Study of nucleobases and nucleosides in light of prebiotic evolution

C. Gonçalves

Abstract

The origin of life is one of humanity's biggest quests. The presence of nucleobases in carbonaceous chondrites suggests that these components of the genetic material would have been originated in the cosmos and later brought to Earth by . In the first part of this work, the possibility of decomposing, nowadays, guanosine, adenosine and cytidine, as a consequence of the energy release due to meteoritic impact, was studied. The samples containing the nucleosides were prepared with some inorganic salts.

Samples containing the above-mentioned nucleosides were prepared by mechanochemical procedures and in aqueous solution with the most common ions in the prebiological environment, such as carbonates, that are also detected in carbonaceous chondrites. The samples produced by mechanochemistry were analysed by PXRD. To understand the role of solar radiation in the reactions, solutions were placed both in presence and absence of sunlight. Monohydrate hydrochloride guanine was obtained after solar exposure of an acidic solution of guanosine and magnesium carbonate (in proportion 2:1), proving this way that the degradation is, indeed, possible.

Samples containing guanosine, adenosine and cytidine with hydroxyapatite, the most abundant phosphate source on Earth, were tested in order to study the possibility of their phosphorylation. ^{31}P NMR spectrometry analyses of the above-mentioned samples were not conclusive, which may be related to hydroxyapatite's limited solubility.

Introduction

Humanity has long wondered how life originated on Earth. Aristotle proposed one of the first hypotheses. He admitted that life originated from the combination of water, air, earth and fire with a vital heat, which he named soul[1].

Later on, Pasteur's experiments showed that life did not originate from non-living matter, as it was previously supposed[2,3]. Subsequent scientific developments allowed the emergence of multiple hypotheses, from which Arrhenius' Panspermia stands out[4]. In this hypothesis, Arrhenius suggested that life arose somewhere in the cosmos and was later brought to Earth by comets, meteoroids and interplanetary dust[1]. However, the survival of living organisms in the adverse conditions of interplanetary space was unlikely. Neopanspermia appeared as a way to respond to this drawback. In this hypothesis, it was proposed that comets and meteoroids transported the fundamental blocks of life, such as simple organic molecules and nucleobases. Carbonaceous chondrites contain calcium and magnesium carbonates as well as adenine, guanine and uracil hence supporting this hypothesis[5,6,7]. Nonetheless, the occurrence of uracil in carbonaceous chondrites is still controversial[8].

Nowadays, the most accepted hypothesis is known as the *RNA World Hypothesis*. It suggests that RNA appeared first, being used to both store genetic information and act as a catalyst known as ribozyme. However, RNA's low catalytic activity, combined with its low stability in aqueous media would have been the key factors that led to the development of the current system, where DNA stores genetic information, proteins act as catalysts and RNA works as an intermediate between these two structures[1,9,10,11,12,13].

A fundamental part of the *RNA World Hypothesis* is to understand how the basic units of ribonucleotides would have reacted in the prebiotic world. It is assumed that inorganic phosphate, ribose and one of the four nucleobases combined themselves to form the ribonucleotides. However this process is not yet clear[14,15].

Although phosphate may react with ribonucleosides through phosphorylation reactions, these reactions are thermodynamically unfavourable in aqueous media. Furthermore, the reaction between these two species required a source of

phosphate on the primitive Earth, that was, most likely, apatite. This mineral presents low solubility in aqueous media as well as low reactivity which would pose difficulty to the synthesis[16,17]. Despite these problems, phosphorylation reactions are already described[18].

Ribose, in biomolecules, occurs as a five-member ring known as furanose. Ribose's low stability is problematic for its use in prebiotic reactions. The problem can be overcome with its stabilization through the reaction with certain ions. Furanose can be stabilized in aqueous media when in the presence of borate and in the solid phase by calcium. It is also important to note that some lanthanides (such as praseodymium, lanthanum, cerium, neodymium and europium) can stabilize the six-member ring form, known as pyranose[2,19,20,21,22,23,24,25,26,27].

Lastly, ribonucleotides are also composed by one of four nucleobases: adenine, guanine, cytosine or uracil. Guanine is, among the four, the most abundant in carbonaceous chondrites[8].

Guanine, whose structure is present in figure 1, is the nucleobase with more tautomeric forms due to its three possible sites for tautomerism that give rise to twenty tautomers. When incorporated in a nucleoside, known as guanosine, one of these locations is lost because of the sugar bonding, so the number of tautomeric forms decreases to ten[28].
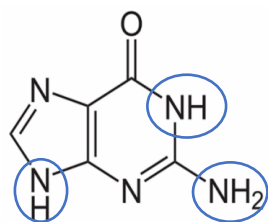
Figure 1 – Schematic representation of guanine molecule where the blue circles represent the possible sites for tautomerism (adapted[29])

There is some evidence of guanosine's degradation into guanine when subjected to the action of cosmic rays, which may explain why only nucleobases are present in meteorites[30]. In this work, the possibility to transform guanosine in the corresponding nucleobase through the reaction with carbonates, was studied.
Perhaps one of the greatest obstacles to ribonucleotides' synthesis, starting from the three basic blocks, is making sure that each component presents the required regioselectivity and stereoselectivity[7]. Besides stabilizing the sugar unit, calcium can also protect positions 2' and 3' formally derived from ribose, promoting the phosphorylation in position 5' (where phosphate is found in ribonucleotides)[31]. In order to test this hypothesis, the possibility of performing the phosphorylation of guanosine, adenosine and cytidine nucleosides using hydroxyapatite, a mineral composed simultaneously by calcium and phosphate, was studied. Hydroxyapatite is also the most common source of phosphate on Earth.

Experimental Part

Materials and Analytical Methods

All reactants were purchased from Baker Analized Reagent Crystal, Carlo Erba, Carlo Tuba, EKA ACS, Euriso-top, Fluka Riedel-de-Haën, MeB Pure Precipitate, Merck p.a, Riedel-de-Haën, Sigma, Sigma Aldrich and Specialty Chemicals Division Morristown and used with no further purification. Hydroxyapatite was synthetized according to the procedure described by *Paz et al*[32].

DRIFT spectra were obtained with a Mattson Research Series 1 Fourier Transform (FTIR) spectrophotometer. The bands were assigned by comparison with the literature[33,34].

^1H and ^{31}P NMR spectra were obtained with a 400 MHz Advanced III Bruker spectrometer equipped with a 5 mm BBO probe.

PXRD diffractograms were collected with a Bruker D8 Advanced diffractometer equipped with copper anode (Cu Kα 1, λ = 1.5406 Å) operated at 40 kV, 40 mA and equipped with LYNXEYE-XE. The data were recorded using Brag-Brentano's geometry.

SCRXD data were collected with a Bruker D8QUEST monocrystal diffractometer with graphite-monochromated radiation (Mo Kα, λ = 0.71073 Å) at 293 K. Crystals were mounted with Fomblin© in a cryoloop. The X-ray generator was operated at 50 kV and 30 mA. The X-ray data collection was monitored by APEX3[35]. The programs SAINT[36] and SADABS[37] were used to correct the obtained data to Lorentzian distribution, polarization and absorption effects. The program SHELXT[38] was used for structure solution while SHELXL-97[39] was used for full matrix least-squares refinement on F[2]. All

programs are included in the package WINGX-Version 2014.1[40,41]. Non-hydrogen atoms were refined anisotropically. A full-matrix least-squares refinement was used for the non-hydrogen atoms with anisotropic thermal parameters. All the hydrogen atoms bonded to carbon atoms were inserted in idealized positions and allowed to refine in the parent carbon atom.

Some samples were centrifuged using a P-selecta centro 8 LusoLab centrifuge.

Some samples were solubilised through sonochemistry using a Transsonic T460 Elma ultrasound equipment.

Degradation of ribonucleosides in the corresponding nucleobases

Samples of guanosine, adenosine, cytidine and guanine with $MgCO_3$, $CaCO_3$, Li_2CO_3, $MgSO_4.7H_2O$ and $CaSO_4.2H_2O$ were first prepared through mechanochemical procedures and then solubilised in aqueous media (acid, basic and neutral).

During the mechanochemical preparation some samples were milled by the mixer mill (with 7 mm stainless steel balls). The gridding time varied from 15 to 40 minutes, depending on the sample.

Solutions were prepared, in neutral media, with different quantities of sample (between 4 and 209 mg), volumes of water (between 4 and 41 mL) and temperature values (from 40 to 95 °C).

Solutions in acidic media were prepared with 0.32 M of HCl and temperatures from 25 to 55 °C.

Solutions in basic media were only prepared for guanine samples. 20 mg of guanine were solubilised in varying concentrations of NaOH (0.050 to 1.2 M) with different temperatures (40 to 50 °C). Then the salt in study was added. Samples prepared by mechanochemical procedures were also solubilised, using concentration of NaOH from 0.20 to 0.47 M. All the experiments were carried out at 40 °C.

After solubilisation the samples were divided into two equal parts (and in some cases, into three). A fraction was exposed to sunlight and another fraction was placed in its absence (using aluminium foil). When there were three fractions, the third was placed in a temperature beneath room temperature (in the fridge, at 4 °C).

Phosphorylation reactions

Samples of guanosine, adenosine and cytidine with hydroxyapatite were prepared by mechanochemical procedures in the proportion 2:1 ribonucleoside: calcium. A sample with both guanosine and adenosine, as well as hydroxyapatite, was also prepared, in the proportion 2:2:1 guanosine: adenosine: calcium.

Samples were first solubilised in neutral media in a total volume of 2.5 mL of water and later on were solubilised using 10 to 11 mL of water and heating to 90 °C. Only in the second case solubilisation was likely achieved. Subsequently, 16 mg of urea were added and 9 cycles (8 cycles in the case with 2.5 mL of water) were performed (between 90 to 95 °C). During each cycle the reagents were 8 hours in solution and 16 hours dry.

Results and Discussion

In this work samples of guanosine, adenosine, cytidine and guanine were prepared with $MgCO_3$, $CaCO_3$, Li_2CO_3, $MgSO_4.7H_2O$ and $CaSO_4.2H_2O$. The salts were chosen to study the effect of carbonate on the ribonucleosides (carbonate is responsible for ribose's degradation[42]) as well as the effect of different cations related with calcium (calcium can stabilize furanose in the solid state[2]).

Mechanochemical procedure

The first study was made by mechanochemical procedure that is the best way to replicate the conditions to which the meteoroids are exposed in the interstellar space.

Guanosine appeared to react with $MgCO_3$ in proportion 2:1. Figure 2 shows the obtained diffractogram for the sample, as well as the diffractograms of the pure reagents.
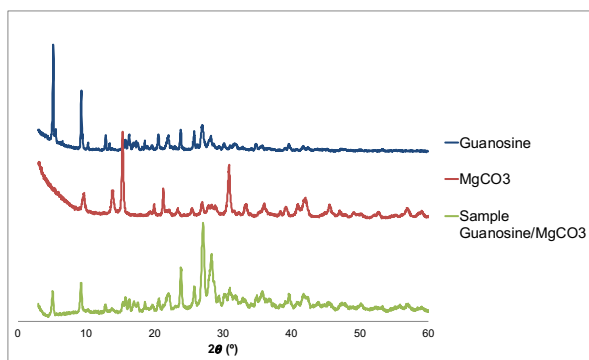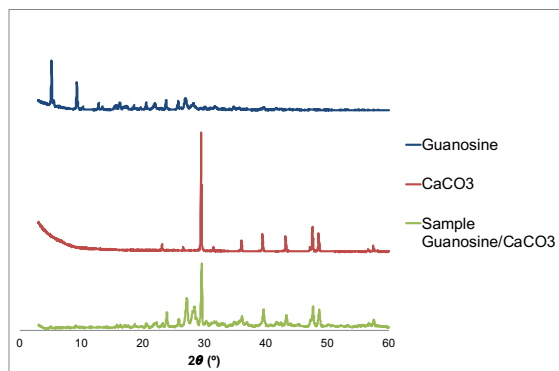
Figure 2 – Diffractogram of the sample containing guanosine and magnesium carbonate (2:1) (in green; for comparative purposes the diffractograms of guanosine and magnesium carbonate are shown in blue and red, respectively)

The use of the mixer mill, in this case, did not lead to significant changes in the diffractogram but made the sample slightly amorphous.

Some samples with guanine were studied for comparative purposes. The observation of the diffractogram of guanine in the presence of $MgCO_3$ (2:1) suggested the occurrence of a reaction, but in this case, it was not as clear as it was for guanosine.

Guanosine appeared to react with $CaCO_3$ (2:1) as the diffractogram in figure 3 shows, while with guanine, in the same conditions, the reaction was not observed.
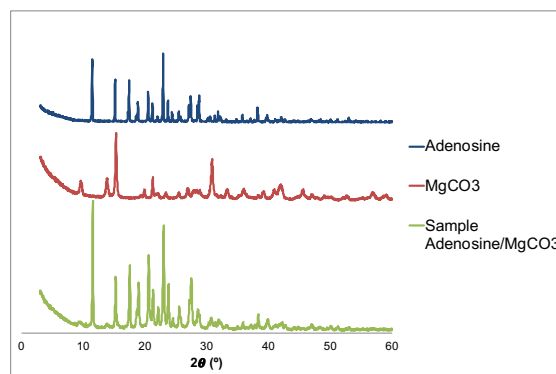

Figure 3 – Diffractogram of the sample containing guanosine and calcium carbonate (2:1) (in green; for comparative purposes the diffractograms of guanosine and calcium carbonate are shown in blue and red, respectively)

Finally, a brief dislocation of the diffractogram (when compared to isolated guanine) was observed for a sample containing guanine and lithium carbonate (2:1), however it was not considered conclusive. For guanosine, in the same conditions, the reaction was not observed.

All of guanine samples treated with the mixer mill tended towards the amorphous state.

Some studies were also performed using cytidine and adenosine. In the first case no conclusive results were obtained, while in the second case adenosine appeared to react with $MgCO_3$ when in proportion 2:1 (as shown in figure 4).

Figure 4 – Diffractogram of the sample containing adenosine and magnesium carbonate (2:1) (in green; for comparative purposes the diffractograms of adenosine and magnesium carbonate are sown and red, respectively)

Neutral Solutions

A sample containing guanosine and magnesium carbonate (2:1) was solubilised in neutral media, leading to the formation (after some days exposed to sunlight) of a green crystal of hexahydrate guanine with magnesium ion as a counterion. This preliminary result, obtained by *Carvalho et al*[43], indicated that the degradation of guanosine into guanine, in the presence of carbonate, was possible.

To understand the conditions that led to the degradation observed, a sample containing guanosine and $MgCO_3$ (2:1), Guanosine/Mg1, was prepared by mechanochemical procedure and subjected to the mixer mill for 20 minutes. After this preparation, 13.7 mg of sample were solubilised in 4 mL of water with heating (50 °C) and stirring. After total solubilisation, the sample was slowly cooled to room temperature. Precipitation was observed at 34 °C. The appearance was similar to the one observed for guanine samples under the same experimental conditions. Guanine presents limited solubility in neutral media. Therefore, it was assumed that the guanosine in the sample did, indeed, degrade into guanine. However, the sample was heated once again to 50 °C, which lead to new sample solubilisation. Since guanine samples do not easily solubilised only by heating it was

assumed the addition of magnesium carbonate promoted a chemical reaction.

Samples of guanosine with $MgCO_3$ (2:1) and guanosine with $CaCO_3$ (2:1) were prepared by mechanochemical procedure with small additions of water, giving rise to a pink colour as shown in figure 5. This colour may be due to the reaction between carbonate and one of guanosine's tautomers. This colour was not observed for guanosine in conjugation with magnesium sulphate and neither for carbonate alone.

Figure 5 – Pink colour observed for guanosine with $MgCO_3$ (2:1) and for guanosine with $CaCO_3$ (2:1)

Samples of guanine with the same salts were prepared for comparative purposes, however solubilisation was not possible. Guanine's solubilisation in neutral media is difficult, and the reasons for this are already described by *Darvishzad et al*[44].

^{1}H NMR spectra for samples with guanine and $MgCO_3$ (2:1) and guanosine and $MgCO_3$ (2:1) did not produce conclusive results. The difficulty in solubilising sufficient quantities of sample only allowed the detection of the HDO's signal.

16.7 mg of a sample composed by adenosine and $MgCO_3$ (2:1), Adenosine/Mg1, were solubilised in 5.3 mL of water with heating (75 °C) and stirring for a period of three hours. The sample was then slowly cooled to room temperature and exposed to sunlight for 36 days. A green precipitate, that seemed to be crystalline, was observed. This colour was already observed by *Carvalho et al*[43] when guanosine degraded to guanine, so, in this case, it was assumed that adenosine did also degraded to adenine. Further studies were not possible, in neutral media, but were carried out in acidic media.

Basic Solutions

Some samples containing guanine were solubilised in basic media (due to the limited solubility of this nucleobase in neutral solutions), however it was not possible to characterise the final structures, so no samples containing guanosine were tested in this media.

The most important result, in this case, was the white precipitate obtained after the solubilisation of guanine and calcium sulphate (2:1) with 1 M of NaOH. This precipitate was washed with water and acetone, to dry, and then analysed by DRIFT. The infrared spectra, shown in figure 6, suggests the coordination of calcium cation with one of guanine's tautomers, but, since it was not possible to obtain additional information, it was not possible to draw definitive conclusions.
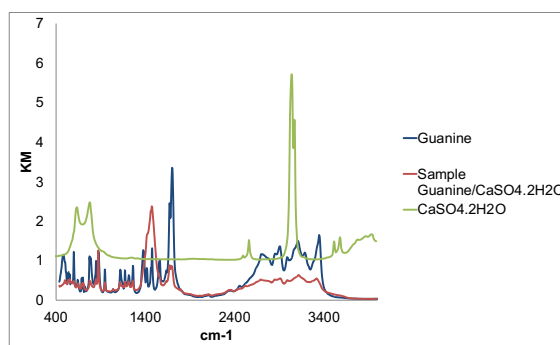
Figure 6 – DRIFT spectra of the sample containing guanine and calcium sulphate (2:1) in basic conditions (in red, for comparative purposes DRIFT spectra of guanine and calcium sulphate are shown in blue and green, respectively)

Acid Solutions

The sample Guanosine/Mg1 was solubilised in acidic media, using 0.32 M of HCl and heating to 25 °C with stirring. After total solubilisation the sample was exposed to sunlight for ten days. The final precipitate was analysed by SCXRD and it was verified to be guanine hydrochloride monohydrate. The final structure is shown in figure 7.
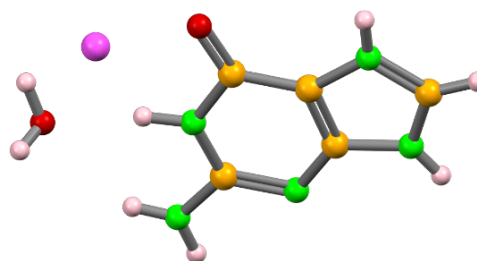
Figure 7 – Structure of guanine hydrochloride monohydrate obtained from the reaction of guanosine with magnesium carbonate (2:1) in acidic conditions. Green shows the nitrogen atoms, yellow the carbon atoms, red the oxygen atoms, purple the chloride atom and pink the hydrogen atoms

The initial hypothesis was verified – the degradation of guanosine to guanine is indeed possible. However, this degradation could have occurred in acidic media. Since there are studies that show guanosine's degradation to guanine in harsher conditions (in acidic media)[45] further studies must be conducted to confirm that the degradation is the result of a reaction with carbonate.

Some samples containing guanine were studied in acidic media for comparative purposes. A sample containing guanine and magnesium sulphate (2:1) was solubilised in sulfuric acid (0.55 M) with heating (45 °C) and stirring. After some days without solar exposure, a precipitate was obtained. It was verified to be, by SCXRD, guanine sulphate hemi-hydrate (whose structure is present in figure 8). Although it was not one of the goals of the present work, this is a new structure, so it deserves to be mentioned.
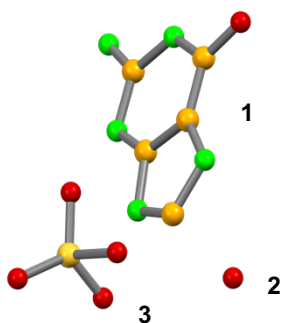
Figure 8 – Structure of guanine sulphate hemi-hydrate obtained from the reaction of guanine with magnesium sulphate (2:1) in acidic media. **1** - Guanine's structure (green shows the nitrogen atoms, yellow the carbon atoms and red the oxygen atoms); **2** – Water molecule shared with another guanine sulphate hemi-hydrate of the unit cell; **3** – Sulphate's structure (red shows the oxygen atom and yellow the sulphur atoms). Hydrogen atoms are not shown for clarity

Finally, Adenosine/Mg1 was also solubilised in acidic media, using 1.37 M of HCl. Since the concentration of acid was superior to the one used in the sample containing guanosine, the solubilisation was possible without heating. It was not possible, at this stage, to obtain a structure and characterise it.

Phosphorylation reactions

The possibility of phosphorylation with hydroxyapatite was tested for guanosine, adenosine and cytidine. Hydroxyapatite and each one of the nucleosides (2:1 ribonucleoside: calcium) were placed in 2.5 mL of water at 90 °C. 8 cycles were performed after which the samples were analysed by ^{31}P NMR spectrometry. It was not possible to obtain conclusive results, which may result from the non-solubilisation of the samples. In order to overcome this problem a bigger volume of water was used. The complete solubilisation of the sample containing cytidine was not achieved, so the study was not pursued. Samples containing guanosine and adenosine with hydroxyapatite as well as a sample containing both nucleosides and hydroxyapatite were apparently solubilised in 10 to 11 mL of water using heating (90 °C) and stirring. Then 16 mg of urea were added and 9 cycles (8 hours solution, 16 hours dry) were performed. The obtained products were solubilised in 1 mL of water and analysed by ^{31}P NMR spectrometry, however it was not possible to obtain conclusive results.

Final Remarks

The structure of guanine hydrochloride monohydrate obtained after the reaction of guanosine with magnesium carbonate in acidic media constitutes the most relevant result of this work. Further studies need to be conducted in order to clarify if the degradation is due to the action of carbonate or the acidic media. However, the pink coloration obtained, in neutral media, for samples containing guanosine with magnesium carbonate and calcium carbonate, as well as the result obtained by *Carvalho et al*[43], indicates that the degradation is promoted by carbonate. One interesting study to conduct is to follow the reaction, by ^{1}H NMR spectrometry, in acidic media, in order to understand how the reagents interact with one another. This study may also quantify the reaction. If the solution is not sufficient concentrate the signal attribute to heavy water may pose difficulties in the reaction quantification. ^{13}C NMR spectrometry can be a way to overcome this problem. Alternatively, HPLC may be used.

The structure of guanine hydrochloride monohydrate was obtained after exposure to sunlight, however, further studies should be performed in order to clarify the action of solar radiation into the reaction.

The sample containing adenosine and magnesium carbonate presented a green precipitate after some days exposed to sunlight. Further studies should be carried out in order to characterise this structure.

As far as the phosphorylation reactions are concerned, ^{31}P NMR spectra did not present conclusive results, which may be due to the deposition of precipitate in the NMR tube. This precipitate could contain unreacted hydroxyapatite. The use of borate minerals to promote phosphate's solubilisation, while still protecting 2' and 3' positions of nucleosides, may lead to their phosphorylation.

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