The abiotic phosphorylation of nucleosides is a major hurdle in origin-of-life studies. We suggest a plausible pathway for the synthesis of adenosine nucleotides from adenosine and NaH$_2$PO$_4$ under radiative conditions mimicking the solar wind in the presence of a meteorite of the aubrite-type. Hydroxyapatite also performed as a mineral heterogeneous phosphorus source. Adenosine polyphosphate derivatives and inorganic polyphosphates were detected in the reaction mixture, highlighting the high reactivity of the system. Both the total yield of adenosine nucleotides and the conversion of adenosine increased upon performing the irradiation in the presence of formamide (NH$_2$CHO) and aubrite. These experiments simulate conditions in space or on an early Earth fluxed by protons from the solar wind, potentially mimicking a plausible prebiotic phosphorylation scenario.

1. Introduction

Phosphorylation is fundamental for cellular growth and survival. It controls metabolic pathways, as well as the structural organization of the cell and provides reactivity. The biosynthesis of nucleotides and polyphosphates is performed in extant systems by several kinases evolved to reach this goal. The intermediate steps between prebiotic and extant biological worlds have been recently discussed. Pre-biological conditions bona fide entailed simpler reagents and chemical conditions. In this context, the attention has been focused on aqueous scenarios under thermal conditions (range of temperature from 333 K to 373 K), using different phosphorus sources, such as orthophosphate, condensed phosphates, mineral phosphates, and reduced phosphorus(III) reagents. These conditions usually required the presence of condensing agents and/or catalysts to overcome the unfavorable thermodynamic balance of the process. Analysis and review of the role of condensing agents and catalysts in abiotic phosphorylation reactions, such as the role of urea in phosphorylation reactions, are reported in the literature. The fact that urea and formamide act as organic catalysts in some of these processes does not detract from the observation that mineral catalysts could have had an important role, as actually shown in this study and in other previous studies on the syntheses of nucleic acids precursors. Mixtures of positional isomers (acyclic monophosphates and cyclic monophosphates), associated to degradation products, were observed in different amounts depending on the experimental conditions. Early studies on the condensation of water-soluble phosphates to polyphosphates and on the phosphorylation, condensation, or polymerization of biomolecules with polyphosphates have been reviewed. Solvents alternative to water, such as deep eutectic and formamide (NH$_2$CHO) and formamide (NH$_2$CHO) have been also used, meeting different degrees of success, NH$_2$CHO being able to contemporaneously solubilize nucleosides, reagents and active phosphorus species. NH$_2$CHO is among the plausible chemical precursors for the prebiotic synthesis of nucleic acids (for the prebiotic relevance of NH$_2$CHO see SI# 1). It has been detected in dense diffuse interstellar clouds, in the galactic habitable zone, in comets and in satellites. Despite the large attention devoted to thermal processes, the data available on the role that other forms of energy present on the primitive Earth (and in space-wise conditions) might have played in the phosphorylation of nucleosides are scarce. As an example, adenosine was phosphorylated in dry-film conditions at room temperature using vacuum ultraviolet (VUV) radiation or γ-radiation, in the presence of sodium orthophosphate (NaH$_2$PO$_4$) or of more complex phosphorus minerals. Recently, we reported that a high-energy proton beam, modelling the solar wind, can be successfully used for the synthesis of variegated and prebiotically relevant panels of compounds,
including four of the natural nucleosides, formed from NH$_2$CHO in the presence of meteorites (iron, stony iron, chondrite, and achondrite types). In addition, the complete panel of nucleobases in RNA and DNA molecules is synthesized from NH$_2$CHO and meteorites in high-energy-density events modeling the extraterrestrial impact of the meteorite on the surface of the primitive Earth. May high energy protons, meteorites and NH$_2$CHO mediate the phosphorylation of nucleosides? Pioneering studies described that the phosphorylation of adenosine in solid-dry film condition under low energy proton irradiation (6.75 MeV) can occur using NaH$_2$PO$_4$ as a phosphorus source. NaH$_2$PO$_4$ has been selected on the basis of the reported phosphate reactivity: NaH$_2$PO$_4$ \( \rightarrow \) Na$_2$HPO$_4$ \( \rightarrow \) Na$_3$PO$_4$. In this latter case, a mixture of acyclic and cyclic nucleotide mono-phosphate isomers was obtained in low total yield relative to the converted substrate. A decrease in the total yield of mono-phosphate isomers was obtained in low total yield experimental conditions: A) adenosine and NaH$_2$PO$_4$ and NWA 2828 powder in dry-film condition under low energy proton irradiation (170 MeV) in the presence of the aubrite meteorite NWA 2828, affording the corresponding nucleotides in high total yield and conversion of substrate. Hydroxypatite also performed as heterogeneous phosphorus source. Both yield and conversion were further increased performing the irradiation in the presence of NH$_2$CHO. Adenosine polyphosphate derivatives ApNs (from adenosine diphosphate ADP to adenosine tridecaphosphate Ap13) and inorganic polyphosphates PNs (from P$_3$ to P$_{14}$) were also detected in the reaction mixture, highlighting the high reactivity of the system. The reaction pathway of the phosphorylation process has been tentatively interpreted on the basis of computational studies focusing on the possible role of NH$_2$CHO and of the sugar residue in adenosine.

2. Results and Discussion

2.1. Phosphorylation of Adenosine

The phosphorylation of adenosine 1 was performed in four experimental conditions: A) adenosine and NaH$_2$PO$_4$ in dry-film condition; B) adenosine, NaH$_2$PO$_4$ and NWA 2828 powder in dry-film condition; C) adenosine and NaH$_2$PO$_4$ in NH$_2$CHO suspension; and D) adenosine, NaH$_2$PO$_4$ and NWA 2828 powder in NH$_2$CHO suspension. NWA 2828 is an achondrite of the aubrite type meteorite. At least 65 different achondrite meteorites have been classified prior to 2015 as members of the aubrite type, including the Allan Hills, LaPaz Icefield, Lewis Cliff, Miller Range, Elephant Moraine and Northwest Africa groups. Examples of comprehensive Reviews are reported in Ref. [34–36]. Aubrites derive from asteroids of the E-type (mainly of the E(II) sub-class), such as the asteroid 2867 Steins, that was the target of the Rosetta mission. The aubrites are considered as a fascinating group of meteorites because they represent, in many of their properties, the end members in the spectrum of conditions of formation of igneous meteorites from asteroids. They formed very early in the history of the solar system, being suggested as geochemical markers for high-speed ejection processes occurring on Mercury, Mars and the pristinet Earth. The prebiotic interest of NWA2828, the reasons for having chosen it in this study, its cosmologic origin and mineralogical characterization are reported in Shi 2. NWA 2828 contains schreibersite ([Fe, Ni]$_2$P), a mineral that was studied in the thermal phosphorylation of nucleosides under radical conditions. Solid films were prepared by air drying of aqueous mixtures (300 μL) of adenosine (0.4 mmol) and NaH$_2$PO$_4$ (50 mg, 0.4 mmol) in the presence or absence of NWA 2828 powder (2.0 mg). As an alternative, hydroxypatite (50 mg) was used as phosphorus source. When required, the irradiation was performed in the presence of NH$_2$CHO (200 μL) under similar experimental conditions. The samples were irradiated at 298 K with 170 MeV protons for 3 min at the Phasatron accelerator facility of the Joint Institute of Nuclear Research of Dubna (Moscow region, Russia). The uniform proton beam was bounded to 10×10 cm$^2$ by the collimator system. The averaged linear energy transfer (LET) was 0.57 keV/μm and the calculated absorbed dose was 6.0 Gy. The solar wind was discovered and measured by the Mecuta probe and by the Mariner 2 spacecraft, and it consists of protons and electrons. The proton irradiation conditions used in this study (170 MeV; dose, 6 Gy) are in the same range of intensity of the solar wind and are of the same order of magnitude as that actually experienced during the phosphorylation of nucleosides in space flight conditions. These conditions were used in ground-based experiments to model chemical transformations on asteroids. The presence of organics in the original sample of NWA 2828, usually observed in the very low ppb range, was prevented as reported. We focused on the detection of nucleotides by reverse-phase ultra-high performance liquid chromatography associated with mass-spectrometry (UHPLC-MS). The structure of products was unambiguously assigned by comparison of the retention time and characteristic mass fragmentation peaks with original commercial samples. The description of the UHPLC-MS procedure, and the UHPLC analyses of adenosine 1, adenosine nucleotides 2–7, and adenine 8, are reported in SI #3.

The formation of acyclic and cyclic adenosine nucleotides was observed irrespective to experimental conditions, the irradiation of adenosine 1 and NaH$_2$PO$_4$ afforded 5'-adenosine mono-phosphate (5'-AMP) 2, 3'-adenosine mono-phosphate (3'-AMP) 3, 2'-adenosine mono-phosphate (2'-AMP) 4, and 2',3'-cycladenosine monophosphate (2',3'-cAMP) 5, in addition to adenine 8, formed as a consequence of the partial breaking of the β-glycosidic bond (Scheme 1, Figure 1). In addition, 3',5'-cycladenosine monophosphate (3',5'-cAMP) 6, and 5'-adenosine diphosphate (5'-ADP) 7, were obtained in reaction B, and reactions B and D, respectively (Scheme 1, Figure 1). The retention time (min) and the specific m/z values corresponding to the targeted molecules are reported in Table 1 (m/z fragmentation spectra of compounds 1–8, and the current ion profile and ESI(−)/MS/MS spectra of reaction D, as selected example, are in SI #3). The very fact that adenosine nucleotides
were observed at the end of the irradiation indicates that the overall balance of the synthesis/degradation process is positive.

The 5'-regioselectivity of the phosphorylation in 7 was confirmed by comparison of the $^{31}$P-NMR (SI# 4) with that of standard available sample.$^{[47]}$ The presence of appreciable amount of 5'-ADP during the phosphorylation of 1 is in agreement with data previously reported on the higher thermodynamic stability of this derivative relative to other
Table 1. Retention time and selected m/z spectrometric data of compounds 1-8.[a]

| Compound        | Retention time [min] | m/z [%] |
|-----------------|----------------------|---------|
| Adenosine 1     | 20.20-22.80          | 268 (5) [M + 1], 136 (100) |
| 5'-AMP 2        | 2.76-2.95            | 348 (4) [M + 1], 136 (100), 97 (6) |
| 3'-AMP 3        | 2.91-3.25            | 348 (3) [M + 1], 213 (2), 164 (4), 136 (100), 115 (2), 97 (5) |
| 2'-AMP 4        | 3.41-3.79            | 348 (4) [M + 1], 213 (2), 164 (8), 136 (100), 97 (4) |
| 2',3'-CAMP 5    | 3.26-3.68            | 330 (18) [M + 1], 312 (2), 136 (100) |
| 3',5'-CAMP 6    | 4.60-5.13            | 331 (8) [M + 2], 330 (38) [M + 1], 312 (5), 232 (3), 204 (1), 136 (100), 97(5) |
| 5'-ADP 7        | 3.20-3.50            | 428 (1) [M + 1], 388 (2), 282 (2), 167 (2), 136 (100) |
| Adenine 8       | 3.65-4.10            | 137 (6) [M + 2], 136 (100) [M + 1] |

[a] The UHPLC analysis was performed by using a C18 column associated with mass spectrometer Q-Exactive (Thermo) in the positive mode (details are in the Supporting Information #3).

Adenosine nucleotide polyphosphates,[46] 5'-ADP accumulates as one of the main reaction products during the thermal phosphorylation of adenosine with KH$_2$PO$_4$ in NH$_2$CHO at 373 K.[11] In addition, 3',5'-cyloadenosine monophosphate (3',5'-cAMP), and 5'-adenosine diphosphate (5'-ADP), were obtained during reaction D, and reactions B and D, respectively (Scheme 1, Figure 1).

The yield of nucleotides and the conversion of adenosine are reported in Table 2. Data on the irradiation of adenosine (1.3 mmol) and NaH$_2$PO$_4$ (1.3 mmol) at 243 K in dry-film condition with slow 6.0 MeV protons are also indicated in Table 2 as a reference (reaction E). As a general trend, the total yield of nucleotides in dry-film condition was significantly higher with NWA 2828 (Table 2, reaction B) than in the presence of adenosine and NaH$_2$PO$_4$ alone, under both high-energy (Table 2, reaction A) and low-energy (Table 2, reaction E) proton beam irradiations. A similar trend was observed performing the irradiation in NH$_2$CHO (Table 2, reaction C versus reaction A and reaction E), suggesting a beneficial role of NWA 2828 powder in the prebiotic synthesis of nucleic acids from adenine and sugars, as a possible result of surface interaction effects.[50] The decisive catalytic role of meteorites in NH$_2$CHO prebiotic synthesis of nucleic acids has been recently reviewed.[51] NWA 2828 and NH$_2$CHO showed a slightly different regioselectivity in the phosphorylation of adenosine. While 2',3'-cAMP prevailed in the absence of NH$_2$CHO (reactions A and B), 5'-AMP becomes the major reaction product in the presence of NH$_2$CHO (reaction C).

This trend was further confirmed when the irradiation was performed in the mixture of NH$_2$CHO with NWA 2828 (reaction D). The prevalence of 5'-AMP is usually observed during the thermal phosphorylation of adenosine with orthophosphate in NH$_2$CHO.[10] As for the other nucleotide isomers, 2'-AMP was detected in yield higher than 3'-AMP, while 3',5'-cAMP was significantly synthesized only in reaction D.

The reactions A–D have been repeated in the described experimental conditions in the presence of hydroxyapatite as

Table 2. Yield of adenosine nucleotides and conversion of substrate during the irradiation of adenosine and NaH$_2$PO$_4$ with a high energy proton beam (170 MeV) under different experimental conditions.

| Entry | Reaction | Conversion [%] | Adenine [b] | 5'-AMP 2 | 3'-AMP 3 | 2'-AMP 4 | 2',3'-cAMP 5 | 3',5'-cAMP 6 | 5'-ADP 7 | Total yield [%] |
|-------|----------|----------------|-------------|----------|----------|----------|-------------|-------------|----------|----------------|
| 1     | A        | 72.3          | 39.8[a]     | 1.5      | 1.1      | 1.3      | 2.1         | –           | –        | 6.0            |
| 2     | B        | 59.8          | 25.0        | 5.7      | 4.9      | 10.3     | 6.2         | –           | –        | 34.8           |
| 3     | C        | 76.7          | 40.8        | 8.3      | 7.4      | 12.0     | 5.9         | –           | –        | 33.6           |
| 4     | D        | 81.1          | 29.1        | 11.2     | 7.9      | 14.0     | 6.7         | 6.5         | 4.8      | 51.1           |
| 5     | C[0]     | 11.5          | 4.0         | 1.8      | 1.4      | 2.1      | 0.9         | –           | –        | 6.2            |
| 6     | D[0]     | 13.1          | 4.1         | 2.1      | 1.5      | 2.7      | 1.4         | 1.0         | –        | 8.7            |
| 7     | E        | 49            | 16.1        | 2.40     | 1.4      | 1.4      | 2.2         | <0.1        | –        | 7.4            |

[a] Adenine. [b] The yield is calculated on the basis of the millimoles of starting adenosine. All irradiations were performed by irradiation of adenosine (0.4 mmol) and NaH$_2$PO$_4$ (50 mg, 0.4 mmol) with 170 MeV protons for 3 min at 243 K. When required, the NWA 2828 powder (2.0 mg) and NH$_2$CHO (200 μL) were used. A: dry-film conditions. B: dry-film conditions in the presence of NWA 2828 powder. C: NH$_2$CHO. D: NH$_2$CHO in the presence of NWA 2828 powder. E: adenosine (1.3 mmol) and NaH$_2$PO$_4$ (1.3 mmol) irradiated at 243 K in dry-film conditions with 6.0 MeV protons (dose 1011 protons/cm²).[51] [c] Hydroxyapatite (50 mg) instead of NaH$_2$PO$_4$. 

[46] M. J. S. Hunter, R. A. van Dorp, P. J. M. van der Pluijm, et al., Nature, 2003, 422, 535.

[11] C. H. Waddington, R. A. van Dorp, M. J. S. Hunter, et al., Proc. Natl. Acad. Sci. U. S. A., 2004, 101, 9356.
an alternative mineral source of phosphorus (Figure 2). In these latter cases, the formation of nucleotides was observed only in the presence of NH₄CHO (Table 2). These results are in accordance with the key role played by NH₄CHO in the solubilizing effect of phosphate from hydroxyapatite, as previously reported by us during thermal phosphorylation processes. The reactions C–D in the presence of hydroxyapatite afforded nucleotides in a total yield lower than that obtained using NaH₂PO₄, probably due to the partial release of active phosphorus from the mineral (Table 2). The 5'-ADP was not detected in the reaction mixtures. A slightly higher yield for nucleotides was observed in the presence of NWA 2828 with respect to NH₄CHO alone (Table 2, entry 6 versus entry 5).

About the regio-selectivity of the reaction, hydroxyapatite showed a selectivity trend similar to NaH₂PO₄, 3',5'-cAMP 6 being obtained only in the presence of NWA 2828 (reaction D).

### 2.2. Synthesis of Adenosine Polyphosphates and Inorganic Polyphosphates

The MALDI-TOF analysis of reactions A–D with NaH₂PO₄ showed, in addition to nucleotides 2–7, the presence of molecular ions corresponding to adenosine polyphosphates (ApNs), including adenosine triphosphate (ATP), adenosine pentaphosphate (Ap5), adenosine heptaphosphate (Ap7), adenosine nonaphosphate (Ap9), adenosine undecapephosphate (Ap11), and adenosine tridecaposphate (Ap13) (Table 3). The MALDI-TOF analyses of reactions A–D, and of nucleotides 2–7, are in SI# 5. As reported in Table 3, ApNs bearing an odd number of phosphates were selectively obtained, suggesting the occurrence of the prevalent transfer of a pyrophosphate moiety to newly synthesized monophosphate derivative. This reaction pattern is of particular interest, since it is chemiomimetic of the extent mechanism of phosphorylation of purine nucleosides by the purine nucleotide pyrophosphotransferases (PNPs), a large family of enzymes which catalyzes the selective transfer of the pyrophosphate moiety from ATP to purine nucleotide monophosphates. In addition to ApNs, the molecular ions of inorganic polyphosphates (PNs) from P₃ up to P₁₄ were also detected by the MALDI-TOF analysis in reactions B–D, the irradiation of adenosine and NaH₂PO₄ in NH₄CHO (reaction B) affording the largest panel of these products. PNs were not produced in reaction A (Table 2). The irradiation of NaH₂PO₄ alone in the presence of formamide (reaction F), and in alternative, in formamide/NWA 2828 mixture (reaction G) was also performed, confirming that polyphosphates were easily produced from NaH₂PO₄ under the reported experimental conditions (Table 4). MALDI-TOF analyses of reactions F–G are in SI# 5. PNs are generally deemed to be key agents in prebiotic evolution having several phosphate residues linked by high-energy phosphorus-anhydride bonds as in ATP and ADP. These polymeric compounds are usually produced by dehydration of orthophosphate at elevated temperatures, and have been synthesized in high yield in plausible prebiotic scenarios, such as volcanic condensates and deep oceanic steam vents. Urea can improve the yield of PNs from NaH₂PO₄ by formation of carbamylphosphate and phosphoramidate intermediates. This effect can be in principle played by NH₄CHO, which has a similar nucleophilic amide moiety available for the interaction with electron-positive phosphate atoms. PNs are also produced from NaH₂PO₄ by radical path-

![Figure 2. Reverse-phase UHPLC profiles of irradiation of adenosine (0.4 mmol) and hydroxyapatite (50 mg) with a high-energy proton beam (170 MeV) for 3 min at 243 K. A) Adenosine and hydroxyapatite in NH₄CHO (200 μL); B) adenosine, hydroxyapatite and NWA 2828 powder (2.0 mg) in NH₄CHO (200 μL).](image)

**Table 3.** MALDI-TOF analyses of adenosine polyphosphates and inorganic polyphosphates.

| Entry | Compound | [m/z] calculated | [m/z] measured |
|-------|----------|-----------------|---------------|
| 1     | Ap₃      | 507.18          | 508.18        |
| 2     | Ap₅      | 667.14          | 668.75        |
| 3     | Ap₇      | 827.10          | 828.10        |
| 4     | Ap₉      | 987.05          | 988.05        |
| 5     | Ap₁₁     | 1147.01         | 1148.03       |
| 6     | Ap₁₃     | 1306.66         | 1307.66       |
| 7     | P₃       | 257.91          | 258.95        |
| 8     | P₄       | 337.88          | 338.88        |
| 9     | P₅       | 417.84          | 418.84        |
| 10    | P₆       | 497.81          | 498.82        |
| 11    | P₁₀      | 817.67          | 818.67        |
| 12    | P₁₂      | 977.61          | 978.612       |
| 13    | P₁₄      | 1137.54         | 1138.54       |

[a] The identification assignments given in the third column of the table represent the exact mass of non-protonated species, in the fourth column are reported m/z values relative to the corresponding [M+1] species.
2.3. Reaction Pathway for the Formation of Polyphosphates

Proton-irradiation-induced formation of PNs observed in our experiments can be rationalized considering a reductive chemistry due to the remarkable amount of atomic H formed from phosphate or formamide under radiolysis conditions.\textsuperscript{[55]} Our computations suggest that orthophosphates may readily bind atomic hydrogen yielding the P-centered $^\text{P(OH)}_4^\text{−}$ radicals with a relatively low activation energy of $+11.3$ kcal/mol in an exothermic chemistry that is accompanied with a reaction free energy change (hereafter abbreviated as $\Delta G_r$) of $−10.4$ kcal/mol (see the computed free energy profile in Figure 3). Subsequent dimerization of two $^\text{P(OH)}_4^\text{−}$ radicals yields a P–P bonded intermediate, having two equivalent phosphorus atoms in the +4 formal oxidation state. After pseudo-rotation and a subsequent internal proton transfer, this intermediate dissociates to phosphoric acid ($^3\text{PO}_4^\text{−}$), phosphorous acid ($^3\text{H}_2\text{PO}_4$), and water in a practically barrierless process in a markedly exergonic chemistry with a $\Delta G_r$ of $−47.2$ kcal/mol.

Note, that the formation of phosphorous acid has also been observed in other radical-assisted phosphorylation reactions conducted using the meteoritic mineral schreibersite.\textsuperscript{[59]} Phosphorous acid, similarly to phosphoric acid, may lose an atomic H leading to a P-centered $^3\text{H}_2\text{PO}_4$ radical, as shown in Scheme 2, in an exergonic chemistry (computed $\Delta G_r = −27.3$ kcal/mol). This radical may then spontaneously recombine with an O-centered $^3\text{H}_2\text{PO}_4$ radical (the latter prevalently forms upon radiolysis of phosphoric acid or its H-containing salts)\textsuperscript{[56,57]} yielding pyrophosphates (Scheme 2).

Further extension of the phosphate-chain may proceed in an analogous manner with participation of the already formed condensed phosphates and the P-centered radicals derived from phosphorous acid upon H-abstraction as shown in Scheme 2. Interestingly, in our experiments polyphosphate formation was enhanced by addition of the NWA 2828

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**Table 4.** Adenosine nucleotide polyphosphates (ApNs) and inorganic polyphosphates (PN) detected during the irradiation of adenosine and NaH$_2$PO$_4$ by the use of MALDI-TOF analysis.\textsuperscript{[59]}

| Reaction\(\textbf{a}\) | Ap3 | Ap5 | Ap7 | Ap9 | Ap11 | Ap13 | P3 | P4 | P5 | P6 | P10 | P12 | P14 |
|---------------------|-----|-----|-----|-----|------|------|----|----|----|----|-----|-----|-----|
| A                   | +   | −   | +   | −   | +    | −    | −  | −  | −  | −  | +    | +    | +    |
| B                   | +   | +   | +   | −   | +    | +    | +  | +  | +  | +  | +    | +    | +    |
| C                   | +   | +   | −   | −   | −    | −    | −  | +  | +  | −  | −    | +    | −    |
| D                   | +   | +   | +   | +   | +    | +    | +  | +  | +  | +  | +    | +    | +    |
| E                   | +   | +   | +   | +   | +    | +    | +  | +  | +  | +  | +    | +    | +    |
| F                   | +   | +   | +   | +   | +    | +    | +  | +  | +  | +  | +    | +    | +    |
| G                   | +   | +   | +   | +   | +    | +    | +  | +  | +  | +  | +    | +    | +    |

\(\textbf{a}\) Reactions were performed by irradiation of adenosine (0.4 mmol) and NaH$_2$PO$_4$ (0.4 mmol) with 170 MeV protons for 3 min at 243 K. When required, the NWA 2828 powder (2.0 mg) and NH$_2$CHO (200 μL) were used. [b] A: dry-film conditions. B: dry-film conditions in the presence of NWA 2828 powder. C: NH$_2$CHO suspension. D: NH$_2$CHO in the presence of NWA 2828 powder (2.0 mg). E: adenosine (1.3 mmol) and NaH$_2$PO$_4$ (1.3 mmol) irradiated at 243 K in dry-film conditions with 6.0 MeV protons (dose 1011 protons/cm$^2$). F: NaH$_2$PO$_4$ in the presence of formamide. G: NaH$_2$PO$_4$ in the presence of formamide and NWA 2828 powder. [c] The symbol indicates the presence (+) or the absence (−) of the expected molecular ion.

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Figure 3. Computed free energy ($\Delta G$) profile for the reduction of phosphoric acid with atomic H formed in the irradiation experiments. Computations were performed at the B3LYP/6-311++G** level.

Scheme 2. Suggested mechanism for the formation of condensed phosphates via the reaction of phosphoric acid and phosphorous acid under proton-irradiation conditions.
meteorite powder. This is in line with the above mechanistic model, because the minerals present in the meteorite (mainly enstatite) may concentrate the radicals formed upon proton-irradiation and may improve the efficiency of their recombination reactions. Similar concentration phenomenon has also been observed in impact experiments performed in the presence of clays reported in Ref. [30].

2.4. Reaction Pathway for the Formation of Adenosine Nucleotides

PNs are most probably involved in the phosphorylation of adenosine and in the formation of the ApNs that we observed in the irradiation experiments. In this context, several studies on the phosphorylation and polymerization of nucleosides and other biomolecules by use of PNs as reagents were performed. In accordance with this hypothesis, the formation of ApNs from AMP by treatment with PNs in buffered NH₄Cl solutions of bivalent metal ions has been reported to occur after 16 days at 310 K. In this reaction, the bivalent metal ions were able to coordinate two oxygen atoms of the PNs phosphate chain, favoring the hydrolysis of the anhydride bonds to yield trimetaphosphate, suggested as the actual phosphorylating agent. Under these experimental conditions, the Ap3 derivative, corresponding to the transfer of the pyrophosphate moiety to starting AMP, was detected as the main component of the reaction mixture. Moreover, the possible transfer of the pyrophosphate moiety from in situ generated ATP to adenosine cannot be completely ruled out.

As an alternative, the possibility of a phosphorylation mechanism of adenosine based on a radical pathway has been evaluated. Indeed, early studies suggest that the main reaction channel of the radiolysis of aqueous NaH₂PO₄ solutions involves the reaction of H₂PO₄⁻ ions with hydrated electrons leading to HPO₄²⁻ radical anion and the release of an atomic H. Reference 59 reports that atomic H is abundantly formed upon the radiolysis of formamide as well. Thus, similar to reference 61, a reductive chemistry associated with the presence of atomic H in the reaction mixture may feature a possible radiation-induced phosphorylation mechanism in our experiments. Thermodynamic driving force for the phosphorylation reaction with phosphate radicals is enormous, because the process is fueled by a highly exergonic radical recombination step between the phosphate radical and H⁺. For example, the computed free energy change balance for the reaction H₂PO₄⁻ + adenosine + H⁺ = 3'-AMP + H₂O is -104.6 kcal/mol, which is far better than that of the reaction between adenosine and pyrophosphates. Note that radical assisted-reactions generally proceed on a much higher energy scale than that of common biochemical reactions. Radiolysis of polyols, like ribose, is known to proceed via H-abstraction from one of the C–H bonds, yielding the C-centered radical I (Scheme 3) or other isomers, differing in the position of the radical center. In the next reaction step radical I recombines with an HPO₄²⁻ radical ion yielding II. In this latter case the addition of HPO₄²⁻ is expected to occur from the alpha-side of the carbonyl moiety in accordance with data previously reported. Compound II is a hemiacetal-type compound and thus it is prone to undergo a proton-assisted Sn1-type water loss leading to the carboxylic intermediate III. Note that proton-irradiation tracks are known to concentrate H⁺ ions accessible for acid catalysis: analogous Sn1-type degradation of acetals upon proton-irradiation is well-known. Theoretical calculations on a simplified

![Scheme 3. Phosphorylation mechanism of adenosine based on a radical pathway. Transiently formed metaphosphoric acid (HPO₄) may act as a phosphorylating agent.](image)
model (for details see the Supporting information SI#6) showed that III (Figure 4) is mainly stabilized by electrostatic interactions: i.e. it rather corresponds to the resonance structure in which the oxidized keto-ribose derivative is electrostatically bound to the partially positively charged phosphorus of a metaphosphoric acid unit. This suggests that intermediate III is prone to lose a metaphosphoric acid, which is a potent phosphorylating agent. Let us note that albeit metaphosphoric acid is obviously not stable in an aqueous environment, our state-of-the-art quantum molecular dynamics simulations (detailed in the Supporting Information) highlight that it may be stabilized by formamide. The various alternatives of the pathways involved are discussed and are all compatible with the observed stimulatory action of formamide and the aubrite. Interestingly, the radical pathway described for the formation of AMP entails the formation of metaphosphoric acid as possible phosphorylating agent. Quantum molecular dynamics simulations suggest that metaphosphoric acid may be stabilized by formamide. The relative promptness, the high yield and the regioselectivity of the phosphorylation products point to the possible prebiotic relevance of the scenario depicted here, which involves proton beams, aubrite and the one-carbon atom compound formamide. Thus, nucleotides may form in the same physical-chemical frame in which the formation of nucleic bases and of polyphosphate chain likely occurs by addition of pyrophosphate units to a starting monomer, anticipating the extant biological reactions carried out by purine nucleotide pyrophosphotransferases. The various alternatives of the pathways investigated are discussed and are all compatible with the observed stimulatory action of formamide and the aubrite. Interestingly, the radical pathway described for the formation of AMP entails the formation of metaphosphoric acid as possible phosphorylating agent. Quantum molecular dynamics simulations suggest that metaphosphoric acid may be stabilized by formamide. The relative promptness, the high yield and the regioselectivity of the phosphorylation products point to the possible prebiotic relevance of the scenario depicted here, which involves proton beams, aubrite and the one-carbon atom compound formamide. Thus, nucleotides may form in the same physical-chemical frame in which the formation of nucleic bases and of polyphosphates has been reported. Unfortunately, the drastic conditions normally used for the analysis of the organic content of meteorites are expected to destroy low-stable organics, such as nucleosides and nucleotides.

This working model might have happened on a planet like Earth in the presence of falling meteorites. The biomimetic nature of the poly-phosphorylation reaction adds to this plausibility. The relevance of proton irradiation as a source of energy for radical chemistry is universal, and should not be dismissed focusing only on the precise geo-evolution of this planet.
Experimental Section

Materials
Formamide (Fluka, >99%) was used without further purification. NWA 2828 was purchased from AZ meteorites.

Adenine, adenosine, nucleotides, NaH₂PO₄, and Hydroxyapatite were purchased from Sigma Aldrich. UHPLC were performed on Ultimate 3000 Rapid Resolution system (DIONEX, Sunnyvale, USA) using Reprosil C18 column (2.5 µm × 150 mm × 2.0 mm). MALDI was performed on Q-Exactive (Thermo).

Preparation of Aubrite Powder

Dust samples of NW2828 (approximately 100 mg) were extracted by a two-step procedure to remove organics. The first consisted in the addition of 1.0 mL 0.1 N NaOH and 3.0 mL of 2:1 chloroform-methanol, the second step in the addition of 1.0 mL 0.1 N sulfuric acid and 3.0 mL of 2:1 chloroform-methanol. Between steps the powder was recovered by centrifugation (6000 rpm, 10 min) and the supernatant phase was decanted. The supernatant contained organics that were soluble in both aqueous and organic solvents at high and low pH ranges, leaving behind the powder of the meteorite. Finally, the powder was pyrolyzed at 600°C to remove the insoluble organic component in the laboratory oven for 1 h.

Irradiation Experiments

General procedure: Solid films were prepared by air drying of aqueous mixtures (300 µL) of adenine (0.4 mmol) and NaH₂PO₄ (50 mg, 0.4 mmol) or hydroxyapatite (50 mg) in the presence or absence of NWA 2828 powder (2.0 mg). When required, the irradiation was performed in NH₂CHO (200 µL) under similar experimental conditions. The samples were irradiated at 298 K with 170 MeV protons for 3 min. The uniform proton field was bounded by the collimator system. The averaged linear energy transfer (LET) was 0.57 keV/µm and the calculated absorbed dose was 6.0 Gy, generated by the Phasotron facility of the Joint International Nuclear Institute (JINR; Dubna, Russia).

Quantum Chemical Calculations

Computations were carried out at DFT-level of theory using the 6–31G** basis set of atomic orbitals and Becke’s three parameters hybrid functional,[60] in combination with Lee-Yang-Parr’s correlation functional.[70–71] Free energy data were derived from frequency calculations performed at 298 K in harmonic approximation. Note that in the past the methodology has been benchmarked against CCSD(T) calculations,[72] and has successfully been used to describe the mechanism of several other radical-based prebiotic reactions.[73] All computations were carried out in gas-phase using the Gaussian09 program package.[74] Further methodological details and comparison with general aspects of other frequently used computational approaches (e.g. metadynamics)[75–76] are addressed in the Supporting Information (SI# 6).

Supporting Information

Prebiotic relevance of NH₂CHO; Prebiotic interest, inorganic composition and cosmo-origin data of NWA 2828; UHPLC-ESI-MS/MS procedure, chromatographic profiles and m/z fragment spectra of compounds 1–8, and current ion profile and ES(I+)-MS/MS spectra of reaction D; [31P NMR of ADP; MALDI TOF analysis of reactions A–F and nucleotides 2–7; technical details of quantum chemical calculations and comparison with general aspects of other frequently used computational approaches, and ab initio molecular dynamics simulations, optimized geometries and energies from B3LYP/6-311 + +G** calculations are available in the Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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