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Structural and Dynamical Studies of the Hydrate, Exchangeable Hydrogens, and Included Molecules in β- and γ-Cyclodextrins by Powder and Single-Crystal Deuterium Magnetic Resonance

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Abstract: To study the degree and time scales of dynamic disorder processes of the hydrate and cavity included molecules in a biomolecule of substantial structural complexity, we have obtained deuterium magnetic resonance spectra of powder and single-crystal samples of β- and γ-cyclodextrins crystallized from deuterated water or solutions containing deuterated molecules. With single-crystal samples, results can be compared directly with the structures obtained by X-ray or neutron diffraction. At room temperature and above, we find that the hydrate, sugar hydroxyl groups, and included molecules (benzyl or ethyl alcohol) are dynamically disordered to varying degrees with reorientational rates that are fast on the deuterium NMR time scale, i.e., rate $>10^6$ s^{-1}. An unexpected finding is that the 11 lattice waters per β-cyclodextrin molecule, which are determined by neutron diffraction to occupy 16 positions,[1] give rise to a single exchange averaged doublet in the NMR experiment showing that they freely move among these sites in a time less than 10^{-6} s. As a consequence of such exchange averaging, the orientation of the residual quadrupole coupling tensor for the water deuterons is expected to reflect the lattice symmetry ($P2_1$) rather than the orientation of the water molecules at individual sites, and this property is experimentally verified. Rapid exchange, which is absent in the less hydrated α-cyclodextrin, is also observed for the more hydrated γ-cyclodextrin. In addition, these results show that included molecules are orientationally disordered to a greater degree than previously thought. These dynamic features are considered in terms of the known esterase activity of cyclodextrins.

On the basis of X-ray and neutron diffraction structural studies, Saenger and colleagues have proposed a very detailed picture for the time averaged structure of the hydrate of β-cyclodextrin (β-CD·11H$_2$O) at both room temperature and 120 K.[1,2] More recently we reported[3] that the exchangeable hydrogens of crystalline β-CD·11D$_2$O and crambin, a small protein, show great similarity in terms of the presence of substantial dynamic disorder as indicated by deuterium spectroscopy. Furthermore, β-CD·11H$_2$O has a proton conductivity similar to that of a hydrated protein[4] which is about 2 orders of magnitude greater than the less hydrated 6-sugar analogue, α-CD·6H$_2$O, in which the hydrate is substantially more ordered.[3] These properties, along with its known esterase activity, make β-CD attractive for studying im-

portant features of enzymatic catalysis and proton conduction in channels both of which are directly related to the molecular dynamics of the aqueous solvate and sugar hydroxyl groups which we study here by solid-state deuterium spectroscopy. Specifically, we examine the exchangeable hydrogens of the cyclic heptasaccharide β-cyclodextrin (β-CD) and its inclusion complexes with water (β-CD·11H$_2$O), ethanol (β-CD·8H$_2$O·EtOH), and benzyl

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alcohol (β-CD·5H$_2$O·BzOH). For comparison, we also present results for the octasaccharide analogue γ-CD·17H$_2$O, and the disaccharide, trehalose·2H$_2$O.

The point of departure of this work is to use deuterium magnetic resonance of powder and single-crystal samples to examine the time scales and types of disorder processes associated with the waters of hydration, the guest molecules, and hydoxyl groups. In closely related studies, we have used these methods to determine the rate at which water molecules undergo tetrahedral jumps in ice,[5] the degree of orientational ordering of the hydrate in crystalline proteins,[3,6] and the allowed orientations of ferrocene included in the hexagonal thiourea lattice,[7] a structural problem which was not solved by diffraction methods.

The room temperature structure of β-CD determined by neutron diffraction shows that most of the sugar and water hydroxyl groups are orientationally disordered and, in some cases, the hydrate is positionally disordered as well. On the basis of indirect evidence, the observation of a thermal phase transition and the lack of disorder in the low-temperature structure,[2] it was proposed that the disorder at room temperature is dynamic.

The question of dynamic versus static disorder is directly answered by solid-state NMR methods and, in the case of the single-crystal deuterium NMR experiment, there is a simple and direct connection between the NMR and diffraction experiments. Given the atomic coordinates which describe the orientation of an O–D group, the projection of the deuterium quadrupole coupling tensor on the magnetic field which determines the doublet frequencies measured in the NMR experiment can be directly calculated without making serious approximations. For example, if the crystal is oriented such that a particular O–D bond is along the magnetic field axis, then its quadrupole doublet frequencies will have their maximum values, $\pm^3/_4$qcc (qcc $\sim$ 216 kHz). In the case of static disorder, the deuterium experiment shows doublets for each of the possible orientations with line intensities proportional to the occupation probabilities. In the case of dynamic disorder, a single quadrupole doublet is observed at the average of the expected frequencies for the different orientations dynamically sampled. Consequently, the maximum frequencies are reduced in comparison to the static case. At the current level of spectral resolution and in a molecule with the complexity of β-CD·11D$_2$O which has $\sim$80 potentially distinct exchangeable deuterons per unit cell, one does not expect to observe resolved doublets for each of the deuterated sites. However, the experimental spectra show some surprising and simplifying features resulting from dynamical averaging which allow us to make these direct comparisons between the neutron structure and the NMR spectra. We also make use of included molecules labeled at a small number of nonexchangeable sites which give simple deuterium spectra and in turn can be directly compared with X-ray or neutron structures. In the examples studied here, we find that the residual quadrupole couplings calculated from the crystallographic structures are larger than those observed, i.e., the system is more disordered than indicated by the X-ray or neutron scattering derived structure.

Experimental Section

Anhydrous β-cyclodextrin (Sigma), γ-cyclodextrin (Fluka). benzyl alcohol. D-trehalose dihydrate (Aldrich), and D$_2$O, C$_2$H$_5$OD, CD$_3$CH$_2$-OH. C$_6$D$_5$CH$_2$OH, and C$_6$H$_5$CD$_2$OH (MSD Isotopes) were used without further purification. Large crystals of β-CD·11D$_2$O were obtained by slow cooling of hot aqueous solutions.[1] Inclusion complexes with ethanol and benzyl alcohol were prepared according to procedures used for the crystallographic work: β-CD·8D$_2$O·EtOD and β-CD·8H$_2$O·CD$_3$CH$_2$OH were crystallized from aqueous ethanol solutions[8] and β-CD·5D$_2$O·BzOD, β-CD·5H$_2$O·C$_6$D$_5$CH$_2$OH, and β-CD·5H$_2$O·C$_6$H$_5$CD$_2$OH were crystal-
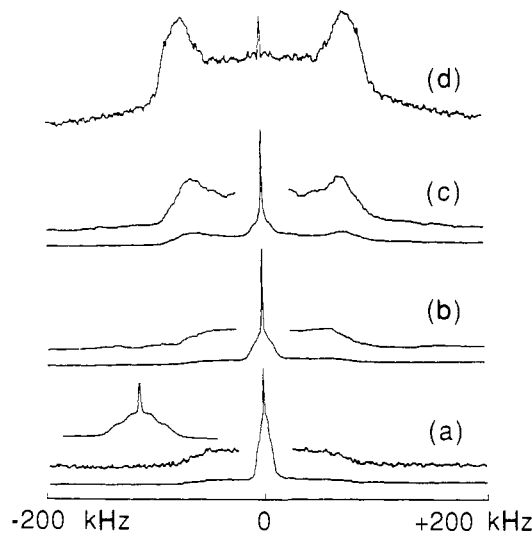
Figure 1. Room temperature ^{2}H NMR powder spectra of (a) β-CD·11D$_2$O, (b) β-CD·8D$_2$O·EtOD, (c) β-CD·5D$_2$O·BzOD, and (d) α,α-trehalose·2D$_2$O. The inset in spectra a is a horizontal expansion from −20 to 20 kHz.

lized from aqueous solutions containing equimolar β-cyclodextrin and benzyl alcohol.[9] α,α-Trehalose·2D$_2$O was crystallized from a saturated 40% C$_2$H$_5$OD/D$_2$O solution. Crystals of γ-CD·17D$_2$O were obtained by slow evaporation of an aqueous solution.

Powder samples were prepared by removing individual crystals from the mother liquor followed by brief drying on filter paper ($\sim$5 min) to remove surface water. We find that the ^{2}H spectra show three spectral features as follows: an extremely sharp peak, with a line width of $\sim$1 kHz and a nearly negligible intensity of $\sim$5% which decreases on drying, a substantially more intense narrow pattern with $\sim$20 kHz width and an intensity of $\sim$60%. and a broad pattern $\sim$150 kHz wide. The intensity of the sharpest peak. which is attributed to surface water, is largely eliminated by drying for periods of 5–20 min without substantially affecting the intensities of the two dominant spectral patterns. After drying. crystals were transferred to 0.25 × 0.5 in. delrin sample tubes and crushed.

Single crystals of β-CD·11H$_2$O and β-CD·8D$_2$O·EtOD were examined by X-ray diffraction to confirm that the space group and lattice dimensions of the samples used here are equivalent to those used in the published X-ray structures and to determine the orientation of the unit cell with respect to the crystal faces for determining the crystallographic orientation of the measured quadrupole coupling tensors. Also. the expected guest host stoichiometry was confirmed for the ethanol and benzyl alcohol complexes by dissolving crystals in D$_2$O and checking their 500-MHz ^{1}H NMR spectrum. We were careful to select small crystals for X-ray work and large crystals for NMR work with the same overall shapes. Our samples, obtained as described above, crystallized in the reported monoclinic space group, $P2_1$, with lattice dimensions measured from 25 reflections in excellent agreement with the published values. The morphology is as follows: the crystals are prismatic with the edges of the basal plane (largest face) the b and c axes and the inclined face is the ab plane. In the case of the β-CD·8D$_2$O·EtOD crystals, the ac plane was not well-developed. Thus well-defined crystal edges correspond to the unit cell axes. Crystals of β-CD·5H$_2$O·BzOH. which has the same space group and nearly equivalent lattice dimensions as the other two isomorphs, were found to grow with the same morphology as the undecahydrate. We have thus assumed that the faces are indexed in the same way.

For single-crystal NMR studies, crystals ($\sim$3 × 2 × 0.5 mm) were removed from the mother liquor and dried briefly ($\sim$1 min) on filter paper and the base (bc plane) epoxied to one face of a 3-sided. hollow cube (4.5 mm^3) and the b and c crystal edges parallel to box edges. To avoid loss of lattice water, the crystal was covered with epoxy cement. In turn, each face of the box is glued to the end of a shaft which is the rotation axis of the NMR goniometer probe, itself perpendicular to the NMR magnetic field. The three faces of the hollow plastic box are orthogonal to one another so that by altering the mounting of the box on the shaft, rotation about 3 orthogonal axes (a^*. b, c) can be performed on the same crystal.

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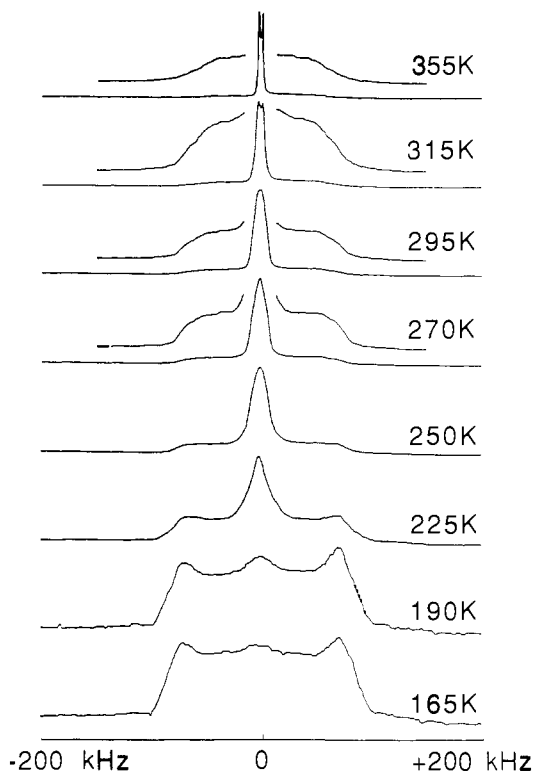
Figure 2. Variable temperature ^{2}H NMR powder spectra of β-CD·11D$_2$O. The overlay traces are expanded 5-fold for the T = 270 and 295 K spectra and 10-fold for the 315 and 355 K spectra.

Figure 3. Room temperature single-crystal ^{2}H NMR spectra of β-CD·11D$_2$O at different goniometer settings.

^{2}H NMR spectra were recorded on a home built 5.9 T spectrometer using a phase-cycled quadrupolar echo sequence. $(\pi/2)_x\text{-}\tau\text{-}(\pi/2)_y\text{-}\tau\text{-}$observe, with $(\pi/2)$ pulse lengths of 2.5 μs and an echo delay τ = 30 μs. In all cases, the pulse sequence was repeated with a sufficiently long waiting delay to ensure that equilibrium signal intensities were observed, typically 100–200 ms for these samples at room temperature.

Results and Discussion

Figure 1 shows the room temperature (287 K) deuterium spectra of powder samples of β-CD·11D$_2$O, β-CD·EtOD·8D$_2$O, β-CD·BzOD·5D$_2$O, and the disaccharide α,α-trehalose·2D$_2$O. The trehalose X-ray structure[10] shows a highly ordered hydrate with the hydroxyl groups from the two glucose units and the two water molecules forming well-defined hydrogen bonds. In hydrates such as Li$_2$SO$_4$·D$_2$O[11] and BaClO$_3$·D$_2$O,[12,13] the presence of rapid 2-fold flip-flop motion of the water deuterons about the water 2-fold axis is well-established by deuterium spectroscopy. This jump-like reorientation which leaves the time averaged structure unaltered is, in principle, not observable in the X-ray work. In this regard trehalose is an interesting hydrated sugar for study by ^{2}H NMR because of differences in hydrogen bonding. One of the waters is tetrahedrally bonded while the other is trigonally bonded. However, the room temperature spectrum, Figure 1d, shows only the wide pattern characteristic of stationary deuterons. Thus, the two waters in this disaccharide are held rigidly in position.

In contrast, the more heavily hydrated and structurally complicated β-CD·11D$_2$O ($\sim$1.5 water molecules per sugar residue) gives a spectrum, Figure 1a, which clearly shows a lack or near absence of stationary deuterons since the entire spectrum is distinctly narrower and contains an intense narrow central pattern. To ensure that spectra with accurate and unsaturated signal intensities were obtained, a recycle delay of 0.2 s at room tem-
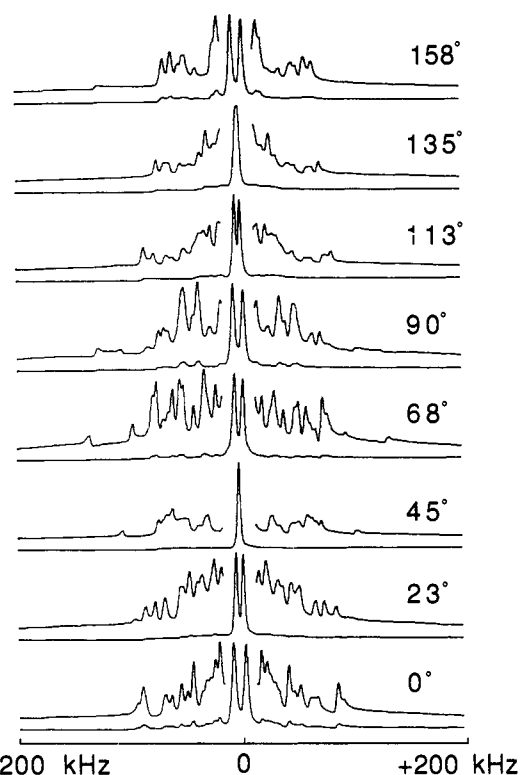
perature was used. Furthermore, spectra with recycle delays up to 60 s were equivalent to those obtained with a 0.2-s delay, indicating an absence of deuterons with long relaxation times in the range of 1–100 s. Thus, the exchangeable deuterons in β-CD·11D$_2$O are dynamically disordered. The temperature dependence of the β-CD·11D$_2$O quadrupole echo spectra, Figure 2, shows that the reorientational dynamics begin to be frozen out on the deuterium NMR time scale only as the temperature is lowered below 200 K. The absence of well-defined powder pattern features like those shown by the trehalose spectrum, Figure 1d, and (more generally) reduced spectrum intensities are indicative of intermediate exchange effects, i.e., dynamical processes with rates within about two orders of magnitude of the static quadrupole coupling constant, qcc $\sim 2 \times 10^5$ s^{-1}. At the lowest temperature examined, 165 K, the pattern is wide but lacks the sharply defined features characteristic of a stationary system and the total spectrum intensity is substantially attenuated when compared to the room temperature value showing that at this temperature reorientational dynamics are in the lower limit of the intermediate exchange range, 10^3–10^4 s^{-1}. The very significantly reduced spectral intensities between 225 and 190 K indicate that reorientational dynamics have rates comparable to qcc (rate $\sim 10^5$ s^{-1}). At the highest temperature, 355 K, the well-resolved central feature suggests that some fraction of the exchangeable deuterons undergo rapid dynamics (rate $> 10^6$ s^{-1}) and are highly disordered since the residual coupling, $\overline{\text{qcc}}$ = 4 kHz, is a small fraction ($\sim$2%) of the static value.

Single-crystal spectra offer the possibility of resolving quadrupole doublets from individual deuteron sites with the number of potentially resolved sites determined by the molecular structure and crystallographic symmetry. The isomorphous series of β-CD inclusion complexes studied here crystallize in the monoclinic space group $P2_1$ with 2 molecules per unit cell related by a 2-fold screw axis. For the general orientation of the crystal with respect to the applied magnetic field, these two molecules are nonequivalent. Thus, since each molecule of the undecahydrate contains 21 distinct hydroxyl groups and 11 water molecules, there are 86 potentially resolved deuteron quadrupole doublets. This number could be increased if some sugar hydroxyl groups are statically disordered or decreased if there are rapid exchange averaging

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processes such as the 2-fold motion observed in $Li_2SO_4 \cdot D_2O$ which makes the water deuterons equivalent in the NMR experiment.

Room temperature single-crystal spectra of β-CD$\cdot$11D$_2$O at selected goniometer settings are shown in Figure 3. Although the crystallographic orientation of the goniometer axis for this sample was not determined, several important features are immediately evident. The spectra show an intense sharp doublet which exhibits a maximum splitting of 8 kHz during a rotation cycle of 180° and vertical expansion of the spectra reveals many sharp lines, only a few of which display quadrupolar splittings approaching the rigid value. The presence of well-resolved sharp lines eliminates the possibility of intermediate exchange effects which result in severe line broadening. The potential number of doublets ($\sim$80) is substantially larger than that observed ($\sim$10), thus a complete structural analysis is not possible at the resolution afforded by these spectra. Consequently, we address the dominant spectral features: the intense doublet with small splitting and the range of splittings observed for the less intense doublets.

The less intense doublets, with a few exceptions, appear within the frequency limits of $\pm$80 kHz. Thus, these lines contribute to the broad feature of the high-temperature powder pattern spreading over the same frequency range, Figure 2. In contrast to water molecules in ice near the melting point, 250–270 K,[5] these deuterons yield well-resolved doublets and thus undergo some form of fast (rate $\gtrsim 10^6$ s^{-1}) and restricted motion which reduces their quadrupole couplings by roughly a factor of 2. Furthermore, subtracting out the narrow central feature from the powder pattern leaves a pattern which in overall breadth and shape approximates that expected for O–D groups undergoing fast 2-fold flip motion. At most, a few doublets are observed which extend beyond this range and approach the maximum expected frequency for stationary hydroxyl deuterons, 162 kHz.

For the following reasons we assign the intense central feature, Figures 1 and 2, or the corresponding intense central doublet, Figure 3, to water deuterons and the less intense doublets primarily to the sugar hydroxyls. A secondary hydroxyl deuteron attached to the rigid sugar structure observed for β-CD$\cdot$11D$_2$O[1] is, at most, disordered by free rotation of the O–D bond about the C–O axis which would reduce qcc by a factor of $\sim^1/_3$ to an averaged value of $\overline{qcc}$ = 54 kHz for tetrahedral geometry. Although not expected on the basis of the neutron structure, primary hydroxyls have an additional degree of internal mobility which could at most reduce the qcc by an additional factor of $^1/_3$ to 18 kHz. Thus we conclude that the sharp central peak which shows a maximum splitting of $\sim$10 kHz corresponds to disordered waters of hydration which do not have the reorientational constraints of the sugar hydroxyl groups imposed by covalent bonding. The intense central doublet is well-resolved for the general orientations, Figures 2 and 3, and shows that a number of hydrate molecules are dynamically equivalent on the time scale of the ^{2}H NMR experiment, $\lesssim 10^{-6}$ s. Finally, 59% of the exchangeable sites in β-CD$\cdot$11D$_2$O are water deuterons. Thus the intensity of the central feature, 60% of the total spectrum integral, is sufficient to account for all of the waters, which indicates that the less intense doublets with residual couplings of $\sim$60 kHz arise primarily from sugar hydroxyls.

The surprising observation of an exchange averaged doublet with small residual quadrupole coupling corresponding to waters of hydration in β-CD$\cdot$11D$_2$O (1.6 D$_2$O/sugar) is not seen in the less hydrated 6-sugar analogue, α-CD$\cdot$6D$_2$O (1 D$_2$O/sugar). If exchange averaging of the water molecules is related to the increased degree of hydration, then we would expect to see the sharp central feature in the 8-sugar analogue, γ-CD$\cdot$17D$_2$O (2.1 D$_2$O/sugar), with perhaps an even higher degree of orientational averaging. Shown in Figure 4 are powder and single-crystal spectra of γ-CD$\cdot$17D$_2$O at room temperature, and they are completely analogous to those described above for β-CD$\cdot$11D$_2$O. The powder spectrum is dominated by an intense central pattern with well-resolved powder pattern showing a single-residual coupling, qcc = 5.5 kHz, and asymmetry, $\bar{\eta}$ = 0.34. The intensity of the sharp feature relative to the total spectrum intensity, $\sim$60%, indicates that most of the water deuterons (62% of the ex-
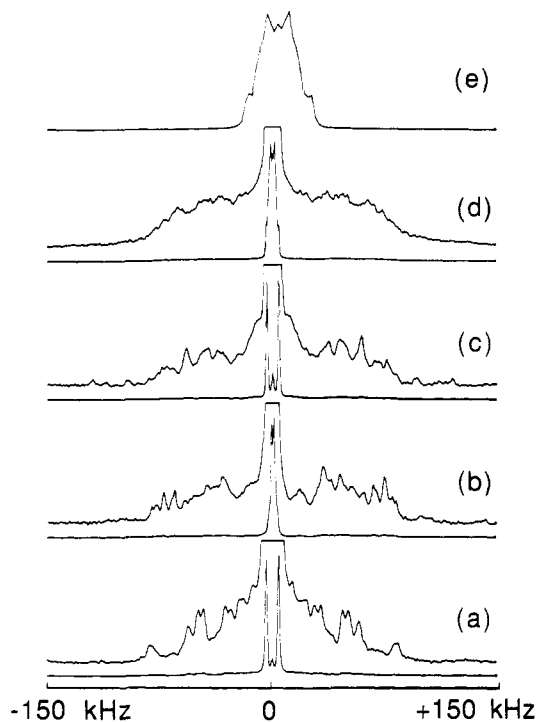

Figure 4. Room temperature single-crystal spectra for several settings (a, b, and c) and powder spectrum (d and e) of γ-CD$\cdot$17D$_2$O. Spectrum e is an expansion, from -30 kHz to $+30$ kHz, of the intense central pattern in spectrum d.

changeable deuterons) contribute to this sharp pattern. The smaller residual coupling compared to β-CD$\cdot$11D$_2$O ($\overline{qcc}$ = 11 kHz) indicates that the water molecules in γ-CD$\cdot$17D$_2$O are indeed more disordered than in β-CD$\cdot$11D$_2$O. Vertical expansions of the single-crystal spectra again show a number of resolved doublets with a range of splittings which in only a few cases approach that of stationary water. The intense central feature corresponding to exchange averaged water deuterons is a single, extremely-well-resolved doublet for the three orientations investigated.

The assignment of the intense central pattern (powder samples) or doublet (single-crystal samples) to waters of hydration is confirmed by studying an isomorphous set of hydrated β-CD inclusion complexes in which the cavity waters are systematically replaced by the increasingly larger guest molecules of ethanol and benzyl alcohol. When crystallized from aqueous ethanol, β-CD forms the isomorphous complex β-CD$\cdot$8H$_2$O$\cdot$EtOH in which three of the eight water molecules and the guest ethanol molecule are located inside the β-CD cavity,[9] or when crystallized from aqueous benzyl alcohol, all six cavity waters are displaced by the benzyl alcohol molecule.[9] In parts b and c of Figure 1 are shown room temperature powder spectra of β-CD$\cdot$8D$_2$O$\cdot$EtOD and β-CD$\cdot$5D$_2$O$\cdot$BzOD, respectively, along with β-CD$\cdot$11D$_2$O, Figure 1a. To avoid removal of lattice water, these samples were dried only briefly (less than 5 min) thereby leaving some surface-bound solvent. The surface water signals are readily distinguished from the intense narrow pattern by their even narrower line width, $\sim$1 kHz, as seen in the horizontal expansion of the inset to Figure 1a. Furthermore, the isotropic solvent peaks have a negligible integrated intensity (less than 5% of the total spectrum). The spectra of β-CD$\cdot$8D$_2$O$\cdot$EtOD and β-CD$\cdot$5D$_2$O$\cdot$BzOD retain the pattern representing sugar hydroxyls seen in the spectrum of β-CD$\cdot$11D$_2$O, but the intensity of the central pattern is systematically reduced in relative intensity. The sharp central pattern is slightly broadened to $\pm$15 kHz in the ethanol complex compared to $\pm$10 kHz for β-CD$\cdot$11D$_2$O. The dominant effect in the series is the decrease in the ratio of the central pattern integral to that of the entire spectrum. The ratio is 62% in β-CD$\cdot$11D$_2$O, decreases to 48% in β-CD$\cdot$8D$_2$O$\cdot$EtOD, and is nearly absent ($\leq$9%) in β-CD$\cdot$5D$_2$O$\cdot$BzOD. This clearly demonstrates the progressive displacement of cavity waters by the guest molecule, ethanol or

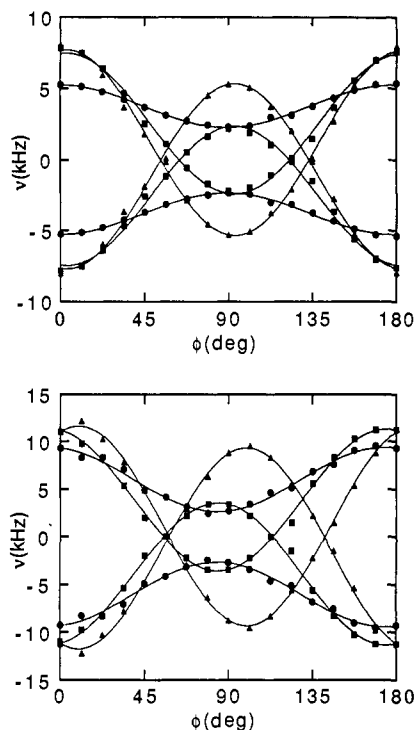**Figure 5.** Graphs of doublet frequencies as a function of goniometer angle for rotation about the a^* (●), b (▲). and c (■) axes for β-CD·11D$_2$O (top) and β-CD·8D$_2$O·EtOD (bottom). For the $\phi = 0$ settings. the axes along the magnetic field are c, a^*. and a^* for the a^*, b. and c rotations, respectively.

benzyl alcohol. As stated above, the intensity of the sharp central peak in the β-CD·11D$_2$O spectrum is sufficiently large to account for all of the water deuterons. Furthermore, the sharp peak intensity change in the isomorphous series is larger than expected on the basis of the number of waters displaced. We conclude that the intense sharp features observed in the powder and single-crystal spectra of β-CD·11D$_2$O correspond to an exchange averaged signal from cavity waters as well as a contribution from interstitial waters, and the replacement of cavity waters has an ordering effect on the remaining hydrate.

Complete single-crystal studies of the intense central doublets for β-CD·11D$_2$O and β-CD·8D$_2$O·EtOD, obtained by rotating the crystals about three mutually orthogonal axes (a^*, b, c), are shown in Figure 5. These results confirm that the signals are single exchange-averaged doublets for the general orientation. The residual coupling, $\overline{qcc}$, and asymmetry, $\bar{\eta}$, are 10.2 (5) kHz and 0.34 (3) for β-CD·11D$_2$O or 17.2 (5) kHz and 0.60 (3) for β-CD·8D$_2$O·EtOD. The increased $\overline{qcc}$ shows that the replacement of 3 waters by a single bulkier ethanol molecule induces a modest increase in the water ordering whereas from the increase in $\bar{\eta}$ one concludes that dynamical averaging is less axially symmetric in β-CD·8D$_2$O·EtOD than in β-CD·11D$_2$O. In both cases the residual coupling and asymmetry correspond exactly to the line shapes of the powder spectra central features.

Interpretation of the water residual tensor orientation requires consideration of the β-CD lattice symmetry. Two symmetry-related molecules are shown in Figure 6a and, to illustrate the herringbone packing, two pairs of symmetry-related molecules are shown schematically as heptagonal rings in Figure 6b. The view is down the c axis onto the a^*b plane with the b axis in the vertical direction of the figure. Each atomic site in the lattice is related to another magnetically nonequivalent symmetry site by a 2-fold screw operation along the crystallographic b axis. Note also that the obliquely oriented β-CD cavities are incompletely closed on either side by the edges of neighboring β-CD molecules. This suggests that if water molecules diffuse, the process is probably not limited to waters within the cavity but includes interstitial waters as was concluded above from the strong intensity of the water doublet. The observation of a single exchange-
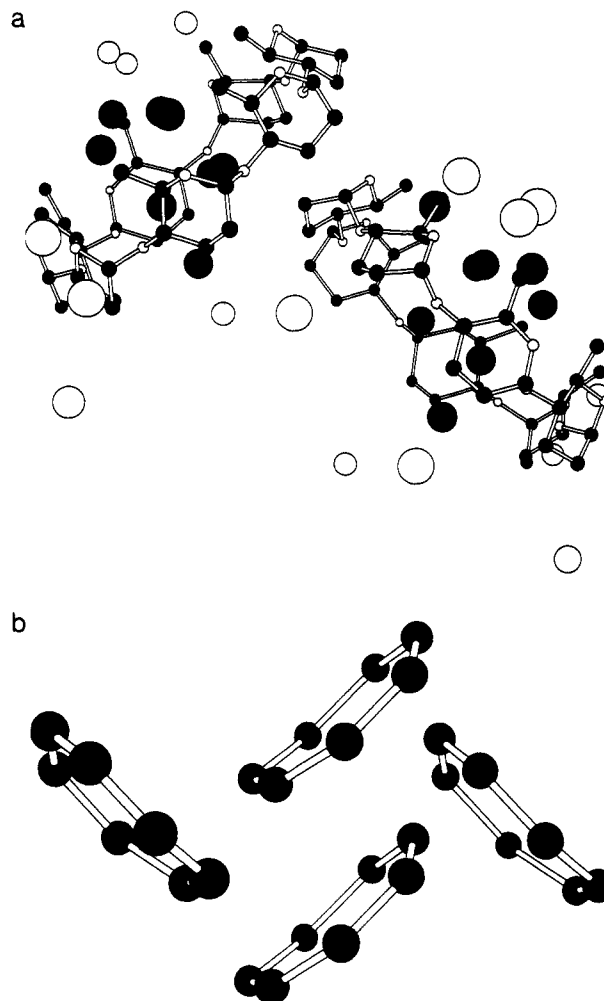

Figure 6. (a) Two symmetry-related β-CD·11D$_2$O molecules according to the structure of Betzel et al. (1984) shown with H atoms eliminated. The large open circles are designated as interstitial waters and the large closed circles are cavity waters. (b) Two pairs of symmetry-related β-CD rings schematically represented as heptagonal rings to show the herringbone packing. The view is the a^*b plane and the vertical axis is b.

averaged water doublet rather than two for the magnetically nonequivalent β-CD molecules is readily explained by the assumption that water rapidly diffuses throughout the lattice thereby exchanging among symmetry-related positions. Water limited to diffusion among sites for either of the symmetry-related molecules will, for the general orientation, produce two doublets corresponding to quadrupole coupling tensors in the (a^*, b, c) frame which have a^*b and bc elements of opposite signs but are otherwise equivalent. This symmetry constraint on the quadrupole coupling tensors is seen immediately from Figure 6a showing that, translations aside, the two molecules are related by a 180° rotation about the b axis. If, as we have suggested, the waters rapidly diffuse among sites related to both molecules, then an averaged residual tensor results in which the a^*b and bc off-diagonal elements will, by symmetry, be averaged to zero. The orientation of the quadrupole coupling tensor principal axes in the (a^*, b, c) frame is readily determined by inspection of the rotation plots in Figure 5. The curves for both the c and a^* rotations of β-CD·11D$_2$O and β-CD·8D$_2$O·EtOD are symmetric about $\phi = 90°$, showing that indeed the a^*b and bc elements, respectively, are zero. For β-CD·11H$_2$O, the a^*c element is also zero but not for β-CD·8D$_2$O·EtOD. These results show that the expected constraints imposed by the lattice symmetry on the orientation of the residual quadrupole coupling tensor for water deuterons moving freely throughout that lattice are observed experimentally.

Inelastic neutron scattering experiments[14] failed to detect translational diffusion in β-CD·11H$_2$O. The negative result was potentially due to insufficient energy resolution.[14] However,
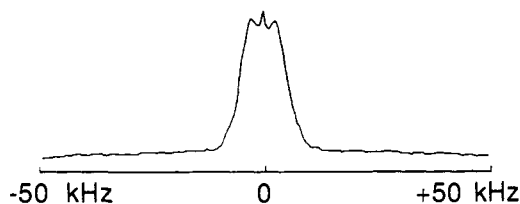
Figure 7. Room temperature ^2H NMR powder spectrum of β-CD·8H$_2$O·CD$_3$CH$_2$OH.

molecular dynamics simulations[15,16] show both the cavity and interstitial waters undergoing translational diffusion from their crystallographic positions in the course of the 20-ps trajectory. The average root-mean-square positional fluctuation for all the water atoms was twice the crystallographic value of 0.5 Å due in part to the observation that 20% of the waters exchanged positions in the course of the simulation. This lack of complete averaging is not inconsistent with our results since the exchange averaging observed by the deuterium NMR experiment must be complete in a time less than about 1 μs, i.e., a time nearly 5 orders of magnitude longer than that examined by the molecular dynamics simulation.

With the assumption of complete positional averaging we can compare the residual coupling and asymmetry measured in the NMR experiment with the room temperature structure determined by neutron diffraction. The β-CD·11H$_2$O structure has the water molecules distributed over 16 sites of which several are rotationally disordered and thus assigned three or more partially occupied hydrogens.[1] Using the atomic coordinates to calculate the corresponding 74 O–D bond orientations in the (a^*, b, c) frame for a symmetry-related pair and assuming that any water deuteron can occupy each of these sites in proportion to the occupation probabilities of the neutron structure, one calculates[17] $\overline{qcc}$ = 15.5 kHz and $\bar{\eta}$ = 0.89 in comparison to the experimental values of 10.2 (5) kHz and 0.34 (3). This comparison is not a strict test on the accuracy of the water positions determined in the neutron structure but reflects the important qualitative point that a water molecule by translating among the available sites with a nearly random set of orientations averages the quadrupole coupling to a small value. Thus, in accord with what is observed, we qualitatively expect that by decreasing the number of sites, as in β-CD·8D$_2$O·EtOD or β-CD·5D$_2$O·BzOD, the residual coupling is increased, or by increasing the number of available sites, as in γ-CD·17D$_2$O, the residual coupling is decreased.

To study the dynamics of a larger guest molecule sharing the cavity with dynamically disordered waters, single-crystal studies of β-CD·8H$_2$O·CD$_3$CH$_2$OH are now described. Given the high degree of dynamical disorder of the cavity waters in β-CD·8D$_2$O·EtOD, it is reasonable to expect that the included ethanol molecule is similarly disordered. This is readily seen from the room temperature powder spectrum of β-CD·8H$_2$O·CD$_3$CH$_2$OH shown in Figure 7. The observed pattern is narrow with $qcc \approx$ 11 kHz. This value is substantially smaller than the 54 kHz expected for a methyl group rotating about its 3-fold axis. Thus the methyl group experiences large-amplitude reorientation of its 3-fold axis. Single-crystal measurements are somewhat hampered due to low resolution resulting from the very small residual coupling and line widths larger than for the hydrate, but the spectra show a single quadrupole doublet for the general orientation with coupling and asymmetry of 11.0 (1) kHz and 0.64 (3), respectively. These results are in agreement with the room temperature powder spectrum and confirm the presence of large-amplitude reorientation lacking axial symmetry. Within experimental error, the residual tensor principal components for
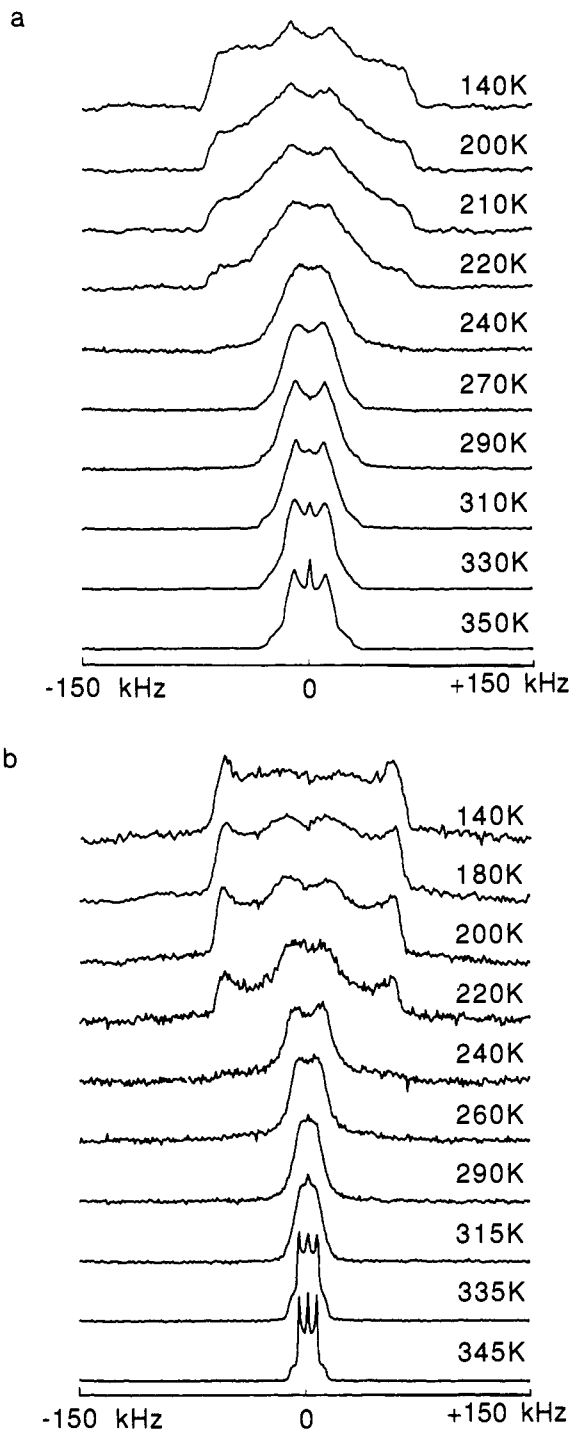
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Figure 8. Variable-temperature ^2H NMR powder spectra of β-CD·5H$_2$O·C$_6$D$_5$CH$_2$OH (a) and β-CD·5H$_2$O·C$_6$H$_5$CD$_2$OH (b).

the ethanol methyl group, like those for exchange-averaged water doublet, are along the axes (a^*, b, c). These results are in qualitative agreement with the room temperature X-ray structure which shows the ethanol molecule disordered between two nearly equiprobable positions. With the assumption of fast methyl group rotation and the two orientations of the ethanol C$_1$–C$_2$ bond from the X-ray structure, we calculate $\overline{qcc}$ = 47 kHz and $\bar{\eta}$ = 0.14 which are in poor quantitative agreement with the experimental values indicating a substantial underestimation of the disorder. The calculated coupling is close to the value for a stationary C$_1$–C$_2$ bond (methyl group rotation only) because the two orientations given for the C$_1$–C$_2$ bonds are approximately antiparallel.

We now consider a large molecule, benzyl alcohol, included in the β-CD cavity thereby displacing all of the cavity waters. In contrast to most cyclodextrin structures dating from the first

published,[18] the X-ray structure of β-CD·5H$_2$O·C$_6$D$_5$CH$_2$OH[9] gives a single set of coordinates for the included molecule. The isotropic B factors for the benzyl alcohol atoms (4–6 Å^2) are, however, about twice as large as those for the sugar atoms and indicate root-mean-square fluctuations of ring carbon atomic coordinates of 0.2–0.3 Å. Spectra of ring-deuterated material are shown in Figure 8a and spectra of methylene-deuterated material are in Figure 8b. For reference, we note that rigid aromatic or methylene deuterons have anisotropies, η, close to zero and quadrupole couplings quite near 180 and 165 kHz, respectively. In the range of 140–200 K, the residual coupling for the benzyl alcohol aromatic deuterons is approximately half the static value, $\overline{qcc}$ = 85 kHz, and the asymmetry is large, $\bar{\eta}$ = 0.9, whereas the methylene deuterons approach the characteristic "rigid" pattern with qcc = 162 kHz as the temperature is lowered to 140 K. The aromatic deuteron pattern is characteristic of 180° ring flips about an approximately stationary axis in the ring plane at an angle of 60° relative to the C–D bonds. These observations are readily explained if the ring is assumed to flip about a nearly stationary para axis, a process which neither interrupts guest–host hydrogen bonding nor is observable in the diffraction experiment. The single para deuteron, in contrast to the four ortho and meta deuterons, is not reoriented and apparently not seen here due to spectral overlap with the observed pattern, an expected longer relaxation time, and the limited signal-to-noise ratio. At the lowest temperature examined, the ring flip rate slows and intermediate exchange effects begin to be seen (Figure 8a), indicating that the ring flip rate is in the range of 10^3–10^6 s^{-1}. Between 200 and 270 K, spectra from both labels narrow and show the onset of additional dynamical freedom with the most dramatic change occurring for both label positions between 220 and 240 K. The patterns at room temperature are not well-resolved, indicating that the additional dynamical modes which begin to be observed at 220 K are still in intermediate exchange. The exchange broadening observed in the patterns of both labels is readily eliminated by warming the samples to 335 K which has the effect of increasing the dynamical rates into the fast exchange limit. Aside from the less sharply defined features, the overall shapes and breadths of the patterns at room temperature (290 K) are similar to those for temperatures above 330 K, showing that the distribution of orientations explored is similar but of slower rate. At 335 K and above, the patterns are well-resolved with residual couplings of 38 kHz for the aromatic and 17 kHz for the methylene deuterons. The residual anisotropy is negligible for the methylene deuterons and $\bar{\eta}$ = 0.24 for the ring deuterons. For room temperature and above, the ^{2}H NMR spectra can be understood by the following model. The additional narrowing of the aromatic deuteron pattern beyond that resulting from ring flipping is accounted for by introducing an additional dynamical mode such as 6-fold in-plane reorientation of the benzyl ring which makes all 5 ring deuterons equivalent in the NMR experiment and reduces the quadrupole coupling to half the static value, i.e., $\overline{qcc}$ = 90 kHz and $\bar{\eta}$ = 0. Note that the 2-fold flip about the para axis probably still occurs but leads to no additional spectral narrowing since that process samples a subset of the orientations sampled by in-plane ring rotation. In-plane ring rotation also reorients the methylene deuterons and decreases their quadrupole couplings by an amount determined by the two deuterons' torsional orientation relative to the ring plane. Since the two deuterons show the same small coupling and vanishing asymmetry, we assume that rather than a fixed orientation of the methylene about the para axis there is free rotation about this axis. These two processes together reduce the quadrupole coupling to $^1/_6$ of its static value, i.e., $\overline{qcc}$ = 28 kHz and $\bar{\eta}$ = 0. Given these two dynamical modes which yield axial residual tensors along an axis perpendicular to the ring plane for both labels, the coupling for the aromatic deuterons is about half the calculated value whereas the observed methylene coupling is 60% of the calculated value. Clearly there is dynamic reori-

entation of the ring normal. The effect of this reorientation will be less for the methylene deuterons since rotation of the ring normal about the para axis will reorient the ring deuterons but not the methylene deuterons since they are already assumed to undergo free rotation about this axis. As an approximation, the required mean square amplitude, $\langle \theta^2 \rangle$, of the ring wobble motion at room temperature and the attendant atomic displacements can be estimated from the residual quadrupole coupling, $\overline{qcc} \sim 44$ kHz, using[3]

$$qcc = qcc \, (1 - \tfrac{3}{2}\langle \theta^2 \rangle) \qquad (1)$$

in which the static quadrupole coupling, qcc, is an effective value including the in-plane reorientation (qcc = 90 kHz). A substantial wobble amplitude of $\theta_{rms} \sim 30°$ is estimated and, if the ring wobbles about its centroid, results in linear root-mean-square displacements of the ring carbon atoms $\sim$0.7 Å perpendicular to and $\sim$0.2 Å parallel to the ring plane. These values are close to but somewhat greater than the values estimated from the crystallographic B factors. However, in disagreement with the X-ray structure, this model reorients the benzyl hydroxyl group relative to the host, i.e., assumes there is no longer hydrogen bonding with fixed donors and acceptors between the guest and host. We note that including dynamical modes observable in the NMR experiment but not observable in the X-ray experiment does not resolve the disagreement between the two methods. We conclude that the rather narrow ^{2}H NMR patterns observed for both the ring and methylene deuterons at temperatures above 240 K are inconsistent with either a well-defined orientation of the benzyl alcohol molecule within the β-CD cavity or fixed hydrogen bonding of the hydroxyl group.

To conclude the discussion we summarize and comment on the primary findings. Whereas diffraction methods are particularly suitable for determining the time-averaged structure of well-ordered systems, solid-state NMR methods more directly address dynamically disordered solids in terms of the distribution of orientations and the time scales of the dynamics. The main result is that the positional disorder of the hydrate in β- and γ-cyclodextrins is dynamic on the deuterium NMR time scale. That is, waters of hydration readily diffuse among most if not all of the hydrate sites in the lattice in a time less than 10^{-6} s. Like a liquid, hydrogen bonding of the lattice waters no doubt occurs but is transient. This dynamic feature of the hydrate would appear to be of kinetic advantage with regard to the ability of β- and γ-cyclodextrins to bind molecules in the cavity as well as catalyze hydrolytic reactions since both processes require solvent to be shuttled into or out of the cavity. Furthermore, large-amplitude jump reorientation of the sugar hydroxyl groups about the C–O bonds, previously assumed to arise from dynamic "flip-flop" hydrogen bonds in which a hydroxyl group reorients between different hydrogen bond acceptors, is confirmed here.

It is interesting to note that a definable neutron structure of the hydrate is consistent with such a high degree of dynamic translational and orientational disorder or conversely that the hydrate structure determined by diffraction method does not necessarily imply a fixed network of hydrogen bonds and the absence of translational or orientational dynamics. The conditions under which a dynamic process is observable in the deuterium NMR experiment and invisible in the diffraction experiment are that a structural transition occurs which leaves the overall system unchanged and that the dynamics are jump like, i.e., the time in transit is short compared to the time in residence. Since the root-mean-square positional flutuations are 0.5 Å or larger for water molecules in β-cyclodextrin, the first condition is somewhat relaxed here. Examples of simple, local orientational transitions like 180° phenyl ring or water flips about a 2-fold axis are well documented; however, translationally permuting waters among the lattice sites is new. In this regard, it would be interesting to compare the residence and transit times of lattice waters moving among the crystallographically determined positions from a long molecular dynamics trajectory ($\gg$20 ps). We note that, calculated from the 20 ps dynamics simulation, occupation probabilities for individual water molecules to be within two standard deviations

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of the crystallographic positions were generally smaller than and in poor agreement with the crystallographic values.[15]

We find that the behavior of the hydrate in β- and γ-cyclodextrin is completely analogous to that observed in more complicated crystalline proteins such as crambin, lysozyme, and ribonuclease[3,6] in terms of the low degree of hydrate order, the temperature dependence of the ordering, and translational diffusion in the lattice, from the perspective of the deuterium NMR experiment. The observations here potentially resolve the apparent disagreement between the deuterium NMR studies and diffraction studies of crambin. The NMR studies indicate a highly mobile and disordered hydrate whereas the X-ray structure was refined to include well-defined hydrate networks.[19]

Finally we note that, in correspondence with the low degree of ordering observed for the hydrate, included ethanol or benzyl alcohol are also orientationally disordered within the β-CD cavity to a substantial degree. The residual deuterium quadrupole coupling, $\overline{q_{cc}}$, and asymmetry, $\overline{\eta}$, calculated from the X-ray structures are larger than the values measured by NMR spectroscopy, showing that the X-ray structures overestimated the

orientational ordering of the molecule included in the cavity. Furthermore, inclusion of NMR visible and X-ray diffraction invisible processes such as methyl group rotations, 180° ring flips, or planar ring rotations does not resolve the discrepancies. Thus, a single hydrogen bonding group and packing constraints do not severely limit the allowed orientations of these molecules stoichiometrically included in the cavity. This is an interesting result in light of the known catalytic properties of cyclodextrins which in part arise from their ability to orient substrates relative to catalytic groups in analogy to enzymes.

Note Added in Proof. A recent quasielastic incoherent neutron scattering study of β-CD·11D₂O by Steiner et al. (*Mol. Phys.* **1991**, *72*, 1211–1232) with higher energy resolution than previously reported[14] shows diffusive motions of the water molecules at room temperature in β-CD·11D₂O in agreement with the results presented here.

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NMR Studies of Iron(II) Nitrosyl π-Cation Radicals of Octaethylchlorin and Octaethylisobacteriochlorin as Models for Reaction Intermediate of Nitrite Reductase

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Abstract: Chemical oxidation of iron(II) nitrosyl complexes of octaethylchlorin (OEC) and octaethylisobacteriochlorin (OEiBC) yielded iron(II) nitrosyl π-cation radicals. The π-cation radicals afforded well-resolved hyperfine-shifted NMR resonances characteristic of an a_2 π-radical state. Non-Curie law behavior of the deuterium NMR resonances was interpreted in terms of a valence isomerization from the chlorin π-cation radical to the $Fe^{II}NO^+$ chlorin complex due to ligation of SbF_6^- to the iron of the π-cation radical as well as a magnetic interaction between the NO and π-radical spins in $(OEiBC)Fe^{II}NO$ π-cation radical. This valence isomerization was confirmed by variable-temperature electronic absorption spectral measurements. Furthermore, ligation of imidazole to the π-cation radicals caused valence isomerization to yield $(OEC)Fe^{II}(NO^+)(Im)$ and $(OEiBC)Fe^{II}(NO^+)(Im)$ complexes.

Introduction

Heme-like prosthetic groups derived from dihydroporphyrins exist in a variety of redox enzymes. The presence of a *d*-type cytochrome in several microorganisms was discovered many years ago, and the green prosthetic group has been found to be an iron chlorin (dihydroporphyrin).[1] Recently, nitrite reductase has also been shown to contain iron hydroporphyrins (iron chlorin and iron isobacteriochlorin) as the prosthetic group.[2-5] The enzyme catalyzes the multielectron reduction of nitrite to ammonia or to nitric oxide. Dissimilatory nitrite reductase having heme d_1 as a prosthetic group catalyzes reduction of nitrite to nitric oxide.[3] Assimilatory nitrite reductase, possessing an iron isobacteriochlorin referred to as siroheme, catalyzes six-electron reduction of nitrite to ammonia.[2] A nitrite reductase which contains iron chlorin has also been isolated from *Aspergillus niger*.[5] Although the whole reduction mechanism (from 1 to 3) for these nitrite reductases

has not yet been established, an iron(II) nitrosyl complex (**2**) has been detected[6-8] as a reaction intermediate in the reduction cycles of nitrite to nitric oxide or to ammonia, as described below.

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