PULSED NMR STUDIES ON Na^+ BINDING TO SIMPLE CARBOHYDRATES

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SUMMARY: Pulsed NMR spectroscopy has been used to study Na^+ binding to several simple carbohydrates in aqueous solution. Changes in the ^{23}Na spin-lattice relaxation time (T_1) were monitored to indicate complex formation between sodium ions and a ligand. It was found that Na^+ interacts with these hydroxy-compounds in a manner similar to other metal cations, but very weakly. Among the sugars investigated, cis-inositol forms the strongest complexes with the stability constant about 1.2 M^{-1} (if 1:1 complexes are assumed). A qualitative study of competition between Na^+ and Ca^{2+} was done, indicating that both cations have the same binding sites.

INTRODUCTION

Several methods have provided evidence for the existence of weak complexes between sugars and cations of alkali metals in aqueous solution. The methods have been reviewed by Rendleman[1] and the general trend for decreasing effectiveness in forming complexes was found to be $Na^+ > K^+ > Li^+$.

Recently Angyal and Davies[2,3] studied the proton NMR spectra of some sugars in D_2O solution and observed changes in signal positions, coupling constants, conformation and equilibrium composition of the sugars when different inorganic salts were added. The authors concluded that sugars containing an ax-eq-ax sequence of three hydroxy groups in a six-membered ring, or cis-cis sequence in a five-membered ring, form complexes with metal ions, particularly with alkaline-earth metal ions. Among the ions investigated, lanthanum(III) forms the most stable complexes.

Alkali metal ions combine far less strongly with hydroxy-compounds and often induce no substantial changes in the ^1H NMR spectra. Nevertheless, complexes between carbohydrates and ions of alkali metals could be biologically significant.

The NMR relaxation of quadrupole nuclei such as ^{23}Na is very sensitive to the electronic enviroment around the nucleus.

If only part of the sodium ions is associated with some ion or molecule, the observed ^{23}Na relaxation time will be shorter than for solutions containing all sodium ions "freely solvated". The method has been found useful for investigation of weak complexes in a number of biological systems[4,5]. We have employed ^{23}Na NMR spectroscopy in order to investigate possible complex formation between sodium ions and several simple carbohydrates in aqueous solution.

MATERIALS AND METHODS

D-arabinose and D-mannose were purchased from Kebo AB, Stockholm. D-ribose and myo-inositol from BDH, 2-deoxy-ribose and D-lyxose from Sigma. Cis-inositol was a gift from Dr Per Garegg, Stockholm University.

The spin-lattice relaxation time (T_1) was measured by 180° - t - 90° pulse sequences on a Bruker BK 322s spectrometer at 23.81 MHz. The temperature of the samples was held constant to 27.0±0.5 deg. C by means of a temperature-regulated stream of dry nitrogen gas. The actual recording of the magnetizations following the 90° pulses was done through a "box-car" integrator. Each point on the plot used to determine T_1 was the visual average of at least 50 separate measurements. Each T_1 was the average of at least two separate measurements.

Viscosities were determined using Ostwald viscosimeter in a constant temperature bath regulated to ± 0.1 deg. C.

RESULTS

The spin-lattice relaxation time of ^{23}Na in 0.2 M NaCl aqueous solution was measured as a function of concentration of added carbohydrates (fig.1). According to the equation for the relaxation of a nucleus undergoing rapid chemical exchange between two sites A and B the observed spin-lattice relaxation time T_1 is given by

$$\frac{1}{T_1} = \frac{P_A}{T_{1A}} + \frac{P_B}{T_{1B}} \qquad (1)$$

where T_{1A} and T_{1B} are the spin-lattice relaxation times of the "free" and bound nucleus, respectively, in the absence of exchange, and P_A and P_B are the probability of finding the nucleus "free" and bound, respectively. When the sodium ion binds to a

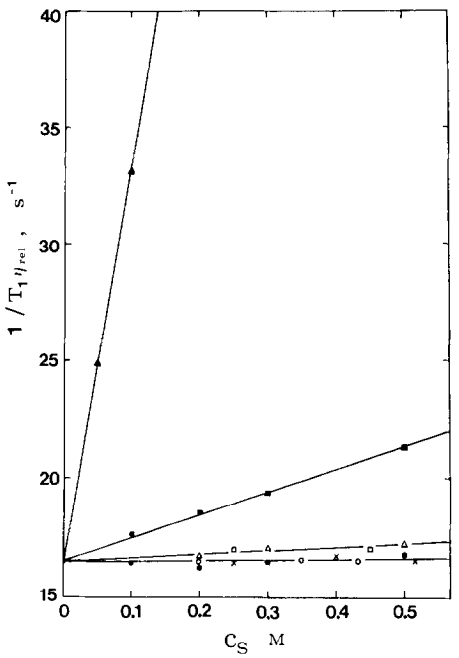

Fig. 1 : The spin-lattice relaxation rate of ^{23}Na corrected for viscosity changes in 0.2 M NaCl aqueous solution as a function of concentration of added sugars (c_S) at 27°C and 23.81 MHz.

$$\eta_{rel.} = \frac{\eta\ c_S}{\eta\ 0.2\ M\ NaCl}$$

(■) D-ribose, (▲) cis-inositol, (x) myo-inositol, (▲) D-mannose, (●) D-arabinose, (□) D-lyxose and (o) 2-deoxy-D-ribose.

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sugar molecule, one or more water molecules associated with the ion is replaced by sugar molecules and the T_{1B} will be much shorter than T_{1A}. The method can be sensitive even for low values of P_B.

Assuming the formation of 1:1 complexes equation (1) can be written

$$\frac{1}{T_1} = \frac{[Na^+]}{c_{Na}} \left(\frac{1}{T_{1A}} + \frac{K\ c_S}{1 + K[Na^+]} \frac{1}{T_{1B}} \right) \qquad (2)$$

where c_S and c_{Na} are the molar concentrations of added sugar and NaCl, respectively, K is the stability constant and $[Na^+]$ the concentration of "free" sodium ions. For weak complexes and low concentrations of sodium ions, the factor product $K[Na^+]$ can

be neglected and $c_{Na} \approx Na^+$. Assuming a rapid exchange, the relaxation rate $1/T_1$ should increase linearly with c_S. If a nucleus undergoes rapid chemical exchange between more than two sites, equations similar to (1) and (2) are valid. The observed relaxation rate is then a weighted composite of the relaxation rates at all available sites. Even in that case $1/T_1$ should increase linearly with c_S.

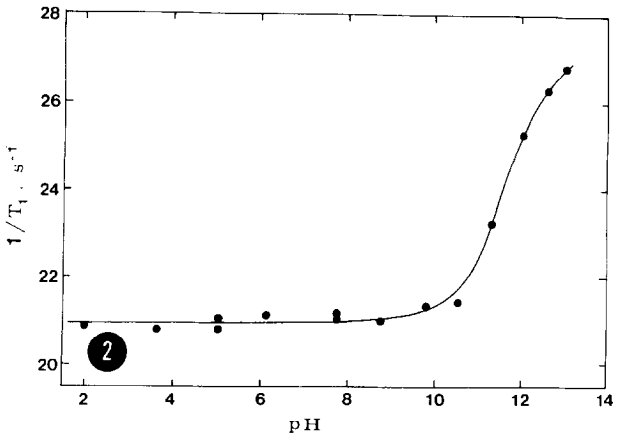
In fig. 1 the sodium relaxation rate is corrected for changes in the macroscopic viscosity upon the addition of sugars. Equation (1), as well as the assumptions implicit in it, seems to be valid. Among the carbohydrates investigated, D-ribose and especially cis-inositol caused recognisable changes in the sodium relaxation rate. The latter has an ideal configuration for binding of metal ions. Up to three metal ions might combine with the compound according to the hypothesis of Angyal and Davies.

The conformation equilibrium of aqueous solutions of D-ribose is known[6,7]. The compound has the required arrangement of hydroxy-groups in the α-pyranose, α-furanose and less stable 1C(D) conformation of β -pyranose form. Even β -D-mannose and β -D-lyxose contain the ax-eq-ax sequence but in their less stable 1C(D) conformations. The importance of certain arrangements of hydroxy-groups in sugars for complex formation is clear also on comparison of D-ribose with 2-deoxy-D-ribose. The latter compound lacks one hydroxy-group in position 2 and its addition to the NaCl solution induces just a very small increase in the sodium relaxation rate, probably due to indirect interactions as manifested in viscosity changes.

Fig.2 gives the spin-lattice relaxation rate of ^{23}Na in aqueous 0.2 M NaCl solution with 0.3 M D-ribose as a function of pH. The figure shows that interactions of sodium ions with D-ribose remain unchanged between pH 2 and 10 and increase in more alkaline solutions presumably due to alcoholate formation.

The second term in equation (1) was studied as a function of temperature in 1 M NaCl with 0.5 M D-ribose and in 0.3M NaCl with 0.5 M cis-inositol and found to decrease with temperature (fig.3), which confirms the initial assumption of rapid chemical exchange. In the case of slower chemical exchange, an additional variable (exchange time) must be put into the equations.

In order to evaluate the stability constants of Na^+ - sugar complexes through the use of equation (2) it is necessary to



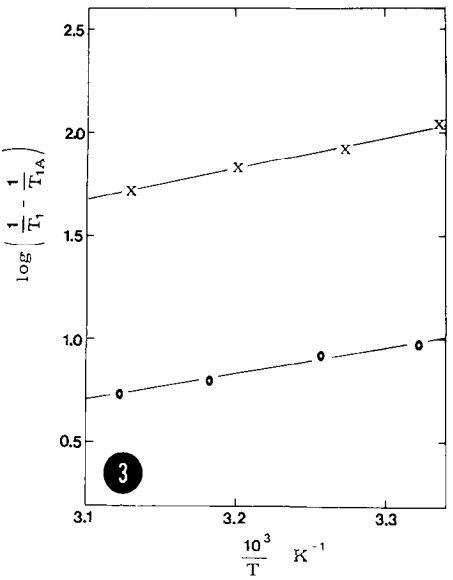


Fig. 2 : The relaxation rate of ^{23}Na in 0.2 M NaCl solution with 0.3 M D-ribose as a function of pH at 27°C.

Fig. 3 : The contribution to the sodium relaxation rate due to complex formation as a function of temperature (between 25 - 45°C).
(x) 0.5 M cis-inositol in 0.3 M NaCl
(o) 0.5 M D-ribose in 1 M NaCl

increase the NaCl concentration and observe changes in the sodium relaxation rate. Unfortunately, this causes small contributions to the ^{23}Na relaxation rate due to ion - ion interactions (see f.ex. Hall et all.[8] and references therein). These contributions can be approximately eliminated in a manner we have used in fig.4. The figure confirms that D-ribose forms very weak complexes with sodium ions. On the other hand, Na^+ - cis-inositol complexes are much stronger. Assuming the formation of 1:1 complexes, the stability constant K = 1.2 M^{-1} can be calculated. This assumption is of course not necessarily valid.

Finally, our measurements show a competition between calcium and sodium ions. Addition of $CaCl_2$ to the solution containing NaCl and D-ribose decreases the observed sodium relaxation rate (fig.4). This signifies that the fraction of bound sodium ions P_B decreases due to replacement by calcium ions. Therefore,

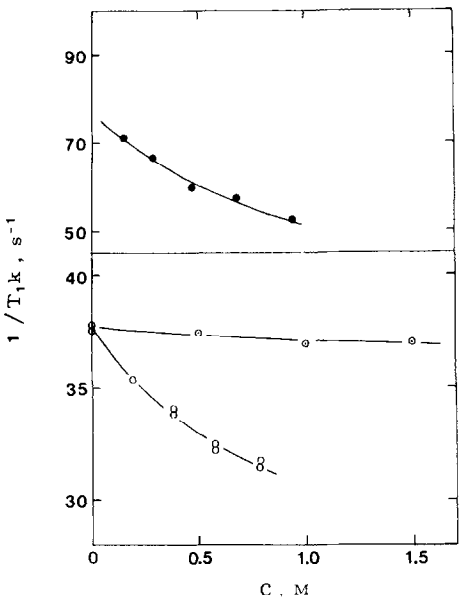


Fig. 4 : The sodium relaxation rate corrected for changes in indirect interactions as a function of concentration of added inorganic salts (c).
(●) 0.255 M cis-inositol - NaCl added
(⊙) 1 M D-ribose - NaCl added
(○) 1 M D-ribose in 0.2 M NaCl - $CaCl_2$ added

$k = \frac{T_1^o}{T_1^c}$ where T_1^c and T_1^o are the ^{23}Na spin--lattice relaxation times in solutions with the same composition and the same macroscopic viscosity as the solutions investigated. In these "model" solutions cis-inositol has been replaced by myo-inositol and D-ribose by D-arabinose. The index c refers to solutions with the concentration of added inorganic salts c and the index o is for the lowest concentration of the salts.

both cations occupy the same binding sites in the sugar molecule. Comparison of the effect of adding NaCl and $CaCl_2$, respectively(to the same solution) on the ^{23}Na relaxation rate also reveals that calcium forms much stronger complexes with D-ribose than sodium.

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