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LETTER

The nature of organic phosphorus in marine sediments: New insights from ^{31}P NMR

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Abstract—Different classes of organic phosphorus compounds have been identified in marine sediment samples taken at depth in cores from pelagic, lower slope, and continental shelf depositional environments using solution and solid-state magic angle spinning (MAS) ^{31}P nuclear magnetic resonance (NMR) techniques. Phosphate esters have been identified as the major form of organic phosphorus in all sediment extracts and in separated bulk organic matter. Phosphonates, a form of organic P previously unreported in marine sediments, were identified in all but one of the samples. The persistence of both phosphate esters and phosphonates at depth in sediment cores suggests that these compound classes include undegradable forms of organic phosphorus that represent an important sink for phosphorus in the ocean.

INTRODUCTION

BECAUSE OF THE IMPORTANCE of the nutrient element phosphorus in marine biological cycles, much effort has been made in recent years to quantify the sources and sinks of phosphorus in the ocean (e.g., MEYBECK, 1982; FROELICH et al., 1982; RUTTENBERG, 1990). One quantitatively important sink is the burial of organic phosphorus in marine sediments (FROELICH et al., 1982; INGALL and VAN CAPPELLEN, 1990). It has been proposed that the burial of organic phosphorus compounds resistant to diagenetic breakdown (refractory organic P) may play a significant role in the overall marine phosphorus budget (FROELICH et al., 1982; INGALL and VAN CAPPELLEN, 1990). Despite the importance of organic phosphorus burial, little is known about the speciation of organic phosphorus compounds in marine sediments. As a direct method of characterizing organic phosphorus in sediment organic matter, both solution and solid-state magic angle spinning (MAS) ^{31}P nuclear magnetic resonance (NMR) techniques were employed.

In a typical down-core profile, organic phosphorus concentrations in marine sediments decrease rapidly in the first few cm and then approach a constant value. The rapid decrease in organic phosphorus concentration reflects the bacterial breakdown and release of phosphorus from the most labile fractions of organic matter (KROM and BERNER, 1981). It has been proposed that during further bacterial breakdown, differential rates of organic carbon and phosphorus release lead to the formation of a phosphorus-enriched residual organic matter made up entirely of refractory (i.e., less degradable) phases (INGALL and VAN CAPPELLEN, 1990). In order to characterize organic phosphorus in marine sediments and to look for potentially refractory phosphorus phases, samples were taken at depth in cores from a wide range of marine depositional environments (Table 1). Freeze-dried phytoplankton also was analyzed in order to characterize one of the many possible organic inputs to the sediments.

METHODS

Table 1 includes sample descriptions and gives references containing additional site information. All samples were pre-treated with a citrate-dithionite-bicarbonate (CDB) solution buffered to pH 7.6 (RUTTENBERG, 1990) to remove paramagnetic iron phases which have been shown to interfere strongly with the collection of NMR spectra (VASSALLO et al., 1987). This treatment is also effective in removing inorganic iron-phosphate phases. Samples were prepared for solution NMR by placing approximately 30 g of dry sediment in 200 ml of 0.5 N NaOH and stirring for 24 h. This treatment extracted 13–44% of the organic phosphorus and 22–42% of the organic carbon from the sediments (Table 1). The resulting solution was centrifuged, to remove suspended solids > 0.2 μm, and then concentrated by evaporation to a volume of approximately 10 ml.

Approximately 5 g of dry sediment were used for solid-state MAS NMR experiments. These sediments were first treated with CDB, washed twice with 1 N HCl, and then repeatedly washed with 20% HF in 1 N HCl. Before decanting the wash solution after each step, samples were centrifuged to remove suspended solids < 0.2 μm from solution. This method dissolved most mineral phases, including inorganic phosphorus compounds, and concentrated the organic matter. Solution samples were analyzed at 11.7 T using a Bruker NMR spectrometer. Solid samples were analyzed at 7 T using a home-built spectrometer equipped with a Doty Scientific high-speed spinning probe. Both NMR spectrometers are housed at the Yale Department of Chemistry.

RESULTS AND DISCUSSION

Samples were chosen to reflect a wide range of marine depositional environments in terms of sedimentation rate. This variable has been shown to be key in terms of sedimentary organic matter preservation (INGALL and VAN CAPPELLEN, 1990; CANFIELD 1989). Depositional age (Table 1), calculated using the sedimentation rate and the depth of the sampled core interval, provides a maximum indication of how long organic matter in a given sediment has been exposed to near-surface diagenetic processing. Samples also cover a wide range of organic carbon and organic phosphorus concentrations and extrabilities (Table 1).

Table 1. Sample information.

Site Name & Description	Location & Core Interval (cm)	Depositional Age (years)[5]	Water Depth (m)	Organic C[6] %	Organic P[7] μmol/g	% of Organic C extracted	% of Organic P extracted
Carmen Basin[1]	Gulf of California 26°28.3'N 110°00.0'W (40-50)	225	527	3.5	9.38	42	35
DOMES[2] Site C 57-58	N. Equatorial Pacific 15°9.5'N 125°54.4'W (7-9)	57,000	4638	0.3	4.33	26	13
SEEP A[3] 83 Gyre 9 Sta. 1	Continental Shelf Mid Atlantic Bight 40°28.1'N 70°54.1'W (15.5-17.5)	19	80	2.9	16.1	22	44
SEEP C[3] 83 Gyre 9 Sta 5	Continental Slope Mid Atlantic Bight 39°10.9'N 70°42.9'W (13.5-15.5)	97	2700	1.1	5.3	40	22
Walvis Bay[4] AII-93/3-15	Namibian Shelf 22°36.0'S 14°07.0 E (40-48)	42	106	4.8		23	

Additional site information: [1]DEMASTER and TUREKIAN (1987); [2]COCHRAN and KRISHNASWAMI (1980); [3]ANDERSON ET AL. (1988); [4]DEMASTER (1979).

[5]Depositional ages calculated using mid-depth of analyzed core interval divided by the reported bulk sedimentation rate.

[6]Determined using the method described in KROM and BERNER (1983).

[7]The method used to determine organic phosphorus concentrations (ASPILA ET AL., 1976) cannot be applied to sediments with high concentrations of inorganic phosphorus such as those from Walvis Bay.

Solution and solid-state NMR spectra are presented in Figs. 1, and 2 respectively. Chemical shifts are reported in ppm using 85% phosphoric acid as the standard. Several things should be noted regarding the interpretation of the spectra. First, peak areas are dependent both on abundance and coupling effects on phosphorus spin systems in the different molecular groups. Since coupling effects were not determined as part of this study, quantitative estimates of various phosphorus species were not attempted. Caution should be applied in evaluating relative peak areas in a given spectrum. Second, the base extracts used in solution NMR experiments only represent a portion of the organic phosphorus (13–44%, Table 1) contained in a sediment; hence, some organic phosphorus phases are potentially missed. Finally, the severe nature of sample extraction necessary to prepare samples for both solution and solid-state NMR results in the alteration of some

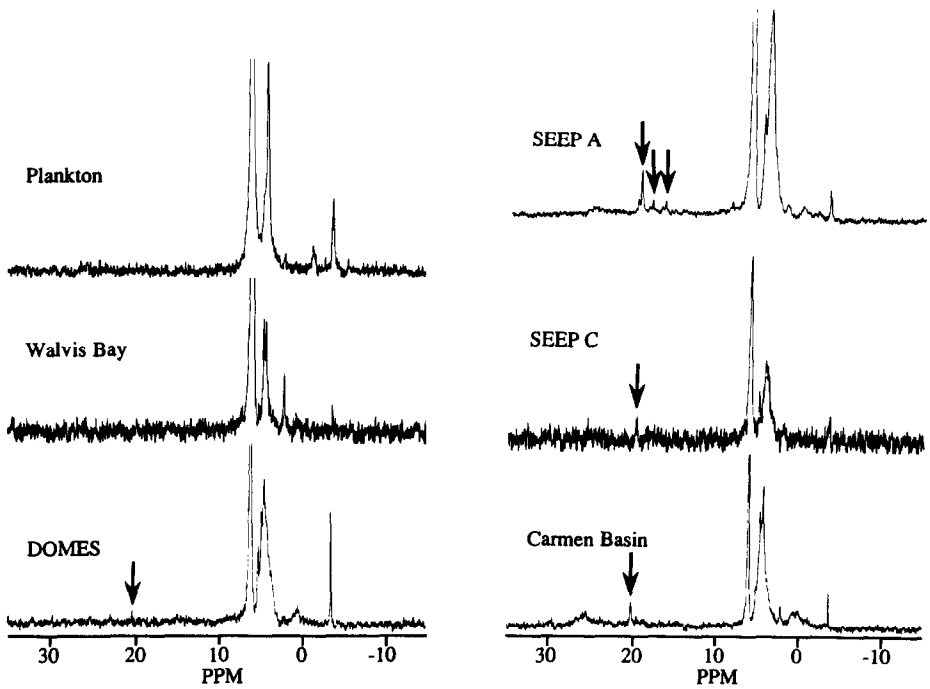

FIG 1. ^{31}P NMR solution spectra for NaOH extracts of marine phytoplankton and sediment samples listed in Table 1. Arrows indicate probable phosphonate peaks. Data collected over 7000–37,000 scans, using a 0.4 sec recycle time, 15 μsec 30° pulse time and 32 K data points.

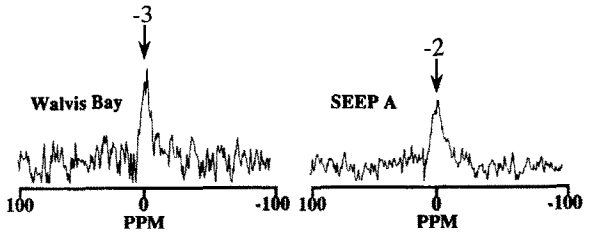

FIG 2. ^{31}P MAS NMR spectra of Walvis Bay and SEEP A organic concentrates at spinning frequencies of 9 and 8 kHz, respectively. Data collected over 750 scans, using a 90 sec recycle time, 4-phased cycled 4 μsec 90° pulse time and 1 K data points. Full width at half maximum is 4 ppm for Walvis Bay and 8 ppm for SEEP A.

organic phosphorus compounds. The nature of these potential extraction effects are considered in the following discussion.

The sharp peak at 6 ppm, highest on all solution spectra (Fig. 1), is characteristic of free inorganic orthophosphate in a strong NaOH solution (HAWKES et al., 1984). This peak probably represents phosphate liberated from mineral and organic phases during base extraction. Spectra for the SEEP A, SEEP C, DOMES, and Carmen Basin extracts, have a small peak at approximately 20 ppm (denoted by the arrows in Fig. 1). In addition, chemical shifts of 18.2 and 16.6 ppm are observed in the spectra of SEEP A. Chemical shifts in this region have been attributed to phosphonic compounds in extracts of soils (HAWKES et al., 1984; TATE and NEWMAN, 1982; NEWMAN and TATE, 1980) and marine organisms (HILDERBRAND, 1983; HENDERSON et al., 1972; GLONEK et al., 1970; QUIN, 1967; KITTREDGE and ROBERTS et al., 1969; KITTREDGE et al., 1969). To our knowledge, these results document for the first time the occurrence of phosphonic compounds in marine sediments.

Phosphonic compounds occur widely in nature (HILDERBRAND, 1983; QUIN, 1967). They have been shown to comprise approximately 3% of the total phosphorus in an undifferentiated sample of marine phytoplankton and microzooplankton (KITTREDGE et al., 1969). The absence of a peak at $\approx$20 ppm in our marine phytoplankton sample probably means that their concentration in the extract was below detection. Phosphonic compounds are unique in that phosphorus is bonded into an organic molecule via a direct carbon-phosphorus (C-P) bond. The chemical stability of the C-P bond makes release of phosphorus from phosphonic compounds difficult even after prolonged acid and base hydrolysis (QUIN, 1967; KITTREDGE and ROBERTS, 1969). It has been shown that while certain marine microorganisms can break down aminophosphonic acids, this process is inhibited in the presence of orthophosphate-containing compounds (ROSENBERG and LA NAUZE, 1967). Preferential decomposition of the orthophosphate-containing organic compounds enriching the sedimentary organic matter in the more resistant phosphonic compounds could explain our observations.

Peaks in the range 5.5 to −4 ppm on the solution and solid-state spectra are characteristic of orthophosphate esters and diesters. It is impossible at the resolution of our method to attribute any given chemical shift to specific phosphorus monoesters or diesters. Naturally occurring orthophosphate monoesters generally appear in the region from 5 to 3.5 ppm in basic solutions (HAWKES et al., 1984; TATE and NEWMAN, 1982; NEWMAN and TATE, 1980; GLONEK et al., 1970). Orthophosphate diesters generally have chemical shifts at lower ppm values relative to monoesters, due to increased shielding of the P nuclei by ester linkages, and could explain the peaks at <3.5 ppm on both solution and solid spectra. One potential candidate for a phosphate monoester that would be potentially stable during early diagenesis would be the inositol phosphates. Inositol phosphates make up a large fraction of the organic phosphorus in soils (STEVENSON, 1986) and have also been shown to occur in marine sediments (WHITE and MILLER, 1976). The chemical shift of inositol hexaphosphate run at the same conditions as the sediment samples consists of a series of closely spaced peaks from 4.8 to 4.1 ppm, approximately the region covered by the largest observed phosphate ester peak in all spectra. Inositol phosphates have been shown to be resistant to acid and base hydrolysis (STEVENSON, 1986; WHITE and MILLER, 1976) and thus are another potential refractory organic phosphorus phase.

Peaks in the <3.5 ppm region both in solution spectra and solid-state NMR spectra for SEEP A and Walvis Bay most likely reflect the presence of phosphate diesters. Of many naturally occurring phosphate diesters, phospholipids are a geologically reasonable candidate. Phospholipids have not been specifically identified in marine sediments, but chemical shifts in the region of −2 ppm have been assigned to phospholipids in base extracts of soils (HAWKES et al., 1984; TATE and NEWMAN, 1982; NEWMAN and TATE, 1980). Interpretation of these peaks as phospholipids would be consistent with organic geochemical studies on SEEP A and Walvis Bay showing that sediment organic matter at these sites contains a high proportion of lipid material (VENKATESAN et al., 1988; DEMASTER, 1979). If present, some of the phospholipids are apparently resistant, during processing for NMR study, to both strong acid (solid-state NMR) and to strong base (solution NMR). Absence of a peak corresponding to orthophosphate in the solid-state spectra, and its presence in the solution spectra, suggests that hydrolysis of some of the ester linkages to orthophosphate occurs during treatment with strong base, as found by others (HAWKES et al., 1984; TATE and NEWMAN, 1982; NEWMAN and TATE, 1980).

CONCLUSIONS

Decomposition of organic matter has been shown to be extensive in pelagic sediments (INGALL and VAN CAPPELLEN, 1990) such as those at the DOMES site. Presence of organic phosphate esters in the DOMES sediment with a depositional age of 57,000 a (Table 1) indicates that some compounds in this class are resistant to long-term diagenesis. In addition, phosphonates, a clearly refractory form of organic phosphorus, were identified in all but one of the sediment samples. The similarity in the spectra of the DOMES sediment and the sediments with depositional ages that are younger by 2 to 3 orders of magnitude suggests that diagenetic release of labile phosphorus phases and the formation of a residual organic matter made up of refractory phosphate esters and phosphonates occur on a geologically rapid time scale.

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REFERENCES

ANDERSON R. F., BOPP R. F., BUESSELER K. O., and BISCAYE P. E. (1988) Mixing of particles and organic constituents in sediments from the continental shelf and slope off Cape Cod: SEEP-I results. *Continental Shelf Res.* **8,** 925–946.

ASPILA K. I., AGEMIAN H., and CHAU A. S. Y. (1976) A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. *Analyst* **101,** 187–197.

CANFIELD D. E. (1989) Sulfate reduction and oxic respiration in marine sediments: Implications for organic carbon preservation in euxinic environments. *Deep-Sea Res.* **36,** 121–138.

COCHRAN J. K. and KRISHNASWAMI S. (1980) Radium, thorium, uranium, and ^{210}Pb in deep-sea sediments and sediment pore waters from the North Equatorial Pacific. *Amer. J. Sci.* **280,** 849–889.

DEMASTER D. J. (1979) The marine budgets of silica and ^{32}Si. PhD. dissertation, Yale University.

DEMASTER D. J. and TUREKIAN K. K. (1987) The radiocarbon record in varved sediments of Carmen Basis, Gulf of California: A measure of upwelling intensity variation during the past several hundred years. *Paleoceanography* **2,** 249–254.

FROELICH P. N., BENDER M. L., LUEDTKE N. A., HEATH G. R., and DEVRIES T. (1982) The marine phosphorus cycle. *Amer. J. Sci.* **282,** 474–511.

GLONEK T., HENDERSON T. O., HILDERBRAND R. L., and MYERS T. C. (1970) Biological phosphonates: Determination by phosphorus-31 nuclear magnetic resonance. *Science* **169,** 192–194.

HAWKES G. E., POWLSON D. S., RANDALL E. W., and TATE K. R. (1984) A ^{31}P nuclear magnetic resonance study of the phosphorus species in alkali extracts of soils from long-term field experiments *J. Soil Sci.* **35,** 35–45.

HENDERSON T. O., GLONEK T., HILDERBRAND R. L., and MYERS T. C. (1972) Phosphorus-31 nuclear magnetic resonance studies of the phosphonate and phosphate composition of the sea anemone, *Bunadosoma,* Sp. *Arch. Biochem. Biophys.* **149,** 484–497.

HILDERBRAND R. L. (1983) *The Role of Phosphonates in Living Systems.* CRC Press.

INGALL E. D. and VAN CAPPELLEN P. (1990) Relation between sedimentation rate and burial of organic phosphorus and organic carbon in marine sediments. *Geochim. Cosmochim. Acta* **54,** 373–386.

KITTREDGE J. S. and ROBERTS E. (1969) A carbon-phosphorus bond in nature. *Science* **164,** 37–42.

KITTREDGE J. S., HORIGUCHI M., and WILLIAMS P. M. (1969) Aminophosphonic acids: Biosynthesis by marine phytoplankton. *Comp. Biochem. Physiol.* **29,** 859–863.

KROM M. D. and BERNER R. A. (1981) The diagenesis of phosphorus in a nearshore marine sediment. *Geochim. Cosmochim. Acta* **45,** 207–216.

KROM M. D. and BERNER R. A. (1983) A rapid method for the determination of organic and carbonate carbon in geochemical samples. *J. Sediment. Petrol.* **53,** 660–663.

MEYBECK M. (1982) Carbon, nitrogen and phosphorus transport by world rivers. *Amer. J. Sci.* **282,** 401–450.

NEWMAN R. H. and TATE K. R. (1980) Soil phosphorus characterization by ^{31}P nuclear magnetic resonance. *Commun. Soil Plant Anal.* **11,** 835–842.

QUIN L. D. (1967) The natural occurrence of compounds with the carbon-phosphorus bond. In *Topics in Phosphorus Chemistry* (eds. M. GRAYSON and E. J. GRIFFITH), Vol. 4, pp. 23–48. J. Wiley & Sons.

ROSENBERG H. and LA NAUZE J. M. (1967) The metabolism of phosphonates by microorganisms. The transport of aminoethylphosphonic acid in *Bacillus Cereus. Biochim. Biophys. Acta* **141,** 79–90.

RUTTENBERG K. C. (1990) Diagenesis and burial of phosphorus in marine sediments. Ph.D. dissertation, Yale University.

STEVENSON F. J. (1986) *Cycles of Soil.* J. Wiley & Sons.

TATE K. R. and NEWMAN R. H. (1982) Phosphorus fractions of a climosequence of soils in New Zealand tussock grassland. *Soil Biol. Biochem.* **14,** 191–196.

VASSALLO A. M., WILSON M. A., COLLIN P. J., OADES J. M., WATERS A. G., and MALCOLM R. L. (1987) Structural analysis of geochemical samples by solid-state nuclear magnetic resonance spectrometry. Role of paramagnetic material. *Anal. Chem.* **59,** 558–562.

VENKATESAN M. I., STEINBERG S., and KAPLAN I. R. (1988) Organic geochemical characterization of sediments from the continental shelf south of New England as an indicator of shelf edge exchange. *Continental Shelf Res.* **8,** 905–924.

WHITE R. H. and MILLER S. J. (1976) Inositol isomers: Occurrence in marine sediments. *Science* **193,** 885–886.