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BORON ENHANCES THE THERMOSTABILITY OF CARBOHYDRATES

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Abstract. We have studied the effect of borate and pH upon the half-lives of ribose and glucose. Under acidic conditions the presence of boric acid increase the thermo-stability of ribose, while under basic conditions glucose is favored.

Introduction

According to one theory the origin of life may have been related to deep sea vents at temperatures as high as 300 °C (Pledger *et al.*, 1994). The fact that some of the hyperthermophiles, presumed to be some of the oldest organisms on Earth (Segerer *et al.*, 1993; Forterre *et al.*, 1995) and thermophiles (Gaucher *et al.*, 2003) grow optimally at relatively high temperatures seems to support the hypothesis that living matter developed at high temperatures. However, some important biomolecules are thermo-labile and thus destroyed as quickly as they are produced (Levy and Miller, 1998). Supporters of the "high temperature origin" theory claim that organic molecules may have been formed at the interface between hot hydrothermal fluids the cold seawater at the bottom of the ocean. The absence of mechanisms protecting carbohydrates at high temperatures remains an important conundrum for the hydrothermal origin of life (Levy and Miller, 1998). RNA-related molecules which may have been important in carrying information and catalysis are also unstable in hydrothermal conditions, with a half-life of just few minutes (White, 1984; Miller and Bada, 1988).

The presence of boric acid, which is due to the hydrothermal springs associated with local volcanic activity (Helvaci and Alonso, 2000) may have led to the formation of stable complexes with organic molecules (Leeman and Sisson, 1996). These include stable esters with "cis-diol" groups from carbohydrates.

In 1999, at the 12th International Conference on the Origin of Life, San Diego, California, we postulated for the first time the thermo-stabilizing role of boron upon the genetic material of the first living cells, based on stable ribose-borate esters (Cimpoiasu *et al.*, 1999; Scorei *et al.*, 1999). More recently, Ricardo *et al.* (2004) brought evidence that borate minerals stabilize ribose. Because of borate-pentose

complexes involving vicinal cis-diols, prebiotic pentoses may have accumulated in the presence of borate (Prieur, 2001; Cimpoiasu and Scorei, 2002).

Complexes with boric acid were formed not only with carbohydrates (sugars and polysaccharides), but also with nucleotides (AMP), with NAD and with vitamins such as ascorbic acid, pyridoxine and riboflavin (Power and Woods, 1997). Kim *et al.* (2003) demonstrated that borate binds to both cis-2,3-ribose diols on NAD^+ forming borate monoesters (1:1 addition), borate diesters (1:2 addition) and diborate esters (2:1 addition), whereas, only borate monoesters were formed with NADH. Generally organic molecules with vicinal cis-diols or with proximal hydroxyls in the correct orientation form borate esters with association constants varying from weak to highly stable. The esterifications of borate with NAD^+ and with NADH were pH dependent, the maximum formation occurring under alkaline conditions. The structures formed between borate and carbohydrates are poorly known due to their complexity and variability. The most stable esters are those in which boric acid forms a bridge between two carbohydrates (e.g. fructose-boron-fructose). Such complexes are found in phloem saps and nectar in plants (Hu *et al.*, 1997). Boron containing polysaccharides (pectins) were found in plant cell walls (Matoh, 1997).

Objective

The aim of this research was to study the proton exchange between solvent (water) and the labile hydroxyl protons of carbohydrates (glucose and ribose) and the potential prebiotic relevance of this process in the thermo-stability of ribose-boron and glucose-boron complexes.

These interactions determine the structure and function of molecule and are important in biological processes such as acido-base catalysis and enzymatic catalysis. In this paper, we discuss only two-site exchanges. The nucleus of interest is assumed to be the exchange between two magnetically distinct environments A (bulk water) and B (exchange sites on sugar). The following scheme is considered: $A \xrightarrow{k+} B$, $A \underset{k-}{\longleftarrow} B$, which correspond to a unimolecular conformation or chemical reaction. In this reaction k_+ and k_- are the forward and reverse rate constants respectively, while the chemical exchange rate constant, k_{ex}, is given by $k_{\text{ex}} = k_+ + k_- = k_+/p_B = k_-/p_A$, where: p_A and p_B are the equilibrium populations of equivalent nuclear spins in sites A and B ($p_B = 1 - p_A$, $p_A \geq p_B$).

Studies of the relaxation rate of the water protons in aqueous solutions have been interpreted in terms of exchange between water protons and exchangeable protons from solute molecules. The dispersion of the transverse relaxation rate R_2 as a function of τ (interpulse delay) from the Carr-Purcell-Meiboom-Gill (CPMG) sequence has been explained in these terms. A general expression for the transverse relaxation rate constant $R_2\,(1/\tau)$ that encompasses all conformational exchange timescales was given in Carver and Richard (1972). This expression for site A ($p_A > p_B$), $R_2(1/\tau)$ is

given by:

$$
\begin{aligned}
R_2(1/\tau) &= \frac{1}{2}\left(R_{2A}^0 + R_{2B}^0 + k_{\mathrm{ex}} - \frac{1}{\tau}\cosh^{-1}[D_+\cosh(\eta_+) - D_-\cosh(\eta_-)]\right), \\
D_\pm &= \frac{1}{2}\left[\pm 1 + \frac{\Psi + 2\Delta\omega^2}{(\Psi^2+\xi^2)^{1/2}}\right], \quad \eta_\pm = \frac{\tau}{\sqrt{2}}\left[\pm\Psi + (\Psi^2+\xi^2)^{1/2}\right]^{1/2}, \\
\Psi &= \left(R_{2A}^0 - R_{2B}^0 - p_A k_{\mathrm{ex}} + p_B k_{\mathrm{ex}}\right)^2 - \Delta\omega^2 + 4p_A p_B k_{\mathrm{ex}}^2, \\
\zeta &= 2\Delta\omega\left(R_{2A}^0 - R_{2B}^0 - p_A k_{\mathrm{ex}} + p_B k_{\mathrm{ex}}\right).
\end{aligned}
\tag{1}
$$

In this equation 2τ is the delay between 180° pulses in the CPMG pulse train.

A first order-order approximation was made by Luz and Meiboom (1963), in the fast exchange limit; the general expression of relaxation rate constant is given by:

$$
R_2(1/\tau) = R_{2w}(1/\tau \to \infty) + (p_w p_B \Delta\omega^2/k_{\mathrm{ex}})[1 - 2\tanh(k_{\mathrm{ex}}\tau/2)/(k_{\mathrm{ex}}\tau)] \tag{2}
$$

where: p_B = the fraction of exchangeable protons of solute ($p_B < 1$);

R_2 depends on the relaxation rate for bulk water R_{2w}, the exchange rate k_{ex}, and the chemical shift difference between these protons and water $\Delta\omega$.

A simple approximate equation for the relaxation rate constant of the resonance associated with site W (here water) that is applicable in the short echo limit ($\tau \ll k_{\mathrm{ex}}^{-1}$) and $p_w \gg p_B$ is given by:

$$
R_2(1/\tau) = R_{2w}(1/\tau \to \infty) + (p_w p_B \Delta\omega^2 k_{\mathrm{ex}})\tau^2/3 \quad \text{(Brooks } \textit{et al.}\text{, 2001)} \tag{3}
$$

Equations (2) and (3) are useful for illustrating major functional features of $R_2(1/\tau)$ that are difficult to discern in Carver and Richard's work because of the complexity of this general expression.

To achieve the main goal of this paper, we study the influence of pH and boron concentration upon the chemical exchange phenomenon in solutions of glucose and ribose, in the presence of phosphate buffer and borate. We present the correlation between the thermal decomposition process (represented by decomposition rate, k_{dec} and half-life $\tau_{1/2}$) and the chemical exchange phenomenon (i.e. the rate of protonation of the OH sites of sugar,the percentage amount of this sitep_B) at different levels of boron concentration, and the c_B for glucose and ribose solutions at different pH's. Finally, we will summarize the measurements and formulate a hypothesis about the prebiotic influence of boron during the proto-metabolic pathways for the synthesis of ribose and glucose.

Materials and Methods

SAMPLE PREPARATION

All chemicals were purchased from Sigma and used without supplementary purification. Our studies were carried out on glucose and ribose solutions (2.5 M) prepared in: bidistilled water (MilliQ), 50 mM phosphate buffer (pHs 5.0, 7.0, 8.0); 0.4, 0.8, 1.6 M borate buffer (pH 8.0); 0.8 M borate buffer (pH 7.0); 0.4 M, 0.8 M, 1.6 M and 2.5 M boric acid solutions (pH 5.0).

All NMR measurements were performed after a mutarotation process of sugars in solution (6 h at 20 °C).

THERMAL DECOMPOSITION

The sample of sugar solution was introduced into a thermostated chamber at 85 °C for 64 h for glucose and for 24 h for ribose. Samples were extracted for NMR analysis at 8 h intervals for glucose and 3 h intervals for ribose. We measured the remaining sugar using NMR titration (James, 1975), and calculated the half-life time for thermal decomposition relative to controls.

NMR MEASUREMENTS

The time domain NMR measurements were performed on a 25 MHz low-resolution pulse H^1-NMR AREMI 78 spectrometer equipped with an audio filter bandwidth of 1 MHz and quadrature phase sensitive detector. All NMR measurements were carried out at 25 °C and the temperature was controlled up to a precision of ±0.2 °C by airflow over an electrical resistance, using the variable temperature unit attached to the spectrometer. The experimental samples were stabilized at 25 °C before analysis. The standard *TD-NMR* (time domain) pulse sequences Carr-Purcell-Meiboom-Gill sequence (CPMG), Meiboom and Gill (1958); Vackier and Rutledge (1996) were used to estimate the spin-spin relaxation time (T_2) values for protons in the sample. The repetition delay (*RD*) was set to 15 sec and the enhancement was 4 (16 scans with $SNR \approx 50$ dB). The CPMG sequence is given by:

$$90^{\circ}_{x} - \tau - (180^{\circ}_{y} - \tau - \text{measurement} - \tau)_n - RD$$

where: τ = the interpulse delay.

In the first set of experiments we study the chemical exchanges between water protons and sugar hydroxyl protons and for this purpose multiple τ values (0.6, 0.8, 1, 1.2, 1.6, 2, 2.4, 2.8, 3.2, 4, 5.6, 11.2 ms) were used. The signals were acquired for the same total duration of 4.9s, corresponding to the various numbers

of experimental points. The duration of these relaxation curves allows a correct characterization of the slow relaxing components (water protons and exchangeable OH sugar protons). We use Equation 1 for the accurate extraction of the p_B and k_{ex} from the experimental curve $R_2(1/\tau)$, using a non-linear regression program based on the Marquardt algorithm (Marquardt, 1963).

In a second set of experiments, we used the standard titration NMR measurement. A single τ value was required (very short, 0.6 ms) combined with various number of points for complete relaxation of the H nuclei. The experimental response of the sample is an electrical signal proportional with the number of nuclei from sample. After detection and A/D conversion, the experimental signal is represented by a discrete number and we can fit these with complex sum of decreasing exponential using the equation:

$$S(t) = \sum_i p_i \exp(-t/T_{2i}),$$

where: p_i is proportional to the amount of protons for the "i" compartment; T_{2i} = spin-spin relaxation time; $S(t)$ = the signal intensity at time t.

We showed previously (Scorei *et al.*, 1997) that the overall relaxation in the sugar samples is characterized by "pondered" T_2 (equal with pondered sum of all spin-spin relaxation times T_{2i} from the samples). The main results of this work are represented by the stability of this parameter (derived by its statistical behaviour) and by the proportionalities with the solute concentration in the sample. This last characteristic was used in the NMR titration measurements to determine the amount of sugar remaining in the samples after thermal decomposition and to estimate the decomposition rate. The half-lives associated with thermal decomposition were calculated from:

$$\tau_{1/2} = t\ln 2/\ln(R_2(0)/R_2(t))$$

where: t = the decomposition time; $R_2(t) = 1/T_2(t)$ = the spin-spin relaxation rate of the entire sample at time t.

Also, based on our work (Steinbrecher *et al.*, 2000), on the stable reconstruction of T_2 distribution applied to low resolution NMR relaxation curve (NMR signal of amount of H nuclei in the sample), it is possible to construct the distribution of spin-spin relaxation times of protons in the sugar solution sample. This distribution is simple (single peak) or complex (separate and mixed peaks), depending on the sugar concentration. Thus, using a single exponential term in Eqs. I and 2 is justified, because for the concentration level used by us our mathematical method can predict a single exponential distribution with good statistical approximation.

Results and Discussion

INFLUENCE OF pH AND BORON CONCENTRATION ON THE CHEMICAL EXCHANGE PHENOMENON

First, we extracted from R_2 (τ) the chemical exchange parameter k_{ex} , p_B and $\Delta\omega$ using Eq.1.The chemical shifts differences $\Delta\omega$ of all samples are not modified by changes in pH. In this paper, we will only examine the exchangeable protons of glucose and ribose, in an effort to elucidate the effect of this exchange on R_2.

For the pH range 5.0–8.0, we observed a good linear dependence of the $log(k_{ex})$ and pH for all solutions (Figure 1). Linear dependence was expected, because the concentration of H^+ in solution manages the protonation-deprotonation reaction of the hydroxyl group of the sugar. The increase with pH for glucose indicated that the equilibrium is displaced in favor of k_+ (i.e. protonation). Ribose shows the opposite relationship with pH relative to glucose, where the rate of deprotonation became dominant at basic pH. The presence of borate in solution is crucial for establishing the rate constant k_{ex}. For glucose, borate acts as an amplifier of k_{ex} (inhibitor for ribose). These two tendencies become important in explaining the thermal decomposition of ribose and glucose in the presence of boron.

The importance of boron concentration in the exchange phenomenon at pH 5.0 and pH 8.0 is shown in Figure 2A which presents the evolution of k_{ex} as a function of the concentration of boron. We also recorded the evolution of k_{ex} for glucose and ribose. Our data show that the interaction of boron with glucose is very different compared to the relationship with ribose. For ribose, the

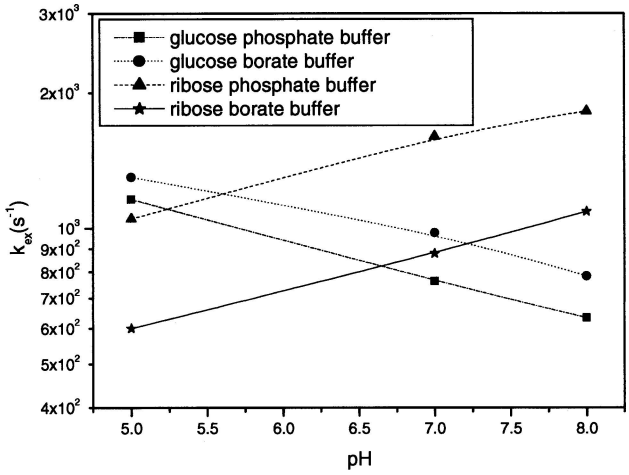

Figure 1. Evolution of the k_{ex} rate constant as a function of pH (50 mM phosphate buffer, 0.8 M boron concentration, 25 °C).

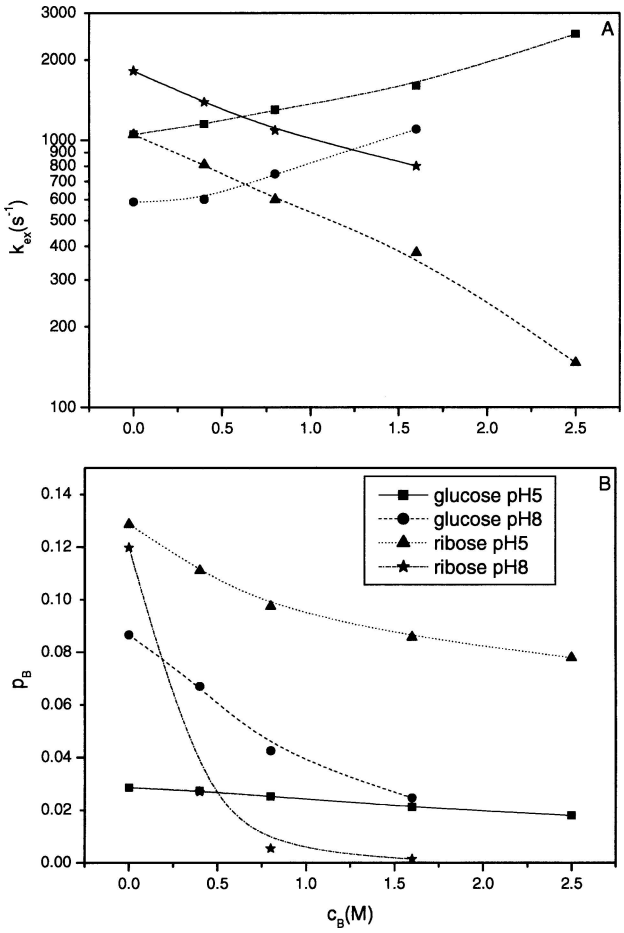

Figure 2. (A) Evolution of the k_{ex} rate constant and (B) the population of exchangeable nuclear sites of sugar p_B, as a function of boron concentration c_B (2.5 M sugar concentration, 25 °C).

complexation with boron leads to the formation of a strong B-O linkage with most exchangeable protons of ribose (from OH groups). The formation of the boron-ribose complex is a consequence of increased boron concentration and results directly in the decrease of the exchangeable proportions of ribose protons p_B in solution (see Figure 2B). For glucose, the high level of boron in solution is followed by the increase of k_{ex} rate and by the decrease of p_B. In acid media, more boron in solution determines the increase of the protonation reaction (k_+ increase with concentration); while in basic media k_+ decreases with the boron concentration because H^+ is bonded more strongly in solution in presence of $B(OH)_4^-$. This suggests that, for glucose, the complexation reaction with boron consist in the formation of labile H-bonds between boric acid/borate and the hydroxyl group of glucose.

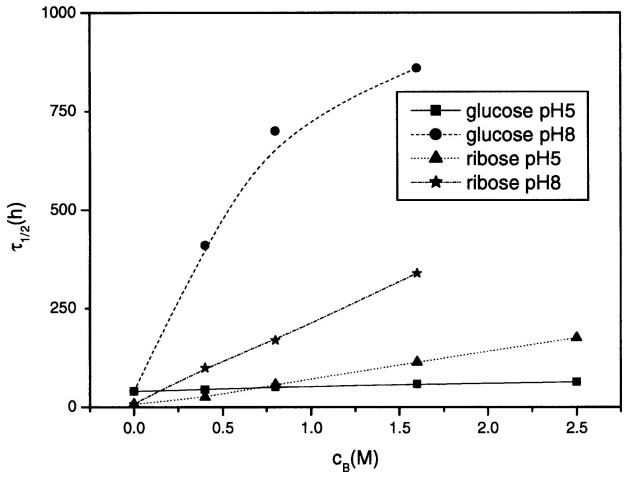

Figure 3. The evolutions of half-life decomposition times of glucose and ribose with boron concentration.

INFLUENCE OF CHEMICAL EXCHANGE PHENOMENON ON THE THERMAL DECOMPOSITION PROCESS

It is known that ribose and glucose decompose easily in acid, neutral and basic solutions, in just a few hours, and that there is a direct link between the decomposition constant and the free aldehyde concentration of sugar (Larralde *et al.*, 1995). Our results support the suggestion that boric acid or borates in solution increase the thermal stability of carbohydrates (Cimpoiasu and Scorei, 2002).

In previous works (Scorei and Cimpoiasu, 1999; Cimpoiasu, 2003) we determined the kinetics of thermal decomposition of glucose and ribose in the presence of boron. Figure 3 presents changes in the half-life ($\tau_{1/2}$) with the concentration of boron. A remarkable effect of boron concentration on the thermal decomposition phenomenon was observed at all pH's, but particularly for pH 8.0.

In Figure 4A and B we present the results of NMR titrations, showing the correlation between the rate constant for thermal decomposition (k_{dec}) with: a) the protonation rate constant k_+ and b) the population of labile protons (p_B) from the –OH groups of ribose and glucose in acid and base media with various concentrations of boron. Glucose at pH 8.0 and ribose at pH 8.0 and pH 5.0 have similar behavior.

Conclusion

Because boron has opposed effects on the thermal stability of ribose and glucose, these two sugars and their associated roles in evolution may have arisen in different environments.

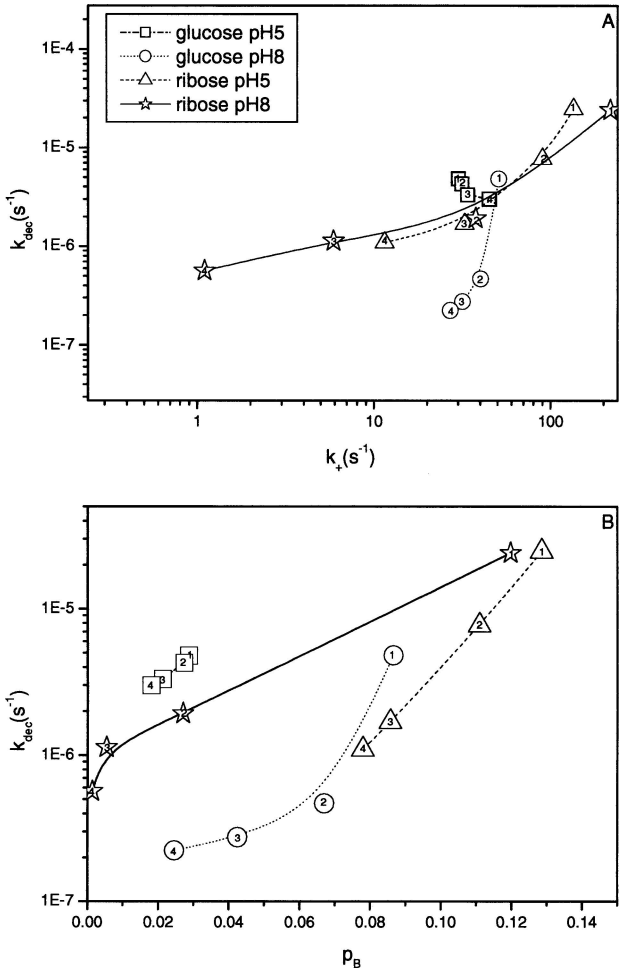

Figure 4. Correlation between of thermal decomposition rate constant k_{dec} of glucose and ribose solution (2.5 M, 25 °C) and: (A) function of protonation rate constant k_+ and (B) function of population of nuclear sites p_B at: pH 5.0 , 1, 2, 3 and 4 represent boron concentration 0, 0.4, 1.6 and 2.5 M respectively and, for pH 8.0, 1, 2, 3 and 4 represent boron concentration 0, 0.4, 0.8 and 1.6 M.

Thus a “pre-RNA” world type of chemistry could have arisen at high temperatures and low pH where furanosyl borate diesters of ribose. On the other hand, environments favoring a ”pre-metabolic world” type of glucose-based chemistry may have displayed high temperatures and basic pH, and were rich in glucose-borates and anion-borates. In the beginning these two different chemical worlds may have had separate chemical evolutions, and were only later associated in more complex biochemical systems.

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