

Surface Adsorption and Enzymatic Hydrolysis of Polyphosphates: Implications for Understanding Phosphorus Cycling

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Abstract:

Phosphorus (P) is a key, yet often limited, nutrient in aquatic environments. Among various species of P, polyphosphate (polyP) constitutes a significant portion of total P in aquatic environments [1]. PolyP is a polymer of phosphate ions joined by phosphoanhydride (P-O-P) bonds of variable chain length ranging from a few to thousands of phosphate units. It is mainly synthesized by bacteria and plankton. Synthesis of polyP by bacteria is exploited in enhanced biological phosphorus removal (EBPR), a process used to remove excess phosphorus during sewage water treatment. Despite its environmental significance, little is known of the transport, transformation, and fate of polyP.

The goal of this project was to characterize the chemical reactions that may control the transport and stability of polyP in order to understand the larger scale P cycling. Adsorption of polyP onto iron and aluminum oxides was studied using quartz crystal microbalance with dissipation (QCM-D). PolyP hydrolysis catalyzed by alkaline phosphatase was studied by monitoring orthophosphate (orthoP) formation using UV-Vis spectroscopy. It was observed that polyphosphate can be degraded through enzyme-catalyzed hydrolysis with the rate and extent dependent on chain length. In the presence of calcium, precipitation of calcium-phosphate (Ca-P) solid phase(s) was monitored by dynamic light scattering, and the precipitates were characterized using scanning electron microscopy with energy dispersive spectroscopy (SEM-EDX), and Fourier transform infrared spectroscopy (FT-IR). FT-IR spectra and elemental analysis from EDX suggested the precipitate to be a non-apatite phase, likely a type of amorphous Ca-P.

Experimental Procedures:

Total Dissolved Phosphate Assay. Determination of total phosphate in solution was done according to the colorimetric protocol developed by Murphy and Riley [2]. This method was used to measure the free orthoP in the adsorption and hydrolysis studies.

QCM-D. Samples of polyP (50 mg/L, 15 and 60 phosphate units) were combined with sodium chloride solution (0.1 M, pH 6). Solutions were flowed into the modules of the Q-Sense E4 QCM-D containing sensors coated with iron oxide, Fe₂O₃, or aluminum oxide, Al₂O₃.

Enzymatic Hydrolysis. Alkaline phosphatase (0.5 units/mL) was prepared in Tris buffer (0.1M, pH 9) containing MgCl₂ (0.005 M). Enzyme solution (9 mL) was combined with polyP (15, 60, and 130P) stock solution to reach a final orthoP concentration of 980 μM. In parallel, CaCl₂ (0.5 M, 0.2 mL) was added to separate polyP-enzyme solutions (15 and 130P) to reach a concentration of 10 mM Ca²⁺. All samples were incubated at 37°C.

Characterization Studies. Nucleation of precipitates during hydrolysis of polyP with calcium was monitored via

dynamic light scattering (DLS) using the Malvern nano-ZS zetasizer. Precipitates were characterized alongside amorphous and crystalline hydroxylapatite (HAP), a model Ca-P species, using the LEO1530 SEM-EDX and ThermoScientific Nicolet iS50 FT-IR ATR with diamond crystal (2 cm step, 64 scans per read).

Results:

Transport. QCM-D results showed the real-time adsorption of 15 and 60P polyP onto Fe₂O₃ and Al₂O₃ (Figure 1). It was observed that polyP is capable of adsorbing onto these naturally abundant mineral species, and due to the fairly uniform adsorbed masses despite the different chain lengths, we hypothesized that the polyP adsorbs on its side instead of terminally. The low loss of mass after the reintroduction of a background solution around 2250 seconds also suggests that the adsorption was irreversible.

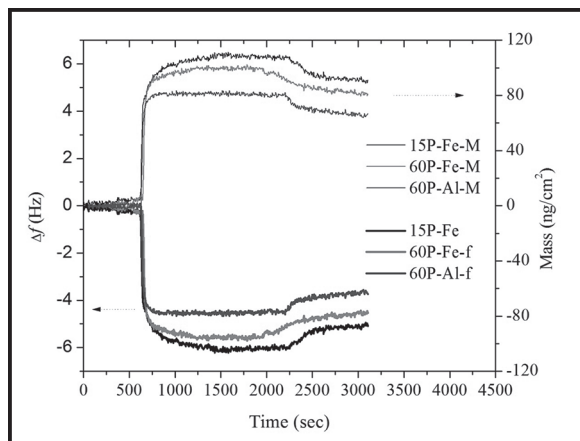


Figure 1: QCM-D monitoring of the adsorption of polyP onto amorphous Al_2O_3 and Fe_2O_3 .

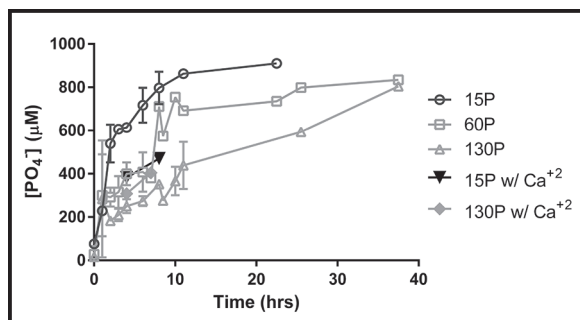


Figure 2: PO_4 produced from APase catalyzed hydrolysis of polyP for varying chain lengths with or without calcium.

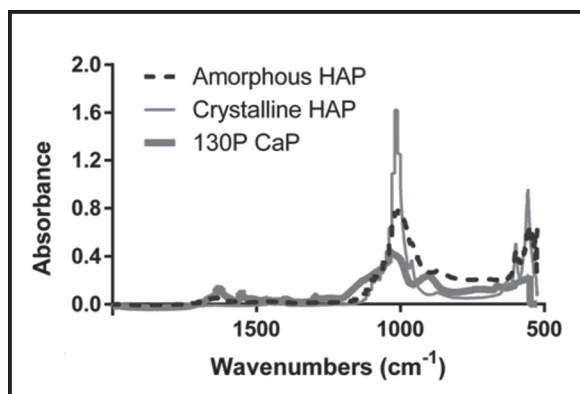


Figure 3: FT-IR spectra of Ca-P and HAP species.

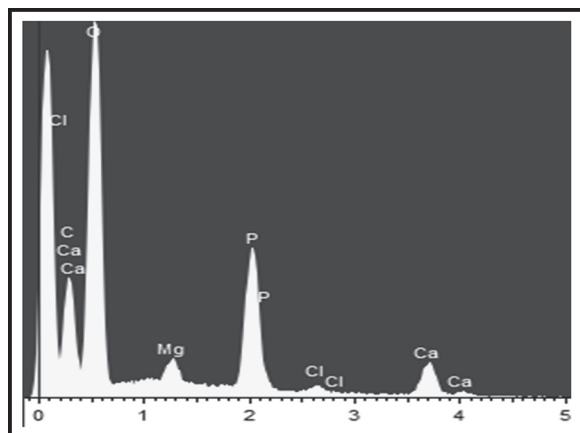


Figure 4: EDX spectrum of Ca-P precipitate.

Transformation. Figure 2 shows orthoP formed over time from different polyP with and without the presence of calcium. Incubation with APase resulted in a faster rate and greater extent of degradation for the shorter polyP compared to that of longer polyP. We believe the hydrolysis was via the terminals of the polyP and followed a consecutive first-order kinetic model. This can be modeled using the equation $[\text{orthoP}] = [\text{orthoP}]_0 (1 - \exp(-k_1 t))$ [3]. The experimental parameters for hydrolysis of 15, 60, and 130P were 0.2204, 0.1049, and 0.05, respectively.

Mineralization. Precipitation of a possible Ca-P mineral was observed during the hydrolysis of all polyP lengths in the presence of calcium. FT-IR spectra of the observed Ca-P and both amorphous and crystalline HAP is shown in Figure 3. Based on the vibrational mode peaks, we can confirm that the precipitate was some type of Ca-P species. EDX elemental analysis is shown in Figure 4. The observed Ca/P ratio is around 1.0, nearly fitting into the amorphous Ca-P ratio of 1.2 - 2.2. The ratio observed in crystalline HAP is 1.67 [4]. Based on these results, the observed Ca-P was most likely some type of amorphous intermediate phase.

Conclusions and Future Work:

PolyP was found to irreversibly adsorb onto Fe_2O_3 and Al_2O_3 , and was hydrolyzed with a rate and extent dependent on chain length. In the presence of calcium and APase, Ca-P precipitates formed. Future work is needed to characterize the effects of more environmentally relevant conditions and different solution chemistries on the behavior of polyP as well as other potential mineralization pathways.

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