Phosphorus, Sulfur, and Silicon and the Related Elements

The Possible Role of Cyclic Pentacoordinate Phosphorus Intermediates in the Origin and Evolution of Life. Are Phosphoric Anhydride and Trimetaphosphates Prebiotic Reagents ?

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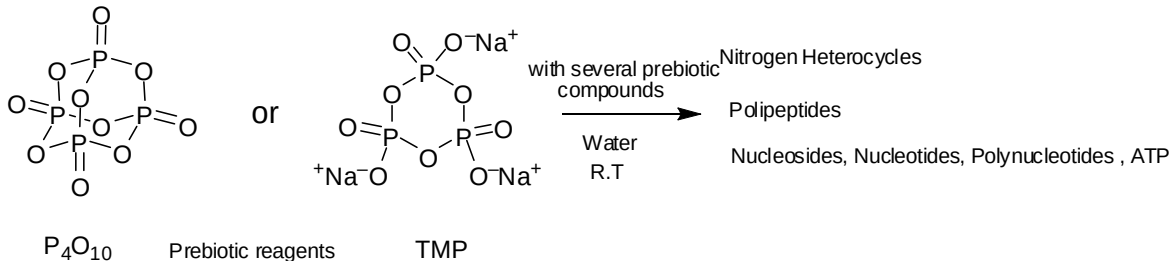
Running head: Pentacoordinate Phosphorus Intermediates in the Evolution of Life.

Dedicated to Professor Dr. Robert R. Holmes in recognition of his pioneering research on phosphorus chemistry and his leadership in the field as Editor-in Chief Emeritus.

ABSTRACT

This mini-review shows that the origin and evolution of life might be governed by a chemical process in which the formation of cyclic pentacoordinated phosphorus intermediates is activated of a factor 10^{6-8} over other similar collateral processes containing acyclic phosphorus compounds. It follows that primordial cyclic phosphorylating reagents such as phosphoric anhydride (P_4O_{10}), produced from volcano magma, and its natural derivative trimetaphosphates (TMP) could be used

to obtain very important biochemical molecules such as proteins, natural heterocyclic bases, ATP and AMP isomers very rapidly in prebiotic conditions. In addition, some results demonstrated that it is possible to generate, with high chemio-, regio-, and stereoselectivity, adenosine monophosphates (AMPs) and their oligonucleotides together with small amounts of ATP, by mixing in a one-pot reaction, D-ribose, adenine and TMP in aseptic water as a solvent and this process might explain the spontaneous generation of pre-RNA molecules in a primordial Earth and the easy formation of ATP.



Keywords: Cyclic phosphorus pentacoordinate, Phosphoric Anhydride , Trimetaphosphates, prebiotic reagents , RNA, evolution of life

INTRODUCTION

Origin of life

How life began on Earth is one of the greatest scientific mysteries. Here we propose some hypotheses about principles that govern several chemical process, that might be important for the origin and evolution of life, which, until a few decades ago were either unknown or not considered. The demonstration that ribosomal peptide synthesis is a ribozyme-catalyzed reaction suggests that there was once an RNA world.[1] The central problem for origin-of-life studies, therefore, is to understand how a protein-free RNA became established on the primitive Earth. Consequently, it has spurred scientists to try to determine if RNA molecules could have spontaneously formed.

In addition, it has been suggested that all the basic molecules for obtaining RNA were found all over the universe or formed in early Earth.[2] However, the question of how simple organic and inorganic molecules go towards life is largely unanswered even if there are many proposed

hypotheses.[1] We believe that the chief obstacle to understanding the metabolic origin of life or RNA-based life is to identify some plausible chemical processes for overcoming the disorder of abiotic chemistry,[1] thus permitting the formation of pre-RNA molecules probably *via* a "self-organized process".

In Search of a Chemical Process that Might Have the Key Role for the Origin of Life

Given its centrality in biology phosphorus was presumably very important for the origin and evolution of life . Just looking at the structure of RNA, DNA and many other molecules of biological interest, we note that the more reactive part of the molecules is the phosphate group. Therefore, the chemical and physical properties of phosphorus, which is also very sensitive to reaction conditions,[3g] may guide all reactions and modifications that arrive at life. This is probably a "self-catalytic" chemical process very sensitive to variations. In my opinion, the process must have other important features that I will briefly describe:

A) It must to be very fast with respect to many other similar possible processes that take place in the same time in the primordial pool, where there are a multitude of other chemical compounds formed over a billion of years. Therefore the presence of a catalyst is not necessary because it might catalyze other collateral processes further increasing the complexity of the primordial reaction mixture.

B) It must be exergonic and therefore does not require energy. It will go very fast even at low temperatures so other possible collateral reactions are minimized. Another important condition is that the water, the unique prebiotic solvent, must be in the liquid state to allow the reagents to be dissolved so the chemical process can takes place.

C) It must explain why adenosine 5'-triphosphate (ATP) and phosphoenolpyruvate (PEP), or c-AMP are called "high energy" compounds. In fact, this definition can be misleading. Many other compounds, for example anhydrides, give reactions with great transfer of energy-but are not called "high energy"compounds. So the true "high energy" compounds such as ATP or PEP or c-AMP have the ability to not only be thermodynamically unstable and therefore lose energy, but also are

kinetically very stable in normal conditions. Then the process must explain this contradictory feature because only in particular conditions these compounds give energy.

D) It should explain the catalytic ability of the ribosome, that is the RNA, to favor certain transformations without the intervention of proteins as occurs with DNA. In other words, it should explain why the DNA needs protein and the RNA does not.

E) It must also explain why RNA folding in a specific position is very important for its chemical functionality. In fact, only a single or a few possible structures usually lead to function; therefore the RNA polymer must avoid the problem of folding into alternative non-functional structures. Finally, it should also explain, in an acceptable manner, the so-called homochirality that is found in the processes of life. Explaining homochirality can be very important in understanding the origin of life.

F) Probably it must be able to synthesize many important compounds necessary for the evolution of life such as proteins, nitrogenous heterocyclic compounds or nitrogen bases, ATP etc , necessary for RNA or DNA formation and functionality, to have the same enormous reaction rate in order not to succumb in the same reaction environment where takes place the spontaneous formation of the RNA .

I believe that we can find a “super-activated “chemical process with these features in the phosphorus chemistry of particular molecules. I will try to explain it by citing some of my works and also some of other important scholars who are sometimes completely forgotten in the tide of publications on this topic.

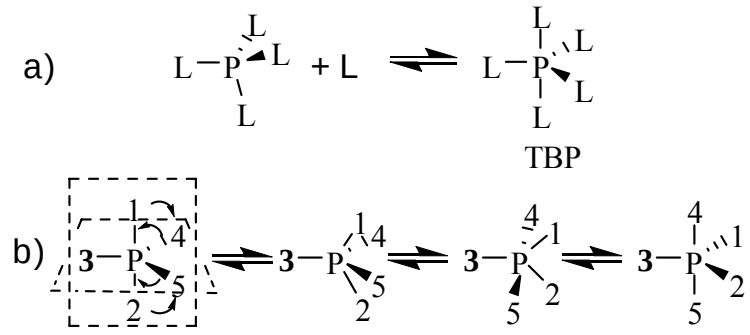
Point A: The Role of Pentacoordinate Phosphorus Intermediates in Biochemistry

Most of the reactions occurring through organophosphorus compounds are guided by the ability of the phosphorus to form “hypercoordinate” intermediates, mainly penta- and hexa-coordinate.[3,4]

For example, phosphoryl transfer reactions, which are basic biological processes, are generally assumed to involve pentacoordinated intermediates, that influence the outcome of the reactions.[5]

The trigonal bipyramidal geometry (TBP) represents the most common structure of

pentacoordinated phosphorus (phosphorane) intermediates.[6] In general, a phosphorane P(L1 L2 L3 L4 L5) with the TBP skeleton can exist as 20 chiral isomers, if all ligands L are different . If two ligands are equivalent, the number of isomers is reduced to 10 which comprise four achiral and six chiral isomers, with respect to the phosphorus configuration. The relative position of the substituents in pentacoordinated compounds depends on their steric hindrance and apicophilicity. Apicophilicity is the relative preference of substituents to occupy the apical positions as opposed to the equatorial positions in trigonal bipyramidal (TBP) structures: a number of experimental results and theoretical calculations have indicated a general propensity of the more electronegative substituents to prefer the apical positions; in addition, bulky ligands prefer the equatorial positions. In addition, the ring size[4h] and its conformational effects[4i] are very important to determine the apicophilicity and stability of pentacoordinated phosphoranes. In particular, pentacoordinated compounds with 5-membered rings are readily formed and fairly stable.[4h] Sufficiently long-lived pentacoordinated intermediates can undergo stereomutation by a Turnstile rotation[6a] (TR) or a by and equivalent process called Berry pseudorotation[6b] (BPR). Both are very rapid processes, since the energy barriers of pseudorotation are usually relatively low.[3,7]



Scheme 1- a) Conversion of a tetracoordinate to a pentacoordinate TBP. b) Berry pseudorotation with ligand **3** as pivot.

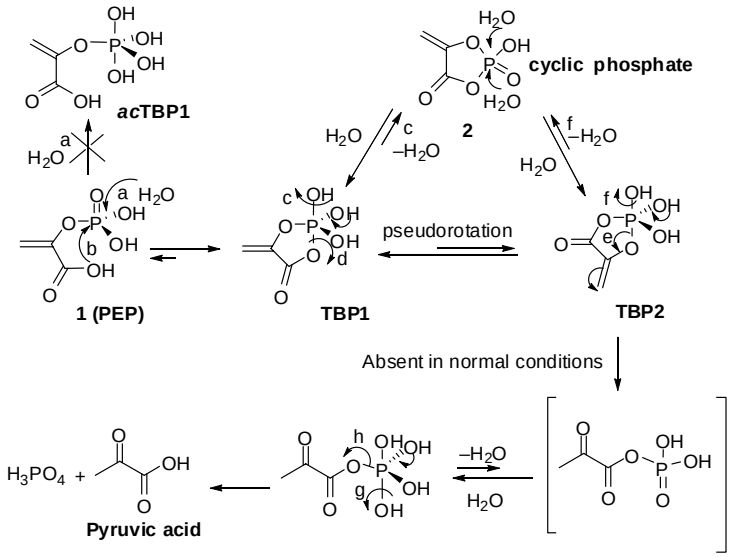
In this manner an isomer can be easily transformed into other isomers; sometime only one isomer collapses to form one final product.[4d] In addition, for these hypervalent intermediates, in solution an equilibrium between their ionic forms may occur. In this latter case, chiral R ligands can be

replaced by S ligands giving two different diastereomers of the hypervalent intermediates. Consequently, from this phenomena a possible homochirality of the final collapsed product may arise. In addition, the stability of these pentacoordinated intermediates strongly depends on their structure; in particular, as reported by Westheimer,[8] when possible, the formation of a ***cycle around the pentacoordinate phosphorus*** intermediate this is favored over that of the corresponding **acyclic** intermediate by a factor of 10^6–10^8. In this way any other possible collateral reaction in which the phosphorus atom belongs to an acyclic pentacoordinate intermediate is practically absent. From these considerations it can be deduced that the **super-activated formation of cyclic pentacoordinate phosphorus intermediates might be a possible candidate for the hypothesized important "self-organized or autocatalytic process"** acting either on simple molecules or on complex molecules in processes in which generally phosphorylation or dephosphorylation reactions are involved. These processes are the centrepiece for the origin and evolution of life. In other words, when we have a cyclic reagent, containing almost a phosphorus atom in the ring, the reactivity of this phosphorus group is more reactive by a factor of 10^6–10^8 with respect to the corresponding acyclic reagent. Many studies reveal that several enzymatic phosphoryl transfer processes such as decomposition of RNA, DNA and many others involving the so-called "high-energy" biomolecules (e.g phosphoenol pyruvic acid, PEP[9] or cyclic adenosyl monophosphate, cAMP[3a]) take place *via* pentacoordinate phosphorus transition states or intermediates.[3e,f,g,h]

Point B and C: Why PEP is a "High-energy" Compound.

PEP[9] is a compound very stable in aqueous solution. In contrast, when PEP is in the presence of an alcohol it becomes unstable and an excellent phosphorylating agent.[10] In order to explain the reason of these contrasting behaviours we recently have studied[9b] the non-enzymatic hydrolysis of PEP by following the reaction course through ^{31}P NMR spectroscopy. We have demonstrated[9b] that PEP in water, at room temperature, exists prevalently as a very stable cyclic pentacoordinated phosphorus compound **TBP1** in equilibrium with other cyclic pentacoordinated forms.(Scheme **2**) It should be

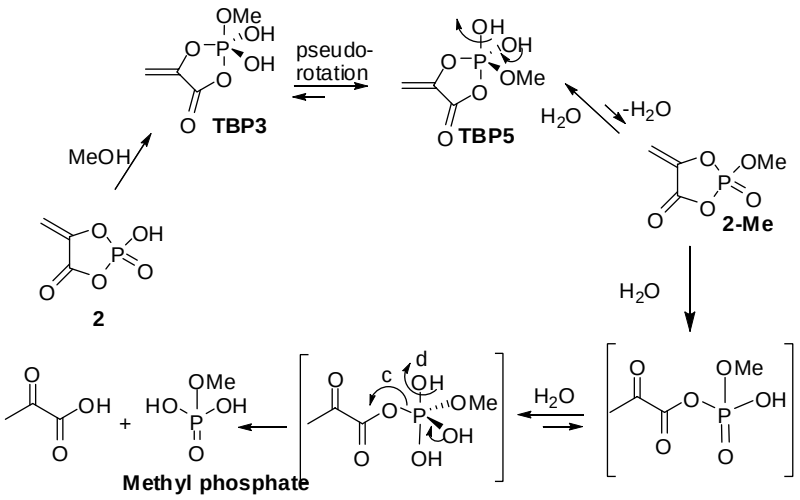
noted that single crystal X-Ray diffraction studies have revealed that PEP is an acyclic compound. [11] Probably, the true powerful phosphorylating agent within the mixture of intermediates is the cyclic phosphate **2**. In fact, addition of water to **2** is very fast because occurs at a phosphoryl group of a cyclic compound which is activated[8] of about 10^{6-8} fold with respect to a correspondent acyclic compound. This mechanism explains that the great stability of PEP in water in neutral conditions is due to the intermediate **TBP1** with respect to the intermediate **TBP2** because PO-CO- group is more apichophilic than POC=C- group. On the contrary, when the reaction is carried out in the presence of Mg++ions, because coordination at the POC=C- this group becomes more apicophilic than PO-C=O. In this case the intermediate **TBP1** can interconvert to the more stable isomer **TBP2,** favouring the hydrolysis of PEP or **2**.



Scheme 2- Mechanism of non-enzymatic hydrolysis of PEP.

Addition of methanol to 2

The dissolution of pure **2** in dry methanol gives rise to phosphorylation with immediate formation, at room temperature, of methyl phosphate (Scheme 3) because **TBP5** is more stable than **TBP3**. This behaviour is due to the fact that an OH group is more apicophilic than OMe group. If the reaction is carried out with an alcohol larger than methanol, this preference becomes higher with respect to methanol.



Scheme 3 - Phosphorylation of methanol starting from pure **2**.

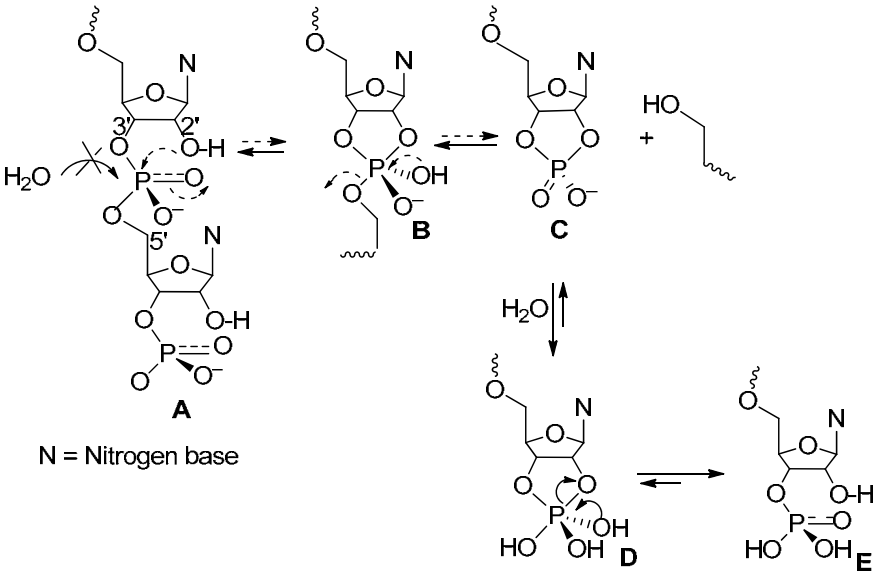
Similar results were obtained starting from pure PEP. Therefore, the results explain why PEP,ΔG^{01} (kJ/mol)= -61.2, is stable in water and prefers to phosphorylate an alcohol. The same stability in water may be extended to all cyclic phosphates. For the "high-energy" compound cyclic phosphates c-AMP ΔG^{01}(kJ/mol)= -50.4, the explanation of the easy formation of phosphorylated products can involve the same mechanism process observed with PEP. Therefore, this process is very sensitive to variations of reactions conditions: the PEP is very stable in water but unstable in the presence of other nucleophiles or Mg++ ions and it becomes an excellent and fast phosphorylating agent. The fact that this excellent phosphorylating agent, PEP, like others cyclic agent, is very stable in water, and therefore not hydrolysable under normal conditions, makes us understand that the water can be the best solvent to use in the primitive Earth. The use of other solvents such as formamide,[12] very unlikely as prebiotic solvent, would allow a high solubility and hence a high concentration of all of the various organic compounds which would have been in that primordial soup. The low water solubility of some molecules necessary for the formation of pre-RNA, such as adenine, that could slow down the process, would be counterbalanced by the enormous rate[8] of reaction of the super-activated process conducted on cyclic compounds of phosphorus.

Point D:The Catalytic Ability of RNA can be Explained by the Reported Mechanism of its Hydrolysis[13]

In the light of the results described above, we wish to propose a revision of the generally accepted[13] mechanism (Scheme **4**) for the self cleavage of RNA. In particular, intermediates **B** and cyclic

phosphodiester **C**, are very similar to intermediates **TBP1** and **2**, involved in PEP. Once formed, **C** can easily undergo an attack, activated by its cyclic form, by the nucleophile H_2O, with formation of the stabilized cyclic pentacoordinate intermediate **D** which then collapses, giving the product of hydrolysis **E**. The intermediate **C** can also undergo the facile elongation and/or polymerization of RNA, which is simply to the reverse (normal arrows in Scheme 3) of the cleavage reaction (dotted arrows). Therefore, the phosphate **C** , similar to the cyclic phosphate intermediate **2** of PEP,[14] could be considered the true "catalyst" or activator of the ribozyme in the so called catalysed transformation of RNA.

It should be noted that the **O-2'** group is more apicophilic and leaving group than **O-3'** in all pentacoordinate cyclic intermediates as those shown in Scheme **4.** This is due to the presence of the N group (N represent one of the four natural nucleotide base moieties), which is more electron-withdrawing than **C-5'**.



Scheme .**4** Proposed mechanism of self-cleavage of RNA with formation of cyclic phosphodiester **C** causing the 3'-5' bond cutting of the RNA chain. Structure **A** represents the 3',5'-phosphodiester linkage in the ground-state configuration. N group represents any of the four natural nucleotide base moieties. Dashed lines depict the continuation of the RNA chain.

By analogy, the latter observation explains the almost exclusive ligation of phosphoryl group in **O-3'** position in the RNA chain. In addition, this mechanism explains both the vulnerability of RNA to

undergo hydrolysis, in contrast with the great stability of DNA. The **2'** -OH group makes RNA less stable but it permits, because the intervention of the cyclic phosphate **C,** to catalyze many transformations without the presence of enzymes.

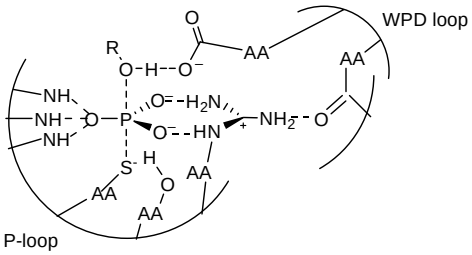


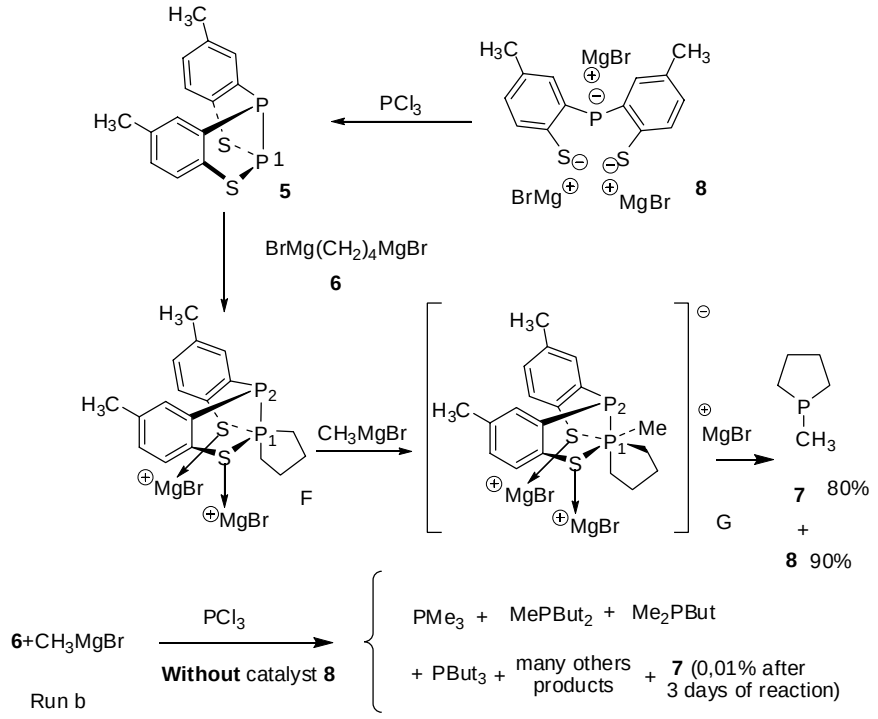
Figure 1-The phospho-enzyme intermediate (E-P) in the action of protein tyrosine phosphatase is assumed to be pentacoordinate.[15]

In contrast, due to the lack of 2'- hydroxyl group, DNA is very stable and for catalyzing some tranformations, as its replication, it needs the assistance of enzymes through their interactions in specific positions of the DNA chain that form the cyclic pentacoordinated phosphorus intermediates as reported[15] in Figure 1.

Point E: Why RNA Folding in a Specific position is very Important for its Chemical Functionality and probable Homochirality[15c] in this Process.

In some of our recent studies,[4f 16a] we noted that, when a compound containing the P atom is polycyclic, the stability of pentacoordinated or /and hexacoordinated phosphorus intermediates[3,4] increases further and the rate of reaction on the P atom remains even at low temperature. In fact,-we found a simple and very fast process of transfer of a phosphorus atom, in order to obtain, from **5** and an incoming mixture of different Grignard reagents (*i.e.* bis-Grignard $BrMg(CH_2)_4MgBr$ (**6**) and CH_3MgBr) easily cyclic tertiary phosphines as **7,** in 80% yield after only 30 min. at room temperature(See Scheme **5**, run a).This process provides an enormous rate enhancement for the formation of **7** compared to the reaction, *run b*, under the same conditions with PCl_3 and the same mixture of Grignard reagents which gives a very complex mixture of acyclic organophosphorus compounds with a very low yield (~0.01%) of compound **7**, after 3 days of reaction (See Scheme ~~4,~~ 5 *run b*). These results clearly demonstrate that the facile formation of the cyclic pentacoordinated **F**

and hexacoordinated phosphorus intermediate **G** is the driving force that allows the disorder of the corresponding un-activated process , *run b*, to be overcome. In addition the molecule **5** has a particular folded structure,[4f 16a] in which the angles around the phosphorus require only a small deformation as the structure is transformed from a tetrahedral to the pentacoordinated trigonal bipyramidal intermediate F. This folded effect in reagent **5** may be helpful to explain the relation between the three-dimensional structure of RNA in a specific position, and its catalytic function and its easy bond rupture. (depicted in Scheme **4**).The first attack of 2'-oxygen to the phosphorus atom, is possible only in a precise position of RNA in which its folded structure facilitates a trigonal bipyramidal intermediate **B** and then only in this position catalysis or bond cutting of the RNA chain , can occur.



Scheme 5 - Reaction and mechanism of **5** with an equimolar mixture of **6** and CH3MgBr. *Run* b, the same reaction with only PCl_3.

In other words, RNA must adopt complex three-dimensional folds that create specific positions with distorted angles around P atom near the angles of a trigonal bipyramidal to facilitate the formation of TBP intermediate to obtain very fast chemical transformations or a breaking of the bond POC at a specific position. It has also been noticed that in these reactions when **5** is treated with asymmetric

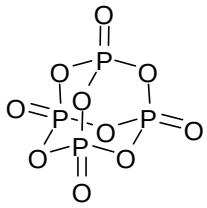
compounds such as **6,** final products with high stereo selectivity are obtained.[4c] Therefore we have a first indication of a possible homochirality[15c] of the final products.

In fact, when racemic ligands are added to racemic form a penta- or hexa- coordinated phosphorus intermediates giving a mixture of diastereoisomers which may have different solubility or stability; by subsequent decomposition of only one, for example the one that remains in solution, it may give final homochiral product.[17]

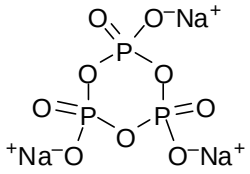
Phosphoric Anhydride and Trimetaphosphates are Primordial Cyclic Phosphorus Compounds and Prebiotic Reagent.

Now, after having found this super activated chemical process, we have to test whether the early Earth had the availability of some cyclic derivatives of phosphorus that might have initiated a process that might have had all the features that we have listed above. The role of phosphorus derivatives and its availability in primitive life forms on the Earth has been the subject of numerous scientific articles. The apparent enigma of the role of phosphorus in chemical evolution, which is the element indispensable in living organisms, is in contrast to its limited availability. But this apparent contradiction shows that today its limited availability is due to the fact that a large part of the phosphorus is concentrated in all living organisms. Then the concentration of phosphorus derivatives in the primitive Earth had to be huge and probably it would be the result from volcanic emissions. In fact, Yamaghata demostrating[18] that the P_4O_{10} is produced from volcano magma is, in this context, very important. In addition, if the volcanic gas containing P_4O_{10} is dissolved in water the main product in the hydration of P_4O_{10} is trimetaphosphate (TMP) which is another cyclic compound containing three phosphate groups. Then the first primordial cyclic reagents are very likely P_4O_{10} and its derivative TMP. Phosphoric anhydride was and is still widely used as a dehydrating agent but only a limited number of studies are conducted in water. Nevertheless several "magic" or unexpected reactions were reported[19] which involved the use of phosphoric anhydride . Probably their interesting and unexpected reactivity reported[19a] in many articles is due to their

cyclic structures. It should be noted that these compounds are stable in water and our explanation is the same that we have given for the PEP. The TMP is very stable and it is hydrolyzed only after several months in water under normal conditions. On the contrary, P_4O_{10} begins to hydrolyze only after several days. P_4O_{10} is known as the most powerful drying agent and this has always discouraged its use as phosphorylating and condensing agent in water.



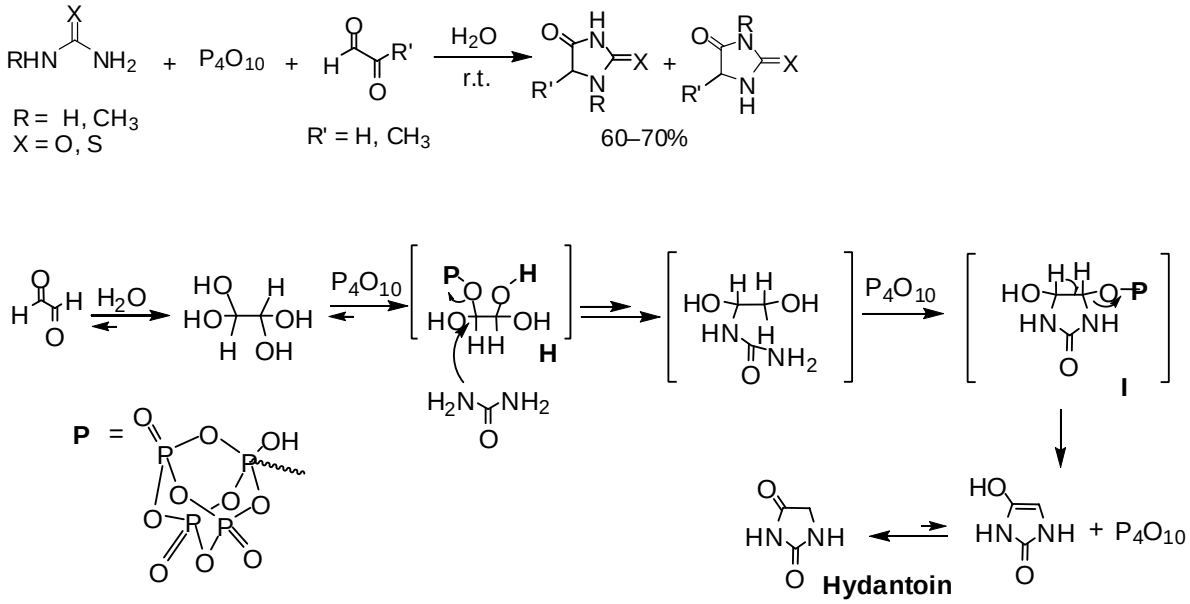
P_4O_{10}



TMP

Point F: Prebiotic Synthesis of Nitrogen Heterocycles , Protein , Nucleotides, Nucleosides , Polynucleotides and ATP

Recently we have reported[19b] that a series of hydantoins and thiohydantoins can be synthesized in water at room temperature from urea (or N-methylurea, or thiourea) and simple aldehydes (as glyoxal, and its simple derivatives) in the presence of phosphoric anhydride. The reaction time is 10 min. by using an equimolar amount of P_4O_{10} with respect to the other reagents, but the reaction occurs also with very small amounts of P_4O_{10}.(Scheme **6**)



Scheme 6 - Synthesis of hydantoins and its proposed mechanism.

The initial step probably involves the hydration of the aldehyde and then the phosphorylation by P_4O_{10} of two hydroxy-groups with formation of the intermediate **H** which is a cyclic, pentacoordinated intermediate. The subsequent nucleophilic attack of urea gives condensation and cyclization with probable formation of intermediate **I** which collapses to hydantoin. In the process there is reformation of P_4O_{10}, as shown in the decomposition of intermediate **I**. The final hydrolysis of P_4O_{10} begins only after several days as evidenced[19b] by P^{31} spectroscopy. Similarly a series of glycoluril derivatives have been synthesized[19c] in water at room temperature from urea and α-dicarbonyl in the presence of phosphoric anhydride. From these reactions it is clear that P_4O_{10} could phosphorylate the OH groups even in water but also favours the next attack of a nucleophile and to give a final condensation. We have little data[19a] on P_4O_{10} used in water as an activating agent to subsequent nucleophilic attack and condensation. In contrast, phosphorylation reactions conducted in water using TMP are numerous, probably because its stability in water is well known. Then after noting that P_4O_{10} and TMP may be used in water, always at room temperature, to phosphorylate and to condense, now we must verify whether we have examples in literature of synthesis, in prebiotic conditions, of nucleosides and nucleotides for a final pre RNA. Almost nothing about the synthesis of nucleosides using P_4O_{10} and TMT in water is found in the literature. The reported[20a] very low yields (4%) of adenosine nucleoside obtained by adenine and D-ribose with TMP at 100°C, for

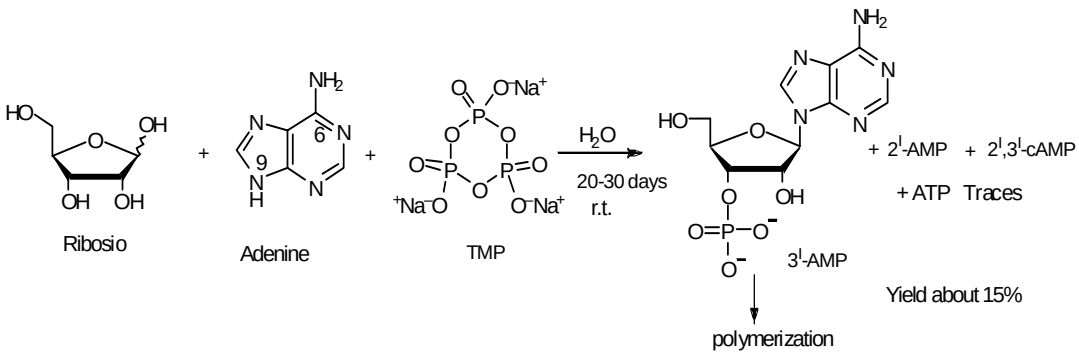
several hours, might be explained, today, by the concomitant formation of adenosine phosphates and its polymerization (or by the decomposition and isomerization of adenosine at 100°C) which were not considered by the authors. In contrast, several papers are reported regarding phosphorylation of nucleosides for obtaining nucleotides. Schwartz [20b](1969) first reported the phosphorylation of adenosine with trimetaphosphate in strong alkaline solution to yield 2'- and 3'-AMP. Saffhill[20c] (1970) and Etaix and Orgel[20d] (1978) investigated more precisely the reaction between nucleoside and trimetaphosphate, and they found the nucleoside 2',3'-cyclic monophosphate in the reaction mixture at lower pH. Tsuhako *et al*[20e]. (1984) demonstrated that all ribonucleosides are readily phosphorylated by sodium trimetaphosphate to form their 2'- and 3'- and 2', 3'c-monophosphates under various conditions and ratio of the reagents. For adenosine at pH 12- 7 and at 70°C after 17- 25 d. gives 37% and 47% respectively of the 2'- and 3'-monophospates and only traces of 2', 3'c-monophosphate and subsequently they decreased gradually. The pH of mixture decreased and reached about 7 after one day. When the reaction is carried at room temperature, with a ratio TMP: adenosine of 5:1 a total yield of 19% after 20 d. was observed. Yamagata *et al.* [20f](1995) discovered that the reaction of trimetaphosphate with adenosine could occur in neutral water solution under the catalysis of magnesium ion to afford mainly 2',3'-cyclic AMP. This is a more plausible condition on the primitive earth, but the yield of phosphorylated adenosine is still low (the limit of the yield is about 9%). Zhao (2002) reports[20g] the phosphorylation of adenosine with trimetaphosphate in aqueous solution, in solid phase and using wet-dry cycles. The main phosphorylated products were 2', 3'-cyclic AMP (10.4%) and 5'-ATP (13.0%). Zhao (2009) reports[20h] also the N-phosphorylation of amino acids by trimetaphosphate in aqueous solution with yields of 60~91%.These compounds are also intermediates in peptide formation at room temperature.

A few months ago, I rediscovered two important articles,[21a,b] which have been almost forgotten: one published in 1958[21a] and another in 1961[21b]. Probably the title and the text were misleading. In fact they report[21a,b] to have used unidentified phosphoric esters , while later they found[21c] that these

latter were TMP esters. In the first paper[21a] it is surprisingly reported that TMP esters react with several aminoacids, to give "protein in mild conditions and without racemizazion". [2d] The second paper[21b] reports that in similar conditions were obtained polysaccarides, nucleosides and nucleic acid by spontaneous polymerization of nucleotides which were difficult to separate. Schramm, for example, prepared adenosine from ribose and adenine in good yield, 20-40 %, relative to the quantity of sugar. The substitution occurred only at the 9-position of adenine and the amino group in the 6-position was unaffected (See Scheme **7**). In order to prevent polymerization of the sugar, the reaction was carried out in the presence of an excess of base and to prevent the sugars decomposition a certain amount of water in the solvent is advantageous. The reaction mixture, ribose, adenine and TMP (in very large excess), in dimethylformamide –water and 0.5 ml of concentrated HCl, is kept at 50°C for 20 hrs. The concentration of adenine was about $7{,}3.10^{-3}$M, in excess respect to the D-ribose. Schramm[21b] reports also the direct formation of "polynucleotides in one step directly from nucleosides by means of phosphorylation of nucleosides and subsequent condensation". The facile polymerization is probably due to the high concentration of nucleotides and a reaction temperature of 50°C. Recently it has been reported[22] by other authors that this polymerization takes place spontaneously even without TMP or other activators. In conclusion Schramm in 1961 understood that compounds containing free amino and hydroxyl groups can be activated by reaction with TMP esters obtaining easily nucleoside from sugars and adenine. If the compounds contain a second functional group, polycondensations are possible; e.g. amino acids give polypeptides, carbohydrates give polysaccharides, nucleotides give polynucleotides. The only problem of the synthesis of Schramm it can hardly be defined as a prebiotic condition being conducted in dimethylformammide –water. Probably he had to use this mixture of solvents because adenine is slightly soluble in water. The concept that guided Schramm experiments is very simple: using TMPesters to activate hydroxyl groups and verifying if these new phosphate esters are activated to undergo a nucleophilic attack with expulsion of the phosphate group. A concept that, already as a student, I used for the synthesis of amides at room temperatures starting from

carboxylic acids and amines using phosphonitrilic chloride as activator of carboxylic acids. [23a] I have always used this concept in researching new synthesis at ambient temperatures of different nitrogen heterocycles such as indoles, [23b] pyrroles[23c] and others[23c] using phosphorus reagents in which cyclic phosphorus pentacoordinate intermediates are involved.

Recently we have reported[24] that adenosine monophosphate isomers are obtained by self-assembling of adenine, D-ribose and trimetaphosphate in aqueous solution and at room temperature in yields of about 15% and in one-pot reaction (See note on ref. 24 and Scheme-**7**). The process is activated if in the solution Mg^{++} ions are present. The reaction was carried out in aseptic water solution (UV, without sodium azide) (pH ~ 7.0÷6.5) with high dilution of the three reagents (1.85 $x10^{-4}$ M). At this concentration value all the reagents were completely dissolved. This concentration is about 40 times more dilute than that used by Schramm[21b] (see above) and for this in our case the polymerization is slow and it permitted the identification of AMPs. The reaction course was followed by HPLC/MS for several days. It has to be noted that the use of $P_4O_{10,}$ instead of TMP, gave a similar behaviour but with minor yields in AMPs because major instability in water that gives rise, after several days, to phosphoric acid. After the first 20-30 days it begins to form a flocculent white precipitate which is probably short oligonucleotides and for this reason the adenosine monophosphate isomers diminish or disappears after longer time. This spontaneous elongation or polymerization was expected as depicted in Scheme **4**.

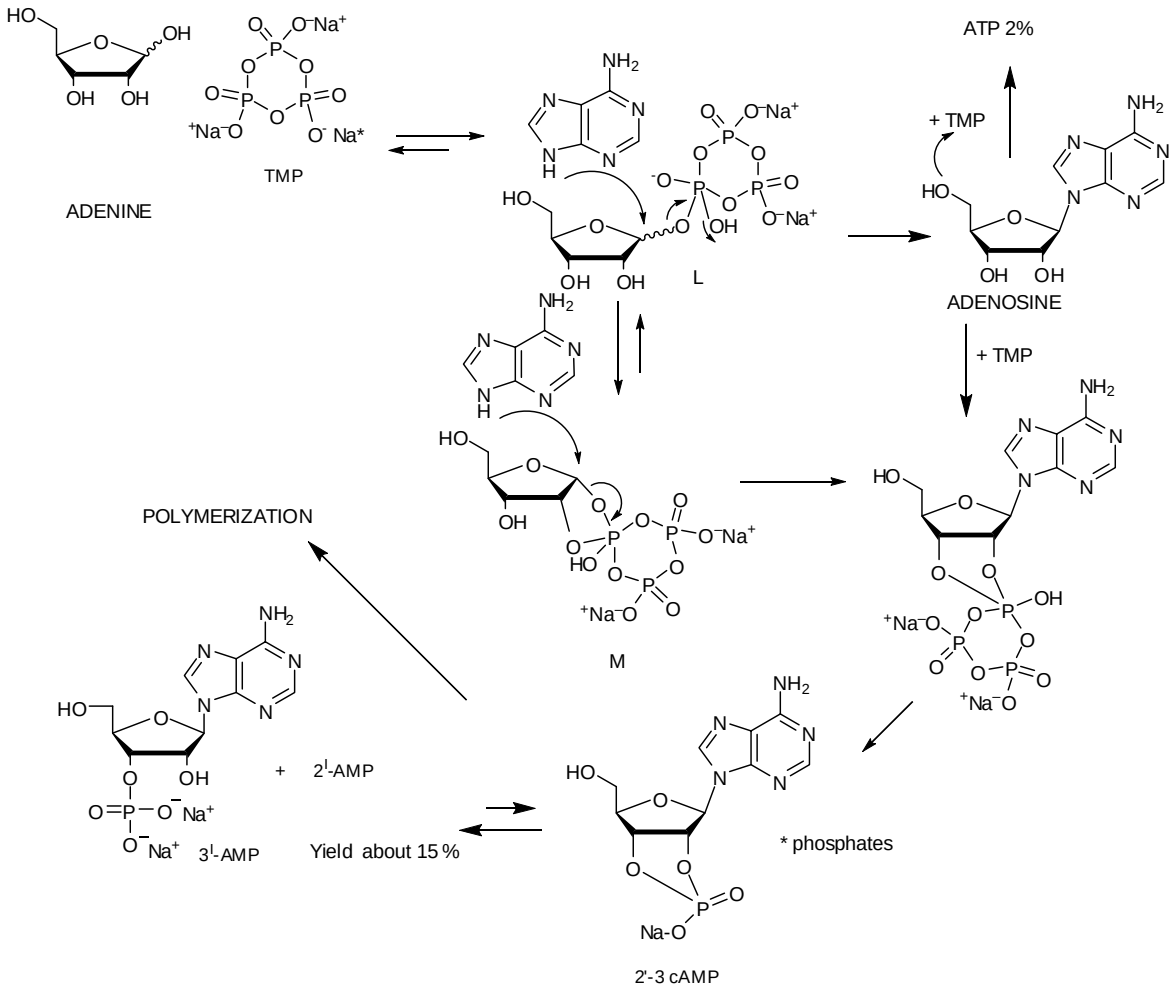


Scheme 7 One-pot generation of adenosine monophosphates in aqueous solution from adenine, D-ribose and TMP.

Our results are in good accord with the findings of Schramm[21a,b,c] that give practically the same yields in adenosine and that, subsequently, it is phosphorylated and polymerized *in situ* making difficult the identification and separation of AMPs. This easy polymerization of nucleosides also explains the low and highly variable yields of AMPs obtained both by us and by other authors. [20] In Scheme **8** we proposed the mechanism of this one-pot synthesis in which is evidenced the probably preferred formation of bicyclic pentacoordinated intermediates **M** and **N** giving AMPs respect the monocyclic intermediate **L** giving probably ATP, obtained in small amounts in this reaction. We should note that Zhao[20g] (see above) obtained with the same reagents in different conditions ATP in 13% yields, always remembering that the yields of these products are highly variable. Then also ATP is a product easily formed in prebiotic conditions with TMP.

CONCLUSION

In conclusion, the origin and evolution of life might be governed also by a chemical process in which the formation of cyclic pentacoordinated phosphorus intermediates is activated by a factor of $10^{6\text{-}8}$ over other similar collateral processes. In other words, it is necessary to find primordial cyclic phosphorylating reagents such as P_4O_{10} and TMP in order to obtain very important biochemical molecules as proteins, natural heterocyclic bases, AMP isomers, ATP ,etc with very fast processes in prebiotic conditions. In addition other results demonstrated that it is very simple to put together the three components D-ribose, adenine and TMP to generate spontaneously in a prebiotic one-pot reaction and with high chemio-, regio-, and stereoselectivity, adenosine monophosphates and its oligonucleotides with also small amounts of ATP. This process might explain the spontaneous generation of pre-RNA molecules in a primordial Earth.



Scheme 8 - Proposed mechanism

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This article has been retracted as requested by the Editor, after solicitation of J.Sutherland, seven months after publication, because the reported yields (37 -45%) of AMPs were not reproducible and that the final polymerization was unproven. Consequently we carried out again a reaction monitored with a HPLC / MS and found yields of about 15% and also the formation of traces, 2%, of ATP that was not found with the previous HPLC analysis. On the light of these results that were communicated to the Editor we should be allowed a revision of the paper.