Evaluation of Oxygen-18 Labeled Phosphate as an Environmental Tracer and Biological Marker

Eric Melby, Douglas J. Soldat, and Phillip Barak
Department of Soil Science, University of Wisconsin – Madison

Abstract

The dynamics of nutrient cycling and tracing of many elements can be accomplished by examining the ratio of different stable isotopes, but unfortunately only one stable isotope of P exists. Phosphorus in the environment is typically found in the phosphate form (PO_4), and it has been hypothesized that evaluating the $^{18}O/^{16}O$ may provide information about the sources and cycling of P in the environment. This research focused on the synthesis and detection of phosphate highly enriched in oxygen-18 and its potential use as an environmental tracer and biological marker. It was found that highly enriched oxygen-18 labeled phosphate (OLP) can be synthesized from either $POCl_3$ or PCl_5 in ambient atmosphere with almost no loss of oxygen-18 enrichment. Furthermore, an electrospray ionization mass spectrometer was used to effectively analyze the synthesized OLP, which provided information on each OLP species and not simply an overall $^{18}O/^{16}O$ ratio. Isotopic fractionation was observed in soils as the result of preferential sorption of OLP species most enriched in oxygen-18. Through a soil incubation study it was determined that OLP would likely not be an effective P tracer due to its rapid delabeling in soil. Because delabeling only occurs in the presence of microorganisms, it is believed that OLP could be used as a biological marker. The synthesized oxygen-18 labeled phosphate (OLP) was composed of a collection of distinct species that contain from zero to four oxygen-18 atoms bonded to the phosphorus atom. As phosphate is utilized by organisms, oxygen atoms are removed and later replaced, which results in delabeling of the OLP.

Objectives

1. **Develop a method to synthesize phosphate that is highly enriched with oxygen-18.**
2. **Develop an analytical procedure to quantify the oxygen-18 enrichment of phosphate in biological samples that can distinguish between phosphate containing varying numbers of oxygen-18 atoms**
3. **Evaluate the effectiveness of OLP as an environmental P tracer by determining the rate of biological OLP delabeling and investigating the possibility of isotopic fractionation of different OLP species.**
4. **Investigate the use of OLP as a biological marker by determining the rate of OLP delabeling in the absence of microbial activity by incubation with sterilized and non-sterilized soils.**

Materials & Methods

Synthesis and Detection of Oxygen-18 Labeled Phosphate (OLP):
OLP was synthesized by reacting either $POCl_3$ or PCl_5 with 97 atom % ^{18}O water. A chemical resistant suit, chemical resistant gloves, and a positive pressure self contained breathing apparatus were worn during the handling of these chemicals and during their reaction with water to prevent any contact with these dangerous chemicals and inhalation of hazardous fumes.

Electrospray ionization mass spectrometry (ESI-MS) was used to establish a ratio of each of the species present in the synthesized OLP using an Agilent 1100 LC-MSD SL single-quadrupole model 1946D mass spectrometer. Table 1 lists the liquid chromatography (LC) and mass spectrometer (MS) conditions used for this analysis. The samples were diluted to approximately 60 mg L^{-1} P prior to analysis. A portion of the OLP extract was used for total elemental analysis using an IRIS Advantage inductively coupled plasma optical emission spectrometer (ICP-OES).

Isotopic Fractionation of OLP Species:
Three replicates of various soils (properties in Table 2) were shaken with a solution containing 65.5 mg L^{-1} OLP-P (1:20 soil:water) on an orbital shaker for 24 h at 80 rpm along with a blank containing no soil. After the mixing period, the suspension was centrifuged for 30 min at 2500 x g, and the liquid portion was analyzed by ESI-MS using the conditions listed in Table 1.

OLP Delabeling in Sterilized and Non-sterilized Soils:
Various soils (selected soil properties are listed in Table 2) were incubated with 250 mg kg^{-1} OLP-P. Non-sterilized Batavia silt loam, Dodge silt loam, Grays silt loam, and Tarr sand were used in the incubation, along with sterilized Batavia, Dodge, and Grays silt loams. Soils were sterilized by autoclaving at 121°C in a thin layer three times for a period of 2 h. Soils were stored at 25°C for 48 h between each autoclaving period. Soils were maintained at 80% of field capacity during the incubation period, and the sterilized treatments received 0.5 mL of toluene twice weekly to maintain sterile conditions. Three replicates of each treatment were removed after 3, 10, 30, and 50 d of incubation. The soils were extracted using a modified Bray extractant (0.03 M NH_4F and 0.025 M HNO_3; 1:10 soil:extractant), and the liquid portion was analyzed by ESI-MS using the conditions listed in Table 1.

Table 1. Liquid chromatography and mass spectrometer conditions used for analysis of OLP.

Parameter	Condition
Liquid Chromatography	
Column	Waters IC-Pak Anion HR, 4.6 x 75 mm, 6 µm particle size
Mobile Phase	Isocratic: 25% acetonitrile, 75% 50 mM NH_4HCO_3(pH 10)
Flow Rate	0.5 mL min^{-1}
Column Temp.	30°C
Mass Spectrometry	
Injection Volume	15 µL
Source Voltage	2250 V
Drying Gas	Nitrogen, 350°C, 40 L min^{-1}, 40 psi
Ionization	Electrospray
MS Detection	Single ion recording (SIR) mode

Table 2. Soil properties from soils used for the fractionation and delabeling studies.

Soil Series	Taxonomic name	pH	Sand	Clay	OM	Bray P	Bray K
			g kg^{-1}			mg kg^{-1}	
Batavia silt loam	Fine-silty, mixed, superactive, mesic Mollic Hapludalfs	5.4	200	170	26	42	119
Dodge silt loam	Fine-silty, mixed, superactive, mesic Typic Hapludalfs	5.9	160	190	28	46	144
Grays silt loam	Fine-silty, mixed, superactive, mesic Mollic Oxyaquic Hapludalfs	4.8	240	100	45	21	104
Kewaunee silt loam	Fine, mixed, active, mesic Typic Hapludalf	7.7	390	110	32	46	84
Plano silt loam	Fine-silty, mixed, superactive, mesic Typic Argiudoll	5.9	170	170	42	51	126
Richford loamy sand	Loamy, mixed, superactive, mesic Arenic Hapludalf	6.7	890	10	19	142	142
Tarr sand	Mesic, uncoated Typic Quartzipsamments	5.0	910	50	13	11	15
Withee silt loam	Fine-loamy, mixed, superactive, frigid Aquic Glossudalf	6.9	230	70	27	23	120

Results & Discussion

Synthesis and Detection of Oxygen-18 Labeled Phosphate (OLP)

Synthesis of OLP with both $POCl_3$ and PCl_5 was carried out in two separate synthesis events in December 2008 and May 2010 (Table 3). The *m/z* values shown in these tables represent the various OLP species that were formed during the synthesis reaction. The different species range from phosphate with no oxygen-18 atoms (*m/z* 97) to phosphate with four oxygen-18 atoms (*m/z* 105). These results show that not only can analysis with a mass spectrometer provide information about overall enrichment but it can also provide detailed information about the individual OLP species formed. Middleboe and Saaby Johansen (1992) stated that a loss of about half of the expected oxygen-18 enrichment could be expected when the synthesis was carried out in ambient atmosphere but the results in Table 3 show that there was no loss in overall enrichment as compared to theoretical synthesis results.

The OLP synthesized in December 2008 was analyzed 16 months later in April 2010, and there was no loss of oxygen-18 enrichment during the storage period. This indicates there was no abiotic oxygen exchange between phosphate and water, which is in agreement with Blake et al. (2005) and McLaughlin and Paytan (2007).

Table 3. Results from the synthesis of OLP from $POCl_3$ and PCl_5 during two separate synthesis events.

OLP Species	Amount of Species Present in OLP Synthesized from $POCl_3$			Amount of Species Present in OLP Synthesized from PCl_5		
	Theoretical	Dec. 2008	May 2010	Theoretical	Dec. 2008	May 2010
m/z	%					
97	0.0	0.0	0.0	0.0	0.7	0.0
99	0.3	1.2	3.7	0.0	0.0	0.0
101	8.5	18.2	23.6	0.5	0.0	0.0
103	91.1	67.7	52.6	11.0	9.3	12.2
105	0.2	13.0	20.2	88.5	90.0	87.8
Total ^{18}O %	72.8	73.1	72.3	97.0	97.0	97.0

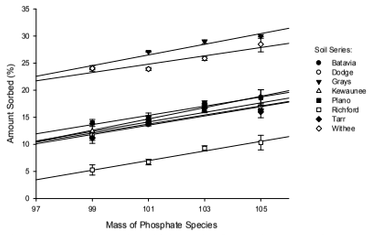

Figure 1. Increase in sorption with increasing OLP species oxygen-18 content.

Table 4. Linear regression analysis of the linear fits for each soil in Figure 1.

Soil Series	Slope	Intercept	R^2
Plano silt loam	0.85 A†	11.95 A	0.94
Kewaunee silt loam	1.04 A	10.51 AB	1.00
Batavia silt loam	0.86 A	10.10 B	0.90
Dodge silt loam	0.88 A	10.60 B	0.88
Tarr sand	0.84 A	10.40 B	0.76
Grays silt loam	0.99 A	22.56 C	0.95
Richford loamy sand	0.89 A	3.46 D	0.98
Withee silt loam	0.77 A	21.73 E	0.86

† Different letters indicate statistically significant differences within a column for each soil ($P < 0.05$).

Isotopic Fractionation

The results of the isotopic fractionation study are shown in Figure 1 and Table 4. There was isotopic fractionation of the different OLP species as the result of preferential sorption of the heavier OLP species. The increase in sorption with increasing mass was the same for all soils tested, as indicated by slopes that were all statistically equal. This isotopic fractionation can be explained by heavier isotopes forming bonds that are more difficult to break (Urey, 1947). The heavier OLP species are more likely to bind to iron or aluminum in the soil through oxygen-18, and as a result these species are held more tightly to the soil. Intercepts were well correlated with the Mechlich-3 phosphorus saturation index; soils with more available phosphorus binding sites had a larger intercept and bound more phosphorus.

Evidence for this sort of isotopic fractionation on a broader environmental scale is provided by McLaughlin et al. (2006). It was found that phosphate in compost contained the largest amount of oxygen-18, followed by soil, and finally a nearby sediment pond. The authors believed this was the result of preferential movement of phosphate depleted in oxygen-18. This study shows that preferential movement of phosphate depleted in oxygen-18 will in fact occur as the result of preferential sorption of phosphate more enriched in oxygen-18.

OLP Delabeling in Sterilized and Non-sterilized Soils

ESI-MS analysis of the modified Bray soil extracts from the non-sterile soil treatments incubated with 250 mg kg^{-1} OLP-P shows exponential decay of the oxygen-18 content of the extracted phosphate (which is synonymous with OLP delabeling) over the 50 d incubation period for the Batavia (R^2 = 0.9946), Dodge (R^2 = 0.9880), Grays (R^2 = 0.9987), and Tarr (R^2 = 0.9694) soils (Figure 2). Based on the exponential decay models, the half life of OLP in the Batavia silt loam, Dodge silt loam, Grays silt loam, and Tarr sand is 19.3, 15.6, 21.7, and 22.4 d, respectively. A parameterized numerical model was used to predict the amount of each OLP species in the various soils, and these numbers were compared to actual values from the soil incubation (Figure 3). This comparison once again highlights the preferential sorption of the heavier OLP species.

In order to confirm that the delabeling of OLP was a function of microbial utilization of OLP as explained above and not some other mechanism, sterile samples of the Batavia, Dodge, and Grays silt loams were also incubated with 250 mg kg^{-1} OLP-P. Analysis of the phosphate extracted from these samples shows that there was no OLP delabeling over the 50-d incubation period in all soils sterilized through autoclaving and toluene treatments (Figure 4), as the slopes of the linear regression fit lines were not significantly different from zero for the Batavia ($P = 0.295$), Dodge ($P = 0.827$), and Grays (0.460) silt loams. These results confirm that delabeling of OLP in non-sterile soils is exclusively the result of microbial utilization of OLP, and as a result OLP may have potential for use as a microbial biomarker.

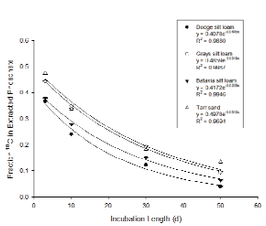

Figure 2. Oxygen-18 content of extracted phosphate over the 50 day incubation period in non-sterilized soil.

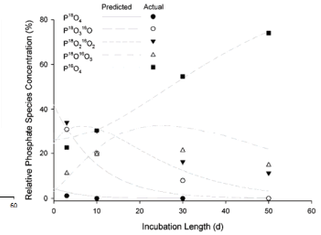

Figure 3. Model predicted and actual values for the amount of each OLP species present during the soil incubation study for the Batavia silt loam.

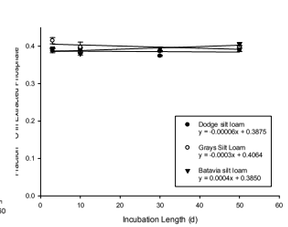

Figure 4. Oxygen-18 content of extracted phosphate over the 50 day incubation period in sterilized soil.

Conclusions

1. **Highly enriched oxygen-18 labeled phosphate (OLP) can be synthesized in ambient atmosphere with almost no loss of enrichment.**
2. **An electrospray ionization mass spectrometer can be used to effectively quantify not only the total phosphate oxygen-18 enrichment but also the amount of each OLP species.**
3. **Isotopic fractionation of the various OLP species occurred as a result of preferential sorption of the heavier OLP species to the soil surfaces.**
4. **OLP is not likely useful as an environmental tracer due to the rapid delabeling of OLP in all soils (half lives 15.6 – 22.4 d), but has the potential to be used as a microbial biomarker as delabeling only occurred in the presence of microorganisms.**

References

Blake, R.E., J.R. O'Neil, and A.V. Surkov. 2005. Biogeochemical cycling of phosphorus: Insights from oxygen isotope effects of photoenzymes. Am. J. Sci. 305:596-620.

McLaughlin, K., B.J. Cade-Menun, and A. Paytan. 2006. The oxygen isotopic composition of phosphate in Elkhorn Slough, California: a tracer for phosphate sources. Estuar. Coast. Shelf S. 70:499-506.

McLaughlin, K., and A. Paytan. 2007. The oceanic phosphorus cycle. Chem. Rev. 107:563-576.

Middleboe, V., and H. Saaby Johansen. 1992. Facile oxygen-18 labelling of phosphate and its delabelling under various conditions. Appl. Radiat. Isot. 43: 1167-1168.

Urey, H.C. 1947. The thermodynamic properties of isotopic substances. J. Chem. Soc. Part 1:562-581.