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Interactions of D-ribose with polyatomic anions, and alkaline and alkaline-earth cations: possible clues to environmental synthesis conditions in the pre-RNA world†

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Received (in Montpellier, France) 6th June 2008, Accepted 1st July 2008
First published as an Advance Article on the web 17th July 2008
DOI: 10.1039/b809636h

Published on 17 July 2008 on http://pubs.rsc.org | doi:10.1039/B809636H
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The interaction of some of the most abundant anions in seawater (borate, sulfate and carbonate/bicarbonate) with D-ribose was studied by ^{1}H, ^{11}B and ^{13}C NMR spectroscopy. The results confirmed that only borate improves the stability of D-ribose and favours significant amounts of the ribofuranose isomer, which is the form occurring in present day living organisms. The effect of cations (Na^{+}, K^{+}, Mg^{2+}, Ca^{2+} and Sr^{2+}) on ribose–borate-bound species was also studied, and it was found that the divalent cations induce a small increase in the relative amounts of ribopyranose isomers in solution and a corresponding decrease in the abundance of the ribofuranose isomers. It was also found that the stability of ribose–borate-bound species, when compared with free ribose over a wide range of temperature and pH values, is higher for compounds with borate, which are stable even at a relatively low pH (6.6) and a relatively high temperature (60 °C).

The stability of ribose under moderate pH and relatively high temperature conditions, in the presence of species that occur in seawater, is important for the viability of the early synthetic steps that led to the first nucleotides, which predated the formation of more complex structures, such as RNA.

Introduction

The interaction of D-ribose with anions is a subject of current interest, due to the probable effect of such interactions in prebiotic and biological chemistries. Ribose participates in relevant biological structures that occur in living organisms, such as nucleic acids, ribozymes and some organic co-factors. The wide distribution of this pentose in life forms suggests that it may have been involved in prebiotic chemistry on Earth. Although the synthesis of ribose by a possible prebiotic pathway has only been achieved in low yields,[1] a long-term accumulation of ribose could overcome this problem. Nonetheless, an additional difficulty is the stability of this pentose, which depends on the physical and chemical conditions of the reaction medium.[2] This fact has stimulated further studies aimed at establishing which conditions are adequate, or even favourable, so that the participation of ribose in prebiotic chemistry becomes both possible and probable.

In 2005, Ortiz *et al.* used ^{1}H and ^{13}C NMR spectroscopy to study D-ribose speciation in aqueous solution and quantified four different isomers (Fig. 1): α- and β-furanose, which account for approximately 20% of D-ribose in aqueous solution, and the more stable α- and β-pyranose isomers, corresponding to the remaining 80%.[3] The open chain forms account for less than 0.2% of the total and are not usually detected.[4]

The relative amounts of the different isomers in aqueous solution are quite different in the presence of sodium borate, when only the ribofuranose–borate isomers are detected.[5] The structures of the ribose–borate-bound species (BBS) are shown in Fig. 2.

More recent studies have reported variations in the thermodynamic stability of ribose with temperature and pH as a consequence of the interaction of ribose with boron species.[6–8]

Boron, which has only one common oxidation state, is currently the 10th most abundant element in seawater,[9] where it occurs predominantly as borate and boric acid, and is essential for several organisms.[10] However, the presence of other anions (such as halogens, sulfate, bicarbonate and carbonate) in higher concentrations[11] poses an intriguing

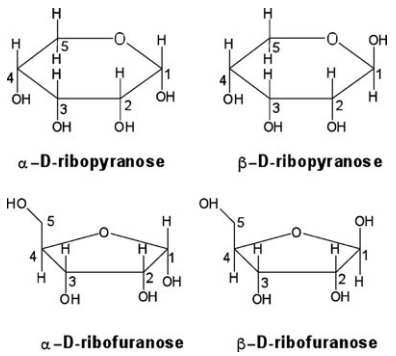

Fig. 1 A schematic representation of the D-ribose isomers present in aqueous solution.

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† Electronic supplementary information (ESI) available: Experimental details and characterization data. See DOI: 10.1039/b809636h

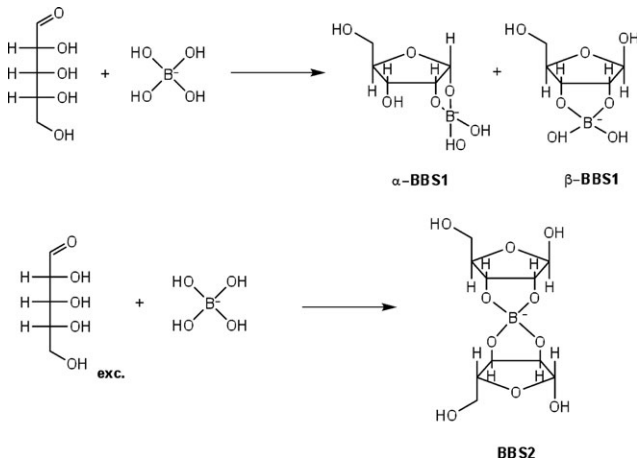

Fig. 2 A schematic representation of compounds of D-ribose with borate in aqueous solution.

question: is the increased stabilization of D-ribose caused by a specific effect of borate or can any of the other anions present in seawater have an identical role?

A previous study concerning the influence of halogen anions (fluoride, chloride, bromide and iodide) also reported a relationship between the concentration of their alkaline salts and the isomer populations of D-ribose in water.[3] In almost all cases, the sugar isomers' populations were affected by the presence of halide salts, but these seemed to favour the pyranose isomers, perhaps as a consequence of interactions between the cations and hydroxyl groups at the C_1 to C_4 carbon atoms, while C_5, the only carbon with a CH_2 group (see Fig. 1), interacted with the anion. The overall effect induced a polarization of the ribose molecule, which led to the predominance of the pyranose over the furanose isomers.

Elucidation of the process of ribose stabilization is a necessary step that would allow us to focus attention on the subsequent stages of nucleotide assembly, such as the phosphorylation of the pentose C_5 hydroxyl and the introduction of nucleobases, none of which have yet been solved adequately.[2] For example, the binding of ribose and phosphate in water requires condensing agents, and the regiospecificity of the reaction (typically phosphorylation at C_1) is not the same as that present in living organisms.[12]

As stated above, ribose occurs exclusively in modern biological structures as the β-ribofuranose isomer. To understand how this takes place in aqueous solution, we have compared the effect of borate on ribose stabilization with the effects of other naturally-occurring anions, such as the polyatomic sulfate and bicarbonate/carbonate anions. We have also tested the effects of the alkaline and alkaline-earth cations, that are predominant in the composition of present-day seawater, in the presence of borate, as well as the stabilities of some of the species we have studied over a wide pH and temperature range. With these models, we have tried to reproduce some plausible environments of primitive Earth where prebiotic structures could have formed, such as in the regions around deep-ocean hydrothermal vents,[13] an environment where the water temperature and pH would allow the solubilization of several salts. The composition of the primitive oceans of Earth is unknown, but it is commonly accepted that some of the relevant ionic components dissolved in modern oceans were also present in significant amounts in the prebiotic period (with the exception of the oxidized forms of some elements, which now occur in higher concentrations).

The results obtained support the idea that the prebiotic synthesis of ribose could indeed have occurred in primordial oceans close to these highly active geological sites. This paper describes results of our ongoing work, aiming to define the conditions that could have allowed the synthesis of block constituents of RNA or its models.

Results and discussion

Effect of sulfate

The presence of sulfate in an aqueous solution of ribose does not interfere significantly with its speciation. All the peaks of the four cyclic ribose isomers are visible in the ^{13}C NMR spectrum, with chemical shift variations lower than δ 0.1 compared to ribose (see Fig. 3, panels: ribose and ribose + Na_2 sulfate). It is important to note that sulfate should have been rare in prebiotic Earth oceans,[14] since its formation in significant amounts would have required an oxidizing environment. The inclusion of sulfate in this study was only justified by its analogies with borate (namely the similarities of their structures, since both are polyatomic ligands with four oxygen atoms), but the distance between the oxygen atoms in sulfate proved to be unsuitable for an effective interaction with the sugar.

Effect of carbonate/bicarbonate

The presence of carbonate/bicarbonate had a rather different effect compared to sulfate. In effect, the solid resulting from D-ribose incubation with sodium carbonate/sodium bicarbonate at pH 9.5 was of a yellow colour. Although the composition of this solid was not investigated, the yellow coloration gives an indication of sugar degradation, presumably as a result of base-catalysed enolization and retro-aldol reactions of the open chain form, followed by autoxidation.[15] The complexity of the ^{13}C NMR spectrum (Fig. 3, panel: ribose + Na bicarbonate + Na_2 carbonate), which displays multiple peaks, including a signal at δ 164.2 that possibly stems from a carboxylic acid or an ester group,[16] is consistent with this interpretation. Not unexpectedly, a similar degradation of ribose was observed when the experiment was conducted at pH 10.9.

Effect of borate

It is well known that borate forms stable compounds with vicinal diol groups.[17] The BBS obtained under our experimental conditions were easily identified by ^{13}C NMR. Since the primary C_5 carbon is not close to the possible binding sites of any of the hemiacetal isomers, analysis of the C_5 chemical shifts provided a simple way of assessing the ribose conformation. Thus, both the α- and β-ribofuranose anomers [δ_C 61.2 ($C_{5\alpha}$) and 63.9 ($C_{5\beta}$)][3] were clearly found to be present. Further analyses, for instance of the anomeric carbon resonance signals, support the formation of α-BBS1 (*i.e.*, interaction through the C_1 and C_2 hydroxyl groups) as the predominant product [δ_C 102.6 ($C_{1\alpha}$)].[5]

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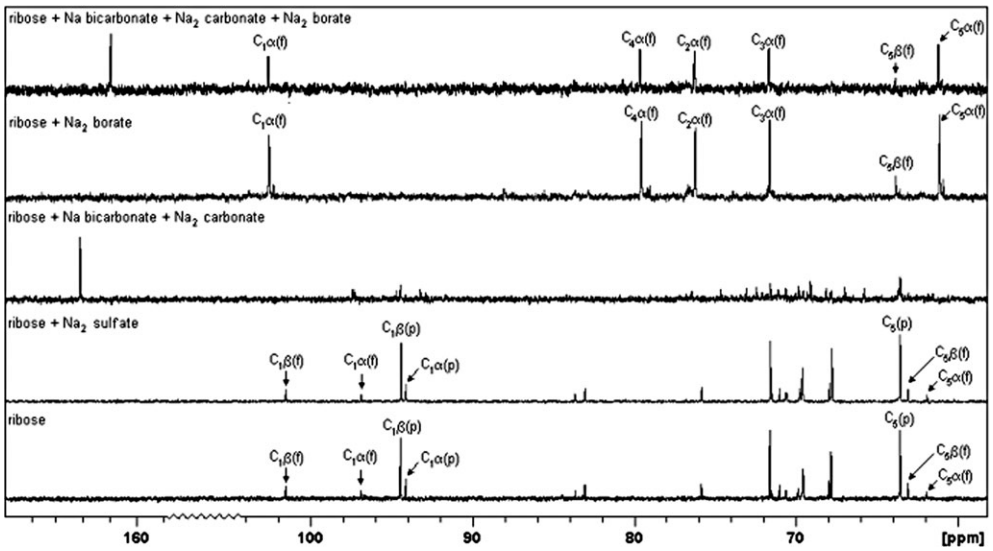

Fig. 3 ^{13}C NMR spectra of ribose, and of ribose solutions with: sodium sulfate, sodium carbonate + sodium bicarbonate, sodium borate, and sodium carbonate + sodium bicarbonate + sodium borate. The symbols 'f' and 'p' represent furanose and pyranose, respectively. The spectra were recorded in D_2O.

Since the carbonate/bicarbonate system is much more common than boron in natural seawater and ribose is degraded by carbonates, could the presence of borate prevent it? D-Ribose incubation tests with a mixture of carbonate/bicarbonate/borate at pH 9.3 indicated the formation of the BBS with little sugar degradation, as evidenced by analysis of the ^{13}C NMR spectrum of the resulting product (see Fig. 3, panel: ribose + Na bicarbonate + Na_2 carbonate + Na_2 borate). In effect, a comparison of the spectra obtained upon the incubation of ribose with either borate (panel: ribose + Na_2 borate), carbonates (panel: ribose + Na bicarbonate + Na_2 carbonate) or a mixture of carbonates and borate (panel: ribose + Na bicarbonate + Na_2 carbonate + Na_2 borate) clearly shows that the resonances assigned to the BBS [δ_C 61.2 ($C_{5\alpha}$), 63.9 ($C_{5\beta}$), 71.7 ($C_{3\alpha}$), 76.3 ($C_{2\alpha}$), 79.6 ($C_{4\alpha}$), 102.6 ($C_{1\alpha}$)] are observed if borate is present, regardless of the presence or absence of carbonates. Furthermore, the spectrum obtained from the solution with borate and carbonates together is almost identical to that recorded from incubation in the absence of carbonates, with the exception of a downfield signal at δ 162.2, presumably corresponding to the carbonate carbonyl. Thus, interaction with borate avoids, at least partially, the carbonate-induced degradation of ribose.

Effect of cations (Na^+, K^+, Mg^{2+}, Ca^{2+} and Sr^{2+}) on the speciation of ribose–borate-bound species

Most boron-containing minerals that exist in nature are associated with alkaline and alkaline-earth metal cations, which are also the most common in seawater,[18] and the most abundant and relevant in the metabolism of modern organisms. Thus, it seemed interesting to investigate several sources of borate and assess the effect of the cation on the reaction between borate and D-ribose. Bearing in mind the purpose of this study, it was decided to evaluate the borates of sodium, potassium, magnesium, calcium and also strontium (which is not very abundant in seawater, but exists in a concentration comparable to that of borate[11]). Note that the first four elements are essential for all organisms and strontium is at least essential for *Acantharea*.[19] It is known that some divalent and trivalent cations can form weak complexes with pentoses and hexoses in water, thus being a possible cause of interference in the reaction of ribose with borate,[20] depending on the cation charge and size. The larger ions can interact with the axial-equatorial-axial arrangement of three consecutive hydroxyl groups of pyranose or the *cis–cis* sequence of furanose, both of which are possible in ribose.[21]

^{11}B and ^{13}C NMR studies were conducted to assess the effect of the alkaline and alkaline-earth cations on the isomers' populations, and their quantification was achieved through integration of selected regions of the ^1H NMR spectra. These analyses confirmed that the nature of the borate counterion affected the relative percentages of ribose isomers (and their bound species with borate) in water, as shown in Fig. 4.

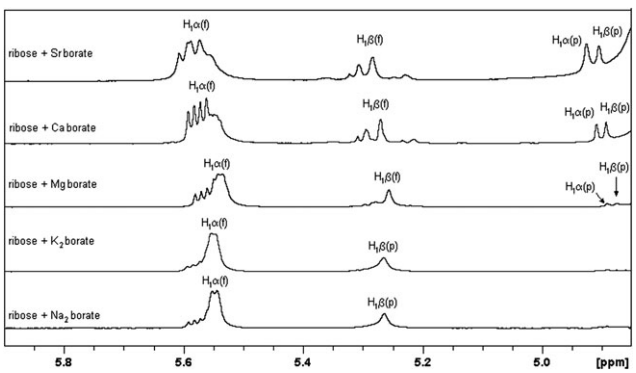

Fig. 4 Detail of the ^1H NMR spectra of BBS synthesized from ribose and sodium borate, potassium borate, magnesium borate, calcium borate or strontium borate. The symbols 'f' and 'p' represent furanose and pyranose, respectively. The spectra were recorded in D_2O.

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Table 1 Ribose isomer populations after incubation with sodium, potassium, magnesium, calcium and strontium borates

Borate counter-ion	α-Ribofuranose, as α-BBS1 (%)[a]	β-Ribofuranose, as β-BBS1 (%)[a]	α-Ribopyranose (%)[a]	β-Ribopyranose (%)[a]
Na^+	72	28	ND[b]	ND
K^+	76	24	ND	ND
Mg^{2+}	75	21	2	2
Ca^{2+}	74	15	5	6
Sr^{2+}	62	15	12	11

[a] Relative percentages of the four isomers discussed, as determined by ^{1}H NMR in D_2O. [b] ND = not detected.

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Two distinct types of interactions are apparent in the expanded region of the ^{1}H NMR spectra, spanning the anomeric proton resonances, as displayed in Fig. 4. Thus, it is possible to distinguish between the sodium, potassium and magnesium borates when compared with their calcium and strontium counterparts: the former group is almost exclusively composed of ribofuranose isomers (although for magnesium, a small percentage of the ribopyranose isomers—around 4%—were also detected), predominantly α-ribofuranose-bound (α-BBS1) species with a smaller percentage of β-ribofuranose-bound (β-BBS1) species. For calcium and strontium, significant yields of ribopyranose isomers were observed, as indicated by the presence of the two upfield resonances between δ 4.8 and 4.9. The structures of the individual isomers were assigned on the basis of the anomeric proton signals: for α-BBS1 and β-BBS1, δ_H 5.6 (1α-H), 5.3 (1β-H); for α- and β-ribopyranose, δ_H 4.9 (1α-H), 4.9 (1β-H).[6] A thorough analysis of the ^{1}H NMR spectra allowed quantification of the observed cation effects by peak integration. In the presence of sodium or potassium borate, all of the ribose in solution was in a bound form, with small differences in the relative concentrations of the ribofuranose isomers (see Table 1). When divalent metals were used, the interaction still occurred, but to a lower extent. In the presence of magnesium, the reaction was almost complete (96% of ribose was bound to borate), but when calcium and strontium were used, only 89 and 77% of the ribose, respectively, was bound to borate.

In agreement with the ^{1}H NMR results, the calcium and strontium borates (and to a lesser degree, magnesium borate) significantly altered the ^{13}C NMR spectra, compared to the spectra recorded in the presence of sodium and potassium borates. In both cases it was possible to identify the ribopyranose isomers, characterized by the signals δ_C 94 ($C_{1\alpha}$p) and 95 ($C_{1\beta}$p), in addition to the predominant ribofuranose-bound species (not shown). These data were further corroborated by ^{11}B NMR analysis of the incubation mixtures containing the divalent cations (magnesium, calcium and strontium), in which, besides the two peaks ascribed to the BBS, δ_B 7.5 (BBS1) and 13.1 (BBS2), a well-defined downfield signal was observed (at approximately δ 16 when magnesium borate was used, and at approximately δ 19 in the case of the other divalent metal borates) that was consistent with unbound borate[22] (see Fig. 5).

The different behaviour of the calcium and strontium borates is unlikely to be only charge-related, given that magnesium has an effect upon BBS that is comparable to the monovalent cations sodium and potassium. Note that the biological molecules occurring in living organisms that contain ribose associated with phosphate (*e.g.* RNA) are anionic at physiological pH, and that they need cations to neutralize their negative charge. Curiously, the alkaline cations, and also magnesium, perform this function in biological structures containing ribose.[19]

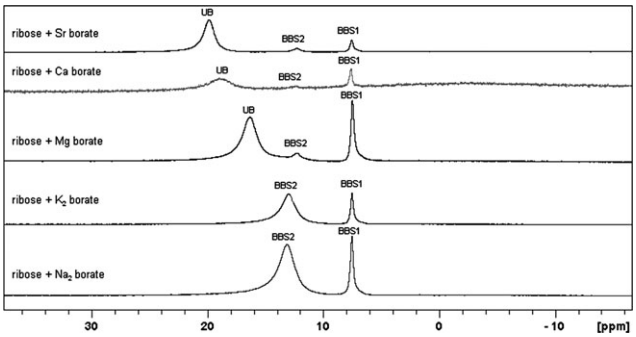

Fig. 5 ^{11}B NMR spectra of BBS synthesized from ribose and sodium borate, potassium borate, magnesium borate, calcium borate or strontium borate. The symbol 'UB' represents unbound borate. The spectra were recorded in D_2O.

Since the amount of BBS varies according to the cation tested, we have tried to detect correlations between this variation and several parameters related to the strength of the chemical bonds involved. One of the most interesting was the correlation between the effective nuclear charge‡ (Z_{eff}), the cation–oxygen distance and the amount of BBS formed (see Fig. 6).

It should be mentioned that the effective nuclear charge according to both Clementi and Froese-Fischer[9] give similar results (not shown). Z_{eff} according to Slater was not considered since, due to its simplifications (namely the assumption of circular orbitals), the conclusions would be more questionable.

The presence of the pyranose isomers can be explained if one takes into account the possibility that the cations compete with ribose for borate, since the association constants of the species formed between the divalent cations and borate, and between ribose and borate, are of similar magnitude.[5,23] Hence, it is possible that the formation of more stable complexes between borate and calcium (or strontium) would decrease the amount of free borate available to interact with ribose. Under these conditions there would be more free ribose in solution, which would preferentially adopt the ribopyranose

‡ Effective nuclear charge (Z_{eff}) is the net charge experienced by an electron of an atom or ion, resulting from the screening of its nuclear charge (number of protons) by its electrons. There are several approaches to calculate this parameter; in this work we have used the values according Clementi.[9]

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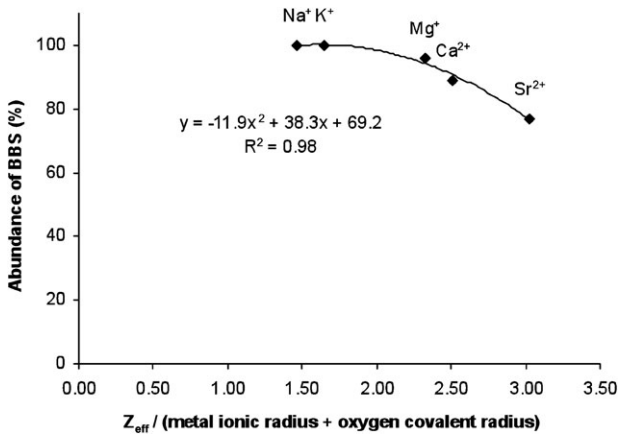

Fig. 6 Correlation of the cation Z_{eff}/(metal ionic radius + oxygen covalent radius) ratio with the percentage of BBS in solutions of sodium, potassium, magnesium, calcium and strontium borates.

form (the most stable ribose isomer in aqueous solution in the absence of borate).

Analysis of Fig. 6 allows the decrease of BBS with increasing [Z_{eff}/(metal ionic radius + oxygen covalent radius)] to be identified. A similar trend is observed with increasing (charge density × atomic weight).§ This could be due to the interaction of the ions with the ribose–borate structure; note that for all cations, the ionic radii[9] are shorter than the distance between the two oxygens of the borate anion (approximately 2.42 Å; calculations based on the borate tetragonal geometry, where the B–O bond length is 1.48 Å).

Effect of temperature and pH on the stability of ribose–borate-bound species

The addition of the D-ribose to borate gives stable compounds, even at room temperature, that occur from the binding of borate to the vicinal hydroxyl groups of the sugar (see Fig. 2), As indicated above, ^{11}B NMR analysis of the reaction mixture provided evidence for the formation of two different BBS, monoester BBS1 and diester BBS2.

The BBS is formed preferentially at pH > 9, since the predominant boron species at lower pH is boric acid.[18] Nevertheless, it was still possible to detect the formation of this compound at a pH as low as 6.6 by ^{13}C NMR spectroscopy, although the compound yield was quite small. Furthermore, when a solution of the compound prepared at pH 9.3 was acidified to pH 7.7, no significant dissociation was observed by ^{13}C NMR (see ESI†).

The conditions adopted in our studies, specifically the approximately equimolar proportion of ribose and borate, are not plausible for primitive Earth (boron, and consequently borate, should have been much more abundant than ribose). As a consequence, even with lower concentrations of borate at a neutral or slightly acidic pH, ribose would be expected to occur almost completely as the BBS.

Another fundamental aspect is the effect of temperature on the stability of ribose. Results concerning ribose degradation by $Ca(OH)_2$ at 25 and 45 °C, which occurs after 1 h and 10 min, respectively, have been reported in previous work.[6] In the presence of borate, the solution remained colourless, even after 2 months. In the present work, the stabilities of the BBS were tested at higher temperatures, T = 40, 60, 80 and 100 °C, but in the absence of base. At a temperature of 40 °C, the compound remained stable after 5 months, as judged qualitatively from the lack of colour change. Even at 60 °C, stability was still observed for more than 45 d. At higher temperatures, the ribose solution turned yellow after a few hours (*ca.* 20 h at 80 °C and 1.5 h at 100 °C). This coloration is characteristic of D-ribose degradation due to products resulting from reactions of the open chain form, which requires prior dissociation of the BBS.

Conclusions

Borate may well have played a key role in the stabilization of ribose, a fundamental step in the prebiotic synthesis of nucleotides. This argument is based on its ability to form stable compounds with ribose that favour the furanose isomers and hamper sugar degradation. An important consideration is that the furanose forms of the pentose, facilitated by borate compounds, are less reactive than the open chain form, given their lack of carbonyl groups.[24] Additionally, the stability range of BBS compared to free ribose, with respect to temperature and pH, is broader for the compounds with borate. In this paper, it is shown that the BBS are stable for long periods of time at temperatures close to 60 °C and that they can be formed at pH > 6.6. Although the presence of the heavier alkaline-earth cations appears to hamper formation of the bound species, an excess of borate can prevent this effect by precipitation due to the sparing solubility of these borate salts (in the range of 0.01 to 1 g per 100 g of water).[25]

The presence of borate in the same regions as the proposed locations of prebiotic ribose synthesis, namely in deep-ocean hydrothermal vents,[26] could be a way of stabilizing this sugar in a form that would allow the assembly of more complex structures, such as nucleosides, nucleotides and nucleic acids. Critics of the hypothesis of deep-ocean hydrothermal vents as plausible sites for the onset of life have primarily emphasized the lack of stability of some of the required constituents, particularly ribose, in these alkaline and high temperature locations. However, the results reported herein show that, even under adverse conditions and environments, borate is still capable of interacting with ribose, inducing a predominance of the furanose isomer and increasing its stability. Curiously, in the BBS, the hydroxyl groups located at carbons C_5 and C_3, which are phosphorylated in biological nucleotides, are 100% and 70% free, respectively.[5] Preliminary results obtained in our laboratory, which will be described in a subsequent paper, suggest that the regioselective phosphorylation of ribose at the biologically-relevant C_5 position is possible under particular conditions.[27]

§ Lithium and caesium were also studied. Neither of these cations occur in present-day living organisms, which, in conjunction with their low current abundance, indicates that their involvement in prebiotic chemistry was unlikely. Their inclusion in this study was decided after some interesting correlations were obtained for the cations Na^+, K^+, Mg^{2+}, Ca^{2+} and Sr^{2+}. To further probe these correlations, additional elements, with a wider range of properties, were incorporated in the study. The results, including lithium and caesium, are presented in the ESI.†

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As stated above, the presence of alkaline-earth cations can interfere with borate and reduce its binding to ribose. This effect could conceivably be used to remove borate (which is acting as a protecting group) from the ribose, which might eventually allow the assembly of structures related to the known nucleotides.

Experimental

Materials and analytical methods

All reactants used were of the highest purity and were purchased from Merck, Sigma-Aldrich, BDH Chemicals, Eka Chemicals, M&B or Carlo Erba.

Calcium, magnesium and strontium borates were synthesised according to a procedure described elsewhere.[28]

^{1}H NMR [300 MHz, D_2O, DOH], and ^{11}B NMR [96.3 MHz, D_2O, $BF_3O(CH_2CH_3)_2$] spectra were recorded on a Bruker Avance III 300 Ultra Shield spectrometer, and ^{13}C NMR spectra [100.6 MHz, D_2O, $CO(CH_3)_2$] were recorded on a Bruker Avance III 400 Ultra Shield spectrometer. All δ values are given in ppm. The assignments of specific proton, carbon and boron signals were achieved by comparison with published data.[3,5]

The pH values were measured with a Model 827 lab Metrohm pH meter.

Study of the interactions between D-ribose species and several anions present in seawater

Under a nitrogen atmosphere, 1.3 mmol of sodium tetraborate was dissolved in 10 mL of deoxygenated water. After complete dissolution, 1 mmol of D-ribose was added, and the reaction mixture was stirred for 2 h. The pH of the solution was 9.3. The solvent was removed under vacuum and a white solid was obtained. Finally, a clear solution of approximately 1 mL of D_2O containing 20 mg of the white solid was prepared, and subsequently characterized by ^{1}H, ^{11}B and ^{13}C NMR spectroscopy.

Similar reactions were conducted by replacing sodium tetraborate with sodium sulfate (a sodium hydroxide solution was added dropwise until the appropriate alkaline pH was reached) or a sodium carbonate/sodium bicarbonate mixture, at pH 9.5 and 10.9, respectively. An additional study was carried out with a mixture of sodium tetraborate, sodium bicarbonate and sodium carbonate at pH 9.3. In all instances, a clear solution of approximately 1 mL of D_2O containing 20 mg of the white solid was prepared, and subsequently characterized by ^{1}H, ^{11}B and ^{13}C NMR spectroscopy.

Effect of cations on ribose–borate-bound species

The quantification of the D-ribose species in the presence of several borate salts (sodium, potassium, magnesium, calcium and strontium) was carried out at pH 9.3 according to the procedure described above. In all instances, a clear solution of approximately 1 mL of D_2O containing 20 mg of the white solid was prepared, and subsequently characterized by ^{1}H, ^{11}B and ^{13}C NMR spectroscopy.

Effect of pH on the formation of ribose–borate-bound species

Under a nitrogen atmosphere, 25 mmol of boric acid was added to 30 mL of a solution of 1.3 mmol of sodium tetraborate prepared in deoxygenated water at pH 6.6. After complete dissolution of boric acid, 1 mmol of D-ribose was added and the mixture was stirred for 2 h. The solution was evaporated under vacuum and a white solid was obtained. Finally, a clear solution of approximately 1 mL of D_2O containing 20 mg of the white solid was prepared, and subsequently characterized by ^{1}H, ^{11}B and ^{13}C NMR spectroscopy.

Effect of pH on the stability of the ribose–borate-bound species

To a solution of the ribose–borate-bound species (prepared according to the procedure described above), a solution of boric acid was added dropwise until pH 7.7 was reached. The solution was evaporated under vacuum and a white solid was obtained. Finally, a clear solution of approximately 1 mL of D_2O containing 20 mg of the white solid was prepared, and subsequently characterized by ^{1}H, ^{11}B and ^{13}C NMR spectroscopy.

The solutions prepared at pH 6.6 and 7.7 were then compared to a solution of ribose–borate at pH 9.3 (obtained by the procedure described above) using NMR spectroscopy.

Effect of temperature on the degradation of the ribose–borate-bound species

The solution containing the compound prepared with ribose and sodium tetraborate was split into four aliquots, which were heated at 40, 60, 80 and 100 °C, respectively, under a nitrogen atmosphere until the solutions turned yellow, indicating ribose degradation. The initial pH of all solutions was 9.3.

Acknowledgements

This research was supported by pluriannual funding from Fundação para a Ciência e a Tecnologia (FCT), Portugal.

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