Prebiotic Chemistry

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Production of Potentially Prebiotic Condensed Phosphates by Phosphorus Redox Chemistry**

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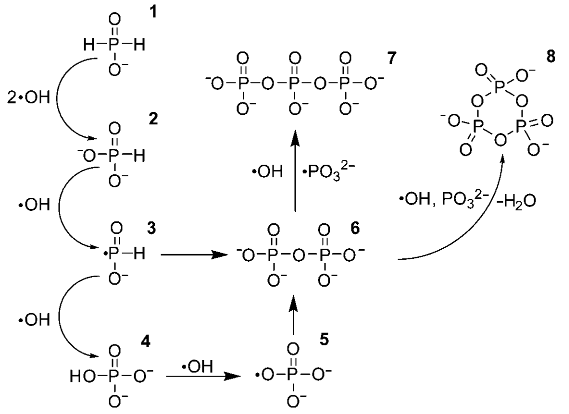
Phosphorus is a biogenic element critical to the development of life. It is present in the backbone of DNA and RNA, it acts to store and transfer energy as ATP, and it serves in cellular structure in phospholipids. Given its centrality in biology, phosphorus was presumably critical for the origin or early evolution of life. Experiments have shown that the formation of phosphorylated prebiotic molecules occurs readily through use of condensed phosphates or by heating with phosphates in the presence of urea.[1] However, with the exception of volcanic vents,[2] plausible sources of these compounds on the early earth have remained elusive. Nearly all phosphorus in the earth's crust is in the form of orthophosphate (**4**; Scheme 1), the major inorganic phosphorus-based compound in marine systems, rocks, and soils.[3] Orthophosphate has a low reactivity towards organic compounds and is insoluble in geologic environments, hence phosphate minerals are poorly suited as prebiotic sources of phosphorus.[4]



Scheme 1. Oxidation pathway to condensed phosphates.

Reduced-phosphorus compounds have been invoked in prebiotic chemistry to compensate for the poor reactivity of orthophosphate.[5] H-phosphonate (HPO_3^{2-}, **2**) and H-phosphinate ($H_2PO_2^-$, **1**) are significantly more soluble yet out of thermodynamic equilibrium with the conditions at the earth's surface, and hence their oxidation could be coupled to the synthesis of reactive phosphate precursors. Recently, we have shown that the reactions of iron phosphide (Fe_3P) or its mineral equivalent schreibersite ($(Fe,Ni)_3P$) with water produce HPO_3^{2-} and $H_2PO_2^-$ as the major phosphorus-based anions in solution.[6] Previous work has also shown that under a reducing atmosphere, high-energy electric discharges reduce phosphate to HPO_3^{2-}, providing a further source of reduced phosphorus to the environment.[7]

A key requirement for calling experiments "plausibly prebiotic" is identification of the geologic setting in which these reactions could occur. Ideally, plausible prebiotic reactions should require minimal workup and should be demonstrated to occur under a variety of conditions.[8] Herein we investigate oxidation reactions of HPO_3^{2-} and $H_2PO_2^-$ in aqueous solution under plausible early Precambrian conditions (i.e. the conditions on Earth 3.0 billion years ago). We mimicked the prebiotic oxidation of HPO_3^{2-} in solution through a Fenton reactor,[9,10] which generates OH and OOH radicals in solution from H_2O_2 using a ferrous/ferric catalyst, and characterized the products of oxidation by ^{31}P NMR spectroscopy. Hydroxyl radicals oxidize HPO_3^{2-} and $H_2PO_2^-$ to form HPO_4^{2-} and a significant yield of condensed phosphates (ca. 34%), including pyrophosphate (**6**), triphosphate (**7**), and cyclotriphosphate (**8**; Figure 1a) at room temperature. The yield of phosphates is proportional to the concentration of H_2O_2 introduced, and the reaction proceeds to completion in minutes. Product identities were established by comparison with ^{31}P chemical shifts in the NMR spectra of authentic samples.[6] Pyrophosphate is detected even in systems with low phosphorus and iron(II) concentrations (0.001 M), solutions of varied pH values, solutions with minerals and calcium salts, and solutions with reducing agents, such as formaldehyde (1.2 M).

As Fenton system oxidation implicates OH radicals, we explored the oxidation of HPO_3^{2-} using a microwave plasma source for the generation of OH radicals from water vapor.[8] Both pyrophosphate and triphosphate are formed in these experiments in significant yields (Figure 1b). These results highlight important characteristics of reduced-phosphorus oxidation; catalytic Fe^{II} is not necessary to oxidize reduced phosphorus to condensed phosphates, and the key reactive

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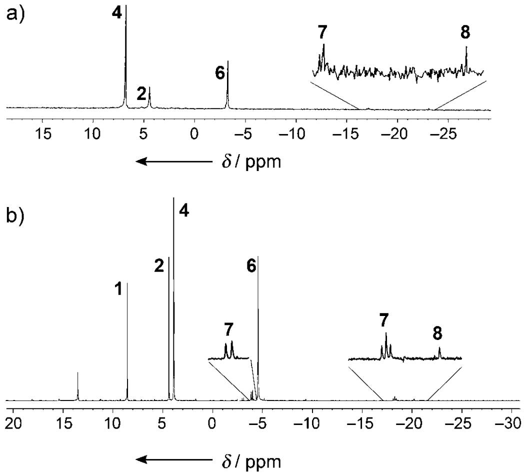

Figure 1. ^{31}P NMR spectra illustrating condensed phosphate formation during oxidation by OH radicals[9] a) formed from a Fenton reactor (spectrum acquired at 121.43 MHz at pH 13), and b) generated by microwave plasma irradiation (H_3PO_3 4.3 mм, NaH_2PO_2 2.8 mм, H_2O, 4 h, spectrum acquired at 202.46 MHz and pH 8.5). The triphosphate (**7**) doublet and triplets have been enhanced in (b). Shifts in orthophosphate (**4**) are due to the difference in pH value between (a) and (b).

intermediate is the OH radical, not a reactive iron intermediate. Experiments lacking OH (substituting N_2 for H_2O plasma) yielded no pyrophosphate.[9] Moreover, the generation of triphosphate and cyclotriphosphate depends on the presence of pyrophosphate.

The oxidation of HPO_3^{2-} and $H_2PO_2^-$ (Scheme 1) involves a series of radical reactions initiated by OH radicals generated by the Fenton reactor or by microwave plasma dissociation of H_2O. These OH radicals oxidize $H_2PO_2^-$ first to HPO_3^{2-} and then to a phosphite radical. Subsequent oxidation of phosphite radicals affords orthophosphate. Orthophosphate in turn can be oxidized to a phosphate radical (**5**) that combines with a phosphite radical to yield pyrophosphate (Scheme 1). As further evidence of this reaction pathway, the addition of orthophosphate to our experimental solutions affords pyrophosphate in similar concentrations as the oxidation of reduced-phosphorus compounds alone. Pyrophosphate itself can be oxidized to a pyrophosphate radical, which subsequently reacts with a phosphite radical to yield triphosphate; this process can potentially continue to yield higher-order phosphates. In this respect, the generation of polyphosphates from phosphorus redox differs significantly from its generation from phosphates, as no heating step is required.[11] We have previously shown that pyrophosphate is generated during schreibersite oxidation;[6] the present scheme shows that pyrophosphate is generated during HPO_3^{2-} oxidation, independent of the source of reduced-phosphorus compounds.

The major oxidants on the early earth were likely OH and H_2O_2, as evidenced by the antiquity of peroxidase enzymes and by model calculations of the early atmosphere.[9,12] Even in reducing atmospheres, these oxidants are capable of accumulating condensed phosphates globally. In reducing atmospheres these oxidants are also accompanied by an appreciable flux of reducing agents like formaldehyde; however, our experiments have shown no significant impediment to the accumulation of condensed phosphates.[9] We estimate that the global production of polyphosphates exceeded 10^9 mole per year under a neutral atmosphere (Figure 2), producing condensed phosphates for up to a billion years of earth's history and affording steady-state concentrations in the range 10^{-8}–10^{-5} м in the mixed zone of the ocean, with localized concentrations across the surface of the earth in excess of 10^{-3} м.[9] These concentrations are high enough to promote the phosphorylation of glycolaldehyde and β-hydroxy *n*-alkyl amines[13] and are comparable to modern cellular phosphorus concentrations.

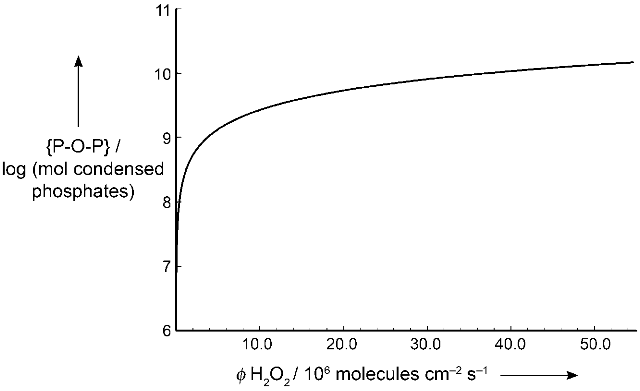

Figure 2. Condensed phosphate production denoted {P-O-P}) vs. flux ϕ of H_2O_2 per year. At our estimate for H_2O_2 flux (5×10^7 molecules cm^{-2}s^{-1}), we calculate a yearly synthesis of greater than 10^{10} mole of condensed phosphates.

These reaction systems provide a facile route to condensed phosphates in relatively high yields (ca. 34%) under simple conditions and from simple reactants, thus suggesting that the phosphorylating agents of the early earth might have been condensed phosphates. This interpretation is contingent on several key points: 1) that the environment was conducive to the formation of oxidants such as H_2O_2 and OH radicals, 2) that HPO_3^{2-} and $H_2PO_2^-$ were formed under conditions on the early earth and in millimolar concentrations, and 3) that these events coincided with the development of an active set of prebiotic reactions. Assumptions (1) and (2) are reasonable given what is known of the early earth environment, and (3) follows from the presumed deep antiquity of life on earth. We note that the reactions explored herein occur regardless of the source of phosphorus, and that if more early Earth pathways leading to reduced-phosphorus synthesis are discovered, then the oxidation of reduced phosphorus will produce even more polyphosphates. These reactions are independent of the ultimate reduced-phosphorus source.

It still remains to be shown whether the polyphosphates generated by this method phosphorylate organic compounds, including key RNA precursors. Furthermore, although a large variety of microorganisms are capable of utilizing reduced phosphorus as their sole phosphorus source,[14] it is unclear whether these organisms couple the oxidation of reduced phosphorus to the generation of metabolic energy. Discovery of a group of organisms capable of forming ATP from ADP and reduced phosphorus would provide strong support for phosphorus redox in early life.

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