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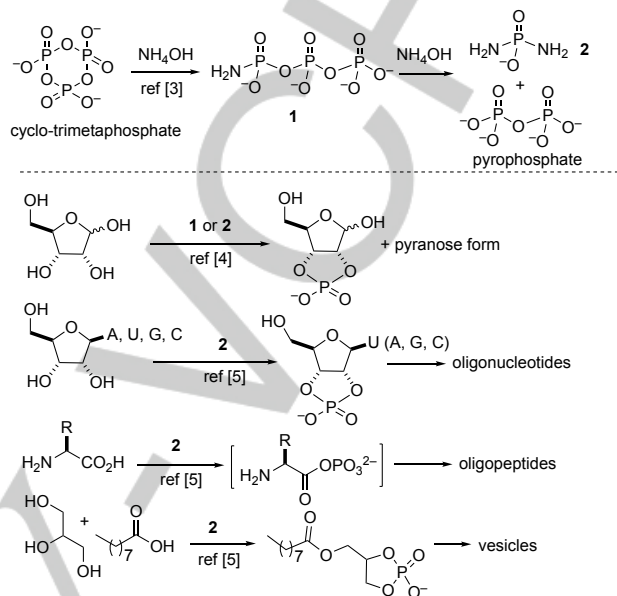
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Geochemical Sources and Availability of Amidophosphates on the Early Earth

Clémentine Gibard^[a], Ian B. Gorrell^[b], Eddy I. Jiménez^[a], Terence P. Kee^{*[b]}, Matthew A. Pasek^{*[c]}, Ramanarayanan Krishnamurthy^{*[a]}

Abstract: Phosphorylation of (pre)biotically relevant molecules in aqueous medium leading to sugar-phosphates, (oligo)nucleotides, (oligo)peptides and lipids/vesicles has recently been demonstrated by the use of water-soluble diamidophosphate (DAP). Naturally, questions arise relating to the prebiotic plausibility and availability of DAP and other amidophosphorus species on the early earth. Herein, we demonstrate that DAP and other amino-derivatives of phosphates/phosphite are generated when Fe₃P (surrogate of mineral schreibersite), condensed phosphates and reduced oxidation state phosphorus compounds that could be available on early earth are exposed to aqueous ammonia solutions. Additionally, DAP is shown to remain in aqueous solution under conditions where phosphate is precipitated out by divalent metals. These results show, for the first time, that nitrogenated analogs of phosphate and reduced phosphite species can be produced (alongside the usual oxygenated versions) and remain in solution, which increase the potential for abiotic phosphorylation reactions by overcoming the thermodynamic barrier in water.

Prebiotic phosphorylation in the context of origin of life studies, by phosphates in aqueous medium, is challenging.^[1] It was recently shown that, instead, by the use of nitrogenous-versions of phosphates (PN, amidophosphates, which could be generated from condensed phosphates, Scheme 1, top), the thermodynamic barrier of phosphorylation in water could be overcome.^[2] For example, sugars could be phosphorylated efficiently in water by the use of amidotriphosphate^[3] **1** and diamidophosphate (DAP, **2**, Scheme 1).^[4] Furthermore, DAP was shown to phosphorylate, in aqueous medium, a suite of (pre)biotically relevant building blocks (nucleosides, amino acids, fatty acids and glycerol) leading to the respective oligomeric or supramolecular self-assembled products (Scheme 1).^[5] Combining this prebiotic phosphorylation proclivity of amidophosphates with the significance of nitrogenous-phosphate intermediates in biochemical phosphorylation pathways, it was argued that amidophosphates (along with phosphates) should be considered as plausible prebiotic reactants – raising the question of what would be the prebiotic sources of amidophosphates.^[2] In this context, we show here



Scheme 1. The phosphorylation capabilities of amidophosphates **1** and **2** under aqueous or moist-paste conditions.

that P-containing Fe₃P (analog of mineral schreibersite^[6] that is present in meteorites), condensed phosphates (P₄O₁₀)^[7] and phosphite^[1] **5** that could be present on early earth, react with aqueous ammonia to yield various nitrogenous derivatives of phosphates and phosphites, such as DAP, monoamidophosphate (MAP, **3**) and amidophosphite, **4** a new species that has been previously hypothesized^[2].

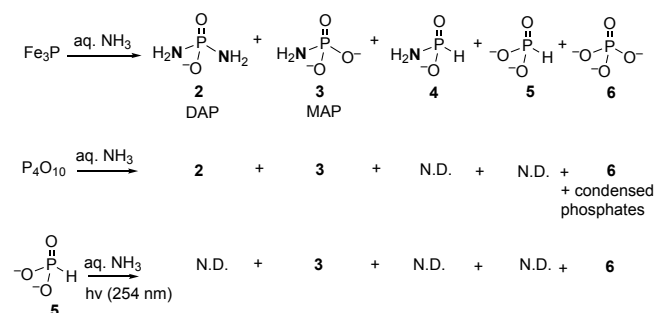
Inspired by the observations that schreibersite was corroded in the presence of water to produce phosphates^[6], we hypothesized that if aqueous NH₄OH was used it may lead to amidophosphates (Scheme 2). Two experiments (one under air and the other under argon) were setup wherein Fe₃P (which is an acceptable surrogate to meteoritic schreibersite)^[6b] was treated with 25% aq. NH₄OH and monitored by ³¹P-NMR. Within a few days, signals around 3.8, 6.2 and 8.8 ppm were observed in the inert atmosphere reaction apart from the formation of phosphite **5** at 3.7 ppm (Supplementary, Fig. S3). Spiking the sample with MAP suggested the peak at 8.8 ppm could be MAP, **3** (Fig. S4). After 12 days, a new peak at 14 ppm was observed, which corresponded to DAP, **2** – as also suggested by spiking with DAP (Fig. S5). Repeating the experiment and taking overnight ³¹P-NMR scans we observed (Fig. 1a) not only DAP and MAP, but also a new species, monoamidophosphite **4**, the nitrogenous analog of phosphite. We also observed phosphate **6** and phosphite **5** as corroborated by the H-coupled ³¹P-NMR. Very weak ³¹P-NMR signals were observed for the reaction under air (possibly due to O₂ quenching radical reactions occurring within or near the mineral^[6]); therefore, all our further experiments were performed under inert atmosphere (Figs. S3 and S6).

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Scheme 2. Reactions for producing amidophosphates under various conditions. Reaction with aqueous ammonia at room temperature (top) with Fe_3P , (middle) with P_4O_{10} and (bottom) with phosphorus acid **5** with irradiation. N.D. = Not detected.

To prove that indeed the amidated species **2**, **3** and **4** are being formed we repeated the above experiment using ^{15}N -labeled 25% aq. NH_4OH . If the nitrogen is attached to the phosphate, then further ^{15}N - ^{31}P splitting should be observed. Indeed, as expected (Fig. 1b, c), the signal assigned to DAP **2** was a triplet ($J = 16.1$ Hz), while the MAP **3** was a doublet ($J = 10.2$ Hz) and the monoamidophosphite **4** signal was a doublet of doublets ($J_{\text{H}} = 576$, $J_{\text{N}} = 6.9$ Hz). When the concentration was lowered to 12.5, 6.3 and 3.2% aq. NH_4OH the amidophosphate species were still observed (Figs. S7-S8) We did not check lower concentrations due to NMR time constraints. The total incorporation of nitrogen in the PN species (**2**+**3**+**4**) calculated from ^{31}P -NMR, under the lower NH_3 concentrations, was about 9 through 4% (Table S1), nearly matching the percentage of ammoniacal nitrogen available in solution – suggesting efficient reaction of NH_3 with Fe_3P . The similarity of the NMR at day 6 versus day 20 suggests that the PN species are stable under these conditions, consistent with known rates of hydrolysis.^[8]

Considering the constraints imposed by the plausible sources and availability of ammonia on early earth^[9], we briefly explored aqueous NH_4Cl (25% by weight) as a source at various pHs (4, 8.5, 10 and 12) but did not observe any PN species

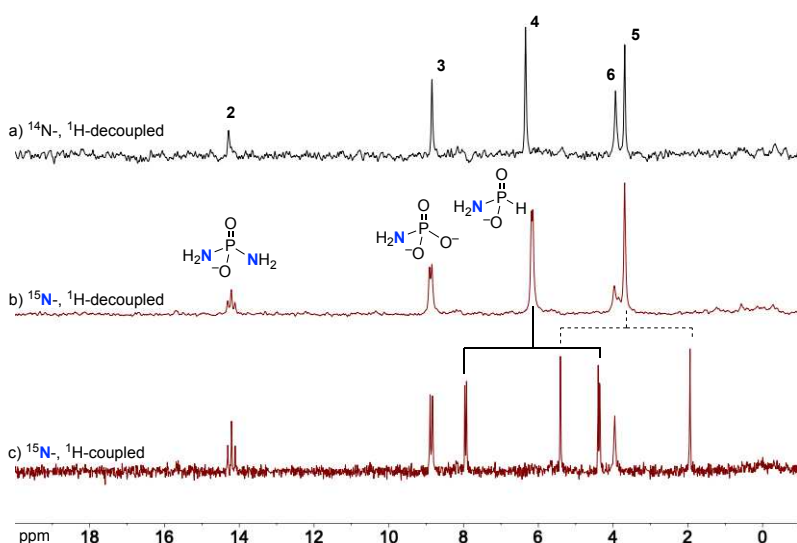


Figure 1. ^{31}P NMR spectra of reaction mixture of Fe_3P in 25% aqueous ammonia at room temperature for 12 days. Top spectrum shows the H-decoupled NMR with unlabeled aq. $^{14}\text{NH}_3$. Middle spectrum shows H-decoupled NMR with labeled aq. $^{15}\text{NH}_3$. The bottom spectrum shows the H-coupled NMR with labeled aq. $^{15}\text{NH}_3$. The coupled signals (doublets and triplet) are the result of the ^{15}N - ^{31}P coupling signifying the formation of amido-phosphate and phosphite species.

within the same time frame by ^{31}P -NMR. At pH 12 we observed only the oxygenated species as previously reported.^[6g] This suggests that there could be a critical concentration of ammonia (at least 3% as suggested by this study), or a critical pH needed for amidophosphates to be generated by the type of chemistries demonstrated above. This may limit the geographical areas where such chemistries may occur, an aspect that needs further investigation. In this context, where schreibersite itself has been shown to phosphorylate nucleosides, it is of importance to note that phosphorylation was observed above the equilibrium concentration only when some nitrogen source was added.^[6h] In fact, the best yield was reported in the presence of NH_4OH suggesting that P-N species may be the species responsible for phosphorylation. Therefore, it may not be surprising if P-N species are also involved in many of the previously reported prebiotic phosphorylation methods that use some form of nitrogen sources with activation.^[2]

We further explored two additional routes for the formation of amidophosphates via reactions in solution: amidation of high energy condensed phosphates, and radical recombinations leading to N-P bonds. In the first approach, P_4O_{10} was slowly added to a solution of 25% aq. NH_4OH . The compound P_4O_{10} has been hypothesized to be formed in dry volcanic vent environments^[7], based on the presence of di- and tri-phosphate in vent fluids. Were this compound formed through volcanic action, then its interaction with ammonium-bearing fluids on the early earth could yield amidophosphates, akin to the reaction of ammonia with trimetaphosphate.^[3] Amidophosphates were formed by amidation of P_4O_{10} , forming PN bonds at the monomer level at about 14% of the total newly formed N-P and O-P bonds (Fig. S13). As in the case for the reaction with Fe_3P , the amount of PN bond formation corresponded closely to the amount of N in solution. In the absence of ammonium, only phosphate and polyphosphates (pyro-, tri-, trimeta- and others) are formed.

A follow-up reaction investigated the formation of amidophosphate by UV-light (254 nm) irradiation of a solution of phosphorus acid (1 M) in 25% aq. NH_4OH in H_2O (Scheme 2). After about 2 weeks of irradiation about 1% of the phosphorus acid oxidized to produce primarily phosphoric acid, but also MAP **3** (~3% of the oxidized product, Fig. S14). These results confirm the stability of phosphite towards oxidation, as suggested previously.^[10] Parallel radiative processes were explored using NH_3 and H_2O -generated microwave plasma experiments, similar to those used previously.^[6b] With Na_2HPO_3 , Na_2HPO_2 and Fe_3P as substrates and H_2O - NH_3 -Ar plasma's (55 W, ca 5% v/v NH_3 , 90 mins) we were unable to confirm the presence of PN compounds, although control reactions demonstrated that the expected PO compounds were present when just a H_2O -Ar plasma was used (see SI, Figs. S1-S2, Table S3).

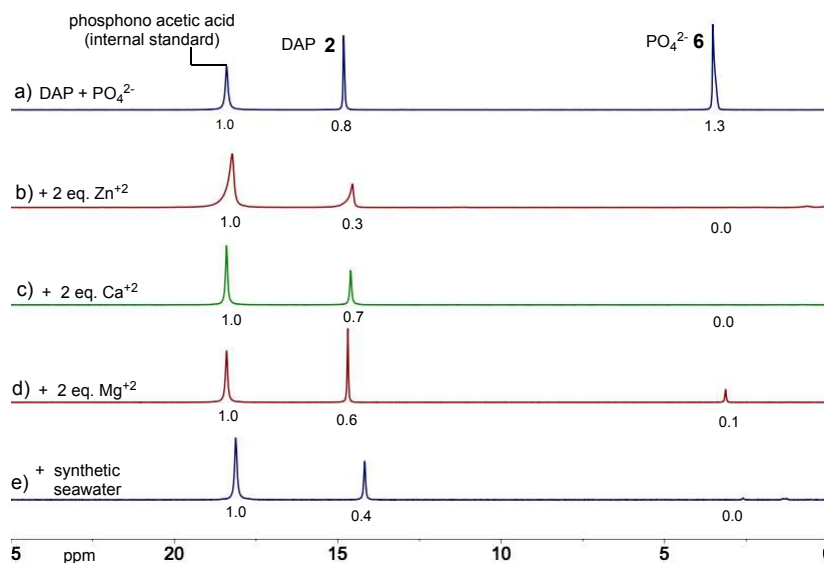


Figure 2. Effects of divalent metals on the availability of DAP versus orthophosphate in solution as monitored by ^{31}P NMR spectra after 15 min. Phosphonoacetic acid (18.4 ppm) was used as internal standard placed within a capillary. a) spectrum of 0.5 M DAP/ Na_2HPO_4 (1:1); b) with 1.0 M, 2 equiv. MgCl_2 ; c) with 1.0 M 2 equiv. CaCl_2 ; d) with 1.0 M 2 equiv. ZnCl_2 ; and e) with synthetic sea water with 0.8 M Mg^{2+} and 0.15M Ca^{2+} (see table S4). The values under the NMR peak refer to the integration values.

A contrasting property of amidophosphate (such as DAP **2**), when compared to its oxygenated orthophosphate counterpart (**6**), is the lower number of negative charges, suggesting that it may be less prone to precipitation by divalent metals. To test this possibility, we monitored solutions of DAP **2** and orthophosphate **6** in the presence of CaCl_2 , MgCl_2 , ZnCl_2 and seawater by ^{31}P -NMR. In each case, DAP was still present in solution while the intensity of **6** had greatly diminished or completely vanished due to precipitation (Fig 2 and Figs. S9-S12). In the case of seawater experiment, we titrated an increasing amount of seawater salt and found that at concentrations where the phosphate was completely precipitated out, DAP still remained in solution (Table S4 and Fig. S15). Taken together, these results imply that amidophosphate species could be available in solution for phosphorylation reaction, more than orthophosphates, and provide a way out of the “phosphate problem”.^[11]

With the results of these experiments in mind, we investigated the thermodynamics of amidophosphate formation. Data was supplemented from the HSC program,^[12] though for monoamidophosphate and diamidophosphate data are limited. However, the formation of DAP and MAP from the amidation of P_4O_{10} and trimetaphosphate,^[13] and the lack of formation of DAP and MAP from amidation of triphosphate and pyrophosphate, provides reasonable constraints on the formation of P-N bonds. The hydrolysis of P_4O_{10} to H_3PO_4 releases about 420 kJ/mol, or about 70 kJ/mol for each bond hydrolyzed, and the hydrolytic opening of the trimetaphosphate ring is about 40 kJ/mol.^[14] The hydrolysis of triphosphate and pyrophosphate release about 20 kJ/mol,^[1] suggesting the hydrolysis of amidophosphates is about 30 kJ/mol. Using this assumption, we calculate the range of stability of various N-P-O-H compounds (Fig. 3). Notably, the presence of amidophosphates should be limited primarily to low water-activity and high ammonia-activity conditions, as might be expected. The formation of amidophosphates hence requires the addition of energy in aqueous solution. This energy addition is accomplished by changes to P speciation upon reaction with

ammonia-bearing solutions. For instance, as stated earlier, the hydrolysis of P_4O_{10} releases about 105 kJ/mol of P hydrolysed (a majority released within the first three hydrolytic steps), enough to also allow for amidation. In addition, the oxidation of schreibersite by water to phosphate, FeO and H_2 gas releases about 100 kJ/mol of P, more than enough to overcome any amidation energy barrier. The oxidation of phosphite to phosphate via water (H_2 as a product) also produces about 55 kJ/mol of P, in addition to the UV energy added.

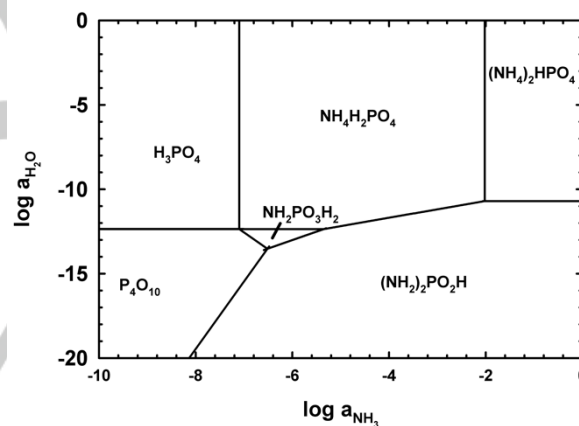
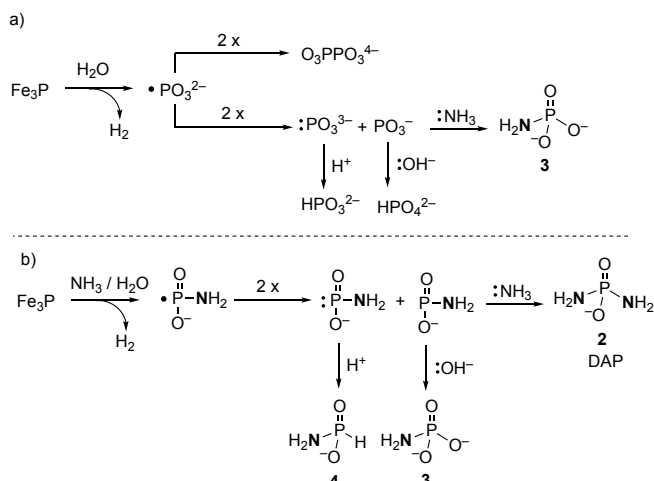


Figure 3. Stability diagram for P-O and P-N species with varying water and ammonia activities (note logarithmic scale). Activities of each compound were assumed to be 1, with the temperature set at 298 K and 1 atm. of pressure.

These results suggest that high energy P sources produce amidophosphates. Furthermore, given that the energy provided per P liberated greatly exceeds the energy barrier for amidophosphate formation for schreibersite oxidation and P_4O_{10} hydrolysis, it comes as no great surprise that the formation of amidophosphates relative to phosphates in these two cases is close to the proportion of N/O in the reactive solutions. In other words, the formation of amidophosphates is due to a kinetic process principally involving whatever nucleophile happens to be nearby, as opposed to a thermodynamics-driven process. In the case of UV photolysis, the lower free energy available means that thermodynamics play a more important role, and hence less amidophosphates (relative to phosphates) are formed. Based on these considerations, coupled with previously proposed pathways for the aqueous corrosion of schreibersite^[6]



Scheme 3. The proposed mechanistic pathways for the production of amidophosphorus species **2**, **3** and **4** by corrosion of schreibersite by aqueous ammonia. This is based on previous studies^[6] and thermodynamic considerations discussed below (see also Fig. S16). Additionally, the failure of the reaction under air may indicate interference in the radical chemistry by O₂.

we outline a plausible mechanism for the formation of amidophosphates (Scheme 3). Some of the predictions of what species will be formed from this mechanism matches well with the observations (Fig. S16). Using the data above, we evaluated the three potential sources of amidophosphates for geochemical relevance (see SI, page S18 for detailed assumptions and calculations and Table S5): schreibersite, volcanic P₄O₁₀, and reduced oxidation state P compounds. It is especially noteworthy that the P₄O₁₀ system affords both PN and PO phosphorylating molecules, both classes being capable of phosphorylating organics under different physicochemical conditions; a scenario that contemporary cellular biology also adopts. Of these, we find that all three could have been relevant to early earth conditions, each generating 10¹⁵-10¹⁹ moles of amidophosphates over the first billion years of earth's history. These data are the first for a planetary environment and complement the observations of PN species in interstellar medium (ISM) diffuse clouds and star-forming regions.^[15]

In summary we have shown that there could be reasonable pathways to various nitrogenated phosphorus (PN) compounds (amidophosphates) that could have been generated and coexisted with the oxygenated counterparts (phosphates) – provided ammonia could have been available at critical concentrations^[9] – in an early earth scenario. This suggests that amidophosphates may have been present in some abundance as a prebiotic reagent on the early earth and could alleviate the problems associated with phosphorylation in aqueous medium.

Experimental Section

See supporting information for description of experimental methods, NMR data, calculations for thermodynamics of amidophosphate formation and geochemical availability of sources for amidophosphates.

Acknowledgements

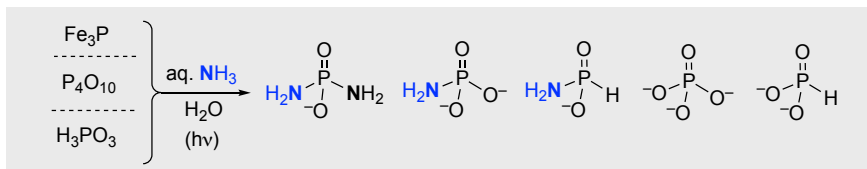
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Keywords: Prebiotic Chemistry • Phosphorylation • Diamidophosphate • Early Earth • Amidophosphate

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COMMUNICATION



Clémentine Gibard, Ian B. Gorrell, Eddy I. Jiménez, Terence P. Kee*, Matthew A. Pasek*, Ramanarayanan Krishnamurthy*

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Geochemical Sources and Availability of Amidophosphates on the Early Earth

Amidophosphates are produced by the same pathways that give rise to phosphates from various phosphorus sources when reacted with aqueous ammonia.

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