Temperature Dependence of the Rotational Mobility of the Sugar and Water Molecules in Concentrated Aqueous Trehalose and Sucrose Solutions

N. Karger and H.-D. Lüdemann

Institut für Biophysik und Physikalische Biochemie Universität Regensburg, Postfach 397, D-8400 Regensburg

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Carbon-13 and deuteron spin lattice relaxation times T_1 of concentrated solutions of trehalose and sucrose in heavy water are given as function of temperature. From the temperature dependence of the T_1 ideal glass transitions temperatures T_0 are derived. For both compounds the T_0 derived for the disaccharides are at high concentrations larger than the T_0 of the water molecules.

Recently Green and Angell [1] studied the experimental glass transition temperatures T_g of several aqueous disaccharide solutions by differential scanning calorimetry and found trehalose to be the most efficient compound in raising T_g. The authors discuss this finding in relation to the protective influence of trehalose upon the quiescent state of anhydrobiosis, in various forms of life in extremely dry habitats [2]. Glass formation can also prevent in cells and organisms crystallization of cellular and intercellular water and protect them from the damaging influence of cold temperatures [3].

It appeared thus of general interest to study in greater detail the dynamic effects occurring in supercooled concentrated aqueous solutions of some disaccharides.

Spin lattice relaxation time studies of the individual carbon atoms in the two disaccharides trehalose and sucrose dissolved in heavy water and deuteron spin lattice relaxation times provide unambiguous information about the rotational dynamics of the two compounds in solution and complement the information obtained by calorimetric methods.

Experimental

Sucrose, trehalose and heavy water (99.75% deuterated) were purchased from E. Merck (Darmstadt, F.R.G.). The solutions (63, 66, and 75% w/w sucrose in D_2O and 69% w/w trehalose in D_2O) were filled into heavy walled 10 mm NMR tubes and degassed by several freeze-pump-thaw-cycles. After degassing the tubes were flame sealed.

The measurements were performed in a Bruker MSL-300 spectrometer operating at a field of 7.05 Tesla. The spin lattice relaxation times were determined by the inversion-recovery pulse sequence [4].

The temperature range studied was from 203 K to 373 K for the deuteron $-T_1$ and from 251 K to 373 K for the carbon-13 data. The temperatures were controlled to ± 0.5 K by a metal sheathed Chromel/Alumel thermocouple. The carbon-13-spectra were obtained under continuous broad-band-proton decoupling.

The accuracy of the data is considered better than $\pm 5\%$, except for the lowest temperatures where the error increases to $\pm 10\%$, due to the low signal to noise ratio and because of the steep temperature dependence of T_1.

Theory

In the high temperature range all different carbons of the two sugars yield individual carbon-13-signals in the 75.4 MHz spectra. At constant concentration and temperature all carbons carrying a single proton yield identical spin lattice relaxation times. Since the individual C–H bonds point into different directions in a molecule fixed axis system this result is a strong indication, that the rotational mobility of the two disaccharides is isotropic. This conclusion was also derived by Allerhand *et al.* [5] for aqueous solutions of sucrose. For the case of isotropic molecular reorientations characterized by a single correlation time τ_c the

Reprint requests to Prof. H.-D. Lüdemann.

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spin lattice relaxation times T_1 for the proton decoupled C-13 atoms are given by [6]

$$\frac{1}{T_1} = \frac{n}{10}\hbar^2 \cdot \gamma_H^2 \cdot \gamma_C^2 \cdot r_{CH}^{-6} \cdot (J_0(\omega_H - \omega_C) + 3J_1(\omega_C) + 6J_2(\omega_H + \omega_C)) \quad (1)$$

$$J_m(\omega) = \tau_C(1 + \omega^2\tau_C^2)^{-1} \quad (2)$$

with n the number of hydrogens bound directly to the carbon, γ_H, γ_C the gyromagnetic ratios of the proton resp. carbon, r_{CH} the length of the C–H bonds, ω_H, ω_C the resonance frequencies of the two nuclei and τ_c the rotational correlation time.

However, all exocyclic-CH_2OD groups could not be fitted by Eqn. (1). As can be seen from an inspection of Fig. 1 their T_1 also reveal a different temperature dependence. This can best be explained by assuming an extra mobility of these groups. The $-CH_2OD$ groups can at the time scale of the NMR relaxation experiment rotate around the C–C bond connecting them to the rings.

For this motion Woessner [7] and Allerhand [5] derived the relaxation rate for a side group fixed to an isotropically rotating molecule at a defined angle by small step diffusion. Into Eqn. (1) a different spectral density function has to be inserted:

$$J_m(\omega) = A\frac{\tau_C}{1+\omega^2\tau_C^2} + B\frac{\tau_B}{1+\omega^2\tau_B^2} + D\frac{\tau_D}{1+\omega^2\tau_D^2} \quad (3)$$

$$\begin{aligned} \tau_B^{-1} &= \tau_C^{-1} + (6\tau_G)^{-1}, \\ \tau_D^{-1} &= \tau_C^{-1} + 2(3\tau_G)^{-1}, \\ A &= 1/4(3\cos^2\Theta - 1)^2, \\ B &= 3\sin^2\Theta\cos^2\Theta, \\ D &= 3/4\sin^4\Theta \end{aligned} \quad (4)$$

with τ_G the correlation time for the internal rotation, and Θ the angle between the C–H vector and the fixed axis of rotation. The tetrahedral angle of 109°28′ was inserted in the numerical evaluation.

The deuterons of the water and the hydroxyl-groups yield under all conditions only one unresolved signal, characterized by a single exponential decay of the magnetization. The results for two concentrations are given in Fig. 2. The chemical exchange between the hydroxyl-groups and water deuterons appears to be rapid on the time scale of the relaxation experiment. Under this condition the experimental T_1 is the weighted sum of the relaxation of the two populations:

$$\frac{1}{T_1} = x\frac{1}{T_1^{\text{Hydroxyl}}} + (1-x)\frac{1}{T_1^{D_2O}} \quad (5)$$

with x the mole fraction of the water deuterons. The relaxation rate of the deuterons is given by

$$\frac{1}{T_1} = \left(\frac{3}{40}\right)\left(\frac{e^2qQ}{\hbar}\right)^2\left(1 + \frac{\eta_Q^2}{3}\right)\left(\frac{\tau_C}{1+\omega^2\tau_C^2} + \frac{4\tau_C}{1+4\omega^2\tau_C^2}\right) \quad (6)$$

with $e^2qQ/\hbar$ the quadrupole coupling constant and η_Q the asymmetry parameter of the electric field gradient [8]. For the quadrupole coupling constant a value of 200 MHz was taken [9]. η_Q for the deuteron is 0.1 and this value can thus safely be neglected in the data analysis. τ_C of the hydroxyl fraction is taken to be identical to the τ_C derived from the C-13 data for the overall rotation.

The analysis of the various spin lattice relaxation rates was accomplished by fitting the experimental results to the most simple forms of the temperature dependence, and by increasing the complexity of the fitting equation, in the cases where the more simple forms failed. The rotation of the hydroxymethyl groups is characterized by a single Arrhenian.

For the rotational mobility of the sugar molecules the VTF-equation [10] had to be applied

$$\tau_C = \tau_o\, e^{\left(\frac{-B}{T-T_o}\right)} \quad (7)$$

where T_o denotes the ideal glass transition temperature. This T_o is usually found to be about 10 to 30 K lower than the experimental glass transition temperature T_g. The deuteron relaxation time of the free water molecules could not be fitted by a single correlation time. Neither an Arrhenian nor the VTF-equation were able to describe the temperature dependence of the deuteron relaxation in the heavy water molecules. For these spins a distribution of correlation times given by the Cole-Davidson-equation [11] had to be used

$$J(\omega) = \frac{1}{\omega}\left(\frac{\sin(\beta\arctan(\omega\tau))}{(1+\omega^2\tau^2)^{\beta/2}} + \frac{2\sin(\beta\arctan(2\omega\tau))}{(1+4\omega^2\tau^2)^{\beta/2}}\right) \quad (8)$$

with β the Cole-Davidson parameter, characterizing the width of the distribution.

Results and Discussion

In Fig. 1 the C-13 spin lattice relaxation times for the two most concentrated disaccharide solutions are given. All carbons carrying one hydrogen are found in the rings of the two sugars. For a given concentration and temperature their relaxation times are identical within the accuracy of the experiment. This experimental fact is most likely explained by the assumption that the molecules reorient by isotropic rotational diffusion. Internal mobilities of the pyranosic rings are thus excluded. In general, five-membered furanosic rings possess a higher conformational flexibility and the activation barriers between the possible conformers are lower than for six-membered ring structures. If conformational transitions would occur at a sufficiently large rate, one would expect the spin lattice relaxation times of the furanosic ring carbons to be longer than the relaxation times found for the pyranosic ring in the same molecule.

The fact that the experimental T_1-values found for the CH-groups in the furanosic fructose ring of sucrose agree with values determined for the pyranosic ring of the glucose moiety of this molecule further supports the assumption that for the analysis of the relaxation data the ring structures can be treated as rigid, and lacking internal flexibility. The lines drawn through the experimental points were calculated for a rigid molecule with the parameters given in Table I. The rotational diffusion of the complete sugar molecule is given by Eqn. (1) and characterized by a single correlation time τ_C. The temperature dependence of τ_C is quantitatively represented by a VTF-equation implying an ideal glass transition temperature T_0 for this mobility.

The relaxation minima for the exocyclic CH_2OD-groups occur compared to the ring structure at higher values of T_1 than would be expected for a rigidly rotating molecule. According to Eqn. (1) the ratio $T_1^{CH}/T_1^{CH_2}$ should be 2 while experimentally a ratio of ~ 1.5 is measured. Also the minima of the relaxation curves are much wider and the slopes are significantly different from the data for the ring moieties. Obviously the rotation around the exocyclic carbon-carbon bond contributes to the spectral density. These relaxation curves are quantitatively described by Eqn. (3) using the correlation times derived from the relaxation data of the ring carbons for the overall mobili-

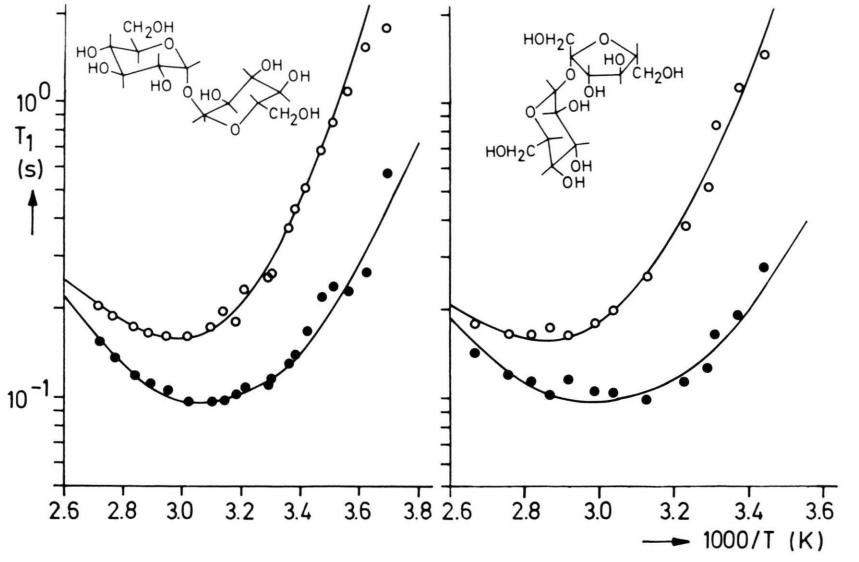

Fig. 1. C-13 spin lattice relaxation times T_1 under proton decoupling for the ring carbons carrying one proton (open circles) and the exocyclic CH_2OD-groups (full circles). Left side: trehalose (69% w/w in D_2O); right side: sucrose (75% w/w in D_2O). The curves drawn through the experimental points result from fitting the ring carbons to Eqn. (1) and (2) and assuming a VTF temperature dependence (Eqn. (7)). The exocyclic CH_2OD-groups were fitted to Eqn. (1, 3, 4) describing τ_C by the results obtained for the ring carbons and assuming an Arrhenian temperature dependence for τ_G.

Table I. Fit parameters derived from the carbon-13 resp. deuteron spin lattice relaxation times.

Substance	Sucrose			Trehalose
Concentration % w/w	63	66	75	69
τ_0 (ring) [s]	5×10^{-12}	1.25×10^{-11}	4.8×10^{-11}	2×10^{-11}
B (ring) [K]	1000	820	500	730
τ_0 (hydroxyl) [s]	6×10^{-17}	2.7×10^{-17}	1.5×10^{-16}	1.5×10^{-16}
B (hydroxyl) [K]	4000	4000	4600	4500
T_0 (ring) [K]	150	150	218	180
τ_0 (D_2O) [s]	–	1.4×10^{-13}	2.3×10^{-13}	4.5×10^{-12}
B (D_2O) [K]	–	1000	1100	700
T_0 (D_2O [K]	–	150	160	148
β (Cole-Davidson)	–	0.24	0.24	0.24
x_{sugar}	–	0.26	0.32	0.29

ty and describing the temperature dependence of the side group rotation by a single Arrhenian. (The parameters for these fits are also compiled in Table I.)

Fig. 2 gives the deuteron spin lattice relaxation times for two sucrose solutions. The shoulder at the high temperature side of both curves is caused by the relaxation of the deuterons at the hydroxyl groups of the sugars. With falling temperature these groups are more rapidly immobilized than the water molecules and thus T_1 for this fraction of the deuterons passes through the minimum at a much higher temperature than the free water molecules. The relaxation behaviour of these deuterons

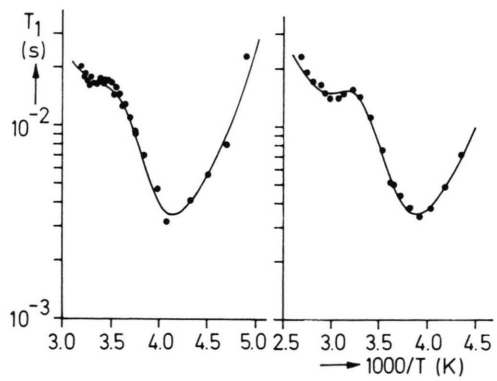

Fig. 2. Deuteron spin lattice relaxation times T_1 for the deuterons in solutions of sucrose (−OD) in D_2O. Right side: 75% w/w sucrose; left side: 66% w/w sucrose. The curves result from fitting the relaxation data to Eqn. (5, 6) and (8). The fit parameters obtained are given in Table I.

can be characterized by the correlation times that were used for the description of the C-13 relaxation of the ring carbons by application of Eqn. (6). The larger fraction of the deuterons, that are found in the free water molecules cannot be represented by a single correlation time. In order to fit the curves in Fig. 2 quantitatively one has to make use of a distribution of correlation times. In our cases the Cole-Davidson-distribution [11] as given by Eqn. (8) had to be used. The curves drawn through the experimental points result from the fitting of the results. The free parameters of these fits are also compiled in Table I.

In Fig. 3 the ideal glass transition temperatures T_0 obtained from our NMR relaxation studies are compared to the experimental calorimetric glass transition temperatures, T_g [1]. The two most concentrated solutions of each sugar reveal a very interesting phenomenon: For these solutions the T_0 for the sugar mobility is much higher than the T_0 for the rotation of the water molecules. For the 75% w/w sucrose solution T_0 (sucrose) is even above the experimental T_g for the complete system.

Obviously one would expect the same observation for the more dilute sucrose solutions, however, since our relaxation data ended well above the T_0 obtained from the fitting procedures a difference of $\Delta T_0 \leq \pm 10$ K could not be considered significant.

The large differences in T_0 observed for the two subsystems could help to explain the stabilizing and protective effect of sugars like trehalose in stabilizing membrane vesicles and complete cells [12].

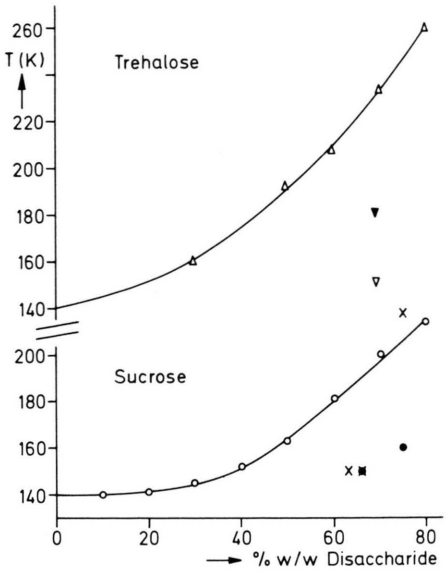

Fig. 3. Glass transition temperatures of aqueous solutions of sucrose and trehalose as function of concentration △, ○: T_g as obtained from the calorimetric studies by Green *et al.*; ▼, x: T_0 derived from the C-13-T_1 of the ring carbons; ▽, ●: T_0 derived for the water molecules.

It appears possible, that on drying the disaccharide molecules form a stable immobile network long before the aqueous subsystem passes into the glassy state and thus preserve the structural and functional integrity of the vesicles and organelles by preventing collapse of the supermolecular structures.

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