UV-RECYCLING OF PHOSPHORUS IN THE ARCHEAN EON

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A thesis submitted to the

School of Graduate Studies

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Master of Science

Graduate Program in Department of Earth and Planetary Sciences

Written under the direction of

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New Brunswick, New Jersey

May 2021
ABSTRACT OF THE THESIS

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Marine productivity relies on a constant flux of essential nutrients in order to fix CO$_2$ and produce O$_2$. Phosphorous, is considered to be a primary limiting nutrient for life. The element is supplied via the continental weathering of silicate rocks - a process which is thought to have been limited by reduced continental exposure during the Archean Eon (4 -2.5 Ga). A lack of consistent P supply would have heavily constrained productivity and the development of the nascent biosphere without recycling. Here, we show that under putative Archean conditions (anoxic, Fe$^{2+}$ rich), UV light quickly degrades biologically ubiquitous organic phosphates (adenosine monophosphate, phosphatidylserine, methyl phosphonate) and thus provides a mechanism for phosphate recycling on the early Earth. We propose that this process promoted stable surface water productivity, bridging the two-billion-year gap between an anoxic and oxygenic phosphate cycle.
Dedication

For Fifo, a fine feline and friend.
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Introduction

Life is limited by nutrient availability. Of the six essential nutrients required for life (CHONPS), phosphorus (P) is the least abundant and potentially limits primary production on geologic timescales\(^1,2\). Its presence is fundamental to the synthesis of the biomolecules that power, code for, and structure life as we know it (ATP, RNA, phospholipids)\(^3\).

Unlike nitrogen, the flux of phosphorus is constrained by the rate of weathering of continental rocks, which can be fixed directly from the atmosphere\(^1,4\). The weathering of phosphate-rich minerals like apatite liberates phosphorous from the continents and delivers a flux to the oceans ranging from 21 - 49 x 10\(^{10}\) mol/year\(^5,6\). The delivery of phosphate to the ocean allows primary producers to produce ribosomes and other organic phosphates that are essential for carbon fixation, growth and, on geologic timescales, O\(_2\). The biological influence over atmospheric composition is thus dependent on phosphorus flux, which has fluctuated over geologic history as varying climate, continental exposure, and sea level acted as controls over terrestrial weathering rates\(^7,8\).

In solution at neutral pH, phosphorus is mostly in the form of PO\(_4^{3-}\) and is subject to removal via flocculation by scavenging particles such as metal oxides, organic compounds, and biological uptake\(^9,10\). In comparison, the adsorption and bioavailability of organic phosphate compounds are much lower\(^11\). In modern oceans 99% of these compounds are recycled via oxidation with the remainder being sequestered in sediments\(^12,13\). Organic phosphate recycling plays a large role in marine productivity as recycling flux is two to three times greater than weathering input\(^6,14\). The ubiquitous presence of oxidants (O\(_2\), SO\(_4^{2-}\), NO\(_3^-\)) in modern oceans support an oxidizing redox state
that promotes an efficient degradation pathway for organic phosphates and phosphorus recycling\textsuperscript{14,15}. This recycling pathway is a phenomenon of an oxidized ocean and prior to the appearance of oxygen during the Great Oxidation Event (GOE) at 2.35 Ga, few sources of electron acceptors are thought to have been available to propel phosphorous recycling\textsuperscript{11,14–16}.

This is problematic for estimating the productivity of the Archean biosphere as the reducing conditions that defined the eon would have limited oxidative phosphorus recycling. Estimates of Archean P flux and corresponding primary productivity are further stymied by a lack of geologic evidence constraining the evolution of continental exposure and the rate of terrestrial weathering. High sea levels and low continental exposure during this time are thought to have resulted in low P flux to the ocean\textsuperscript{11,16–18}. To the limited extent that phosphate could be recycled, released phosphorous would be quickly adsorbed to abundant iron minerals in the ferruginous ocean\textsuperscript{17,19}. Various models have attempted to interpret Archean P flux in spite of these limitations with estimates ranging between 0.01 to 10\% of modern values\textsuperscript{11,20,21}. Recent work estimates the sum of weathering and recycling fluxes leading to a total P flux of 18-154 x 10\textsuperscript{10} moles P/yr and a corresponding productivity estimate of 0.001\% of modern values\textsuperscript{14}. Prior research has argued that this diminished rate of phosphate recycling served to limit early marine productivity until the advent of oxygenic photosynthesis around 2.4 Ga\textsuperscript{11,14}. However, these previous estimates have failed to recognize photooxidation as a viable pathway for organic phosphate recycling, which could increase estimates of primary production.

Ultraviolet radiation was a possible energy source for the photo-degradation of organic phosphorus during the Archean. Prior to the appearance of the ozone layer following the
GOE, unfiltered UV light in the region of 200-315 nM reached the surface and interacted with surface chemistry and likely penetrated ocean water down to a depth of 80m \(^{22-28}\). High energy photons in the ultraviolet region have been shown to drive many chemical reactions far from equilibrium and the geochemical record of these interactions has yielded information regarding the redox state of the ancient environment\(^ {25,29}\). The photochemical oxidation of Fe\(^ {2+}\) to Fe\(^ {3+}\) is understood to have contributed to the early iron redox cycle and the formation of the banded iron formations (BIF) during the GOE\(^ {30-32}\). Other oxidized metal species, like Mn\(^ {2+}\), were thought to be geochemical indicators of the presence of oxygen but have experimentally been revealed to be susceptible to photooxidation as well\(^ {33,34}\). Photooxidation has even been shown to oxidize reduced (Fe,Ni)P\(_3\) minerals derived from meteorites, a major source of P input during the Hadean\(^ {35}\). The photooxidation of the surface ocean likely governed the availability of essential nutrients like P in addition to organics and trace metals\(^ {16,36}\).

The photochemical degradation of organic phosphates is not a novel idea; however, it has only been studied in the context of environmental remediation as phosphorous-based containments are common pollutants in drinking water. Diazinon is a common pollutant found in fertilizer runoff and is found to quickly degrade and release phosphate under the presence of UV light and oxygen\(^ {37,38}\). The cause of this degradation is attributed to direct photolysis of the organophosphate bond in addition to the formation of oxidative radicals such as OH\(^ -\) in the presence of UV light and various photosensitizers. Oxidants such as Fe\(^ {3+}\) and NO\(^ 3-\) have been shown to photosensitize organic phosphates, increasing the rate of degradation under UV light\(^ {37,39}\). Conversely, the addition of HCO\(^ 3-\) or C\(_3\)H\(_8\)O has been shown to reduce the rate of degradation due to the scavenging of oxidative radicals like
OH−37. The effect of redox state, compound complexity, Fe2+ concentration, and wavelength of light are all important variables when considering the fate of organic phosphates in an early Earth environment, and these have yet to be studied.

In this study, we investigate the photodegradation of several representative organic phosphorus compounds, including adenosine monophosphate, methyl phosphonate, and phosphatidylserine under putative Archean ocean conditions. Our experiments simulated the degradation by irradiating quartz reaction cells containing the compounds at room temperature in anoxic, saline solutions. The effect of the addition of various salts and light filters were used to constrain the effect of radical formation and to quantify the photon flux at specific wavelengths, necessary to reconstruct probable Archean conditions. Our experimental data demonstrate that the photodegradation of organic phosphates occurs under Archean conditions and that photooxidation was able to drive a significant amount of marine phosphorus recycling during the Archean. These observations imply that photooxidation of organic phosphorus might have provided a major phosphorus source for biological productivity in the early photic zone.
Results and discussion

Fig 1. Phosphate released by the UV degradation of adenosine monophosphate (AMP), phosphatidylserine (PS), and methyl phosphonate (MP).

Our experiments showed that anoxic solutions of adenosine monophosphate (AMP), phosphatidylserine (PS), and methyl phosphonate (MP) irradiated with UV light result in the total release of phosphate.

AMP was chosen as our principal model for organic phosphates due to its universal role in life as the base unit of energy transfer and as a complex organic molecule with a variety of bonds potentially susceptible to photolysis. AMP is composed of three distinct molecular components: a nucleoside, ribose ring, and phosphate group. AMP has a strong UV signal at 260nM due to the presence of its nucleoside ring, and the analysis of its degradation over time was followed by observing the diminishing of that signal’s
intensity in figure S3. The ratio of the rate of phosphate release to the loss of signal intensity indicated that the molecule is destroyed during photooxidation rather than the preferential lysis of the C-O-P bond. The other organic phosphates tested present different phosphate bonds, complexity, and lack an UV active component.

A constituent of cellular membranes, PS is a glycerophospholipid, a complex organic molecule comprising two fatty acid chains attached to a glycerol group bound to serine with a phosphodiester bond. We observed that the irradiation of PS released phosphate at a faster rate than AMP. The complex structure of the compound likely lends to its fragility under UV exposure.

The simplest organic phosphate tested was MP, composed of a sole organophosphate bond. Its biological function is ill-defined, and it makes up only <1% of organic phosphorus flux yet is most represented (>20%) in the organic phosphate found in sediments\(^{13}\). More interestingly it has been found in significant quantities within meteorites, thus representing a possible abiotic source of organic phosphate flux during the early Archean \(^{40}\). The compound’s recalcitrance was affirmed in our experiments as it resisted photooxidation, releasing phosphate at a much slower rate than AMP. The degradation of these molecules under UV light alone suggests the efficacy of UV-mediated phosphate recycling.

Further experiments were conducted to examine the effect of dissolved salts on the rate of phosphate release. Modern seawater is comprised of a variety of salts which include NaCl, MgCl\(_2\), CaCl\(_2\), and MgSO\(_4\). These salts were individually examined and were found to have no effect on the rate of phosphate release or the stability of AMP.
A major constituent of Archean seawater was Fe$^{2+}$, with various attempts at reconstruction placing Fe$^{2+}$ concentrations between 0.01 and 1 mM $^{14,19,29,31}$. Ferrous iron is thought to have been a major source of electrons during the Archean and its oxidation would have promoted the release of H$^+$ and the recycling of phosphorous in the early oceans$^{16,20}$. We observed that the addition of 0.2 uM Fe$^{2+}$ did not significantly change the rate of phosphate release despite its photooxidation and the precipitation of Fe$^{3+}$ oxides. Prior studies into the photosensitization of organic phosphates show that Fe$^{3+}$ significantly increases the rate of degradation, which was observed in solutions containing 0.2uM Fe$^{3+}$. Archean seawater pH was thought to be slightly acidic, ranging between pH 6 and 7; however, it likely varied depending on environmental influences$^{14}$. The addition of HCO$_3^-$ was observed to raise the pH and slightly decrease the rate of
phosphate release. The ferruginous oceans would likely contain a significant quantity of ferrous iron precipitates, which was observed with the addition of HCO$_3^-$ and Fe$^{2+}$ resulting in a reduced rate of phosphate release. Iron precipitates are thought to have strongly controlled trace metal and nutrient fluxes in the Archean, which is seen in modern analogues like the sub-oxic waters of Lake Matano$^{41,42}$.

Archean recycling of phosphorous would be complicated by the sequestration of phosphate by these iron precipitates. Silica, which competes for absorption to iron minerals is thought to have reduced the extent to which phosphate is sequestered$^{17,19}$. Microbial dissimilatory iron reduction has been hypothesized to act as a control on the primitive marine iron cycle which would have allowed this sequestered iron to be biologically recycled, releasing sequestered P$^{43}$. The Fe$^{3+}$ produced during this process would have also serve as a photosensitizer for additional P photo-recycling$^{39}$.

To understand the difference between the Archean and modern marine P recycling, we compared the release of phosphate in anoxic solutions to oxygenated solutions containing H$_2$O$_2$. We observed a 41% decline in the initial rate of phosphate release for AMP and no change in the rate of release for methyl phosphonate. It is known that oxidized solutions result in a substantial generation of OH$^-$ under UV light which contributes to the degradation of complex organic compounds$^{37}$. The organophosphate bond in methyl phosphonate displays a strong resistance to oxidation, implying its rate of phosphate release is a result of photolysis only. The effect of radical formation was diminished in separate reactions using isopropanol and HCO$_3^-$ as scavengers of OH$^-$ as well as using long pass and band pass filters which diminished or removed wavelengths of light responsible for most of the radical formation$^{44}$. 
<table>
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<tr>
<th>Compound</th>
<th>Filter (nM)</th>
<th>Additive</th>
<th>Rate (mol/L/hr)</th>
<th>Quantum Yield</th>
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<tr>
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<td>( y = 1 \times 10^{-6}x - 2 \times 10^{-6} )</td>
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Table 1. Initial release rates of phosphate under all conditions tested.

The photo-recycling capacity of the Archean sun is dependent on the flux of sunlight able to reach the surface. While a UV-absorbing ozone layer was not yet present, CO\(_2\), SO\(_4\), and CH\(_4\) would have controlled the absorbance of UV light at the time\(^{24}\). Recent models of Archean atmospheric composition depict a pCO\(_2\) at 3.8 Ga lying between .08 to 1.2 bar, which offers a wide range of UV absorbances\(^{23}\). In the best-case scenario, low pCO\(_2\) would allow nearly 100% of UV reach the surface while conversely a high pCO2 would effectively block at least 80% of it\(^{45}\). A middle ground of 0.24 bar is considered a reference standard and does allow most UV light to pass through.

Using bandpass filters to isolate specific wavelengths of light we found that UV light at 230 nM had the highest quantum yield relative to the other wavelengths tested, 200 and
260 nM. Using the calculated quantum yields from three wavelengths (200, 230, 260) we estimated the total possible P photo recycling based on Archean surface flux with an atmospheric pCO2 of 0.24 bar resulting in $4.82 \times 10^{10}$ moles P/yr. While conservative with our calculations of possible P degradation based on AMP, we find that even our lowest predicted photo-recycling flux could recycle a significant amount of P per unit area. This lends support to higher recycling flux estimates of $\sim 120 \times 10^{10}$ moles P/yr, compared to a modern recycling flux of $3710 \times 10^{10}$ moles P/yr$^{-1}$,$^6,^{14}$ This would significantly increase phosphate available for marine productivity.

This has an interesting implication as phosphorus limitation is considered to have limited the total biomass of the early biosphere until the advent of oxygenic photosynthesis, neatly squaring away organic carbon isotopic excursions present in the geologic record$^{11}$. If photooxidation led phosphorus recycling was not a limit for early life as suggested by our findings, then the discrepancy remains unresolved. While phosphorus recycling may have not been the limit it is prudent to be open to all possible limits to the productivity of the early biosphere. Our experiments suggest the Archean UV flux was likely destructive to unprotected organic molecules at the surface of the ocean. UV light is effectively absorbed by the presence of Fe-precipitates and is mostly attenuated at depths of 80 m in analogue systems, possibly creating a refuge for early life in the lower photic zone$^{46}$. Adaptations to UV flux have been found in modern environments, yet the full extent to which the primitive biosphere adapted to such hostile conditions remains to be elucidated$^{47,48}$. It may be possible that the sun, despite recycling phosphorus, restricted life from inhabiting the surface oceans and banished them to the twilight below.
Our results suggest that despite the mildly reducing conditions, Archean marine productivity was likely not limited by a lack of phosphorus recycling as UV flux was sufficient to recycle a significant amount of organic phosphate. Photooxidation was a strong force shaping the geochemical and biological environment of the early Earth. The Archean sun performed a paradoxical role as both the enabler and executioner of early life, with its high energy photons fertilizing a nursery for biological productivity through the destruction of essential biological compounds. It is likely that the primitive Martian and Venusian oceans received comparable surface fluxes early in their history, thus allowing for phosphorus recycling whether there was life there to receive it or not.
Methods

Reactor contents:

Solutions were prepared in a purged anerobic chamber (Coy Laboratory Products) by dissolving (50uM) of Adenosine 5′-monophosphate monohydrate, 1,2-Diacyl-sn-glycero-3-phospho-L-serine, or Methylphosphonic acid to solutions containing NaCl, CaCl₂ x 2H₂O, MgCl₂ x 6H₂O, FeCl₂ x 4H₂O, HCO₃⁻, H₂O₂, and C₃H₈O. Solutions were transferred to several quartz bottomed reaction cells and were sealed with butyl stoppers and aluminum crimp caps to prevent gas exchange with the atmosphere. They were then purged with 99% N₂ (Airgas) to remove hydrogen from the headspace. Gas chromatography with a thermal conductivity detector (Model 310, SRI Instruments) was used to determine presence of O₂, H₂, and CH₄ throughout experiments.

UV irradiation:

A 450-W Hg vapor lamp (Hanovia PC451.050) in a photochemical quartz immersion well was used to irradiate the samples. The suspensions were irradiated over 24-250 hours while the quantum yield experiments required irradiation up to 400 hours. The dark controls were placed in N₂ purged serum bottles wrapped in aluminum foil. UV bandpass filters (Andover :230 nm, Semrock 260, 200 nm, LP) were used to determine the rate of degradation at specific wavelength ranges.

Analyses:

Samples were collected at intervals ranging over the course of 1 to 400 hours for determination of phosphate, hydrogen production, and Fe²⁺ concentrations. Aliquots were removed from the reactors via syringe and stored in microcentrifuge tubes at 8C
prior to analysis using the Molybdenum Blue method for phosphate determination and the Ferrozine method for iron\textsuperscript{49,50}. A Thermo Scientific Orion 9863BN Micro pH Electrode was used to measure the pH.

Quantum Yield:

The bandpass filters (200, 230, 260 nm) were used to determine the apparent quantum yield of phosphate release. The apparent quantum yield ($\xi$) to produce phosphate was calculated using the equation $\xi = \nu_p/I_o$ where $\nu_p$ is the moles of $\text{PO}_4^{3-}$ produced per unit time and the $I_o$ is the moles of incident photons per unit time. This was determined using ferrioxalate actinometry\textsuperscript{51}. Initial rates were compared and used to calculate for quantum yield.
Supplemental figures

Figure S1: AMP degradation

Figure S1 shows photoinduced degradation of AMP in anoxic solution. Addition of H2O2 represents effect of oxygenated water, resulting in rapid degradation of AMP and release of phosphorous. Isopropanol reduces effect of OH- led degradation.

Figure S2: AMP degradation with various salts
Figure S2: Addition of MgCl, MgSO4, CaCl, show no effect on the photoinduced degradation of AMP.

Figure S3: AMP degradation UV spectrum

Figure S3: AMP has a UV signal at 260 nM due to its nucleotide ring, its intensity proportional to the concentration of the compound in solution. Continuous exposure to UV light destroys the ring structure of AMP resulting in decreasing peaks at 260nM until all AMP is fully degraded.
References


47. Gauger, T., Konhauser, K. & Kappler, A. Protection of phototrophic iron(II)-oxidizing bacteria from UV irradiation by biogenic iron(III) minerals: Implications


