Microsecond to Subnanosecond Molecular Relaxation Dynamics of the Interaction of Ca^{2+} with Some Carbohydrates in Aqueous Solution

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Ultrasonic absorption spectra in the frequency range 1−500 MHz are reported for solutions of methyl-β-D-arabinopyranoside and of 1,6-anhydro-β-D-glucopyranoside, both with and without added Ca^{2+} and Ba^{2+} ions. These carbohydrates have been chosen because neither monosaccharide shows any relaxation process, over the same frequency range, in salt-free aqueous solutions. Spectra for both carbohydrates in aqueous solution with added $Ca(ClO_4)_2$, $CaCl_2$, or $Ba(ClO_4)_2$ can be represented by an asymptotic high-frequency contribution and a Debye relaxation term centered between 50 and 200 MHz, corresponding to a process with a relaxation time of approximately 3−4 ns. Spectra for solutions of $Ca(ClO_4)_2$ + 1,6-anhydro-β-D-glucopyranoside also exhibit a second relaxation process at lower frequencies, with a relaxation time of approximately 30 ns. These results clearly indicate cation−carbohydrate complex formation. The observed relaxation terms are interpreted as a modified Eigen−Winkler-type multistep process, associated with formation of monodentate cation−carbohydrate complexes and, where possible, subsequent rearrangement to tridentate complexes.

1. Introduction

Interactions between carbohydrates and mono- or bivalent cations have been intensively investigated in the past because they are ubiquitous and of fundamental importance in biochemistry and biology. However, attention has so far been given predominantly to equilibrium properties.[1] Recently, some of us have studied[2] the molecular relaxation dynamics of some simple carbohydrates in aqueous solution and have found evidence that the ultrasonically active relaxation process, observed in the 10−100 MHz frequency range, is due to a $-CH_2OH$ rotation of the C6 carbon atom of glucose and methyl-β-glucopyranose. This finding confirms an earlier interpretation[3] of the same phenomenon, which, however, was not an uncontested opinion.[4] Preliminary measurements of ultrasonic absorption spectra of aqueous glucose solutions with different salts added[5] have indicated a possible interference of the cation with the carbohydrate relaxation process, characterized by relaxation times in the nanosecond range. Some spectra, shown in Figure 1, illustrate the effect of added salt, depending substantially on the cation. In that diagram, the normalized sonic absorption (α/f^2) vs frequency f is given for a glucose solution with and without NH_4Cl or $CaCl_2$ added. For $f > 10$ MHz, the spectra exhibit relaxational behavior, i.e., $d(\alpha/f^2)/df < 0$. While the monovalent cation tends to slightly reduce the (α/f^2) values, the addition of $CaCl_2$ clearly increases the absorption amplitude

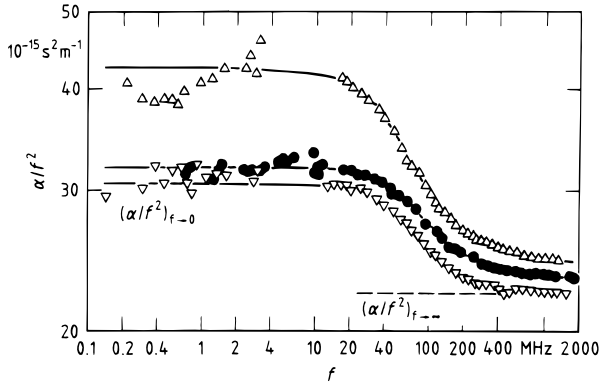

Figure 1. Ultrasonic absorption spectra $\{\alpha/f^2\}$ for aqueous D-glucose solutions (0.5 mol dm^{-3}; 298 K) with added $CaCl_2$ (0.5 mol dm^{-3}; △) and NH_4Cl (0.5 mol dm^{-3}; ▽) and (●) without additional salt. Data from ref 5.

$\{(\alpha/f^2)_{f\to 0} - (\alpha/f^2)_{f\to\infty}\}$. It is difficult, however, to draw clear-cut conclusions regarding the molecular mechanism from this observation. On one hand, the addition of salt may alter the $-CH_2OH$ rotation of the hexose in an unknown way. On the other hand, the complexation of the cation with the carbohydrate could establish an additional relaxation process, contributing to the sonic spectrum in our frequency range. It appears that the effect of the addition of NH_4Cl is mainly to depress slightly the amplitude of the relaxation spectrum. On the contrary, for the $CaCl_2$ solutions, a separate relaxation process, reflecting the complexation of Ca^{2+} with the carbohydrate D-glucose, is calculable from the data. Work in this direction is in progress in our laboratories. For the time being, to eliminate the $-CH_2$-

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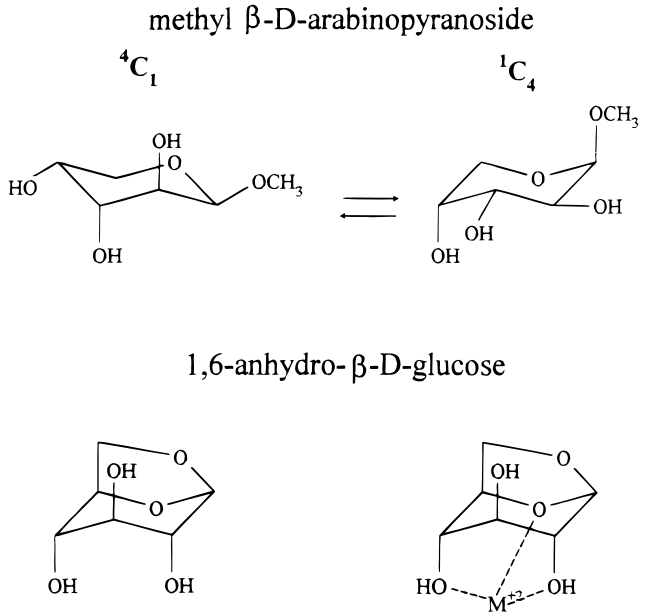

Figure 2. Structures of monosaccharides used in this study. For 1-methyl-β-D-arabinopyranoside, the $^4C_1$ and $^1C_4$ chair conformations are depicted. For 1,6-anhydro-β-D-glucopyranoside, the cation Me^{2+} interaction is indicated on the right.

OH rotation at the C6 atom, possibly obscuring the cation relaxation, we have studied the interaction of Ca^{2+} (and Ba^{2+} at times) with methyl-β-D-arabinopyranoside and 1,6-anhydro-β-D-glucopyranoside. The former carbohydrate is a pentose in the pyranose form with the $-CH_2OH$ group missing; in the latter, an ether bridge connects C1 and C6 (see Figure 2). Consequently, no noticeable relaxational ultrasonic absorption has been found[2] for these two carbohydrates in aqueous solution, resulting in (α/f^2) being constant in our measuring frequency range. The present study intends to demonstrate the significance of the carbohydrate structure for the complexation with bivalent cations.

2. Experimental Section

Resonator (1−10 MHz) and pulse-modulated traveling wave (10−500 MHz) methods have been employed to measure the ultrasonic spectra of carbohydrate plus cation solutions (concentration range, 0.25−1.5 mol dm $^{-3}$). Equipment and procedures for such ultrasonic measurements have been described previously.[6,7] At present, resonator methods yield only relative absorption values with respect to a reference liquid; velocity-matched NaCl aqueous solutions have been used for this purpose. Measurement errors $\Delta\alpha/\alpha < 0.15$ are reached for $f <$ 15 MHz, and $\Delta\alpha/\alpha < 0.03$ for $f >$ 15 MHz.

The carbohydrates methyl-β-D-arabinopyranoside (Sigma Chemical Co., crystalline) and 1,6-anhydro-β-D-glucopyranoside (Fluka Inc., claimed to be 98% pure), have been used as received from the manufacturers. Doubly deionized water, passed twice through purifying columns in order to eliminate ions and organic residuals, has been used for all solutions; in the final state, the electrical conductance of water was close to 5×10^{-5} S/m.

$Ca(ClO_4)_2 \cdot 4H_2O$ and $CaCl_2 \cdot 2H_2O$ have been obtained from Aldrich Chemical Co.; $Ba(ClO_4)_2$, anhydrous, was from Alfa-Thiokol Co. Each electrolyte was weighed in a volumetric flask and was added to a preweighed quantity of carbohydrate; the total weight was rechecked, and the volumetric flask was then filled with water in steps, first dissolving the solutes and then diluting the solution up to the fiduciary mark. $Ba(ClO_4)_2$ was redried in vacuo (∼0.1 Torr) at room temperature for 2−3 h with negligible weight loss. All other salts were used as received, and the solutions were prepared in correspondence to the procedures described above. No attempt at altering the pH of the solution by the addition of acids, determining their effect on the ultrasonic spectra if any, was done in the present work.

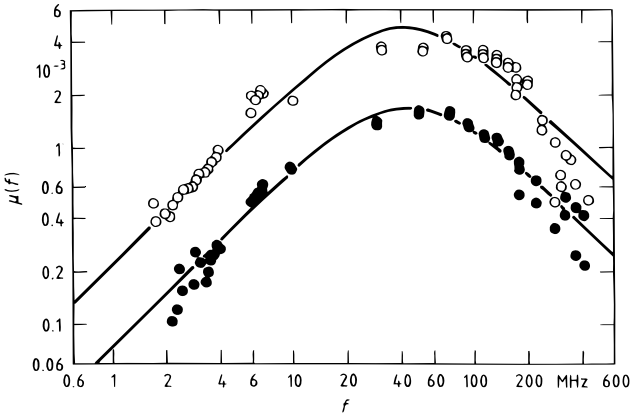

Figure 3. Ultrasonic excess absorption per wavelength $\mu(f)$ spectrum for aqueous solutions (298 K) of $Ca(ClO_4)_2$ + 1-methyl-β-D-arabinopyranoside (●: $c_{Me^{2+}} = 0.5$ mol dm^{-3}, $c_{ch} = 0.5$ mol dm^{-3}. ○: $c_{Me^{2+}} = 1$ mol dm^{-3}, $c_{ch} = 1$ mol dm^{-3}).

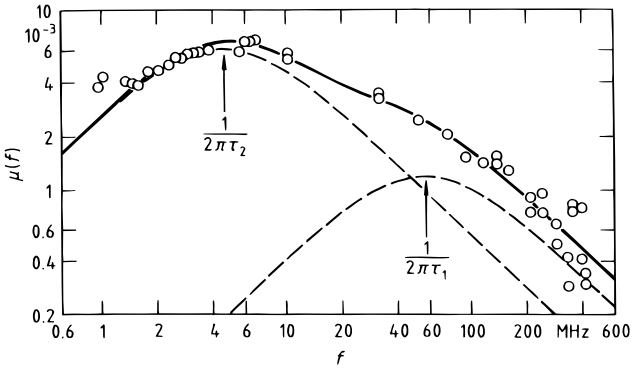

Figure 4. Excess absorption $\mu(f)$ spectrum for an aqueous solution (298 K) of $Ca(ClO_4)_2$ + 1,6-anhydro-β-glucopyranoside ($c_{Me^{2+}} = 0.77$ mol dm^{-3}, $c_{ch} = 0.75$ mol dm^{-3}). Dashed lines (- - -) indicate the separation of $\mu(f)$ into two Debye-type relaxation terms; the full line (—) shows the sum of both terms.

3. Results

The structures for methyl-β-D-arabinopyranoside (in both the $^4C_1$ and $^1C_4$ conformations) and 1,6-anhydro-β-D-glucopyranoside are depicted in Figure 2. At 25 °C and carbohydrate concentrations $c_{ch} \leq 1$ mol dm^{-3}, both carbohydrates alone, in aqueous solution do not exhibit a recognizable ultrasonic absorption of relaxational origin between 1 and 500 MHz.

However, solutions of $Ca(ClO_4)_2$ + methyl-β-D-arabinopyranoside and of $Ca(ClO_4)_2$ + 1,6-anhydro-β-D-glucopyranoside at concentrations of 0.5−1 mol dm^{-3} and at various molar ratios $r =$ [cation]/[carbohydrate] with $1 \leq r \leq 2$ show a relaxational profile in their ultrasonic spectra. This profile is illustrated by Figures3 and 4, with the data given as excess absorption per wavelength $\mu(f) = \{\alpha_{exc}\lambda\}$ vs frequency, to accentuate the high-frequency section of the spectra. $\mu(f)$ is calculated from that part of the sonic absorption coefficient that exceeds the "backgound" absorption $B'f^2 = (\alpha/f^2)_{f\to\infty}f^2$ (see Figure 1). We therefore have

$$\mu(f) = \alpha\lambda - Bf = \alpha\lambda - B'c_S f \qquad (1)$$

Here c_S denotes the sound velocity, $\lambda = c_S/f$ is the sonic wavelength and $B = B'c_S$ represents the asymptotic behavior of $\mu(f)$ at high frequencies.

In conformity with the example given in Figure 3, all excess absorption spectra of methyl-β-D-arabinopyranoside solutions with $Ca(ClO_4)_2$ or $CaCl_2$ added show one relaxation region, characterized by $d(\alpha/f^2)/df < 0$ and $\mu(f) > 0$. This situation is different for $Ca(ClO_4)_2$ + 1,6-anhydro-β-D-glucopyranoside. As indicated by the spectrum in Figure 4, there is a second relaxation process at lower frequencies, in addition to the process found with the methyl-β-D-arabinopyranoside solutions. Replacing the perchlorate ions by chloride ions in the $Ca(ClO_4)_2$ + methyl-β-D-arabinopyranoside system {0.75 M; $r = 1$} keeps the $\{\alpha_{exc}\lambda\}$ spectrum nearly unaltered.

For solutions with $Ba(ClO_4)_2$ added, one relaxation region emerges for 1 mol dm^{-3} solutions of both carbohydrates at $r = 1$ within our range of measurements. With respect to the corresponding $Ca(ClO_4)_2$ solutions, the relaxation regions in the spectra of $Ba(ClO_4)_2$ solutions are noticeably shifted toward higher frequencies.

Summarizing, all sonic excess absorption spectra can be described either by one relaxation term or by a sum of two terms. To refer adequately to the various frequency regions in which relaxation processes occur, we have used a model relaxation function, with an allowance made for two excess absorption terms. Both of the terms (indices "1" and "2") represent processes with a higher or lower relaxation frequency, respectively. It turns out that, within the limits of experimental accuracy, each of the different processes can be described by a discrete relaxation time. Hence a sum R(*f*) of two Debye-type relaxation terms[8] and an asymptotic "background" absorption term can represent all observed relaxation spectra analytically.

$$R(f) = \sum_{i=1}^{2} \frac{\Delta\mu_i \omega\tau_i}{1 + (\omega\tau_i)^2} + Bf \qquad (2)$$

In this equation $\omega = 2\pi f$ and τ_i and $\Delta\mu_i$ are the particular relaxation times and amplitudes, respectively. The spectral function $R(f)$ has been fitted to the measured $\{\alpha\lambda\}$ spectra, using a Marquardt algorithm[9] to minimize the variance.

$$\chi 2 = \frac{1}{N - P - 1} \sum_{n=1}^{N} w_n[\alpha(f_n)\lambda - R(f_n)]^2 \qquad (3)$$

Here N denotes the number of frequency points f_n per measured spectrum and P is the number of adjustable parameters in $R(f)$. The w_n ($n = 1, ..., N$) are weighting factors, set inversely proportional to the experimental uncertainties of $\Delta\alpha(f_n)$. To enhance the significance of the parameter values, the amplitudes of nonrelevant relaxation terms have been fixed at 0 in the final run of the regression analysis.

The parameter values for solutions of methyl-β-D-arabinopyranoside and of 1,6-anhydro-β-D-glucopyranoside, resulting from the regression analysis, are collected in Tables 1 and 2, respectively.

TABLE 1: Parameters of the Relaxational Spectral Function *R* (Eq 2) for Aqueous Solutions of Methyl-β-D-arabinopyranoside with Different Salts Added ($T = 298$ K; $f_1 = (2\pi\tau_1)^{-1}$)

$c_{Me^{2+}}$ [mol dm^{-3}] (±0.2%)	c_{ch} [mol dm^{-3}] (±0.2%)	B [ps] (±5%)	c_S [m/s] (±1%)	$\Delta\mu_1 \times 10^3$ (±10%)	τ_1 [ns] (±10%)	f_1 [MHz] (±10%))
			$Ca(ClO_4)_2$			
0.5	0.5	37.5	1582	3.4	3.6	44
1	0.5	37.7	1579	4.8	3.5	45
0.75	0.75	40.6	1571	6.6	3.8	42
1	1	48.3	1609	9.3	4.1	39
			$CaCl_2$			
0.75	0.75	43.7	1628	6.3	4.3	37
			$Ba(ClO_4)_2$			
1	1	46.4	1572	8.4	0.8	200

TABLE 2: Parameters of the Relaxational Spectral Function *R* (Eq 2) for Aqueous Solutions of 1,6-Anhydro-β-glucopyranoside with Different Salts Added ($T = 298$ K)

$c_{Me^{2+}}$ [mol dm^{-3}] (±0.2%)	c_{ch} [mol dm^{-3}] (±0.2%)	B [ps] (±5%)	c_S [m/s] (±1%)	$\Delta\mu_1 \times 10^3$ (±10%)	τ_1 [ns] (±10%)	$\Delta\mu_2 \times 10^3$ (±10%)	τ_2 [ns] (±10%)
			$Ca(ClO_4)_2$				
0.5	0.5	35.3	1533	0.5	3.3	6.4	22
1	0.5	36.4	1560	1.5	2.2	7.9	23
0.77	0.75	37.2	1581	2.8	3.6	12.8	34
1	1	39.5	1572	3.5	2.5	16.5	26
			$Ba(ClO_4)_2$				
1	1	48.4	1540	33.9	2.4		

4. Discussion

Within the four series of solutions of calcium salts and carbohydrates in water, the relaxation times τ_1 and τ_2 are roughly independent of concentration (see Tables 1 and 2). This finding suggests that the relaxation terms "1" and "2" result from a reformation of carbohydrate−Ca^{2+} complexes, assuming the terms to reflect appropriate steps in a modified Eigen−Winkler reaction scheme.[10]

$$Ca^{2+} + ch \underset{k_{-0}}{\overset{k_0}{\rightleftharpoons}} Ca^{2+}\cdots ch \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Ca^{2+}-ch \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Ca^{2+}\equiv ch \qquad (4)$$

Here ch symbolizes the carbohydrate, $Ca^{2+}\cdots ch$ denotes a solvent separated species, $Ca^{2+}-ch$ denotes a monodentate complex, and $Ca^{2+}\equiv ch$ represents a tridentate complex, as sketched in Figure 2. The first step leads to a solvent separated species, $Ca^{2+}\cdots ch$ sometimes symbolized by $Ca^{2+}(H_2O)ch$, whereas the second step results in a monodentate contact species, in which the cation interacts with the lone electrons of preferably one carbohydrate oxygen. The third step, finally, leads to a species in which the cation is complexed to three oxygens of hydroxyl or ether groups of the carbohydrates, as has been discussed in the literature.[11]

The first step of reaction scheme 4 may contribute a high-frequency relaxation process (index "0") in the solutions, above our frequency range of measurements. Indeed, up to 500 MHz, the presence of an upper relaxation process was undetectable. In any case, let us assume the formation of solvent-separated species to be fast as compared to the subsequent steps in the cation−carbohydrate association mechanism, and let us also assume the concentration of the $[Ca^{2+}\cdots ch]$ species to be high compared to that of the $[Ca^{2+}-ch]$ and the $[Ca^{2+}\equiv ch]$ complex. The formation of the monodentate and tridentate complex may then be considered to be monomolecular reactions, which are almost decoupled from the first step of the cation−carbohydrate approach. For monomolecular reactions, coupled to each other, concentration−independent relaxation times are predicted,[12] in conformity with our experimental observations.

We now wish to compare the data for $Ca(ClO_4)_2$ and $CaCl_2$ + methyl-β-D-arabinopyranoside with those for $Ca(ClO_4)_2$ + 1,6-anhydro-β-D-glucopyranoside. The former systems show a

single Debye-type relaxation with a relaxation time around 4 ns. On the other hand, $Ca(ClO_4)_2$ + 1,6-anhydro-β-D-glucopyranoside exhibits a spectrum that can be interpreted by a sum of two Debye terms ($\tau_1 \approx 3$ ns; $\tau_2 \approx 30$ ns). From the literature it is known[11] that Ca^{2+} binds 1,6-anhydro-β-D-glucopyranoside to three oxygens (two of the OH groups), including the O5 in the ring, as sketched in Figure 2. No such binding seems to occur in methyl-β-D-arabinopyranoside, as the hydroxyl groups of the C2, C3, and C4 positions do not have the required arrangement of oxygen atoms for optimal complexation of cations with an ionic radius[13,14] near 1 Å. We therefore advance the hypothesis that the process with relaxation time τ_1 corresponds to a ligand rearrangement establishing a monodentate contact species Ca^{2+}−ch from an outersphere complex $Ca^{2+}\cdots$ch. This hypothesis is supported by the observation of nearly identical τ_1 values for both the $Ca(ClO_4)_2$ + carbohydrate series and a significantly smaller τ_1 for $Ba(ClO_4)_2$ + methyl-β-D-arabinopyranoside than for $Ca(ClO_4)_2$ with the same carbohydrate. Ba^{2+} has a more labile solvent coordination shell than Ca^{2+}; the ratio of their ionic radii is $r_{Ba^{2+}}/r_{Ca^{2+}} \approx 1.36$, and the bigger cation is less tightly bound to the oxygen lone pair electrons. From Table 2, it is evident that the only relaxation process visible for $Ba(ClO_4)_2$ 1.0 M + 1,6-anhydro-β-D-glucopyranoside 1.0 M solution has a relaxation time comparable to τ_1 for $Ca(ClO_4)_2$ plus the same carbohydrate solution (even if with a much larger $\Delta\mu_1$). The lack of a slow relaxation process for Ba^{2+} may indicate either the nonexistence of a tridentate complex $Ba^{2+}\equiv$ch or more likely, a process not involving the existence of an intermediate Ba^{2+}−ch, because of the lability of the solution shell around Ba^{2+} when compared to that of Ca^{2+}.

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