A molecular vestige of the origin of life on minerals: phosphoribosyl-pyrophosphate

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Abstract: In this contribution, we report the prebiotic formation of phosphoribosyl pyrophosphate (PRPP) as a molecular precursor in the one pot synthesis of a canonical nucleotide, namely adenosine monophosphate (AMP) from its building blocks (KH_2PO_4 or "Pi", adenine, D-ribose), on a fumed silica surface. The “on the rocks” approach[1] has been successfully applied to the simultaneous phosphorylation and glycosylation of ribose. The one pot formation mechanism of AMP involves a two-step pathway going through an activated intermediate, namely PRPP, obtained by multiple ribose phosphorylations upon mild thermal activation.

The quest for the origins of life is a very popular topic that has been extensively studied since the 1920s. One of the main questions concerns the nature of the primordial molecules that have allowed the emergence of metabolism and all biochemistry. The aim of prebiotic chemistry is to develop reasonably simple chemical scenarios that are compatible with the geochemical properties of the early Earth. In the first place, it is important to limit the number of chemical steps, and successive reactions must be carried out in the same environment: the ideal process should be a “one pot synthesis” from simple building blocks.

It was soon realized that among biomolecules, RNA is a plausible precursor for both informational and catalytic functions: this is the “RNA-world” hypothesis[2]. This scenario demands that the monomers of RNA, i.e. nucleotides, be formed first.

Indeed, elegant work has been carried out demonstrating the possibility of nucleotides synthesis from simple organic species[3]. For example, the group of J. Sutherland has proposed the formation of nucleotides from a precursor common to the base and the sugar, namely glyceraldehyde.

To the chemist, a nucleotide appears as made of three components: a nucleobase, a sugar (D-ribose) and a phosphate group. Phosphate could have been available in solution[4] and the prebiotic syntheses of sugars and nucleobases, although not trivial, have been extensively studied. Ribose can be formed in the “formose reaction” through successive condensations of formaldehyde[5]. Nucleobases can also be formed, e.g. from formamide[6]. Both formamide and formaldehyde are simple primitive organic compounds, likely present on the early earth.

However, the synthesis of a nucleotide by condensation of its three components (implying two condensation reactions, phosphorylation and glycosylation) is unlikely in aqueous solution. The main problem is that condensation reactions are thermodynamically unfavorable in water[7]. In addition, ribose is rather unstable[8]. Thus, every reported successful nucleotide synthesis from these precursors has involved either the use of activating agents[9] or of non-canonical nucleobases[10]. This is why alternative scenarios to aqueous phase condensation have been explored. A recent theoretical work has proposed that ribose diphosphate could be formed first as a high energy intermediate in nucleotides formation[11]. If such phosphorylated sugars are indeed a crucial intermediate, it is important to take into consideration phosphorylation processes as proposed by Schwartz[4b] or Pasek[12].

Carrying out condensation reactions on mineral surfaces could avoid the thermodynamical problem by stabilizing and confining molecules during a drying process[13], when the low water activity would shift the condensation equilibria to the right. Minerals have indeed been used for oligonucleotides formations. Orgel and Ferris have formed nucleotides[9] up to the 55mers on clay minerals; however they needed to use imidazole activated precursors. In a related reaction, we have previously demonstrated that scenarios incorporating mineral surfaces allow the polymerization of amino acids into peptides[14] through a related condensation reaction. Finally, it has been established that silica can stabilize ribose and also promote the condensation of mono- to polyphosphates[15].

In the present work, we have studied the reactivity on silica surfaces of mixtures containing the three components of nucleotides, namely Adenosine (A), D-Ribose (R) and inorganic monophosphate (P). The first elements of information come from ^{31}P NMR. This technique can identify different environments of phosphate groups. It is highly responsive to the existence of P-O-P bonds, and less so to P-O-C bonds. As a result, one can easily differentiate monophosphates (Q^0) from polyphosphates: among the latter, terminal phosphate groups resonate in the Q^1 region (one P-O-P bond), while central phosphates (present in ATP, but not in the species considered in this communication) resonate in the Q^2 region (two P-O-P bonds). Monoesters such as AMP can be discriminated from ADP, ATP, but the chemical shift differences between different monoester species are less important, and their interactions with silica surface groups may

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induce further shifts leading to difficulties for their NMR characterization. Therefore pristine references were also analyzed in ^{31}P NMR spectroscopy; they include Pi/SiO_2, R5'P/SiO_2 (ribose-5' phosphate) and AMP/SiO_2.

In a dry P/SiO_2 powder (i.e. a sample where phosphate ions were deposited without organic molecules), three phosphate environments are observed (two Q^0 and one Q^1). The Q^0 peaks, at 0.8 and 1.5 ppm, are characteristic of adsorbed monophosphates interacting with different surface groups. The Q^1 environment at -8.0 ppm is characteristic of adsorbed pyrophosphate (diphosphate) groups, indicating that concentration and drying are sufficient to form activated phosphoric anhydride (P-O-P) groups by P-OH condensation[15 b]. Upon activation at higher temperatures, the monophosphate contribution decreases and the diphosphate increases, while the appearance of a Q^2 peak betrays the appearance of longer phosphate chains.

The reference AMP/SiO_2 was studied next. Its spectrum shows a single Q^0 peak centered at 3.9 ppm; R5'P/SiO_2 (Figure 1A) also has a single Q^0 peak, at the slightly different position of 2.9 ppm. Since regioselectivity is important for our purposes, we tried to determine how sensitive the ^{31}P chemical shift is with respect to the position of the phosphoryl group. As we did not have genuine samples of ribose phosphorylated on other positions, we simulated the ^{31}P spectra of 1', 2', 3' and 5'-phosphorylated ribose using the ACDLab program. The calculated resonance positions for R-1'P, R-2'P, and R-3'P were separated by 2 to 3 ppm from that of R-5'P. Thus, we can consider that a peak in the 2.9 to 3.9 ppm range is diagnostic of a phosphoryl group on the 5' position of ribose.

We have then prepared a sample containing ribose and monophosphate on silica without adenine (R+P/SiO_2) in order to study possible phosphorylation reactions. The ^{31}P NMR spectrum of this sample showed two new peaks around 2.9 and -10.1 ppm (compare Figure 1 B and C). The peak at 2.9 ppm, as remarked earlier, would be diagnostic of a phosphoryl group on the 5' position of ribose, as in R5'P, but also in PRPP (+3.7 ppm in a solution of the genuine compound). Moreover, the new environment at -10.1 ppm may correspond to the phosphate in the "α" position in the di-phosphorylated ribose, which is observed at -11.0 ppm in aqueous solution. The phosphate group in the "β" position was not observed: in fact, it is probably not resolved from the signal of the inorganic diphosphate groups, since it is expected to resonate at -5.4 ppm. As a matter of fact, a decomposition of the Q^1 signal indicates a significant increase of the intensity in this region, which may indicate a contribution of the β phosphate group. The other two contributions that we tentatively assigned to PRPP, at 2.9 ppm and -10.1 ppm, have the same relative intensity as expected (4.6 % of the total phosphorus).

We tried to modify the reaction conditions in order to evaluate the effect of these changes on the amount of the suspected PRPP component. Increasing the reaction temperature, from 70 °C to 130 °C caused an increase in the signals assigned to PRPP (Figure 1C). This would be compatible with the endothermic nature of the PRPP formation if the reaction is under thermodynamic control; however, additional experiments would be necessary to disentangle this effect from the effect of of variable drying efficiency, inducing variable water activity. We also increased the amount of inorganic phosphates by a factor of two, and the apparent yield of PRPP increased accordingly.

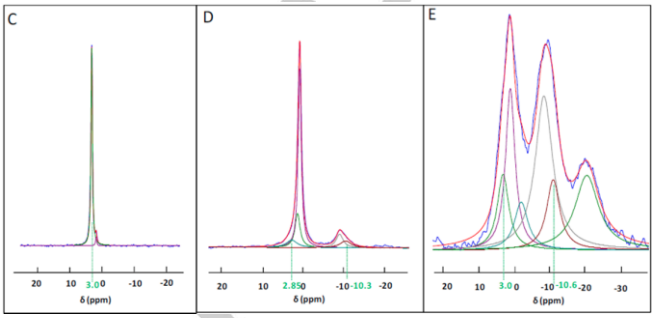


Figure 1 ^{31}P NMR (1 pulse) RP5'/SiO2 (A) ; (R+P)/SiO2 (B) ; (R+P)/SiO2 at 130 °C (C)

After co-adsorption of ribose, adenine, and inorganic phosphate on silica (sample A+R+P/SiO_2) and drying at 70 °C, Q^0 and Q^1 were predominant with a small amount of Q^2. The Q0 environment could be decomposed into three Gaussian components at 3.9, 1.2 and -0.7 ppm representing 10, 46 and 14 % of the total 31P respectively (Figure 2B). The existence of a peak at 3.9 ppm was confirmed by cross-polarization, which showed a clearly separated signal at this position. In comparison with the reference samples discussed above, the latter two peaks can be assigned to adsorbed monophosphates, while the peak at 3.9 ppm is suggestive of the existence of AMP molecules.

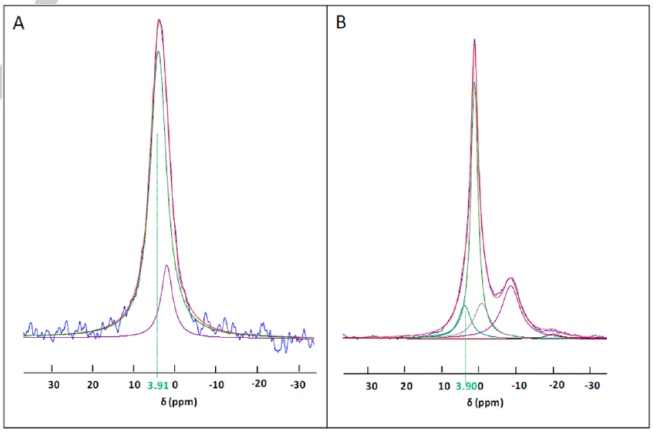


Figure2 ^{31}P NMR (1 pulse) of AMP/SiO2 (A) ; (A+R+P)/SiO2, dried at 70°C (B).

To confirm this conclusion, the organic matter was desorbed from silica, and mass spectrometry (MALDI) was performed in positive and negative mode (Figure 3A,B). In the positive mode we observed protonated AMP ($C_{10}H_{14}N_5O_7P$-H^+, signal at 348 amu), and protonated adenosine (which of course cannot be detected by ^{31}P NMR; a signal at 268). In the negative mode, a signal at 346 amu was assigned to deprotonated AMP ($C_{10}H_{13}N_5O_7P^-$). By comparison of the relative intensities with

those in solutions of genuine compounds, the yield of adenosine formation could be evaluated at approximately 20 %, which would be 10 times higher than literature results in a similar system[16]. This semi-quantitative assessment is based on the comparison of the relative MALDI peak intensities in our sample with those in a reference mixture of adenine and adenosine. We did not attempt to assess the AMP yield, but it is far from negligible.

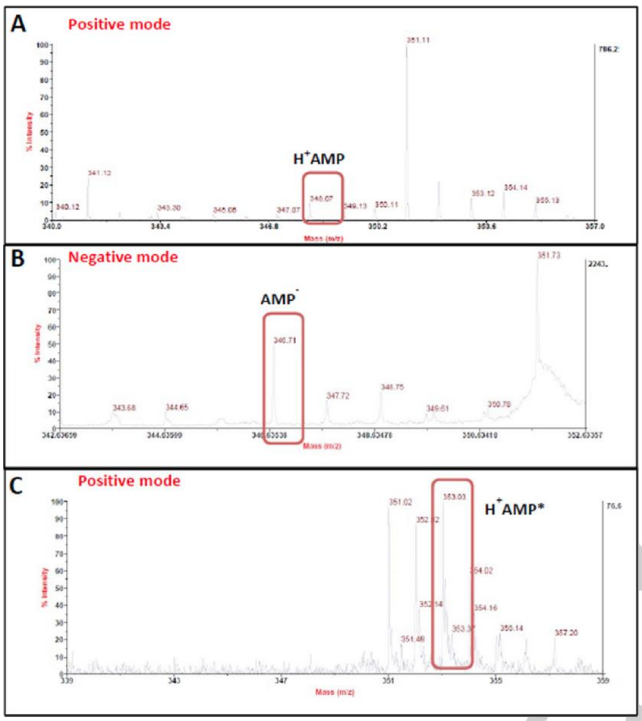


Figure 3 Mass spectrometry of A+R+P/SiO2 after desorption. A: positive mode; B: negative mode; C positive mode of (A+R*+P)/SiO_2 where R* corresponds to a ^{13}C-labeled ribose.

A further confirmation of the formation of AMP was provided by isotopic labeling. A sample was prepared starting from fully ^{13}C labeled ribose, and the desorption solution was analyzed by MALDI in the positive mode (Figure 3C). The peak at 348 amu corresponding to protonated AMP was no longer present, but a new peak was observed at 353 amu, corresponding to a protonated AMP containing five ^{13}C. A similar observation was made for the adenosine signal.

If we accept that AMP has been formed, the theoretical work of Sponer et al.[11] is useful to understand its possible formation mechanism. These authors were looking for a pathway that could make the formation of nucleosides exergonic. Their calculations showed that if the ribose sugar (R) is first phosphorylated to give ribose diphosphate (RPP), the second step of glycosylation is then thermodynamically favored because the diphosphate PP is a good leaving group. It is straightforward to adapt this mechanism in order to explain the formation of AMP:

R + 3P = PRPP + 3 H_2O
PRPP + A = AMP + P-P

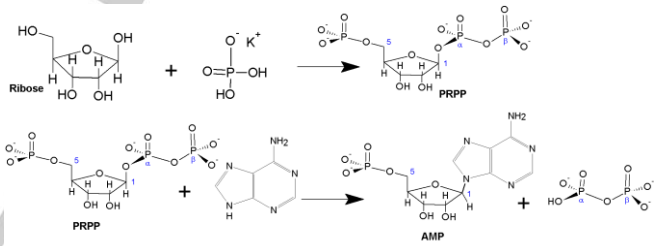
Based on these results, we propose that when the three precursors are co-adsorbed on the silica surface, AMP is formed in two successive steps, following the sequence:

(R + 3P)/ SiO_2 = PRPP/SiO_2 + 3 H_2O
(PRPP+ A)/SiO_2 = (AMP + P-P)/SiO_2

The first step produces a chemically activated molecule, PRPP. This can be considered as a "one-pot" synthesis since no intervention is needed to isolate the intermediate or for any other purpose. While the mechanism proposed here appears reasonable, alternative ones are possible, involving the formation of triphosphate intermediates; additional research will be needed to determine which is correct.

The effect of the activation temperature was checked for (A+R+P/SiO_2), as it had been for (R+P/SiO_2). The activation temperature was decreased from 70 °C to 40 °C. Since the second reaction step is exothermic, a decrease of the reaction temperature should increase the yield of glycosylation. Mass spectrometry indicates that the intensity of the protonated AMP peak increases about twofold, in conformity with the expectations.

In summary, we propose that the synthesis of nucleosides (such as adenosine) and nucleotides (such as AMP) could have occurred from the nucleobase, ribose and monophosphate as precursors in a mineral surface scenario.



Scheme 1 Two steps reactions of AMP formation

The proposed reactions are very simple since the number of different molecular species is small (scheme 1), as well as the number of steps, and since no activating agent is needed other than silica. Moreover, the reaction occurs in a one-pot process and is quite compatible with relevant prebiotic scenarios. PRPP is a crucial intermediate in modern metabolism and from our results it might well be considered as a vestigial molecule from prebiotic times. We will leave open the question of how silica activates ribose phosphorylation: this could be due to the lowering of water activity upon drying, but another possibility is that the pyrogenic silica we used plays the role of a sacrificial activator through the reaction of high-energy surface defects such as strained 3-tetrahedra rings (“D2 defects”), as suggested in a recent work by Rimola et al[17].
Once this molecule is formed, glycosylation is thermo-dynamically allowed and could occur on the surface or even in solution.

Experimental Section

Materials

Potassium dihydrogenophosphate (KH_2PO_4 = P), 5' adenosine monophosphate sodium salts (AMP), 5' ribose phosphate, D-ribose (D) were purchased from sigma Aldrich.

Aerosil 380 silica (BET surface area = 380 m^2/g) was purchased from Degussa. These particles are produced by the high-temperature pyrolysis of silicon tetrachloride.

Adsorption procedure

Co-adsorption of Ribose, inorganic phosphate and adenine was carried out by the method of impregnation with excess water. In this procedure, 15 ml of a solution containing the reagents were added dropwise to 300 mg of dry amorphous silica. This solution contains an amount of ribose which corresponds to 3 weight percent with respect to dry silica, together with 1 molar equivalent of phosphate and the same equivalent of adenine. The fluid dispersion obtained was dried overnight in an oven, typically at 70°C, under room humidity. The sample was stored in a desiccator containing silica gel before analysis. This sample is designated (R+A+P)/SiO_2. Samples (R+P)/SiO_2 and P/SiO_2 were prepared in a similar manner, but using only the mentioned precursors.

Analytical methods

Solid State NMR : single-pulse and CP-MAS sequences were recorded at room temperature with a Bruker Avance 500 spectrometer with a field of 11.8 T equipped with a 4 mm MAS probe with a spinning rate of 10 kHz. The pulse angle was 30°, decoupling was applied at 50 KHz, and the recycle time was 10 s. The contact time was 1000 µs for CP measurements.

Mass spectrometry : Mass spectra were generated using a 4700 Proteomic Analyzer MALDI-TOF/TOF (Applied Biosystems) instrument fitted with a Nd:YAG laser (λ = 355 nm; pulse duration, 4 ns; repetition rate, 200 Hz). All MALDI-TOF spectra, resulting from the average of a tens of or a hundred laser shoots, were obtained in positive and negative ions using the reflector mode in the m/z range of (10–4000). The final solution was vortexed for 1 min at high speed prior to deposition on the MALDI plates. No residual adsorbed organic matter was detected by TG

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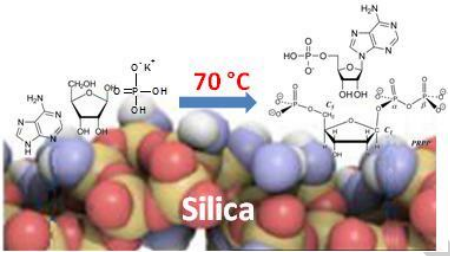

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