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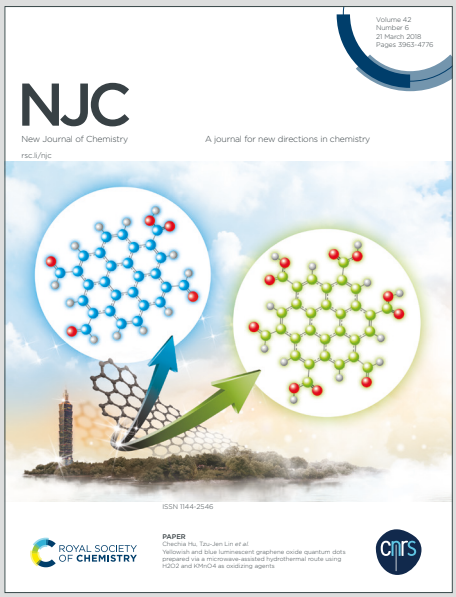

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COMMUNICATION

Synthesis of Ribonucleotides from the Corresponding Ribonucleosides under Plausible Prebiotic Conditions within Self-assembled Supramolecular Structures

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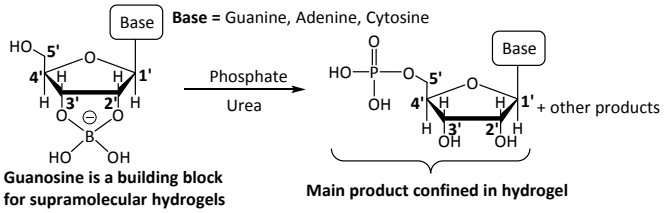
Abstract: Abiotic synthesis of ribonucleotides, mainly at the 5' position, from the corresponding ribonucleosides within self-assembled supramolecular structures, based on guanosine:borate hydrogels, were carried out in the range 70 ºC-90 ºC, using yet urea and a source of phosphate (K_2HPO_4 or hydroxyapatite). Phosphorylation is possible at initial concentrations of guanosine lower than 20 mM and it is more efficient using wet/dry cycles. Monoamidophosphate (and, eventually diamidophosphate), diamidodiphosphate and pyrophosphate are intermediates in the synthesis of ribonucleotides. These conclusions are supported by NMR spectroscopy and mass spectrometry analysis of samples. On the other hand, after reaction, hydrogels can produce globular aggregates by addition of water and decreasing temperature, thus confining ribonucleotides that, once activated under suitable conditions, could form polyribonucleotides.

Phosphorylation of ribonucleosides is a key step for prebiological evolution by "RNA world hypothesis".[1] This challenge began at least in the sixties of the last century.[2] Some methods (in different conditions, e.g., aqueous solutions and dry environments) using diverse sources of phosphate have been suggested for all ribonucleosides, leading to phosphorylation at different carbon atoms and, typically, with higher yields to uridine.[3-14] However, in all the cases, the synthesis of more complex prebiological molecules would be very limited because the products could be easily dispersed. This might be overcome with supramolecular hydrogels as reaction matrix, since they could confine prebiotic molecules.[15,16] Phosphorylation of a ribonucleoside at C5' position is not very efficient, due to competition of the two other free –OH groups of the organic molecule. However, by protection of positions C2' and C3' of ribonucleoside, this reaction could be improved with a prebiotically plausible reagent, such as borate, since it stabilises ribose (constituent of the framework of ribonucleosides) in those positions.[17] Guanosine forms diesters with borate, leading to the self-assembly of supramolecular hydrogels, based on stacked hydrogen-bonded guanosine quartets, which have high thermal stability.[18,19] Their structure is related with BBS (Boron Bound Species, i.e., ribose borate esters),[1Error! Bookmark not defined.] with only one free hydroxyl group (C5'). However, direct phosphorylation of nucleosides is not effective without an activator, and urea has been suggested as a plausible reagent.[11,20] The occurrence of urea in the interstellar space[21] supports its involvement in prebiotic reactions, since it could have been delivered to Earth.[22]

Herein is reported phosphorylation, under prebiologically plausible conditions, of ribonucleosides, within self-assembled structures (guanosine-borate hydrogels), see Scheme 1. These conclusions are supported by NMR spectroscopy and mass spectrometry analysis of samples based on reactions with guanosine supramolecular hydrogels or solutions containing urea and phosphate.



Scheme 1 – Phosphorylation of ribonucleosides under plausible prebiotic conditions.

Phosphorus signals of ribonucleotides by ^{31}P NMR spectroscopy exhibit chemical shifts at ca. 4 ppm.[23] Figure 1 is a ^{1}H-^{31}P HMBC spectrum[24] from reaction of a hydrogel of guanosine with borate containing urea and K_2HPO_4, showing phosphorylation of the nucleoside, mainly in position C5' (^{31}P signal at 3.90 ppm has cross-peaks at 3.78 ppm, which are assigned to the protons of the CH_2 group, unique of nucleotide). Additional ^{31}P signals at 3.49 and 4.02 ppm with cross-peaks at 4.89 and 4.58, respectively, are assigned to phosphorylation in positions C2' and C3', in this order.

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This should result from borate that reduces phosphorylation in positions C2' and C3', and simultaneously induces the stability of the hydrogel.

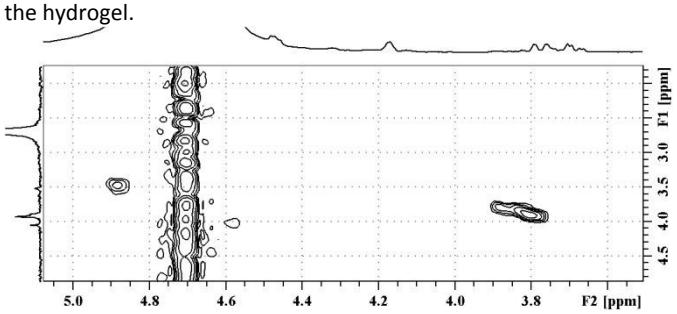

Figure 1 - ^{1}H-^{31}P HMBC spectrum of a guanosine hydrogel with borate after 4 wet/dry cycles (2 days of reaction) at 90 °C with K_2HPO_4 and urea run in a 400 MHz spectrophotometer.

A similar reaction was carried out for a longer period (12 cycles, i.e. 6 days) with a yield of 5% to 5'-ribonucleotide (major species formed) determined by ^{31}P NMR spectroscopy (see Figure 2 and Experimental Section in Electronic Supplementary Information). This yield is significant compared with previous results, since our reactions typically need lower concentrations and number of reagents, i.e. they are better adjusted to the prebiological environments.[3-14]

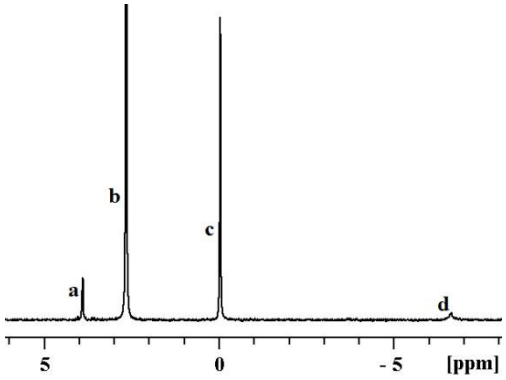

Figure 2 - ^{31}P NMR spectrum of a guanosine hydrogel after 12 wet/dry cycles of reaction at 90 °C with K_2HPO_4 and urea, run in a 400 MHz spectrophotometer; $Cr(acac)_3$ and D_3PO_4 were relaxation agent and internal standard, respectively; (a) guanosine-5'-phosphate, (b) inorganic phosphate, (c) internal standard (D_3PO_4) and (d) pyrophosphate.

The level of phosphorylation increases with the number of wet/dry cycles of reaction, Figure 3. Concomitantly, a structural change on the hydrogel occurs. In fact, the ^{11}B NMR spectra in Figure ESI1 show that the boron bonds are altered during reaction, suggesting that guanosine-borate monoesters become more probable than diesters. This could be due to the decrease of the stability constants of borate bound to nucleosides derivatives with phosphorylation.[25]

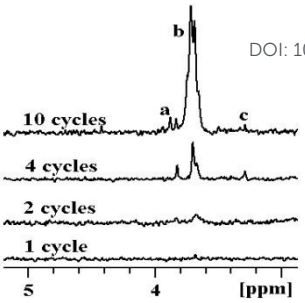

Figure 3 - ^{31}P NMR spectra of guanosine hydrogels after (from bottom to top): 1, 2, 4 and 10 wet/dry cycles of reaction at 90 °C with K_2HPO_4 and urea, run in a 400 MHz spectrophotometer; (a) guanosine-3'-phosphate, (b) guanosine-5'-phosphate and (c) guanosine-2'-phosphate.

On the other hand, samples with two daily additions of water and without any cycles have chemical shifts at 3.72 ppm and -6.33 ppm, assigned to guanosine-5'-phosphate and pyrophosphate signals, respectively, while for the sample without dryness period the former signal is absent (see Figure 4). Therefore, reaction conditions with wet/dry cycles are more efficient than just under dry conditions. This is in agreement with a recent publication.[26]

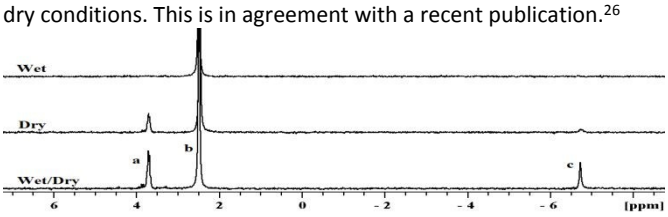

Figure 4 - ^{31}P NMR spectra of guanosine hydrogels after (from bottom to top): 10 wet/dry cycles, dryness and wetness, respectively, for an equivalent amount of time (5 days), at 90 °C with K_2HPO_4 and urea, run in a 400 MHz spectrophotometer. Signals were normalised with (b), inorganic phosphate; (a) guanosine-5'-phosphate and (c) pyrophosphate.

Urea is also required for activation of phosphate, since the reaction of a guanosine hydrogel with K_2HPO_4 in the absence of urea does not lead to the phosphorylation of guanosine (spectrum not presented). This suggests the formation of activated species (required to phosphorylation of nucleosides) from the reaction of urea and phosphate (see Figure ESI2). In Figure 4 (wet/dry cycles and dry conditions) another phosphorus species is presented, pyrophosphate, as it was previously observed.[27] This species was also detected by ESI-MS(-) of a solution prepared after a phosphorylation reaction by water addition and adjusted at pH 1, with a *m/z* 177 [pyrophosphate-H], and confirmed by MS/MS fragments 159 [$P_2O_5OH^-$] and 79 [PO_3^-] (see Figure ESI3).[28] Pyrophosphate formation was previously detected in different reaction conditions,[29] including at a similar temperature to our highest value, under more acidic conditions and with nitrogen-rich compounds.[30] Herein, the synthesis of activated phosphate species is also detected at a lower temperature, i.e. 50 °C (see Figure ESI4). On the other hand, in the ^{31}P NMR spectrum (not shown) of urea and K_2HPO_4 without heating, the signal corresponding to pyrophosphate was absent. Pyrophosphate was proposed as a phosphorylation reagent[29,30] and it is now suggested as an intermediate in phosphorylation of guanosine.

Pyrophosphate formation has as intermediate monoamidophosphate. This species was detected by ESI-MS(-), *m/z* 96 [monoamidophosphate-H] (see Figure ESI5) and by ^{31}P NMR spectroscopy ca. 8.8 ppm (see Figure ESI6) that is according with a recent publication.[31] On the other hand, diamidophosphate was suggested as a prebiological phosphorylation reagent, although its synthesis was unlikely from a prebiotic standpoint;[27] the synthesis of this species was recently proposed[31] using other phosphorus compounds as starting material, although they are much rarer on Earth's crust than phosphate salts.[32] In an aqueous solution (pH 8) prepared from a solid-state reaction of K_2HPO_4 and urea, characterised by ESI-MS(-)(not shown) a *m/z* 95 corresponding to diamidophosphate was identified (not detected by ^{31}P NMR spectroscopy, since the detection limit of this technique is higher than by mass spectrometry), suggesting that in absence of a substrate to phosphorylate (or, probably, with a higher ratio urea:phosphate) this species could be formed. Additionally, the ^{31}P NMR spectrum of phosphorylation reaction with a guanosine hydrogel after 1 wet/dry cycle (Figure ESI7) has another signal (-3.43 ppm) assigned to diamidodiphosphate,[27] another intermediate in the pyrophosphate formation. Our results and also from[27]suggest that upon reaction of urea with phosphate under dry conditions monoamidophosphate (and, eventually, diamidophosphate) is formed, which, by reaction with another phosphate molecule, leads to diamidodiphosphate. This is hydrolysed to pyrophosphate, even with low amounts of water. The formation of activated phosphorus species suggests that phosphorylation could have happened as a continuous process during the prebiological period in at least the temperature range 70 °C – 90 °C without degradation of nucleosides or nucleotides. Their confinement into hydrogels with high thermal stability would promote the reaction.

Other ribonucleosides were included into guanosine hydrogels (see Experimental Section in Electronic Supplementary Information). Subsequently, hydrogels with guanosine/adenosine, and guanosine/cytidine were tested concerning phosphorylation. All hydrogels require guanosine. In its absence gelation is not achieved, since self-assembling is supported by hydrogen bonding[18] that is stronger to guanosine (from its additional groups with this capacity) than to the other ribonucleosides. Figure ESI8 and mass spectrometry data show that other ribonucleosides are also phosphorylated. Samples from these reactions solubilised in water and pH adjusted to 4 (to avoid gelation) with HCl were analysed by ESI-MS(-). The mass spectra were very complex, but *m/z* 322 [cytidine monophosphate-H], 346 [adenosine monophosphate-H] and 362 [guanosine monophosphate-H] are only detected in samples containing as starting material cytidine, adenosine and guanosine, respectively. An amplified effect on the phosphorylation is observed by the presence of another ribonucleoside in the hydrogel, Figure ESI8, with special emphasis to cytidine (complementary base pair of guanosine).

Minerals containing a source of phosphorus would have been reagents of prebiological phosphorylations. However, some minerals containing hydroxidotrioxidophosphate(2−) (hydrogenphosphate) are typically from biological material.[33] Prebiological synthesis of crucial molecules for the prebiotic evolution was hindered by the low solubility product of naturally occurring phosphate minerals.[32] However, using hydroxyapatite as source of phosphate for phosphorylation, solubilisation of phosphate was detected by ^{31}P NMR spectroscopy, as well as traces of ribonucleotide, see Figure ESI9, after 15 days (30 cycles) of reaction, despite the low solubility of hydroxyapatite[34] at pH of reaction, i.e., 1000-10000 lower than the concentration in the reactions with K_2HPO_4. This suggests that hydroxyapatite as source for phosphorylation of ribonucleosides could have occurred in a short period as compared with the overall prebiological era.

Finally, we observed that hydrogels with guanosine and borate, upon heating, followed by water addition and temperature decrease, form globular aggregates (see Figure ESI10). Generally, covalently bound hydrogels are quite robust against temperature fluctuations.[35] However, guanosine-borate hydrogels stabilised by K^+ are non-covalent, since the guanosine-borate esters self-assemble into G_4 sticky nanowires by hydrogen bonding and π-π stacking, and the wires entangle to produce a fibrous network.[36] Guanosine:borate mono or diesters may react, when temperature rises.[37] For instance, N1 could no longer be an H donor, affecting the stability of the G_4-quartets. Thus, the gel network could be partially altered by heating. An alternative explanation for the partial destruction of the guanosine:borate hydrogels upon heating and adding water could be the reversibility of covalent bonds, such as by the borate ester hydrolysis**Error! Bookmark not defined.** (compare with Figure ESI1).

How could guanosine:borate hydrogels have formed under prebiotic conditions? The mixture of aqueous solutions of nucleosides with potassium and borate minerals (e.g. kaliborite or santite) and subsequent water evaporation until the formation of hydrogels could be the answer. These hydrogels are stable (over a significant range of temperatures) and, in case of dryness, their properties could be recovered in the presence of water by self-assembly, as consequence of their supramolecular characteristics. This behaviour facilitates wet/dry cycles towards phosphorylation in the presence of a suitable and activated phosphate source. This reaction is possible at temperatures, in the range of 70 - 90 °C or probably at lower temperatures (in this case, with a higher number of wet/dry cycles of reaction, compare with Figure ESI4), would lead to the formation of globules containing ribonucleotides that, once activated, might have led to polyribonucleotides. Moreover, these gel particles have external boundaries that could promote reactions at the external or internal surfaces and thus the prebiological evolution.

Acknowledgments

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Conflicts of interest

There are no conflicts to declare.

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