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DYNAMICS OF CYCLOHEXAAMYLOSE (ALPHA CYCLODEXTRIN)·6 H_2O BY ^1H SOLID STATE N.M.R. SPECTROSCOPY

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ABSTRACT

In the present ^{1}H-n.m.r.-studies, we investigated the dynamics of the spins associated with the narrow component of the absorption lineshape of solid cyclohexaamylose (alpha cyclodextrin)·6 H_2O. The presence of dipolar interactions in both the narrow and the broad components of the lineshape is demonstrated with the detection of double quantum coherences. On the assumption of a dominant relaxation mechanism, the temperature dependence of the lineshape and the spin–lattice relaxation time (T_{1d}) in local dipolar fields yield, at −35°, a distribution of motional correlation time with a mean of 13.0 ±0.7μs and an apparent energy of activation of 3.25 ±0.33 kcal/mol. The "freezing" of the motions of the distribution occurs around −60°, with a corresponding measured T_2^* of 6.8 ±0.3 μs. The calculation of the second moment of the lineshape due to ^{1}H homonuclear dipolar interactions from the crystal structure gives a T_2 value of 6.5 μs. The assignment of motion causing the T_{1d} minimum and the transition of a composite single absorption lineshape is discussed in light of the present and previous n.m.r. studies, crystallographic data, and molecular dynamics simulations.

INTRODUCTION

Cyclohexaamylose (alpha cyclodextrin) is a cyclic oligosaccharide consisting of six α-(1→4)-linked D-glucopyranosyl residues. With a cavity inner diameter of 4.5–5.5 Å, this torus-shaped molecule forms inclusion complexes with a variety of guests[1]. In view of the binding and catalytic properties of alpha cyclodextrin and some of its derivatized forms, the topology of the guest–host interactions has served as an enzyme model[1,2]. Crystallization of alpha cyclodextrin from water produces two forms of alpha cyclodextrin hexahydrate having the same space group $P2_12_12_1$

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but with different unit-cell parameters and packing arrangements[3–5]. Form I, which grows preferentially, accommodates two water molecules within the cavity and four water molecules in interstices between the macrocycles. In form II, one water molecule and the primary hydroxyl group of an adjacent alpha cyclodextrin are included within the aperture, and five water molecules are located on the outside of the ring. Although n.m.r. methods have been applied to the study of cyclodextrin complexes in solution[1], it is only recently that molecular reorientation of some guest molecules in the solid inclusion-complexes has been reported[6].

In previous work[7], we showed, with a variety of n.m.r. transient techniques, the absence of domain structures of size $>\sim 11$ Å in powders of alpha and beta-cyclodextrin, dextran B512F, and their deuterated analogs wherein deuterium replaced exchangeable protons. At room temperature, the ^{1}H-n.m.r. lineshapes of these systems all display a narrow line superimposed on a broad line. Composite lineshapes arise in a number of situations, such as domain structures[8], distribution of correlation times[9], cross-correlation effects in the anisotropic motion of molecular segments[10], and cross-relaxation between two spin species intermingled at short distances from each other[11].

In the present ^{1}H-n.m.r. studies of solid alpha cyclodextrin·6 H_2O, the motions of the spins associated with the narrow component of the ^{1}H-n.m.r. lineshape are investigated. Measurements of the lineshape and spin–lattice relaxation times in local dipolar fields as a function of the temperature, and detection of multiple quantum coherences, permit monitoring of the dynamic and the static components of the observed lineshape. From these measurements, we obtain for the motions associated to the lineshape's narrow component a mean correlation time at −35° and an apparent energy of activation. In the discussion, some possible assignments of motions are suggested.

EXPERIMENTAL

The ^{1}H-n.m.r. experiments were performed at 220 MHz on a homebuilt spectrometer, the design of which is similar to one described previously[12]. Free induction decays and the measurements of the spin–lattice relaxation times along local dipolar fields (T_{1d}) *via* the Jeener–Broekaert method[13] were obtained with the following conditions: 1.5-μs 90° pulses, non-quadrature detection with a 5-MHz digitization rate, 325 kHz filter, and accumulation of a 2048-point transient every three seconds. Phase cycling of the r.f. pulses in the various sequences minimized receiver ringdown and baseline artifacts. Absorption lineshapes were obtained by Fourier transformation of the 2048 data points zero-filled to 8192 points and were fitted by using a nonlinear least-squares routine[14]. A similar numerical procedure permitted the analysis of two-component decays of the magnetization in the relaxation measurements.

Variable temperature measurements were done by blowing dried and cooled nitrogen gas over the sample. The temperature was monitored with a chromelalumel thermocouple at one cm from the sample in a homebuilt probe, and controlled manually by gas flow to within ±1°. With a probe designed by Doty-Scientific Instrument, the temperature was regulated to ±1° with a copper–constantan thermocouple.

Multiple quantum free-induction decays were measured with an off-resonance Jeener–Broekaert[15] pulse program, $90_{+x} - t_1 - 45_{+y,-y} - t - 45_{+y} - t_2$, where t is a variable evolution period. The magnetization was sampled at $t_2 = t_1 = T_2^*$ with a 24-kHz offset for the long T_2^* (67 μs) component, and a 40-kHz offset for the short T_2^* (9 μs) component. When the signals obtained with the first 45° pulse along $-y$ are added to the signals observed with the same pulse along $+y$, odd quantum coherences are detected by an oscillation of the magnetization, of frequency $\eta\Delta\omega$, where η is the order of the coherence, and $\Delta\omega$ is the resonance offset. The difference of these signals gives even quantum coherences.

The sample of alpha cyclodextrin·6 H_2O (recrystallized from water) was generously provided by Dr. P. Braun and Professor J. F. Robyt of the Biochemistry–Biophysics Department at Iowa State University. Crystals of alpha cyclodextrin grown in water produce two forms of hexahydrate having distinct shapes[3–5]; form I is as stout and prismatic crystals, while form II is tabular and flaky. Direct observation confirmed that the powdered sample used in this work consisted of form I.

RESULTS

In Fig. 1 is presented the ^{1}H-n.m.r. lineshape of alpha cyclodextrin·6 H_2O as a function of the temperature. At low temperature, a broad lineshape is observed; as the temperature is increased, there is a gradual slight narrowing of the linewidth, with the appearance, at −50°, of a narrow component the intensity of which increases at the expense of the broad component. The full width at half height of a Gaussian lineshape is given by[16].

$$\Delta\nu = \frac{(2\ln 2)^{1/2}}{\pi T_2^*}\,(\mathrm{s}^{-1}) \tag{1}$$

where T_2^* is the time decay constant of the corresponding free induction decay. The onset of linewidth narrowing of the minor component occurs at a T_2^* (broad component) of 6.8 ±0.3 μs, on the reasonable assumption of a Gaussian lineshape.

In order to demonstrate that static ^{1}H homonuclear dipolar interactions contribute to the narrow component of the lineshape, component selective multiple quantum coherences were excited at room temperature. The reliability of the off-resonance Jeener–Broekaert method[15] was examined with the double quantum response arising from the selective excitation of the broad component of the lineshape. The results of these experiments are shown in Fig. 2 and Table I. Both lineshape components generated single (data not shown) and double quantum coherences (see Fig. 2). The excitation of double quantum coherences shows that

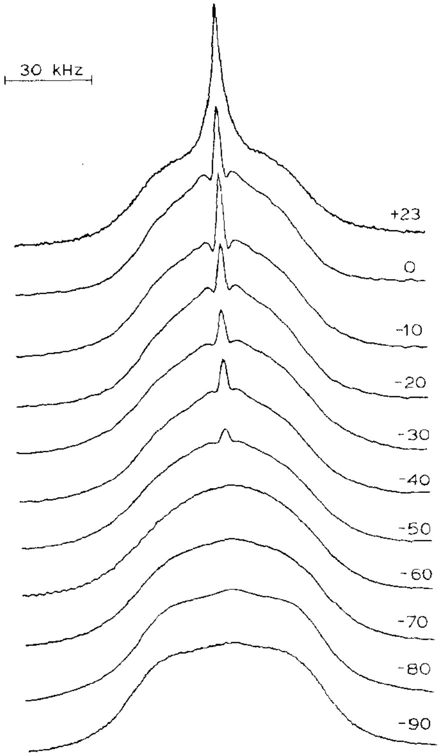

Fig. 1. ^{1}H absorption lineshape of alpha cyclodextrin·6 H_2O as a function of the temperature in °C.

dipolar interactions contribute to both components of the lineshape. The decay of the coherences is due to proton spin fluctuations during the evolution period. Higher order coherences could in principle be excited with more-complicated multiple-pulse sequences, but at the expense of experimental simplicity of the off-resonance Jeener–Broekaert technique.

To determine if a dynamic component could be associated with the lineshape transition around $-50°$, spin–lattice relaxation times (T_{1d}) along local dipolar fields were measured on-resonance as a function of the temperature. Bi-exponential decays of the magnetization of the Jeener–Broekaert echoes were observed: 71% of the decay exhibited a T_{1d} of 21 μs, and 29% displayed a T_{1d} of 1.9 ms, at room temperature. The relative errors on the time decay constants T_{1d} and the ratio of proportions of decays have been estimated at $\pm 10\%$. In Fig. 3 is presented the temperature variation of the long T_{1d} component. From a linear, least-squares fit of the data on each side of the V-shaped curve, the slopes yield an apparent energy of activation of 3.25 ± 0.33 kcal/mol. From the T_{1d} minimum, a mean correlation time of 13.0 ± 0.7 μs is determined (see discussion). We can confidently associate the long T_{1d} minimum at $-35°$ and the onset of linewidth narrowing of the lineshape's

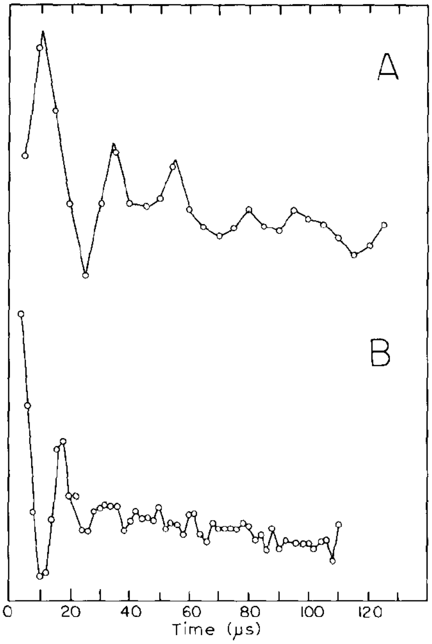

Fig. 2. Double quantum coherence free induction decays in alpha cyclodextrin·6 H_2O. [Circles are experimental points, while the solid lines serve to guide the eye. Excitation of narrow component (A) and broad component (B) of the absorption lineshape.]

minor component at around −50° with the same motional processes. Technical limitations prevented acquisition of the short T_{1d} component over the whole temperature range displayed in Fig. 3. Preliminary results indicate that the short T_{1d} component "freezes" at a temperature lower than −60°.

Paramagnetic impurities can cause lattice defects that affect values of relaxation times. E.s.r. measurements carried out at 4° and 298° K on an IBM/Bruker 200D instrument operated at 9.5 GHz revealed no detectable signals. These results were interpreted as indicating the absence of paramagnetic impurities in our sample.

TABLE I

OFF-RESONANCE JEENER–BROEKAERT OSCILLATIONS' PERIODS (IN μs)

Type		*Long* T_2^*	*Short* T_2^*
Single quantum	calculated	42	25
	found	43 ±2	25 ±1
Double quantum	calculated	21	12.5
	found	22 ±2	12 ±1

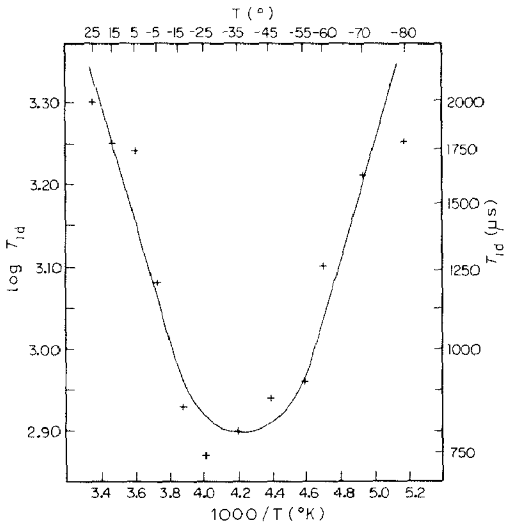

Fig. 3. Plot of log T_{1d} *versus* (temperature, °K)$^{-1}$. Crosses are experimental points; the solid line serves to guide the eye.

DISCUSSION

The interpretation of n.m.r. experiments requires an understanding of the relevant mechanisms that affect lineshapes and relaxation behavior. The generation of dipolar order with the Jeener–Broekaert sequence (see Fig. 3) and the observation of the double quantum coherences (see Fig. 2) both establish the presence of ^{1}H homonuclear dipolar interactions. The anisotropy of the ^{1}H magnetic shielding interaction (chemical shift) can also contribute to the lineshape and relaxation. In powdered ice at −100°, this mechanism broadened[17] the ^{1}H absorption lineshape by 34 p.p.m. At the same temperature in our 5.2-T Zeeman field, this would correspond to a 7.5-kHz broadening. The full width at half height of the ^{1}H lineshape of alpha cyclodextrin·6 H_2O is 63 ±3 kHz (see Fig. 1) at −90°. therefore, as a first approximation, we consider only the dipolar interaction as the relevant mechanism in our experiments.

Dipolar spin-couplings modulated by lattice fluctuations give rise to spin–

TABLE II

CORRELATION TIME τ_c DEPENDENCE OF T_{1d}^{-1} (VAN STEENWINKEL[18])

Regime	*Condition*	τ_c *dependence of* T_{1d}^{-1}
Short correlation time weak collision	$\tau_c < T_2$	τ_c
Long correlation time strong collision	$\tau_c > T_2$	τ_c^{-1}
Intermediate case	$\tau_c \simeq T_2$	$\frac{\tau_c}{1 + (\omega_L \tau_c)^2}$

lattice relaxation. In local dipolar fields H_L, Van Steenwinkel[18] showed how the spin–lattice relaxation rate (T_{1d}^{-1}) depends on the relative values of the correlation time (τ_c) of the fluctuations and the observed second moment of the lineshape (or an operational T_2 as defined in Eq. *5*); see Table II. There it may be noted that the maximum rate is observed when $\omega_L \tau_c = 1$ with $\omega_L = \gamma H_L$, where γ is the magnetogyric ratio. For a Gaussian lineshape, the full width at half height is given by

$$\Delta\nu = \frac{(2\ln 2)^{1/2} M_2^{1/2}}{\pi} \ (s^{-1}), \tag{2}$$

where $M_2^{1/2}$ (rad/sec) is the square root of the second moment of the lineshape[16]. M_2 can be expressed in terms of the local dipolar fields[9] as

$$M_2 = 3\omega_L^2. \tag{3}$$

With Eqs. *2* and *3*, we obtain at the T_{1d} minimum

$$\tau_c = \omega_L^{-1} = \frac{(6\ln 2)^{1/2}}{\pi\Delta\nu} \tag{4}$$

The linewidth of the broad component at the T_{1d} minimum and Eq. *4* yield τ_c = 13.0 ±0.7 μs. This approach is valid for a single correlation time τ_c or the average value of a distribution of correlation times. The fraction of the spins in the distribution whose $\tau_c \leqslant T_2$ are associated with the narrow component of the lineshape, while spins whose $\tau_c \geqslant T_2$ cannot average the local dipolar field and give rise to the broad component. In principle, the T_2 value of the latter spin is independent of τ_c, and is represented by T_{2R} for the rigid lattice be havior. While the spin–spin relaxation time T_2 cannot be well defined for strongly dipolar coupled spin systems, an operational definition of T_2 through a Gaussian lineshape's second moment is given[16] by

$$M_2^{1/2} = \frac{1}{T_2}\left(\frac{\text{rad}}{\text{sec}}\right) \tag{5}$$

When several interactions contribute to the lineshape one obtains an effective T_2*. From Fig. 1, it is apparent that, at −60°, all the spins display a rigid-lattice type of behavior. With the linewidth at −60° and Eq. *1*, we find $T_2^* = 6.8 \pm 0.3\ \mu$s. For a powder of microcrystallites oriented at random, the lineshape's second moment due to homonuclear dipolar coupling can be calculated[16] from

$$M_{2R} = \frac{6}{5} I \frac{(I+1)\,\gamma^4\hbar^2}{N} \sum_{j<k} r_{jk}^{-6}, \tag{6}$$

where I is the spin angular momentum quantum number, γ the magnetogyric ratio,

$\hbar$ Planck's constant divided by 2π, N the number of spins, and r_{jk} the distance between spins j and k. From the crystal structure[3], we find $M_{2R} = 2.36 \times 10^{10}$ (rad/sec)^2, which corresponds to $T_{2R} = 6.5$ μs on the assumption of a Gaussian lineshape. This result compares favorably to the experimental value of T_2^*. The room-temperature crystal structure[3] of alpha cyclodextrin·6 H_2O yields thermally averaged atomic positions; the slight gradual broadening of the lineshape observed when lowering the temperature below −60° implies that the "true" rigid lattice behavior, *i.e.*, "fixed nuclei" with T_2 independent of τ_c, occurs at a lower temperature.

The nature of the motions responsible for the T_{1d} minimum and the lineshape transition cannot be unambiguously identified solely on the basis of the present data. We suggest two plausible assignments of motion consistent with our n.m.r. data, the results of crystallographic studies, and molecular-dynamics simulations. The fact that the narrow component of the absorption lineshape still persists, even after the replacement of exchangeable protons by deuterium[7], indicates the absence of a direct role for the hydroxyl groups and water molecules. Nevertheless, fluctuations of the extensive hydrogen (or deuterium) bonding ring structures and chain-like networks in alpha cyclodextrin hexahydrate[3–5] could allow for backbone oscillations, in spite of the macrocyclic ring constraint, and be responsible for relaxation. Molecular-dynamics simulations[19] have demonstrated the presence of anisotropic backbone oscillations and also of the puckering of the individual sugar rings. Such localized motions would be consistent with our observation[7] of heterogeneity limited to a length scale <11 Å. The extent of three-dimensional spin diffusion can also be easily shown[7] to be restricted to such small length scales with the T_{1d} values of Fig. 3. Point defects such as lattice vacancies or orientational fault sites present at crystal grain boundaries could also lead to localized relaxation. Indeed, a refinement of the crystal structure using neutron diffraction[4] indicated the presence of twofold disordered sites (with 92% occupancy for one site and 8% for the other) for a particular primary hydroxyl group of the alpha cyclodextrin ring.

CONCLUSION

In the present work, we have studied the dynamics of the spins associated with the motionally narrowed component of the ^{1}H absorption lineshape of solid alpha cyclodextrin·6 H_2O. We showed, with the detection of double quantum coherences, the presence of dipolar interactions in the composite lineshape at room temperature. Measurements of the lineshape and T_{1d} as a function of the temperature yielded an average of 13.0 ±0.7 μs for the distribution of motional correlation time, and an apparent energy of activation of 3.25 ±0.33 kcal/mol on the assumption of a single relaxation mechanism (*i.e.*, homonuclear dipolar interactions). While our studies do not permit unequivocal assignment of motion, the interpretation of our results with crystallographic investigations, molecular-dynamics simulations, and previous solid-state n.m.r. work on carbohydrates is consistent with motions localized on a length scale <11 Å.

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