

The Curious Case of Phosphate Solubility

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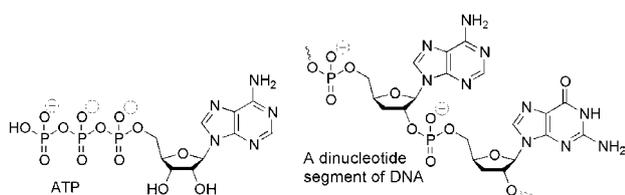
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Introduction

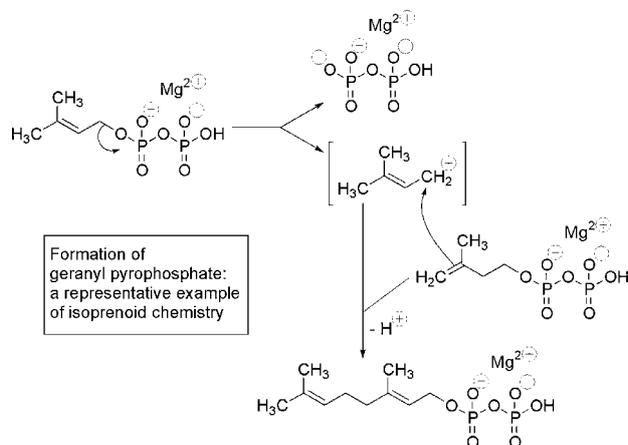
Phosphate is an essential chemical for life. In many marine environments it is a biolimiting macronutrient. The limitations in phosphate availability are related to the insolubility of important phosphate salts. Indeed, the relative solubilities of iron(II) and iron(III) phosphates have been widely considered as a key determinant in the phosphate cycle. The importance of understanding phosphate availability for addressing contemporary environmental issues, such as lake eutrophication (nutrient pollution), has led to more sophisticated analyses of phosphate sedimentation. The dynamics of iron phosphate solubility carry implications beyond contemporary environmental issues. It is likely that the interaction of iron and phosphate, along with sulfide and other species, has been important throughout the evolution of the biosphere. It is suggested that the sparingly soluble nature of iron(II) phosphate has played a key role in the biosphere from the emergence of life to the present day.

Why Nature Chose Phosphates

In an excellent review, Westheimer¹ laid out the chemical basis of the utilization of phosphates by living systems. Phosphate, a strong, tribasic acid, is ionized at neutral pH in aqueous environments. It can link up to two groups, as esters, *e.g.* in nucleic acids, and remain ionized. All these ionized species are retained within cells because of the inability of charged species to traverse non-polar cell membranes readily.



Phosphate esters and anhydrides, *e.g.* ATP, are thermodynamically unstable with respect to hydrolysis, but their negative charge repels nucleophiles and renders them kinetically stable. The combination of thermodynamic instability coupled to kinetic stability is a hallmark of life. Nucleic acids are polyesters that are sufficiently durable in water to house and transmit genetic information, but sufficiently labile thermodynamically to allow their subsequent recycling—polyphosphates such as ATP accomplish the impressive job of being water-compatible dehydrating agents for life! When needed, these kinetically stable species are activated by appropriate enzymes acting in concert with multivalent metal ions. Finally, phosphate derivatives, as the conjugate bases of strong acids, are good leaving groups, *e.g.* they are utilized as carbocation precursors in isoprenoid chemistry. Pyrophosphate, complexed to magnesium ions, is a ubiquitous leaving group in biochemistry.



The Hassle with Choosing Phosphates: Solubility

The many desirable chemical features of phosphate come with a cost: the limited solubility of phosphates in the presence of many multivalent metal cations. As Williams has pointed out, this factor has been a major issue in the evolution of phosphate biochemistry² and raises interesting questions about how phosphate came to play such an integral part in core metabolism.

The chemistry of phosphate within cells is primarily mediated by magnesium ions as the corresponding salts are soluble in aqueous media. By contrast, calcium phosphate is insoluble. Cells maintain low levels (10^{-7} M) of intracellular calcium, in part, because they must avoid the adventitious precipitation of calcium phosphate salts. Having adapted to export excess calcium ions, organisms have gradually evolved the ability to exploit the corresponding concentration gradients and solubilities. With higher concentrations of calcium outside cells, the temporary influx of calcium ions is now exploited as a biochemical signalling device. Furthermore, animals deposit extracellular calcium phosphate as bone: not just a useful material but also a dynamic store and buffer of both calcium ions and phosphate. Calcium phosphate, as apatite minerals, is a major sink of phosphate. The insolubility of this and other phosphate salts, control phosphate availability in many aqueous habitats.

The Phosphate Cycle: Interactions with Iron

The phosphate cycle plays a key role in governing the productivity of the planet.³ As such, phosphate availability has a critical impact on the environment. Control of phosphate levels in the environment is the subject of ongoing discussion. Thus, it has been suggested that fertilizing the oceans with phosphate might be used to stimulate algal growth and hence to reduce carbon dioxide levels in an effort to address climate change issues.

Phosphate levels are controlled by solubility processes: continental weathering is the most important source of bio-

available phosphate, whilst the steady state levels of phosphate are subsequently controlled by mineral deposition. For example, it has been known for many decades that oxic lake sediments retain phosphate more efficiently than anoxic sediments, and that oxic sediments release large amounts of phosphate when they become anoxic. These observations led Einsele⁴ and Mortimer⁵ to develop a model for the phosphate cycle in lakes, in which the redox chemistry of iron species in sediments is coupled to phosphate availability. In this model, phosphate is sequestered by adsorption onto the surface of iron(III) oxide hydroxide (Fe(O)OH) in aerobic sediments. When these sediments are buried, the iron(III) is reduced to iron(II). Iron(II) phosphates have a greater solubility than those of the corresponding iron(III) species and it is assumed that this differential solubility leads to the release of soluble phosphate.

All chemists in NZ are aware of the incredible impact of superphosphate on agricultural productivity. The downside of this has been the environmental impact of enriched phosphate levels in waterways due to fertilizer run-off. This has generated a pressing, international environmental issue: the excess phosphate accelerates growth and is an important factor in anthropogenic eutrophication of rivers and lakes. Efforts are being made to control phosphate levels in waterways both by decreasing inputs, through more careful fertilizer use and irrigation, and by maximizing the trapping of phosphates by sedimentation processes. Thus, there has been a long standing effort to address the eutrophication of Lake Sempach in Switzerland, both by decreasing phosphate inputs and decreasing phosphate release from sediments. Based on the iron phosphate redox cycling model of Einsele and Mortimer, the latter issue was addressed via artificial oxygenation of the hypolimnion (bottom waters) in an effort to minimise anaerobic release of phosphate from sediments. After a 15 year study, it is clear that diminishing phosphate inputs has reduced eutrophication. However, hypolimnion oxygenation has not resulted in a lowering of lake phosphate levels.⁶ The problem is likely due to an oversimplified notion of iron phosphate solubilities. It is important to understand the limitations of the Einsele and Mortimer model in order to inform detailed analyses of the cycling of phosphate in such lake environments, and in the wider biosphere.

Why is phosphate released when an iron(III) phosphate-containing sediment becomes anaerobic? It is not a simple case of the relative solubilities of iron phosphates. Although iron(II) phosphate is formed in such sediments, and is significantly more soluble than its iron(III) counterpart, it is still only sparingly soluble. Unless iron is precipitated as an even less soluble mineral it can easily accumulate to levels that lead to efficient precipitation of iron(II) phosphate. The solubility product of vivianite, $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ ($\log K_{s_0} = 36$), is sufficient for μM levels of iron(II) to deplete phosphate to μM levels also. The most likely mineral to trap iron(II) in anaerobic sediments is sulfide, which is generated in such sediments by microbial reduction of sulfate. Iron(II) sulfide is significantly less soluble than iron(II) phosphate and so can sequester iron(II) that is formed by reduction. It is likely that it is the combination of iron(III) reduction and sulfate reduction that leads to the sequestration of iron as

iron(II) sulfides and the liberation of phosphate. From this perspective, the observed uptake of phosphate by aerobic sediments is likely to be due to diminished sulfide levels, rather than surface iron oxidation. Based on these ideas it has been proposed that the ratio of iron(II), phosphate and sulfide in the anoxic sediment controls phosphate retention and not the oxic sediment surface.⁷

This type of analysis illustrates the complexities of dealing with environmental issues when dealing with complex chemical equilibria. They also illustrate the shifting of environmental processes that can occur by perturbing the solubility of sparingly soluble salts. However, the relevance of these analyses go far beyond the contemporary environment; they inform us of constraints facing the adoption of phosphate as a key metabolite for life.

Phosphate, Iron, and Sulfide in the Origins of Phosphate Metabolism

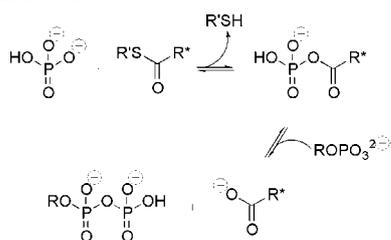
Soluble phosphates are present in cells at mM concentrations. By contrast, only μM concentrations of phosphate are present in the sea, due to phosphate mineral deposition. This is a first indication of the challenge facing the adoption of phosphate as a core metabolite. However, the discussion of phosphate release from anaerobic sediments hints at a much more substantial problem facing the origin of life. The most plausible habitats for the emergence of life (hydrothermal systems) are awash with iron. Not surprisingly, given the previous discussion, these environments are denuded of soluble phosphate. Furthermore, magnesium, phosphate's partner in contemporary biochemistry, is also scavenged from the hydrothermal fluids to form silicate minerals.⁸ How could phosphate-dependent life arise in such an environment? In order to answer this question we have to back-track a little and examine why hydrothermal systems are plausible habitats for early life, and to evaluate whether the biochemistry of phosphate is compatible with such conditions. Understanding the opportunities and constraints of phosphate chemistry under these conditions has the potential to inform our understanding of the emergence of life on earth.

One of the most plausible scenarios for the origin of life is that it emerged more than three billion years ago at hydrothermal vent systems on an anaerobic earth.⁹ At these mid-ocean ridge sites seawater percolates into a fragmentary crust and is heated to more than 300°C. Once superheated, the water leaches minerals from the crustal rocks and deposits them in mineral mounds at the surface. These unlikely sites are often luxuriant oases in the middle of an almost sterile deep ocean. The iron sulfide minerals provide a continuous input of redox energy to fuel the ecosystems.¹⁰ Iron sulfide can spontaneously form porous micro-compartments that have the capacity to act as reaction chambers, fulfilling many properties of cells such as redox and pH gradients, and compartmentalization.¹¹ Iron sulfide systems have also been found to catalyze a range of biomimetic transformations¹² including carbon¹³ and nitrogen fixation.¹⁴ These processes mimic contemporary iron/sulfur-dependent processes of anaerobic metabolism.¹⁵

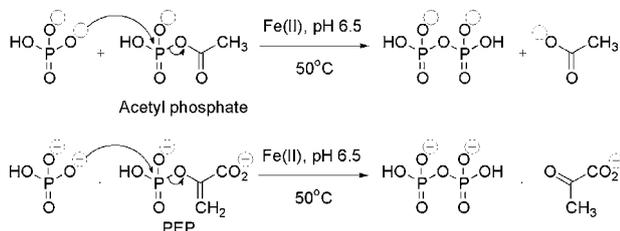
Phosphate is only present at vanishingly low levels in hy-

drothermal fluids, but it is not absent from the hydrothermal environment. It is present, admixed with iron, sulfide, and other prebiotic chemicals, as a precipitate rather than free in solution. Precipitation of phosphate species at hydrothermal vent sites¹⁶ provides a possible concentration mechanism. Hence, one possible way in which phosphate could have been incorporated into an emergent iron/sulfur-based life is via heterogeneous chemistry. We have undertaken a study of key biomimetic phosphate chemistry under such conditions which lends support to this possibility.

Our studies have focused on the biomimetic generation of polyphosphates in aqueous media.¹⁷ As indicated above, phosphate anhydrides, notably ATP, are used in biochemistry as dehydrating agents in water. Once made, polyphosphates are ubiquitous phosphoryl donors for metabolism. Simple inorganic polyphosphates, such as pyrophosphate, can be utilized in an analogous way to ATP.¹⁸ In contemporary biochemistry ATP is generated both by exploiting concentration gradients at cell membranes and via direct *substrate-level* phosphorylation, involving phosphate transfer to phosphoryl donors, notably acyl phosphate and phosphoenolpyruvate (PEP). Acyl phosphates are, in turn generated from thioesters produced via primary metabolism. Thioesters, acyl phosphates and PEP are all plausible prebiotic molecules.



Phosphate and activated phosphoryl donors are efficiently precipitated from aqueous solution by the addition of iron salts at mM levels. In contrast to simple solution chemistry, the resulting co-precipitates facilitate the efficient production of pyrophosphate from inorganic phosphate and activated phosphoryl donors; pyrophosphate being formed from acetyl phosphate and PEP in yields of up to 25 and 14%, respectively. Pyrophosphate formation is most efficient at pH 6.5 and 50°C, conditions that are compatible with many biochemical processes.



The co-precipitation of both phosphate and a phosphoryl donor on iron(II) diminishes electrostatic repulsion between the reactants, in much the same way as magnesium ions in phosphoryl-transfer enzymes. As is required in hydrothermal systems, the catalysis of pyrophosphate formation is tolerant of, but not insensitive to, the presence of sulfide ions, *e.g.* pyrophosphate is produced from acetyl phosphate in 12% yield in the presence of equimolar amounts of sulfide. These reactions mimic the extant biosynthesis of ATP from acetyl phosphate or phosphoenolpyruvate. Interestingly, under similar conditions, these minerals also retard

the hydrolysis of pyrophosphate; allowing the accumulation of polyphosphates in aqueous media – a key requisite for foundational phosphate metabolism. Perhaps then, it is the sparingly soluble nature of iron(II) phosphate that was initially important in its biochemical exploitation. Co-precipitation of iron(II) phosphate species concentrates them, brings them into close proximity, and alleviates electrostatic repulsion. However, because the resulting salts are still sparingly soluble, a dynamic interaction with the aqueous environment is maintained; the products can then be utilized by other (bio)chemical processes in the surroundings.

Iron(II) Phosphate and the Importance of Being Sparingly Soluble

Phosphate solubility has been a key issue for life since its origins. The interplay of phosphate with iron has been a dynamic issue throughout life's history and, for all the changes in the environment of the earth from anaerobic to aerobic, it continues to the present. The sparingly soluble nature of iron(II) phosphates makes them fascinating, dynamic, components of the biosphere, a fact that life may have recognized from the very start.

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