. 2. Solid-state ^{31}P CP-MAS spectra of UDOM from Station Aloha stacked according to depth in the water column. Asterisks denote spinning side bands. Also indicated are the major peaks and the functional groups represented by them. NMR operating parameters: contact time 1 ms; recycle delay 4 s; 8000 transients. (a) ^{31}P NMR spectra of UDOM from 20 to 300 m. (b) ^{31}P NMR spectra of UDOM from 500 to 4000 m.

depth in the water column. UPOM from other geographic locations also show a single solid-state ^{31}P NMR peak due to P-esters (Clark et al., 1998, 1999; Kolowith et al., 2001).

5. Discussion

5.1. C:N:P ratios of UDOM and UPOM

The atomic C:P and N:P values (Table 1), together with atomic C:N ratios (Hernes and Benner, 2002; Sannigrahi et al., 2005) clearly indicate differences in the bulk chemical

composition of UDOM and UPOM. UDOM from all depths deviate substantially from the Redfield C:N:P ratio of 106:16:1, especially in terms of the C:P ratios. It is not known whether UDOM is produced in non-Redfield stoichiometry or if the observed ratios are a result of preferential remineralization of P relative to C. Various studies have reported C:N:P ratios of bulk DOM from Station Aloha to be much higher than Redfield ratios, especially in deeper waters, indicating preferential remineralization of P over C and N (Thomson-Bulldis and Karl, 1998; Church et al., 2002). At Station Aloha's 1000 m reference

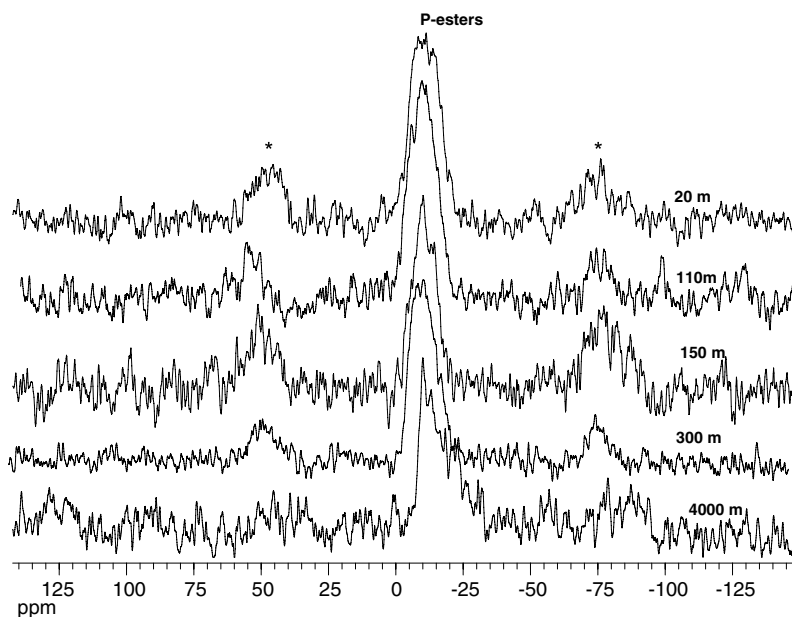


Fig. 3. Solid-state ^{31}P CP-MAS spectra of UPOM from Station Aloha stacked according to depth in the water column. Asterisks denote spinning side bands. Also indicated are the major peaks and the functional groups represented by them. NMR operating parameters: contact time 1 ms; recycle delay 4 s; 32,000 transients.

depth, the estimated 9 year average C:N:P ratio of bulk DOM is 2600:129:1 (Thomson-Bulldis and Karl, 1998). The C:N:P ratios of bulk DOM are, in general, higher than those for UDOM, which represents the high molecular weight (>1000 Da) fraction (Thomson-Bulldis and Karl, 1998; Kolowitz et al., 2001; Church et al., 2002; Hopkinson and Vallino, 2005).

One possible explanation for the difference between our UDOM C:N:P ratios and those reported for bulk DOM above is that the low molecular weight (LMW; <1000 Da) fraction of DOM, which is not recovered during ultrafiltration, may be highly depleted in P. Extreme P depletion in the LMW fraction would result in high overall C:N:P values for bulk DOM. Another explanation may be related to the potential for large errors in sub-euphotic zone DOP measurements. As mentioned previously, DOP concentrations are most commonly estimated as the difference between TDP and DIP measurements (Karl and Björkman, 2002). Because DOP concentration is typically less than 10% of TDP concentration in the sub-euphotic zone, determining DOP as the difference between two relatively large values (TDP and DIP) can lead to large errors in calculated DOP values and C:P ratios (Aminot and Kerouel, 2004; Karl and Björkman, 2002). In contrast, organic P concentrations in UDOM and UPOM are measured directly and the problems inherent in determining concentrations by difference can be avoided.

DOP measurements and associated C:P ratios in the deep ocean show no consistent pattern which is likely a reflection of measurement difficulties. Reported deep water DOP concentrations (below 800 m) range from 10 nM in the middle Atlantic Bight (Hopkinson et al., 2001) to 170 nM in the

Southern Ocean (Loh and Bauer, 2000). The variations in reported values of C:P ratios of DOM are even greater. While C:P ratios as high as 3100:1, 4400:1 and 4768:1 have been reported from the N-E Atlantic Ocean, middle Atlantic Bight and the north Pacific Ocean, respectively (Aminot and Kerouel, 2004; Hopkinson et al., 2001; Hopkinson and Vallino, 2005), such high values were not observed in detailed depth profiles from the eastern North Pacific or Southern Ocean (Loh and Bauer, 2000). In contrast to the extremely high C:P values mentioned above, the highest deep water DOM C:P ratio reported by Loh and Bauer (2000) was 688 in the Southern Ocean.

The average C:P and N:P values of UPOM were closer to Redfield stoichiometry than UDOM, suggesting a planktonic source. Similar results from other locations in the Pacific Ocean have been reported by Kolowitz et al. (2001). A substantial contribution of terrestrial OM to UPOM at Station Aloha has been inferred from $\delta^{13}\text{C}$ and lignin measurements (Benner et al., 1997; Hernes and Benner, 2002; Sannigrahi et al., 2005). Depending on the type of vegetation, such as plants with soft tissues vs. woody tissues, terrestrial OM shows a wide range of C:P and N:P values. In general, terrestrial plants are relatively poor in P and N with C:P ratios ranging from 300 to 1300 and N:P from 10 to 100 for soft tissues (Ruttenberg and Goni, 1997). C:P ratios for woody tissues can be greater than 1300, while their N:P values range from 100 to 1000 (Ruttenberg and Goni, 1997). Given the large range in C:P and N:P ratios of terrestrial materials, it is not clear if this terrestrial component of UPOM would be reflected in the observed C:P and N:P values.

5.2. P composition and cycling of UDOM and UPOM

P esters are the dominant P functional group observed in the solid-state ^{31}P NMR spectra of UDOM as well as UPOM (Figs. 2 and 3). Phosphate monoesters are present in biomolecules such as mononucleotides, sugar phosphates and inositol phosphate, whereas diesters occur in phospholipids and nucleic acids (Paytan et al., 2003). The abundance of P esters in natural organic matter is undoubtedly related to the synthesis of these compounds by all living organisms and they are typically the most abundant compound class seen in studies from both marine and terrestrial environments. Phosphonates occur widely among biogenic and anthropogenic compounds in the form of phosphonolipids and as side groups on polysaccharides and glycoproteins. The strong covalent C–P bonds in phosphonates make them resistant to chemical, thermal and photolytic degradation (Ternan et al., 1998; Kononova and Nesmeyanova, 2002). Though their presence in marine DOP had been proposed earlier (Kittredge et al., 1969; Cembella and Antia, 1986) phosphonates have only recently been recognized as a significant component of marine UDOM (Clark et al., 1998, 1999; Kolowith et al., 2001). ^{31}P NMR results from sinking particles and sediments from the Cariaco Basin show up to 23% phosphonates in sediments and up to 18% in sinking particulate material (Benitez-Nelson et al., 2004). Solution NMR spectra of sinking particulate material have shown phosphonates to comprise a much smaller percentage of the P (up to 6%) in sinking POM (Paytan et al., 2003). ^{31}P NMR analyses of UDOM and UPOM isolated from laboratory cultures of several marine primary producers detected P-esters as the only organic P compound (Clark et al., 1999). Phosphonates were also not detected in ^{31}P NMR spectra of bulk plankton tow samples from the coastal Pacific Ocean and Cariaco basin (Paytan et al., 2003; Benitez-Nelson et al., 2004). The sources of phosphonates in the ocean are yet to be ascertained and the dynamics by which they become a major fraction of UDOM is not known. The widespread distribution of phosphonates in UDOM seen here and over a range of depths, latitude and longitudes in the Pacific and Atlantic Oceans (Kolowith et al., 2001) suggests that phosphonates originate from a widely distributed group of marine organisms such as bacteria.

Dyhrman et al. (2006) studied the utilization of phosphonates by the marine diazotroph *Trichodesmium*. In a detailed study, using data from genome sequences, laboratory cultures and field samples from the North Atlantic Ocean, they showed that *Trichodesmium* has the capacity to utilize phosphonates via the C-P lyase pathway. The C-P lyase encoding genes *phnD* and *phnJ* were expressed by laboratory cultures as well as field samples of *T. erythraeum*. The ability to utilize phosphonates via the C-P lyase mechanism would enable *Trichodesmium* to hydrolyze a wide range of phosphonate compounds. This broad substrate specificity is distinct from that mediated by phosphonate enzymes and thus represents an apparently unique

niche adaptation that could explain the abundance of *Trichodesmium* in low DIP systems such as the NPSG and the Sargasso Sea (Dyhrman et al., 2006). Further, the apparent absence of the *phn* genes in the other marine cyanobacterial genomes (including the diazotroph *C. watsonii*), suggests that *Trichodesmium* could have a competitive advantage with respect to DOP utilization in the many oligotrophic regimes where they co-exist.

There are clear differences in the ^{31}P NMR spectra (Figs. 2 and 3) and C:N:P ratios (Table 1) for UDOM and UPOM from Station Aloha. A ^{13}C NMR study of the same materials also revealed clear differences with respect to carbon in the overall composition of UDOM and UPOM (Sannigrahi et al., 2005). Solid-state ^{13}C NMR spectroscopy indicated that at all depths, UPOM had higher amino acid and lower carbohydrate contents compared to UDOM. The differences in UDOM and UPOM composition with respect to both C and P suggest that UDOM does not originate from simple solubilization of UPOM or alternatively UDOM does not aggregate to form UPOM.

Depth-related compositional variations of UPOM and UDOM are quite distinct for C and P. Both UDOM and UPOM show little compositional variation with respect to organic P with depth (Figs. 2 and 3). In contrast, solid-state ^{13}C NMR and molecular level analyses indicate clear compositional variations in organic C with depth (Sannigrahi et al., 2005). The percentages of carbohydrate-C in UDOM and UPOM decreased with increasing depth, whereas the percentages of lipid-C increased with depth. The percentage of amino acid-C in UDOM remained fairly constant with depth but decreased in UPOM. These depth-related compositional trends were also reflected in the molecular-level carbohydrate and amino acid analyses. Decreases in the relative abundance of carbohydrates relative to other compound classes with depth are indicative of selective degradation processes.

The selective decomposition of certain organic carbon compound classes with depth in the ocean may be indirectly related to the abundance of DIP in the deep ocean. In the deep ocean, growth of heterotrophic organisms is likely limited by the availability of suitable carbon substrates rather than nutrients such as P. Hence, there is selective remineralization of certain carbon compounds like carbohydrates, which are presumably better substrates for heterotrophic microorganisms. This idea is supported by isotopic studies of oxygen ($\delta^{18}\text{O}$) associated with DIP at Station Aloha (Colman et al., 2005). Profiles of these oxygen isotopic ratios with depth suggest that DIP remineralization in the deep ocean is incidental to the microbial consumption of OM as an energy and C source, rather than to fulfill cellular DIP requirements.

5.3. Association of P with biomolecules in UDOM and UPOM at Station Aloha

In order to explore the biomolecular origins of P, relationships between the concentrations of C in carbohy-

drates, lipids and amino acids (estimated using ^{13}C NMR spectroscopy, Sannigrahi et al., 2005) and P concentrations in UDOM and UPOM were examined. Results of linear regression analyses show a strong positive correlation ($R^2 > 0.87$) between P concentrations and carbohydrate-C and amino acid-C concentrations in UDOM (Fig. 4). These analyses indicate that a large fraction of the P in UDOM is associated with carbohydrates and amino acids. As seen from the ^{31}P NMR results above, P-esters and phosphonates are the two organic P functional groups present in UDOM. The association of P with carbohydrates is not unexpected given that they constitute a major fraction of the C in UDOM (Benner et al., 1992; Aluwihare et al., 1997; Sannigrahi et al., 2005) and P-esters can be associated with carbohydrates in a variety of biomolecules such as nucleic acids (DNA and RNA), nucleotides (e.g., ATP) and sugar phosphates. The strong relationship between amino acid-C and P in UDOM is however more intriguing. The association of P with amino acids may be in the form of a class of biomolecules known as phosphoamino acids.

At Station Aloha, P in dissolved nucleic acids (DNA plus RNA) was estimated to account for 10–12% of the DOP in the euphotic zone (Karl and Bailiff, 1989; Karl and Björkman, 2002; Björkman and Karl, 2005). P is in the form of diesters in DNA and RNA. RNA is more abundant than DNA in organisms and in the dissolved

as well as particulate fractions at Station Aloha, with RNA to DNA ratios ranging from 3 to 10 in the dissolved fraction (Karl and Bailiff, 1989). Thus, a fraction of the P in UDOM is likely associated with carbohydrates (ribose and deoxyribose) in RNA and to a lesser extent DNA. The relative contributions of the other P bearing carbohydrate biomolecules to DOP have not yet been ascertained.

Phosphoamino acids are formed by the addition of phosphate groups to amino acids via a P-ester linkage by phosphate donors such as ATP. This modification of amino acids is carried out by the protein kinase group of enzymes and is a ubiquitous regulatory mechanism in prokaryotes as well as eukaryotes (Hong et al., 2003). Intracellular phosphorylation of amino acids by protein kinases to form phosphoproteins provides a mechanism for the cell to switch on or off many processes including cell signaling, growth, metabolic pathways, membrane transport and gene transcription (Hong et al., 2003). In bacteria, the amino acids histidine, aspartic acid and glutamic acid, are usually favored for phosphorylation (Yan et al., 1998). It is possible that the P associated with amino acids is also linked with carbohydrates via the phosphoenolpyruvate:sugar phosphotransferase system (PTS). PTS is an energy driven transport system for hexose sugars, which involves phosphorylation of proteins and ultimately the hexose sugar. Its presence has been detected in marine bac-

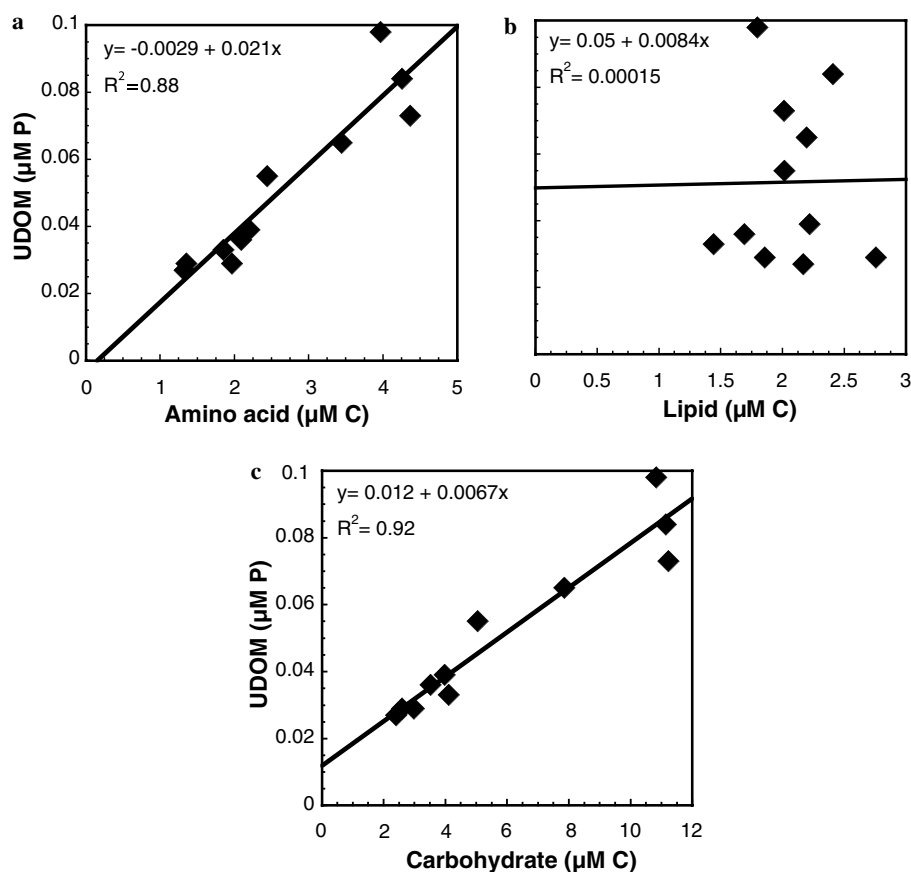


Fig. 4. P contents of UDOM (µM) plotted against the concentration of C (µM) present in amino acids; lipids and carbohydrates. Biomolecule contents were obtained from ^{13}C NMR results (Sannigrahi et al., 2005).

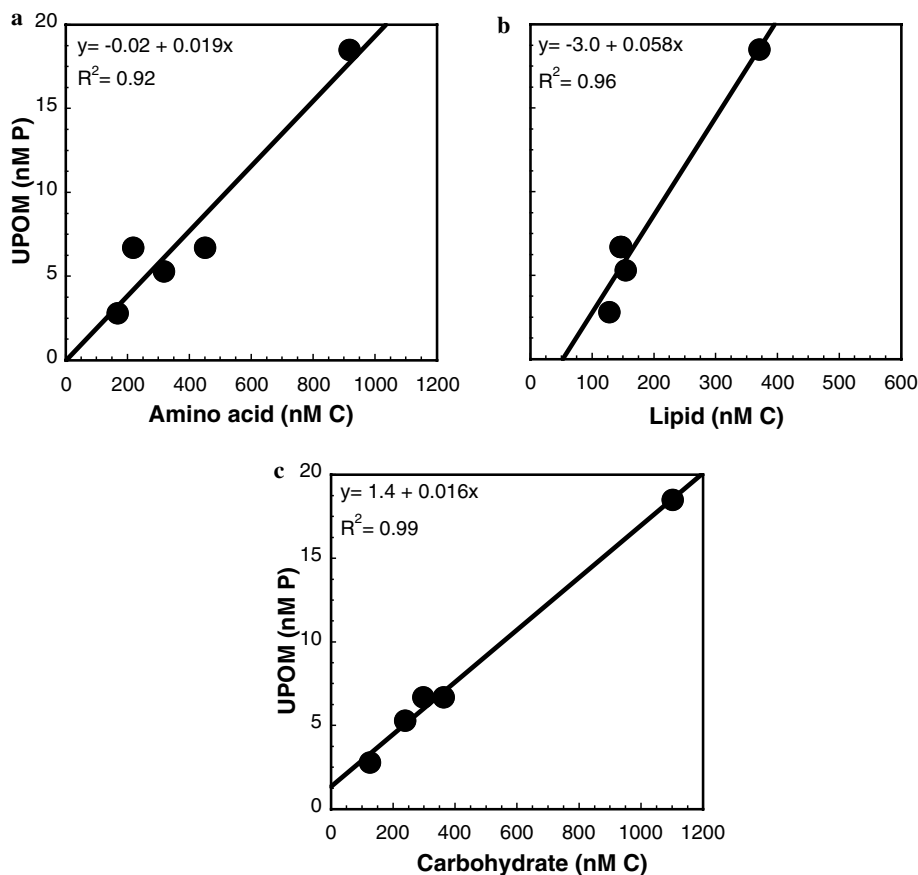


Fig. 5. P contents of UPOM (nM) plotted against the concentration of C (nM) present in amino acids; lipids and carbohydrates. Biomolecule contents were obtained from ^{13}C NMR results (Sannigrahi et al., 2005).

teria (Hodson and Azam, 1979). The coordination and regulation of cellular metabolism are key ecological processes and large portions of the bacterial and archaeal genomes appear to be used for regulation. Therefore it is possible that regulatory compounds such as phosphoamino acids are constantly produced and used by marine microorganisms and these compounds could, in theory, be selectively retained in the DOM pool following exudation or cell death (Karl and Björkman, 2002).

Phosphonates, the other P functional group in UDOM, can also be associated with carbohydrates and amino acids in the form of side groups on glycoproteins and polysaccharides (White and Metcalf, 2004), but little information is available on the composition and abundance of these compounds in the marine environment. P concentrations in UDOM do not exhibit a significant correlation with lipid C (Fig. 4). The lack of a strong relationship between lipid-C and P in UDOM suggests phospholipids and phosphonolipids are minor components of UDOM.

All three biomolecule classes in UPOM show strong relationships ($R^2 > 0.92$) with P concentrations in UPOM (Fig. 5). The dataset is smaller for UPOM, because the low C and P contents of certain samples did not allow NMR spectra to be acquired in a reasonable amount of time. However, the data available are representative of the entire range of sampling depths. The main distinction between UDOM

and UPOM in terms of biomolecule versus P correlations is the additional association of P with lipids in UPOM. P associated with lipid C can be present in the form of phospholipids, which are widely present in *Bacteria* and *Eukarya* and are important structural components of biological membranes (Karl and Björkman, 2002). P-esters in UPOM can also be associated with lipopolysaccharides, which are composed of amino sugars and fatty acids, and are components of the cell wall of Gram-negative bacteria. The presence of lipopolysaccharides relates to the correlation observed between P and lipids as well as carbohydrates. The strong positive relationship between P and lipid-C concentrations in UPOM corroborates our earlier observation that UDOM and UPOM are compositionally distinct.

6. Conclusions

Results from ^{31}P NMR analyses together with atomic C:N:P ratios clearly indicated differences in the bulk chemical compositions of UDOM and UPOM at Station Aloha. These results also suggested that aggregation of UDOM does not result in significant production of UPOM and conversely UDOM is not produced simply by the solubilization of UPOM. UDOM is composed of P-esters (75%) and phosphonates (25%), whereas UPOM is composed predominantly of P-esters. Regression analyses indicated

a large fraction of the P in UDOM is associated with carbohydrates and amino acids, but not with lipids. Similar analyses for UPOM indicated that P is associated with carbohydrates, amino acids and lipids. P in UDOM and UPOM is likely present as altered biopolymers of RNA, DNA, sugar phosphates and phosphoamino acids. P is also likely present as phospholipids and lipopolysaccharides in UPOM.

7. Future recommendations

Many unanswered questions regarding the marine organic P pool will continue to keep ocean scientists involved in studying the biogeochemical cycling of P, in particular its coupling with carbon and nitrogen. Several avenues worth exploring in future research efforts are identified below:

- (1) Identifying the source of phosphonates to marine DOP,
- (2) Improved methods for DOP measurements especially for low DOP samples and inter-laboratory comparisons of existing methods,
- (3) Application of advanced spectroscopic techniques such as two-dimensional ^{13}C - ^{31}P NMR to discern the connectivities between C and P functional groups,
- (4) Characterization of the low-molecular weight (<1000 Da) fraction of DOP and studying the biomolecular associations of C and P in this fraction.

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