

Phosphorus Redox Geochemistry on Rocky Worlds: Exploring Phosphite Oxidation and Organic Phosphorylation Catalyzed by Iron and Manganese Oxyhydroxides. L. E. Rodriguez¹, L. M. Barge¹, J. Major^{1,2}, M. A. Pasek³, and D. Vander Velde⁴. ¹NASA Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena, CA (laura.rodriguez@jpl.nasa.gov). ²University of Tulsa, Dept. of Geoscience, Tulsa, OK. ³University of South Florida, Dept. of Geology, Tampa, FL. ⁴California Institute of Technology, Pasadena, CA.

Introduction: Phosphate is a critical component of nucleic acids, coenzymes, and membrane lipids. Given the importance of phosphate to life, understanding the geochemical cycles of phosphorus—especially in how this relates to phosphorylation of organics on early Earth, Mars, and ocean worlds—would better inform us which environments are most promising to search for evidence of past or extant life.

One approach to elucidating the geochemical fate of phosphorus is to study how Fe and Mn oxyhydroxides influence the availability and reactivity of phosphite. Fe and Mn oxyhydroxides have been identified in a wide-range of environments, including Mars [1-2], meteorites [3], and hydrothermal vents [4]; there is also evidence to suggest that these minerals, along with phosphite, had accumulated in Earth's early oceans [5]. Furthermore, previous work by Pasek et al. [6] showed that—via hydroxide radicals generated from Fe^{2+} in solution (i.e. Fenton chemistry)—phosphite can be oxidized to the phosphorylating agent, trimetaphosphate. Nevertheless, it remains to be investigated whether Fe or Mn oxyhydroxides can—akin to free Fe^{2+} —catalyze the oxidation of phosphite, and in so doing, facilitate the phosphorylation of organics.

Methods: Solutions were made using ultrapure water deoxygenated via bubbling with N_2 . Samples were prepared and incubated at 25°C or in a 70°C water bath in an anaerobic chamber filled with N_2 gas. Reactions were run using 25 mM phosphite, 500 mM H_2O_2 , and 250 mM FeCl_2 or MnCl_2 at pH 5, 6.5, 8, and 10. In parallel experiments, 25 mM of glycerol was added at the start of each reaction to investigate the possibility of coupling phosphite oxidation with organic phosphorylation. Aliquots (1 mL) of each reaction were taken after 24 h, 48 h, 72 h, and 1 week.

Preparation for NMR analysis. To each aliquot, 0.5 mL of 5 M NaOH was added and the resulting solution centrifuged to precipitate out any dissolved metal. The supernatant was spiked with phosphonoacetic acid in D_2O and analyzed via NMR. To identify products bound in the precipitate, the residual pellet was redissolved in 0.5 mL of 10 M HCl. Ethylenediaminetetraacetic acid (EDTA) was added to chelate any dissolved metal and the solutions were centrifuged. To the supernatant, 0.5 mL of 10 M NaOH was added and the sample recentrifuged. The supernatant was spiked with phosphonoacetic acid in D_2O for NMR analysis.

Identifying structures and determining yields. Phosphorus species were identified by comparing with standards. The stability and NMR shifts of the standards were investigated by processing each using the methods outlined above. To ensure accurate yields, ^{31}P T1 relaxation experiments were conducted.

Preliminary Results: Stability experiments showed that triphosphate and trimetaphosphate are unstable under our analytical conditions. Within 48 hours after prepping for NMR, 32% of the starting triphosphate had degraded to pyro- and ortho-phosphate; all the trimetaphosphate had hydrolyzed. Neither phosphite or orthophosphate degraded.

Relaxation experiments revealed that the standards had comparable T1 delays, with phosphonoacetic acid having the longest at 9 seconds. Thus, it was deemed sufficient to calculate yields by comparing ^{31}P integrals of products with that of phosphonoacetic acid.

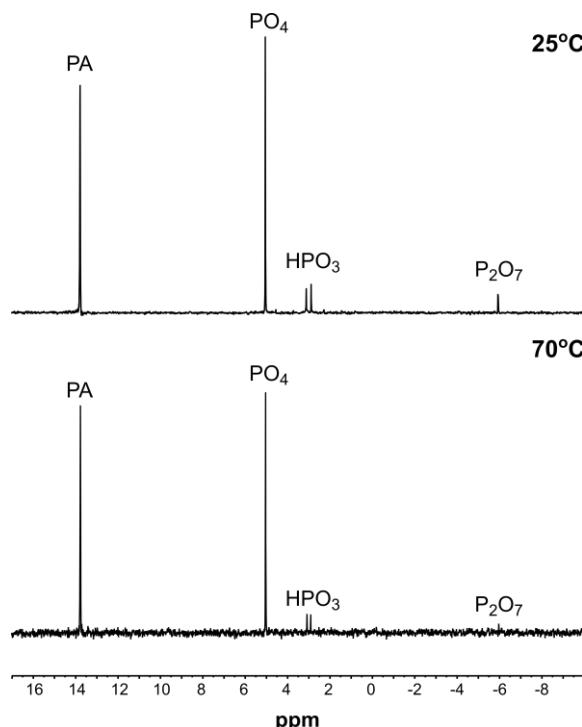


Figure 1. ^{31}P NMR reveals that $\text{Fe}(\text{II})\text{OH}$ catalyzes phosphite (HPO_3) oxidation via the Fenton reaction. The reaction generates both orthophosphate (PO_4) and pyrophosphate (P_2O_7). PA is phosphonoacetic acid.

Phosphite Oxidation. In peroxide solutions containing both dissolved Fe^{2+} (pH 6.5) and insoluble ferrous oxyhydroxide (pH 8), phosphite was oxidized to orthophosphate (69–86% yields) and pyrophosphate (8–9% yields; Fig. 1). Raising the temperature to 70°C improved orthophosphate yields, but pyrophosphate was generated in only trace amounts. No products were observed in the absence of peroxide, even at 70°C.

We also investigated whether Mn^{2+} could catalyze Fenton chemistry and found that neither solutions containing Mn^{2+} as dissolved ions (pH 6.5) or as oxyhydroxides (pH 8) facilitated the formation of pyrophosphate. Orthophosphate may have been generated in pH 6.5 solutions; however, the peak is very weak and cannot be confidently discerned from the noise.

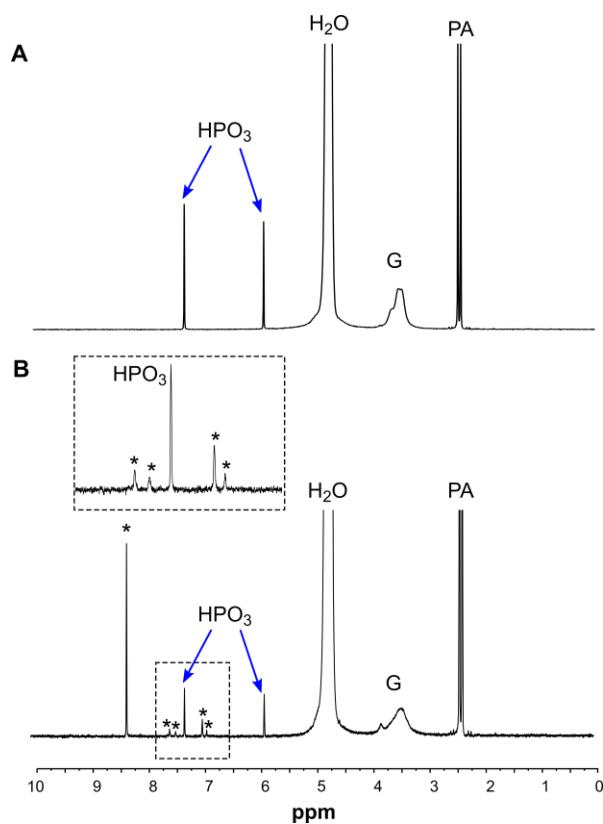


Figure 2. ^1H NMR of solutions (25°C, pH 8) containing ferrous oxyhydroxide, phosphite (HPO_3), and glycerol (G) without (A) or with (B) peroxide (i.e. Fenton reaction). Peaks likely from glycerol degradation are indicated by *. Note that the phosphite peak is split because of H-P coupling. PA is phosphonoacetic acid.

Phosphorylation of Glycerol. Phosphorylated glycerol was not detected via ^{31}P NMR, however new peaks were identified in the ^1H spectra (Fig. 2). These peaks are downfield from the protons of glycerol,

found only in solutions where Fenton chemistry was observed, and did not correspond to either the ^1H spectra of glycerol-1-phosphate or glycerol-2-phosphate. Given this, these peaks are likely the product of glycerol degradation.

Discussion: Previous work by Pasek et al. demonstrated that Fe^{2+} facilitates the oxidation of phosphite at pH 7 [6]. Here, we replicated these results at pH 6.5, and importantly demonstrated that these reactions still generate phosphate and pyrophosphate at pH 8, even though the majority of the ferrous iron had precipitated out as an oxyhydroxide mineral; formation of trimetaphosphate and triphosphate cannot be ruled out as our analytical conditions promoted their hydrolysis. Fenton reactions were also carried out at pH 5 and 10 and will soon be analyzed; the results will be presented.

In addition, our results suggest that, unlike orthophosphate, formation of pyrophosphate is not favored at higher temperatures. One possibility for this could be that higher temperatures facilitate the hydrolysis of pyrophosphate more so than it does its formation.

We also found that even after 1 week, Mn^{2+} did not catalyze Fenton chemistry; however, a potential orthophosphate peak was identified in pH 6.5 solutions. Ongoing work involves concentrating the samples to determine if the peak is indeed real. Nevertheless, this work demonstrates that Fe^{2+} is a much more efficient Fenton catalyst than Mn^{2+} .

Finally, attempts to phosphorylate glycerol during these reactions were unsuccessful in solutions at pH 6.5 and 8. Instead, ^1H NMR suggests that the Fenton reaction degraded glycerol. Indeed, Fenton reactions are used to promote the degradation of organic contaminants during waste water treatment [7]. Thus, while Fenton chemistry may facilitate the formation of phosphorylating agents, it also catalyzes organic degradation. Conditions potentially more conducive for the formation and stability of trimetaphosphate (i.e. using metals that better promote Fenton chemistry, lower temperatures, or utilizing minerals that better adsorb the polyphosphate) may help facilitate the coupling of phosphite oxidation with organic phosphorylation. Alternatively, phosphorylation may require the organic to be added only after the Fenton reaction is complete.

References: [1] Klingelhoefer G. et al. (2005) *Hyperfine Interact.* 166, 549–554. [2] Lanza N. L. et al. (2014) *Geophys. Res. Lett.* 41, 5755–5763. [3] Lee M. R. et al. (1996) *Meteorit. Planet. Sci.* 31, 477–483. [4] Mottl M. J. and McConachy T. F. (1990) *Geochim. Cosmochim. Acta* 54, 1911–1927. [5] Pasek M. A. et al. (2013) *PNAS* 110, 10089–10094. [6] Pasek M. A. et al. (2008) *Angew. Chem. Int. Ed.* 47, 7918 –7920. [7] Wang Y. et al. (2015) *Catal. Today* 252, 107–112.