Chapter 6
The Origin of the Ionized Linker: Geochemical Predestination for Phosphate?

Matthew A. Pasek

Abstract A major event in the origin of life on the earth must have been the formation of self-replicating polymers [e.g., Gilbert (Nature 319(6055):618, 1986)]. It is likely that any robust self-replicating polymer would have needed an ionized linker to slow hydrolysis and prevent diffusion. In modern life, the ionized linker is phosphate. In this chapter, I consider other alternatives to phosphate as linkers prior to the evolution of modern RNA/DNA. From a chemical and geological perspective phosphate is suggested to be the most likely molecule capable of performing the key activities of an ionized linker within a nucleic acid.

6.1 Polymers in the Origin of Life

A major event in the origin of life on the earth must have been the formation of self-replicating polymers (e.g., Gilbert 1986). A self-replicating polymer, if also bearing some catalytic ability, could have allowed selection from materials otherwise governed by organic chemistry and chemical evolution, leading to survival based on fitness and biological, Darwinian evolution. In modern life, the self-replicating polymers are a combination of DNA, RNA, and protein, which either store information as DNA, or provide the basis for chemical selection (protein) or act as the intermediary between the two (RNA) in a role that is likely ancient.

A major problem in our understanding of the origin of life is the formation of polymers such as these. Polymers form from repeating monomer units, such as proteins from amino acids, nucleic acids from nucleotides, and polysaccharides from sugars. These three polymers are the most ubiquitous across all modern forms of life.

The initial success of the Miller-Urey experiment (1959) provided a prebiotic synthesis of amino acids from high energy discharge in a reducing atmosphere, and

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hence proteins were viewed as possibly being the earliest polymer present at the origin of life (Fox 1969). In part this perception—of the primacy of proteins—was a product of its time. Proteins and the structures of enzymes had been known for many years in biochemistry and were well-known to play key roles in life. In contrast, the Watson-Crick DNA model (1953), which showed how DNA could structurally hold genetic information, was contemporaneous with Miller's work, published only 20 days apart from the report of Miller's first experiment (1953).

In the 1980s it was shown that RNA could also serve as an enzyme (Cech and Bass 1986). This finding demonstrated that the key role of proteins—catalysis—could be accomplished by a material that looked almost identical to DNA. With this finding, in addition to an accumulation of evidence suggesting that RNA was a key part in numerous biochemical functions as coenzymes (White 1976), the RNA world gained traction as a leading hypothesis in the origin of life, carrying with it an idea that nucleic acids were the critical polymer for prebiotic chemistry.

Thus two types of polymers have competed for the role of original biopolymer: the nucleic acids and proteins. Modern life also includes polysaccharides; however, these are principally structural in function and have little catalytic or genetic potential; hence it is unlikely they were part of the prebiotic chemistry-biochemistry transition. Of proteins and nucleic acids, the formation of monomers of the proteins appears to be easier. The synthesis of amino acids requires reduced nitrogen compounds such as NH_4^+ and CN^-, reacting with an aldehyde via the Strecker synthesis to give an amino acid. All of these compounds are produced in CH_4/N_2 atmospheres with water when a high-energy driver—such as a spark discharge—is added to overcome kinetic barriers (Miller 1953).

In contrast, nucleotide formation occurs at the intersection of formose chemistry to make ribose and reduced nitrogen chemistry to make the nucleobases, and reports of their synthesis rely on compounds with varying carbon oxidation state (Powner et al. 2009). When formaldehyde and ammonium compounds are added together, amino acid synthesis is the major pathway, instead of nucleotide synthesis. In addition, these reactions must also occur in an environment where phosphorylation is also plausible, an environment typically viewed as being low in water activity.

The formation of polymers from amino acids or nucleotides also experiences numerous difficulties with side reactions. Synthesizing polymers of RNA and DNA in modern life is done by the reaction of a nucleotide triphosphate with the 3-′-hydroxyl group of the polymer, building nucleic acids. Before the enzymes that promote this reaction arose, other condensation routes were likely active, including cyclization of the phosphate, or attachment to other OH groups. Such routes may not always occur through 5′, 3′-linkages and can react by the 2′-linkage of ribose as well. The formation of polypeptides by amino acid condensation also struggles with the reactivity of amino acids to form cyclic dimers called diketopiperazines, demonstrating that problematic side reactions are not unique to nucleic acids.

Peptides do not perform replication at the sequence level, and what replication that does appear to occur with peptides is limited to changing other peptides (e.g., Prions, Prusiner 1991). Thus nucleic acids are likely the original polymer storing genetic information. However, due to the numerous difficulties in the abiotic

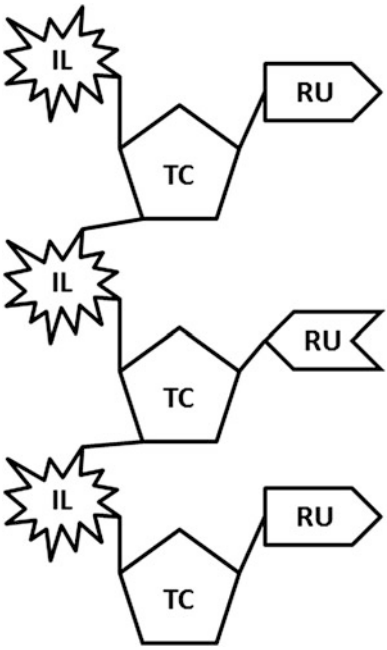

Fig. 6.1 Schematic representation of a nucleic acid. RU is recognition unit (e.g., nucleobase), TC is trifunctional connector (e.g., ribose), and IL is ionized linker (e.g., phosphate)

synthesis of RNA and DNA (e.g., Orgel 2004), it is plausible that an alternative to RNA and DNA—similar to these molecules structure and form—preceded them.

Hud et al. (2013) broke down genetic material into three major parts (Fig. 6.1). The "Recognition Units" (RU) are the nucleobases in RNA and DNA. These are the materials that carry genetic information via coding and can be stabilized by Watson-Crick base pairing. Alternatives to A, G, C, and U/T have been considered in the prebiotic literature for some time (Benner and Sismour 2005; Menor-Salván et al. 2009; Callahan et al. 2011; Cafferty et al. 2016a). Furthermore, biochemical nucleic acids are rather tolerant of alternative nucleobases, with variations common to RNA (e.g., pseudouridine, inosine, and methylguanosine), thus suggesting that other nucleobases could have been used prior to the locking in of codon translation.

The other two units of genetic material are the "Trifunctional Connector" (TC) and "Ionized Linker" (IL). Both of these form the backbone of nucleic acids and consist of ribose/deoxyribose and phosphate, respectively. The trifunctional connector serves to form a bridging polymer together with the ionized linker, and the trifunctional connector also binds the nucleobase through a glycosidic bond. In modern biology the trifunctional connector consists only of ribose and deoxyribose, with no other sugars filling this role.

The ionized linker is exclusively phosphate in modern biology, though some bacteria are known to have thiophosphate as a modified linker, formed after replication by unusual enzymes that modify the phosphate backbone to make thiophosphate (Wang et al. 2007). Such a modification is attributed as a defensive mechanism that protects DNA from nucleases (Chen et al. 2010) and remains the only known modification of the ionized linker in modern life that occurs naturally.

6.2 Why Is an Ionized Linker Necessary?

An ionized linker is likely an intrinsic part of nucleic acids that are dissolved in water. The ionized linker establishes the fundamental structure of a nucleic acid. The ionization repels the monomeric units within the backbone away from each other to promote base-pairing, and the charge prevents diffusion through lipid membranes, protects the nucleic acid from hydrolysis, and, at the monomer level, promotes dissolution of the nucleotides.

The ionized linker, along with the trifunctional connector, forms the backbone of nucleic acids. Since the ionized linker carries a charge, and since like charges repel, the backbone of two strands of nucleic acids also repel. When a strand of nucleic acid meets is complementary base-pair sequence, then hydrogen bonding from the nucleobases determines the structure of the nucleic acid, making helices. Were the backbone not ionized, then the structure of a nucleic acid would be driven by other noncovalent bonds, increasing the "messiness" of the nucleic acid.

The ionization of the linker has a further major benefit: it prevents hydrolysis of the nucleic acid backbone. The negative charge confers stability toward hydrolysis, specifically nucleophilic attack, an important feature of a nucleic acid that may have millions of ester bonds (Westheimer 1987). As long as the rate of hydrolysis of ester bonds is less than the number of bonds divided by the life span of an organism, the nucleic acid can be considered to be sufficiently stable as genetic material.

Another important property of having a charged nucleic acid is the fact that charge decreases the diffusivity of a nucleic acid across a membrane (Westheimer 1987). Since nucleic acids get their name from being found within a cell nucleus, the retention of genetic information is critical to biological evolution. Were genetic information capable of diffusing through a cell boundary, evolution would not have the ability to select upon unique sequences manifested in individuals. In aqueous solution, a charged polymer cannot diffuse through an organic membrane composed of lipids; hence, individual identity is retained along with the chance of inheritance.

At the monomer level, ionization promotes dissolution in water. For instance, the solubility at room temperature of adenosine is about 0.07 g/L in water (Merck 1996), whereas adenosine monophosphate is soluble to about 8 g/L (Wang et al. 2009). This is due to the polar character of water, and the enhanced solubility better allows the formation of nucleic acids in solution.

As highlighted by these characteristics, an ionized linker such as phosphate in nucleic acids may be presumed to be necessary if life develops in a polar solvent, such as water. By enhancing the stability of the nucleic acid, increasing the solubility of its monomeric units, stabilizing the structure of the nucleic acid, and preventing its diffusion out of a membrane, the ionized linker is a critical part of the structure of nucleic acids. Given the presumed ubiquity of water as a solvent throughout the universe, it is likely that ionization is universally an important facet of biological genetic material.

6.3 Phosphate as the Ionized Linker

Why then did life specifically chose phosphorus as phosphate as its ionized linker? Several works have addressed the reasons for phosphate, including Westheimer (1987), Kamerlin et al. (2013) and Wohlgemuth et al. (2017). The findings of these papers will be reviewed at the end of this chapter. Phosphate is capable of doing things that, at first glance, might be replaceable with other molecules, for instance arsenate.

Most alternatives to phosphates seek to replace phosphate with either something similar to phosphate or, if suitably chemically different, something much more abundant than phosphate. Why then is phosphate suspected as presenting difficulties to the prebiotic chemistry of nucleic acid synthesis? Why might phosphate be questioned as the original ionized linker?

The difficulties faced by phosphate are threefold: the first is that phosphate is rare. Compared to the other biogenic elements, phosphate is most rare of the elements used in nucleic acids with respect to cosmic abundance and ocean chemistry (Pasek and Lauretta 2005). The former is due to the nucleosynthetic processes that resulted in the elemental distribution of the solar system (and elsewhere). Phosphorus, being an odd atomic number element, is difficult to synthesize by helium atom fusion, which forms even atomic number elements preferentially. The second issue, of solubility, is due to the high stability of common phosphate minerals, such as the mineral apatite, $Ca_5(PO_4)_3(OH,F,Cl)$. These minerals are poorly soluble in water, and hence phosphate is buffered by their low dissolution. In the modern ocean, phosphate has concentrations between 10^{-8} and 10^{-6} molar. Such low concentrations are inhibitory for phosphorylation reactions (e.g., Pasek 2017).

Thirdly, phosphate is not reactive. The formation of both organic monoesters and diesters of phosphate requires the addition of energy and/or the loss of water, which can prevent the phosphorylation of organics to form the key constituents of nucleic acids. Multiple studies, including that of Burcar et al. (2016), have attempted to find solutions to this issue, and though significant progress has been made, especially in the spontaneous formation of monoesters, formation of diesters under plausible prebiotic conditions is still considered difficult. Given that orthophosphate diesters are the backbone of nucleic acids, this highlights the issues of phosphate as the original ionized linker.

The backbone of RNA and DNA consists of six repeating bonds: -O-P-O-C-C-C-. If a nucleic acid similar to RNA or DNA were present on the early earth, then it is plausible, if not likely, that the polymeric chain would have also consist of between five and seven repeating bonds, due to several molecular constraints (Cafferty et al. 2016b). In a search for an alternative to phosphate as an ionized linker, these repeating polymeric units should form the basis of any plausible polymer.

6.4 Alternatives to Phosphate as Ionized Linkers

There have been several proposed replacements of phosphate with other substances, in an attempt to circumvent (1) the low solubility of phosphates and (2) the poor reactivity of phosphates toward organics to make diester nucleic acids. Broadly, these replacements may be considered to fall into three main groupings: inorganic ion replacements, organic replacements, and phosphorus oxyacid replacements. The first two were examined in part by Westheimer (1987), and the last one is new to this work.

6.4.1 Inorganic Replacements

6.4.1.1 Nitrogen

Replacing the phosphate with other elements within its column can be viewed through the lens of science fiction, with silicon-based lifeforms being encounter by future space explorers, and is based in the argument of element periodicity: elements within the same column have similar chemical behaviors. Phosphorus has two contenders: immediately above it is nitrogen and immediately below is arsenic.

The exchange of nitrogen with phosphorus results in much more significant change than the exchange of phosphorus with arsenic, both in terms of biological functionality and in terms of potential toxicity (e.g., Hughes 2002). Nitrogen as nitrate is hard-pressed to replace phosphate. Nitrate does not form monoesters readily, and it does not form diesters while retaining a charge. This is due to nitrogen lacking accessible d orbitals. Nitrogen is unable to have more than four bonds under typical conditions, whereas phosphorus as a default has five bonds. In this respect, fundamental chemistry prevents nitrate from substituting for phosphate: it is unable to form diesters while retaining a charge.

An alternative route may be to consider nitrogen as an amine instead of nitrate. In this case, the ionized linker would be a diamine. Diamine compounds could feasibly form an ionized backbone at neutral pH, albeit with opposite charge from present day. Westheimer (1987) notes that positive charge may be detrimental as positive charges increase the rate of nucleophilic attack, and hence decrease the stability of the nucleic acid. Additionally, other backbone modifications would be necessary, as condensation with ammonium would result in a four bond -C-C-C-N- repeating unit, likely too short for a typical nucleic acid. The trifunctional connector would either have to connect differently or would have to be significantly modified to allow NH^+ to be an adequate ionized linker. An example of a successful nucleic acid based on nitrogen is peptide nucleic acids, which do away completely with both sugar and phosphate and have been considered as precursors to RNA and DNA (Nelson et al. 2000). Such nucleic acids are not charged and hence may not gain the many benefits of charge as outlined in the prior sections.

6.4.1.2 Arsenic

The possibility of replacing phosphate with arsenate in nucleic acids received its greatest support from Wolfe-Simon et al. (2011). This study purported to show the incorporation of arsenic into the DNA backbone of a microbe from Mono Lake, California, USA. Response to the paper was significant and rapid, mostly arguing that the findings were interpreted incorrectly (e.g., Benner 2011; Schoepp-Cothenet et al. 2011), and, eventually, most researchers concluded that the findings were incorrect (Erb et al. 2012; Reaves et al. 2012; Elias et al. 2012).

On paper, the idea seems reasonable enough. The pK_as of arsenic acid (H_3AsO_4) are very close to the pK_as of phosphoric acid (H_3PO_4), and both ions are similar in size. Arsenate does substitute for phosphate in some enzymes (Mukhopadhyay et al. 2002); such interchangeability is the root of some of its toxicity. If arsenate were to replace phosphate in the backbone of a nucleic acid, then it should be presumed that, absent other factors, the nucleic acid would be similar to RNA/DNA.

These other factors do indeed play a significant role, though. Arsenate esters (Fekry et al. 2011) are significantly less stable than phosphate esters (e.g., Williams and Wyman 2001). Fekry et al. (2011) estimate the hydrolytic half-life of DNA as about 30×10^6 years, compared to 0.06 seconds for the corresponding arsenate-replaced DNA. Arsenic oxyacids also readily exchange oxygen with water, especially in contrast to phosphorus oxyacids (Fekry et al. 2011 and references therein), suggesting low stability of arsenate esters.

In addition to the low kinetic stability of arsenate esters, arsenic is also redox sensitive over the range of terrestrial redox-pH conditions that are commonly found on the surface of the earth (see Sect. 6.5). Arsenate reduces to arsenite, which has significantly different properties compared to arsenate.

6.4.1.3 Borate

Borate as an ionized linker could be justified due to its strong propensity to react with sugars. This propensity has been used in prebiotic syntheses of the current trifunctional connector, ribose (Ricardo et al. 2004; Benner et al. 2012). In these reactions, borate spontaneously links to the 2′ and 3′ hydroxyl groups on ribose, forming a cyclic borate ester. Borates are also known to spontaneously link across natural carbohydrates, forming borate ester polysaccharides (e.g., O'Neill et al. 1996). Borate also acts as a phosphate mimic in some enzymes, replacing phosphate in dihydroxyacetone-phosphate aldolases (Sugiyama et al. 2006).

Boronic acid nucleotides have been used to replace nucleotides in nucleic acid with some success (Martin et al. 2011, 2013). The synthesis of these molecules, however, invokes chemistry unlikely to be present on the early earth, due to the use of solvents other than water and boron reagents other than borate (e.g., diisopinocampheylborane; see Martin et al. 2009).

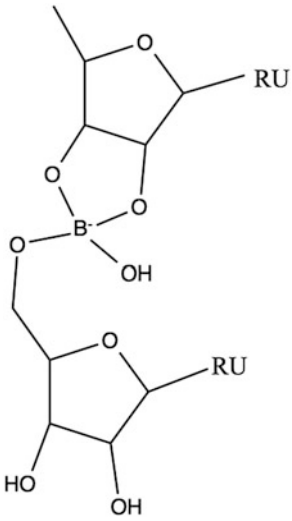

Fig. 6.2 Borate-based nucleic acid, formed of cross-linked ribose with borate. RU is the recognition unit

Borate spontaneously forms cyclic esters more easily than phosphate and other ions due to its small size. However, forming nucleic acids from ribose and borate can be envisioned (Fig. 6.2), though the conformation of such a nucleic acid may be rather strained and has a longer backbone than current nucleic acids. Additionally, given the extent of boron research in prebiotic chemistry, the fact that such structures have never been reported indicates these polymers do not form spontaneously.

6.4.1.4 Aluminate

Beneath boron on the periodic table is aluminum, with aluminate, $Al(OH)_4^-$, being a natural analog of borate $H_2BO_3^-$, might be expected to behave similarly to boron, and might overcome some difficulties with the low abundance of boron in crustal rocks. However, aluminate does not appear to form esters to any extent, likely due to their low stability toward hydrolysis.

6.4.1.5 Silicon

In contrast to aluminum, silicon as silicic acid (H_4SiO_4) readily forms esters, many of which form staples of organic chemistry labs (e.g., tetramethylsilane). Silicon esters of organics are described as quick to hydrolyze (Westheimer 1987) and do not appear to form ionized linkages at neutral pH. The first deprotonation of H_4SiO_4 occurs under strongly alkaline conditions; hence, silicic acid is an ionized linker only at high pH.

6.4.1.6 Sulfur

The sulfate ion, in contrast to arsenate and vanadate, is stable over most of the conditions that may have been present on the developing earth. Additionally, sulfur is the only element that is known to substitute for phosphorus in phospholipids, which are the material that comprise cell membranes. Sulfolipids bear a $C\text{-}SO_3^-$ moiety and are found principally in phosphorus-limited ecosystems (Van Mooy et al. 2006). Additionally, the reactions associated with sulfur redox in biological systems (from sulfate to sulfide) proceed through linking a sulfate molecule to ATP, indicating a relationship between the building blocks of nucleic acids and sulfate. However, the ATP is acting in this chemistry in its metabolic role, as opposed to its nucleic acid building block role. Phosphatase enzymes are also known to hydrolyze organosulfates (and vice versa with sulfatases), hinting that there is some biochemical similarity between the two molecules (Pabis et al. 2016).

However, sulfate is unlikely to act as an ionized linker as the diester of sulfate bears no charge. Furthermore, biologic organosulfur compounds are typically thiols, as opposed to oxyanions, and hence there's little role for sulfur in nucleic acids as an ionized linker.

6.4.1.7 Vanadate

Oft-forgotten but of significant interest as a phosphate replacement is the ion vanadate (VO_4^{3-}). Vanadium is known to replace phosphate in biochemical reactions (Lopez et al. 1976; Bornscheuer and Kazlauskas 2004), as the vanadate ion behaves similarly to phosphate and has similar pK_as and ionic sizes, akin to arsenate. The esters of vanadate appear to be generally stable over a short term and occur in equilibrium with alcohols (Tracey and Gresser 1988; Tracey et al. 1988).

Potential problems with vanadate are twofold and include the redox instability of vanadate over a large redox region and the low abundance of vanadium. In this respect, vanadate is akin to arsenate, suffering from many of the same problems with redox, abundance, and possibly long-term ester instability.

6.4.2 Organic Replacements

6.4.2.1 Citrate

Westheimer (1987) first suggested citrate as an organic replacement for phosphate, though it was dismissed in that work, as the ionization K_As were too close to have a major positive effect on preventing hydrolysis. Furthermore, the large size of the citrate molecule minimizes the benefits of having a negative charge, as the negative charge is further away from the linker and doesn't help prevent hydrolysis as well.

6.4.2.2 Glyoxylate

Bean et al. (2006) explored the possibility of replacing phosphate with glyoxylate ($O_2C\text{-}CH(OH)_2^-$). This study found that glyoxylate spontaneously links nucleosides, while retaining the negative charge from the $\text{-}CO_2^-$ group. The proximity of the $\text{-}CO_2^-$ group to the linked center also overcomes the issue of distance between ester bonds and ionized charged that citrate face and means the $\text{-}CO_2^-$ group helps prevent hydrolysis, in contrast to citrate where the ionized group is too far from the linked chain.

The spontaneous formation of nucleosides linked by glyoxylate occurs because the acetal bond formation is exothermic. The reaction occurs most easily when the activity of water is low, and products include some dinucleotides, and appears to require metal cations such as Mg^{2+}. Not all nucleosides reacted with glyoxylate; this was attributed to differences in nucleoside solubility.

Unlike most of the other ionized linkers discussed, glyoxylate is prochiral. Prochiral linkers result in a new chiral center on reaction, and this effect was addressed by Bean et al. (2006), who suggested that, as long as the chirality was random, there would be little net effect on nucleic acid structure.

A prebiotic route to glyoxylate was identified by Mohammed et al. (2017), consisting of a transamination of glycine and formaldehyde at 50–70 °C, and this route addresses the question of whether glyoxylate was present on the early earth. Some amount of glyoxylate would presumably have been present, provided there were both glycine and formaldehyde, which are presumed to be relatively abundant prebiotic feedstock molecules for the early earth.

6.4.3 Phosphorus Oxyanion Replacements

Phosphate, while amenable to being the modern ionized linker, may not have been the only phosphorus oxyanion present on the early earth. Other phosphorus oxyanions may have also been present (Pasek and Lauretta 2005), including hypophosphite ($H_2PO_2^-$), phosphite (HPO_3^{2-}), pyrophosphate ($P_2O_7^{4-}$), and hypophosphate ($P_2O_6^{4-}$). Substitution of these ions for phosphate may have overcome some of the difficulties typically associated with prebiotic phosphorylation, including the low solubility and reactivity of phosphate.

6.4.3.1 Thiophosphate

The only known actual substitute for phosphate in DNA is thiophosphate (SPO_3^{3-}). Thiophosphate is formed by enzymes that act specifically on the DNA backbone of some organisms, selectively removing one oxygen atom and replacing it with a sulfur atom (Wang et al. 2007). DNA appears to suffer no significant conformational

changes with this replacement, which appears to be a defensive mechanism against environmental oxidants such as H_2O_2, preventing DNA break damage (Xie et al. 2012).

Thiophosphate, as such, should be a relatively successful replacement for phosphate as an ionized linker. The issue with thiophosphate is its abundance. There are no known thiophosphate minerals, and outside of some biochemical sources, thiophosphate is not generated naturally by any known natural reactions.

6.4.3.2 Phosphite

Gulick (1955) first proposed that alternatives to phosphate—phosphite and hypophosphite—could have provided reactive P for the first biological organisms. Building on this hypothesis, De Graaf and Schwartz (2005) demonstrated the synthesis of nucleoside phosphonates (bearing a HPO_3^{2-} ion instead of a HPO_4^{2-} ion). However, Peyser and Ferris (2001) had earlier demonstrated that nucleic acids formed of phosphorous acid (H_3PO_3) were susceptible to rapid hydrolysis and breakdown. Additionally, once formed the nucleic acids are not charged as phosphite lacks a third hydroxide group compared to phosphate.

Phosphite has some clear benefits over phosphate as it is more soluble and it is more reactive than phosphate. The demonstration that phosphite is a natural product of the corrosion of meteoritic mineral schreibersite (Pasek et al. 2007) and can be found in natural samples (Pasek and Block 2009; Pasek et al. 2013) indicates that the ion is geochemically available. If phosphite nucleotides could be formed and then subsequently quickly oxidized as suggested by De Graaf and Schwartz (2005), then phosphite nucleosides would work well as a phosphite replacement.

6.4.3.3 Phosphonates

In contrast to the phosphites, which utilize inorganic phosphite as the phosphorus oxyanion, an alternate route to synthesizing nucleic acids is to incorporate C-P bonds into the ring structure of molecules of interest. Two such possibilities are discussed in the literature. De Graaf et al. (1998) investigate sugar phosphonates formed by the reaction of phosphonoacetaldehyde with formaldehyde via the formose reaction. The products of this reaction are six-membered rings with two phosphite groups. A nucleic acid formed from this material was envisioned by De Graaf et al. (1998) but necessarily has diphosphonate linkages (Fig. 6.3). Such a system ties the ionized linker and trifunctional connector together. Diphosphonate linkages likely are as stable to hydrolysis as pyrophosphate, which has hydrolytic life spans of 1000 years or so (Pasek et al. 2008).

Another route to forming nucleic acids using phosphonates comes from Bryant et al. (2010). By reacting hypophosphite ($H_2PO_2^-$) with pyruvate via a phosphoaldol addition, a lactone phosphinate is produced that incorporates phosphorus into the ring structure. Although it is unlikely that such a structure could form a nucleic

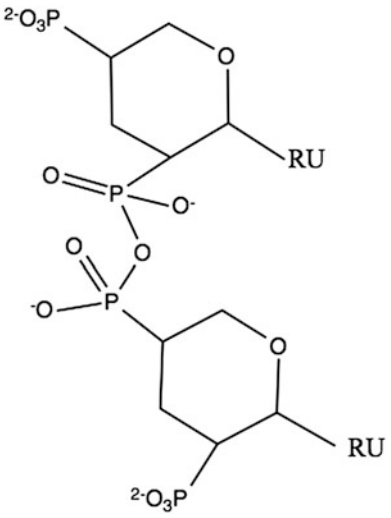

Fig. 6.3 De Graaf et al. (1998) proposed phosphonate ribose-ring structure

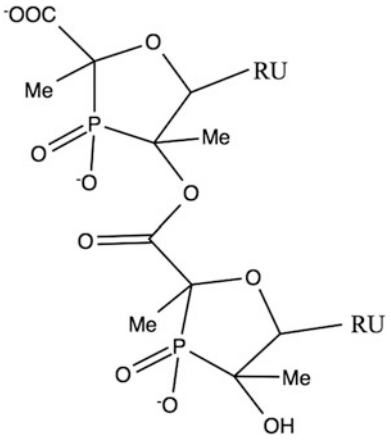

Fig. 6.4 A lactone-phosphinate compound was reported in Bryant et al. (2010), from reaction of hypophosphite with pyruvate. If the lactone is reduced at the carbonyl, and the reduction provides as a site to attach a recognition unit, then the nucleic acid below could be envisioned. As in the De Graaf et al. (1998) phosphonate bond, such a structure merges the ionized linker with the trifunctional connector

acid, as linking such a structure to a nucleobase would not proceed through an obvious route, if the carbonyl formed by this reaction is reduced, it may provide a good site for a glycosidic bond (Fig. 6.4). Although the rate of hydrolysis of esters is faster than that of phosphoesters, the proximity of the ionized P to the ester may help

stabilize this structure. However, as of yet, this structure has not been synthesized and serves mostly to provide another example of a potential phosphonate chemistry that could combine the ionized linker with the trifunctional connector, akin to the De Graaf et al. (1998) model.

In both cases, these phosphonates are reaction products of inorganic reduced phosphorus compounds (phosphite and hypophosphite) with organic reagents. A high abundance on the early earth of these compounds is not guaranteed and would be contingent on concentrating the reagents in a suitable environment. Esters formed from these compounds could be linked by a P-O-P bond, which can be remarkably stable.

6.4.3.4 Hydroxymethylphosphonate

A specific phosphonate, hydroxymethylphosphonate or HMP, can be envisioned as an ionized linker that expands the length of the linker. Such a system could work well if the trifunctional connector were smaller, for instance, a replacement of ribose with threose as the backbone sugar. HMP also overcomes some of the issues with phosphites in that the linker remains ionized when forming nucleic acids, and it does not have the problems associated with the phosphonates as the linker is not part of the trifunctional connector. However, the linkage through the hydroxymethyl group is not an ester and hence may not be as stable as the phosphoester bond.

HMP is formed relatively readily by reaction of reduced P compounds, such as phosphite, with formaldehyde (Pasek et al. 2007). It is one of the simplest organophosphonates to form from inorganic phosphorus compounds and may have been present in high concentrations in some systems. The stability of these compounds is not well known, though esters of alpha-hydroxyphosphonic acids tend to be pretty stable (Wuggenig and Hammerschmidt 1998).

6.4.3.5 Hypophosphate

The hypophosphate ion ($H_2P_2O_6^{2-}$) is an unusual ion formed by reaction of metal phosphides with water and is typically the third of fourth most common ion in these experimental solutions (Pasek et al. 2007). It has a P-P bond and is one of the few phosphorus compounds with this linkage that occurs (presumably) in nature on the surface of meteorites. Its presence is indicative of phosphite radicals (PO_3^{2-}) as reactive intermediates (Pasek et al. 2015). Hypophosphate is stable in solution and in solid form for periods lasting years or more.

Much akin to HMP reactions, hypophosphate could form a linkage between a smaller trifunctional connector such as threose (Fig. 6.5). The backbone of such a nucleic acid would be quite different compared to RNA/DNA and would likely be

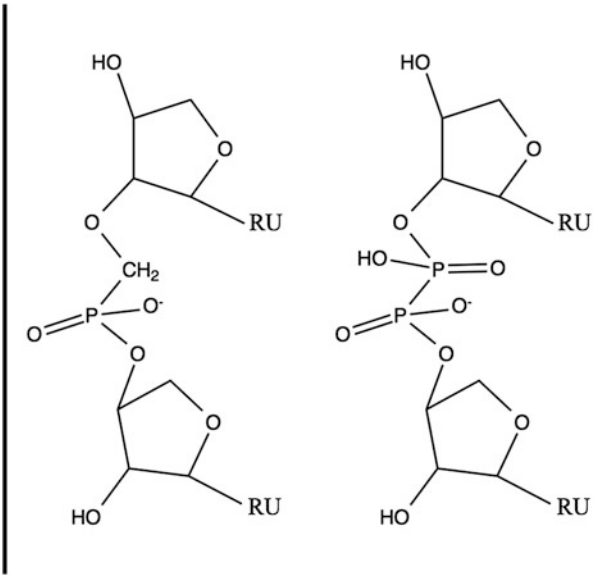

Fig. 6.5 Potential alternative nucleic acids employing (**a**) hydroxymethylphosphonate and (**b**) hypophosphate ionized linkers. The trifunctional connector may change to threose to have the same bond number per repeating unit as RNA and DNA: six in RNA-CCCOPO vs (**a**) CCOCPO or (**b**) CCOPPO. RU is the recognition unit

even more negatively charged, though the final pK_a of hypophosphate is rather large (12–14).

The stability of hypophosphate esters is unknown, though in this case it is not a question of their low stability but likely of the relatively low research that has been done on this ion. Hypophosphate does not form esters easily, in contrast to phosphite, and hence it is possible that the esters of this compound may not be stable. In biochemical studies, hypophosphate can interfere significantly with phosphate chemistry due to the difficulty of breaking the P-P bond (Pawlowska et al. 2016).

6.5 Why Then Phosphate?

Phosphate may be the preferred ionized linker for several key reasons. Phosphate is more abundant than many of these alternatives. Hence, if a replacement is possible, it may not be likely if the replacement is rare. Furthermore, several of these potential replacements are not stable over the varied terrestrial conditions potentially present on the early earth. They may become oxidized or reduced depending on the environment. Additionally, empirical evidence suggests that replacing the phosphate with different materials just doesn't work so well—the esters may be or have been shown to be quite unstable. Finally, some of the ions are prochiral: they induce the nucleic acid to have another chiral center, which adds to the molecular complexity of their synthesis. Whether or not this is an issue is unclear for the development of life on the earth.

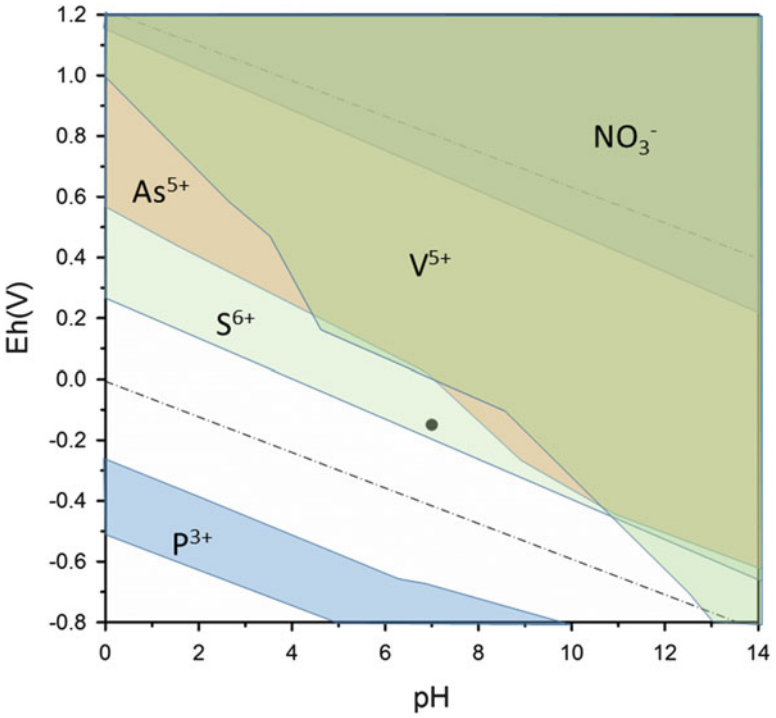

Fig. 6.6 Redox and pH conditions at 298 K necessary for the stability of some of the proposed ionized linkers. The black dot represents the most oxidizing conditions for ammonium at pH 7. For sulfate (S^{6+}), arsenate (As^{5+}), vanadate (V^{5+}), and nitrate, the stability zone technically extends to the most oxidizing conditions as well; hence, these are stable in air and overlap. The two dashed lines represent the typical oxidizing conditions of surface water (top, in contact with O_2 in air) and the presumed reducing conditions where water breaks down to H_2. Stability fields come from Takeno (2005)

The stability of phosphate over a large redox (Eh) and pH range is a key feature of why phosphate may have been employed as a nucleic acid ionized linker. The stability fields for nitrate, vanadate, arsenate, phosphite, and sulfate are shown in Fig. 6.6. Borate and silicic acid are stable over this entire range, with the exception of the possibility of forming BH_4^- under very low redox conditions (unlikely on the early earth). Included on this diagram is a likely boundary point for prebiotic chemistry—the point where ammonium is dominant (vs. N_2) at pH 7. Given the role of reduced nitrogen species in modern life, it is likely that NH_4^+ was an important species that should have been stable on the surface of the earth. It is apparent that nitrate is too oxidizing to likely have been present on the early earth and that arsenate and vanadate may be problematic as they also require more oxidizing conditions relative to the reducing conditions necessary for NH_4^+. Phosphite requires extremely reducing conditions, but, if formed, appears to be

kinetically stable for a long enough period of time to participate in reactions (Pasek et al. 2013).

From this diagram, it is apparent that several of the proposed alternative ionized linkers are not stable, if the redox conditions on the early earth fluctuated significantly, for instance, by becoming significantly more reducing perhaps within early cells. If these ionized linkers are unstable, then nucleic acids formed with these linkers as the backbone may spontaneously degrade, destroying the nucleic acid. Arsenate reduction kinetics appear to be fast (Rochette et al. 2000), whereas sulfate and vanadate reduction is slow (Wanty and Goldhaber 1992; Goldhaber and Orr 1995), though still shorter than phosphate ester hydrolysis rates.

Most of the inorganic linkers have a significant advantage over the organic linkers and even several of the phosphorus oxyacid linkers: they are all achiral. Given the difficulty already faced by prebiotic chemists in understanding the origin of chirality (Blackmond 2010), the addition of one more chiral center from the ionized linker may cause new issues. Phosphate serves well in this respect as an ionized linker because it is achiral, as the two oxygen atoms on phosphate not bound to ribose share the negative charge through resonance. However, if one of these oxygen atoms is replaced with a hydrogen (as in phosphite) or a sulfur (as in thiophosphate), then the backbone linker becomes chiral. In some cases, this may not matter, for instance, the sulfur in thiophosphate may be able to share the negative charge with oxygen, and hence the net electronegative effect would be minimized. In contrast, phosphite has no chance of sharing charge between O and H; thus, there will be electronic effects to replacing an O with an H, which would result in structural changes. Structural changes may not be important for single strands of nucleic acid, but if duplex were to form, they might cause significant issues.

Chirality may be especially problematic for organic replacements as, like phosphite, they are not capable of resonance stabilization of charge between the two unlinked units. As such, these linkers may induce folding of the nucleic acid, especially if they all assume the same chirality, perhaps if formed enzymatically. Bean et al. (2006) argued that, in the absence of a chiral selecting mechanism, random assembly will not significantly affect duplex formation and neither would alternating assembly (S, R, S, R, etc.).

A summary of the characteristics of various ionized linkers is provided in Table 6.1. The rates of hydrolysis of esters of these ionized linkers demonstrate that phosphate is unique in its ability to form stable diesters. Most other esters of ions are unstable, with diesters or polymers (when data is available) lasting only for seconds (arsenic) up to years. These fast hydrolysis rates are due to large differences in the ionized characteristics of various esters, and those without charge (e.g., boric and silicic esters) do not bear charge at neutral pH. The ionization helps prevent hydrolytic attack of the ester.

The relative abundances of the proposed replacement ions are shown in Fig. 6.7. These calculations omit the organic replacements, whose abundance would depend on prebiotic environment, and omit phosphorus oxyanion alternatives, which should be a subset of the total P abundance. It is clear from these graphs that phosphorus is much more abundant than some of the linkers, principally arsenate, vanadate, and borate, but is less abundant than Al and Si, the major rock-forming elements on the

Table 6.1 pK_as, bond lengths (Å) ester half-lives, and prochirality of the proposed alternative linkers (and phosphate)

Linker	Prochiral	Atom-O bond length	pK_a 1	pK_a 2	pK_a 3	Ester half-lives	Reference
H_3AsO_4	N	1.7	2.26	6.76	11.29	Seconds	Fekry et al. (2011)
H_3VO_4	N	1.7	3.8	8.3	13.1	Seconds	Borden et al. (2006)
H_3BO_3	N	1.4	9.24	>14		Minutes	Steinberg and Hunter (1957) (triesters)
$H_4Al(OH)_4$	N	1.7	4.8	5.1	6	Not measured	
H_3PO_4	N	1.52	2.15	7.2	12.35	Millennia	Westheimer (1987)
H_4SiO_4	N	1.6	9.9	11.8	12	Hours	Guthrie (1978), Ossenkamp et al. (2001)
H_2SO_4	N	1.49	<0	2.15		Hours to days	Guthrie (1978)
HNO_3	N	1.25	<0			Years	Guthrie (1978)
Phosphite	Y	1.5	1.3	6.7		Hours to weeks	Peyser and Ferris (2001), Mitchell et al. (1998)
Phosphonate	Y	1.5	2	7		Hours to years	Niemi et al. (1999)
HMP	N	1.5	0	8		Unknown, likely years	
$H_4P_2O_6$	N	1.5	2.1	6.8	9.5	Unknown, likely years	
Glyoxylate	Y	1.4	3.3			Likely years	Westheimer (1987)
Citrate	Y	1.4	2.92	4.28	5.21	Years	Westheimer (1987)
H_3PSO_3	Y	1.5	1.2	5.6	11.5	Millennia	

earth's crust. Although such calculations could be repeated for ocean abundances, the abundance of phosphate in the modern ocean is strongly affected by biological processes, and the composition of the ocean prior to the oxygenation of the atmosphere is unknown.

Given that neither aluminate nor silicate is effective at forming esters due to low stability, this leads us to a conclusion about phosphate: that it is the most abundant ionized linker capable of doing its job. Alternatives to phosphate are either too rare, add too much complexity, or are too reactive to fill the job as well as phosphate can. That is not to say that some of original linkers may have been playing a role in early nucleic acid synthesis, but once nucleic acids started to become selected for stability and ease of formation (including linker rarity and lack of chirality), then phosphate is likely to have taken over. Whether this happened before the onset of Darwinian evolution—and hence the origin of life—is unknown. If it happened during the chemical selection/evolution stage, then phosphate may have been the first ionized linker in life.

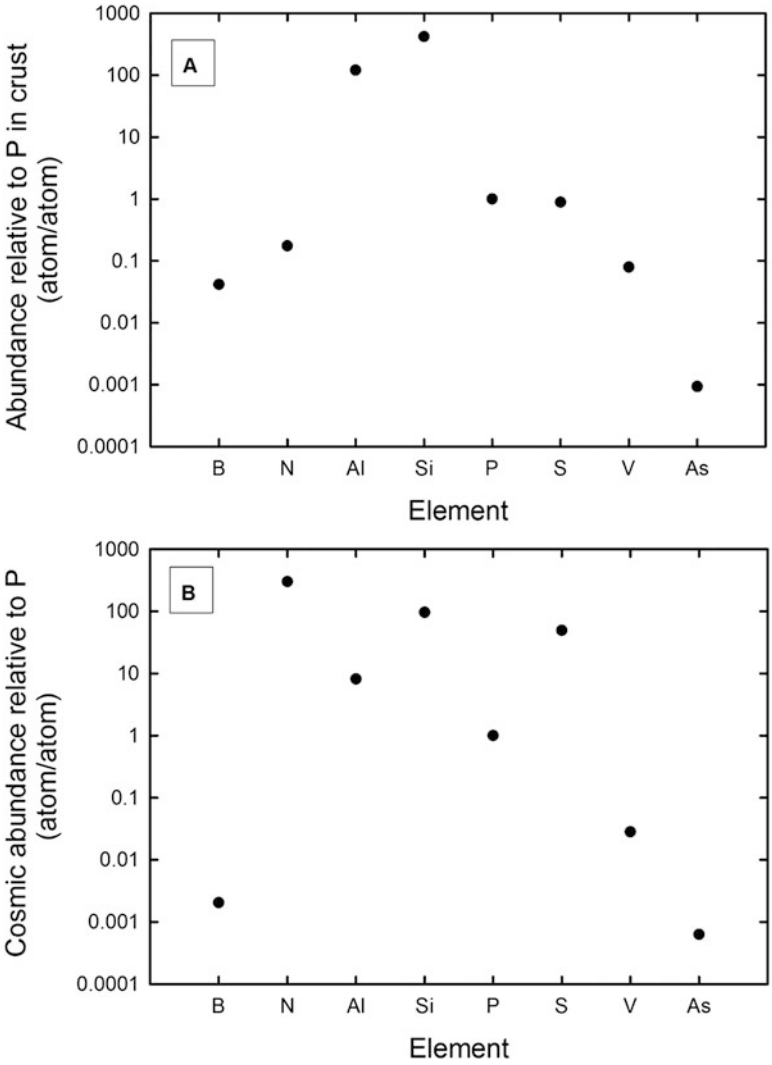

Fig. 6.7 Relative abundances of the proposed linkers, (**a**) based on the composition of the earth's crust (Taylor and McLennan 1995) and (**b**) on cosmic abundances (Anders and Grevasse 1989). Both are normalized to one P atom

6.6 Why Nature Chose Phosphate

Phosphates are important constituents of natural polymers because they are stable to hydrolysis, they are achiral, and they are ionized. Phosphate is the most common potential ionized linker that might occur in water; hence, its choice as the ionized linker appears to have been geochemically predestined.

This is not to say there couldn't be alternatives in early polymers. Glyoxylate, borate, and possibly other linkers have several significant advantages over phosphate in that they spontaneously link to sugars and may form dimers with some ease. In contrast, phosphate does not form polymers spontaneously. It is possible that one of these linkers preceded phosphate, and then natural or chemical selection pushed polymers linked by ionized species such as phosphate to dominate.

The principal difficulty of phosphate as an ionized linker is dimer formation. One of the successes of prebiotic chemistry of the past 15 years has been the identification of a number of routes to forming organophosphates monomers from simpler starting reagents. Although nucleic acids have yet to be synthesized with any of these routes, nucleotides have been synthesized both through use of energetic phosphorus-bearing minerals (Gull et al. 2015) and by dissolution in low water activity solvents (Burcar et al. 2016). These results indicate that formation of phosphate monoesters is not problematic. Formation of the diesters remains a challenge and, if such a reaction was prebiotic, likely proceeded through some sort of activation of the monoester, for instance, by linking a second or third phosphate to the molecule, such as by nucleotide triphosphates.

Indeed, the prevalence of phosphate in metabolic molecules belies a potential reason for its dominance: it is part of the main energy storage molecule in life, ATP. The triphosphate linkage in ATP carries chemical energy for metabolic reactions (as do other nucleotide triphosphates, such as GTP, though with much less frequency). The triphosphate group of ATP and other nucleotide triphosphates is the business end of the molecule. Energy is stored within the phosphoanhydride bond. This has led some to propose that nucleotide triphosphates, were they prebiotic, would have produced nucleic acids by assembling these monomers (e.g., Yamagata 1999). The difficulty with this assumption is that nucleotide triphosphates have highly complex organic molecules attached to the relatively simple triphosphate. These include molecules with specific stereochemistry (ribose) and specificity of the nucleobases. In contrast, simplifying the triphosphate to an energetic polyphosphate is consistent with modern metabolic energy storage molecules in some primitive organisms (Achbergerová and Nahálka 2011). The storage of energy in polyphosphates again is something that appears to be unique to phosphate, as polyarsenates and polyvanadates are not energy-storing (Klemperer et al. 1992).

For these reasons it is apparent that phosphate is the best at what it does: making stable nucleic acids. If early in life's history instability could have been useful (as per Bean et al. 2006), then phosphate may not have been part of the first nucleic acids. However, since there is such interplay between metabolic reactions involving polyphosphate and the polyphosphate-based building blocks of nucleic acids, phosphate was likely incorporated early in the history of life. Routes to forming phosphorylated nucleic acids should still be a goal of prebiotic chemistry research, as there appears to be an adequate ability to make phosphate monoesters. Ideally, a prebiotic route that could demonstrate nucleic acid formation from energetic phosphorus molecules (e.g., triphosphate) could provide strong evidence for forming the original nucleic acids.

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