Dynamics of H/D and D/H exchanges in β-cyclodextrin dodecahydrate observed in real time: effects from zero-point vibrational energy

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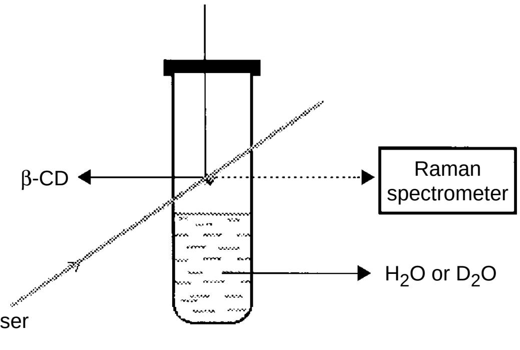
Cyclic H/D and D/H exchange processes in crystalline β-cyclodextrin dodecahydrate exposed to D_2O (or H_2O) vapour, monitored in real time by the integrated intensity of the O–H Raman stretching band, exhibit different rate constants and activation energies ascribed to different zero-point vibrational energies of OH and OD.

Crystalline hydrates of β-cyclodextrin (cyclomaltoheptaose, β-CD) are good models for the study of hydration processes in biomolecular structures.[1–8] The water molecules contained in the crystal[1,2] and most of the 21 β-CD hydroxy groups display appreciable positional and/or orientational disorder, with jump rates up to 10^{10}–10^{11} s^{-1}.[4,5] At atmospheres with humidities $< 100\%$, the water content is reduced, and equilibration to changes of humidity takes place within minutes. This shows that, despite the absence of permanent channels, rapid water diffusion takes place in the crystal lattice.[2] If crystalline β-CD hydrate is exposed to an $H_2{}^{18}O$ atmosphere, all water molecules are replaced by the labelled species, showing that there are no 'fixed' water sites.[6] If a sample is exposed to a D_2O atmosphere, complete H(O)/D(O) exchange takes place. Both exchange mechanisms occur on the same timescale, showing that long-range transport must be by diffusing intact water molecules, but since the H/D exchange also extends to β-CD hydroxy groups which are sterically inaccessible to diffusing water molecules, there must be local chains of proton transfer processes.[6] The previous studies were based on measurements on samples in equilibrium at room temperature. In the present work, we present, for the first time, real-time observations on the H/D exchange, and also report on temperature-dependent experiments.

In an H(O)/D(O) exchange experiment, the quantity of H(O) in the sample can be measured by the relative integrated intensity of the O–H Raman stretching band (I_{OH}).[7] Recently, we reported on a Raman spectroscopic real-time method to monitor I_{OH},[7] which is here applied to the observation of the H/D exchange kinetics in β-CD dodecahydrate. The experimental setup is shown in Scheme 1.



Scheme 1

Crystalline β-CD dodecahydrate† was placed in a closed cell over a reservoir of D_2O, and the Raman spectra of the region of O–H and O–D stretching vibrations were recorded at short time intervals (by use of a CCD detector, the 2100–3800 cm^{-1} region could be recorded within *ca.* 1.5 min).‡ As D_2O diffuses into, and H_2O out of, the sample, and H/D exchange processes at the β-CD hydroxy groups take place, I_{OH} continuously decreases, and, in parallel, I_{OD} increases.§ A typical series of spectra is shown in Fig. 1; the invariant central peak represents a complex band of C–H stretching vibrations and is conveniently used as an internal reference intensity. During H(O)/D(O) exchange, the relevant kinetic variable is the fraction of remaining H(O) atoms, θ, which is experimentally measured by I_{OH}/I_{OH}(max.). As shown by the inset of Fig. 1, the relative H(O) concentration decreases according to approximate first-order kinetics (here with 50% exchange within 43 min). After equilibration, the D_2O reservoir of the experimental cell was quickly replaced by an H_2O reservoir,¶ so that the reverse exchange process [D(O)/H(O)] could be monitored on the same sample. After equilibration, another H(O)/D(O) exchange experiment could follow, and so on. The results obtained from a two-cycle sequence of experiments H/D → D/H → H/D → D/H are listed in Table 1.

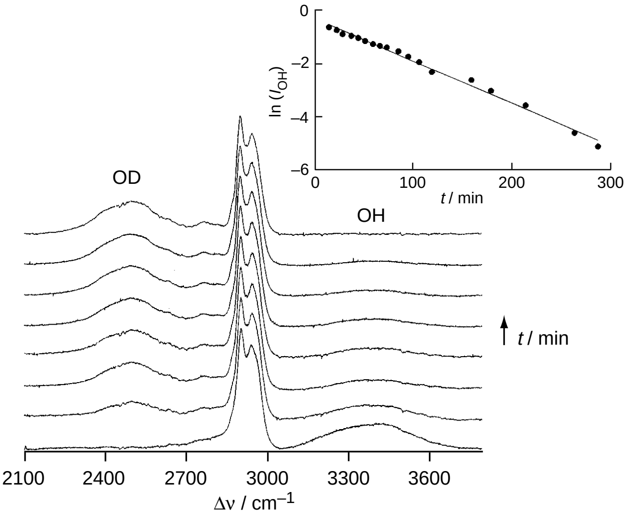


Fig. 1 Examples of Raman spectra in the relevant spectral region 2100–3800 cm^{-1}, for on-line observation of H/D exchange

Table 1 Results for a two-cycle exchange/back-exchange experiment: sample quantity *ca.* 20 mg, *n* is the number of spectra used, *k* the effective rate constant, and $t_{\frac{1}{2}}$ the time for 50% exchange[9]¶

Experiment	*n*	k/min^{-1}	$t_{\frac{1}{2}}$/min	$t_{\frac{1}{2}}$ (95% confidence)
First cycle, H → D	18	−0.0160	43	40–46
First cycle, D → H	15	−0.0126	55	52–59
Second cycle, H → D	18	−0.0175	40	37–43
Second cycle, D → H	13	−0.0116	60	54–67

In all experiments, the exchange processes follow first-order kinetics. Although the determination of the $t_{\frac{1}{2}}$ values suffers some uncertainty, the data indicate different rate constants for the H/D and D/H exchange processes (the H/D and D/H values are each clearly outside the other 95% confidence interval), with the D/H process being the slower. Comparing the first and second cycles, the two H/D values are within each other's confidence intervals, as are the two D/H values, showing that the process as a whole can be reproduced cyclically.

As mentioned previously,[6] the macroscopic rate constant k is sample dependent: owing to shorter diffusion paths, the exchange must be faster in samples with larger surface-to-mass ratios. Therefore, when repeating the above experiment with a different sample, the same general behaviour with somewhat different absolute $t_{\frac{1}{2}}$ values must be expected. This was in fact observed for another set of experiments, where again H/D exchange was observed to be faster than the D/H back-exchange.

These observations are in consonance with recent quantum mechanical calculations which indicate that OD hydrogen bonds are stronger owing to a lowering of the zero-point vibrational energy (ZPVE).[10] Therefore, OD hydrogen bonds should be more difficult to break, and D(O)/H(O) substitution should be slower than the reverse process. A consequence is that intact water molecules, which are the carriers of H or D through the lattice, diffuse more slowly when of the D_2O type than of the H_2O type (DHO should be in between these two extremes).

In order to assess the effect of temperature on the exchange rate constants, a different set of experiments was performed. At a number of defined temperatures, crystalline β-CD(H_2O) dodecahydrate was exposed to the atmosphere over a reservoir of D_2O, for 30 min. Then, small samples were removed, sealed in capillary tubes and Raman spectra were recorded (batch method).‖ The fraction of remaining H(O) was determined from I_{OH}, allowing the calculation of k and $t_{\frac{1}{2}}$ [from $I_{OH} = I_{OH}(\text{max.}) \exp(-kt)$ with $t = 30$ min]. Analogous experiments were performed for β-CD(D_2O) exposed to the atmosphere over H_2O. Numerical results are given in Table 2: as expected, the exchanges are appreciably faster at elevated temperatures. In a plot of $\ln|\ln\theta|$ *vs.* $1/T$, linear correlations are obtained for both processes (Fig. 2), suggesting Arrhenius type dependences of the rate constants. Linear regression calculation obtains activation energies, E_a, of 26 kJ mol^{-1} for H/D exchange, and 33 kJ mol^{-1} for D/H back-exchange. Again, this is in consonance with the theoretical calculations mentioned above,[10] which indicate that substitution of H(O) by D(O) lowers both the intramolecular and the intermolecular ZPVE.

Table 2 Temperature-dependent H/D and D/H exchange experiments

Experiment	T/K	$t_{\frac{1}{2}}$/min
H → D	279	26
	291	16
	308	10
	343	3
D → H	280	47
	299	23
	315	13
	341	3

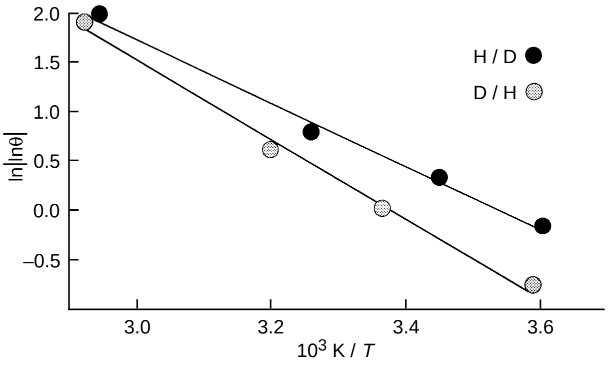


Fig. 2 $\ln|\ln\theta|$ *vs.* $1/T$ for H/D [$\theta = I_{OH}/I_{OH}(\text{max.})$] and D/H [$\theta = I_{OD}/I_{OD}(\text{max.})$] exchange experiments

The experimental setup used (Scheme 1) allows the real-time observation of cyclic H/D and D/H exchange processes. Since the data are obtained from the same sample under the same conditions, the macroscopic rate constants for H/D and D/H exchange can be compared directly. The results indicate different rate constants for H/D and D/H exchanges in crystalline β-CD dodecahydrate. Presumably, the origin of this phenomenon is found in the different zero-point vibrational energies of OH and OD. It is of interest that this microscopic effect leads to noticeably different behaviour in the macroscopic diffusion properties.

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Footnotes

† β-CD, kindly donated by Wacker Chemie, München (Germany), was recrystallized by cooling concentrated aqueous solutions in a Dewar flask from *ca.* 80 °C to room temperature. Crystals were taken from the solvent and exposed to an atmosphere of moderate temperature and humidity (*ca.* 25 °C and 60% relative humidity). After equilibration with the atmosphere, crystals were ground, not sieved, to obtain a microcrystalline powder. Care was taken that all samples were prepared similarly.

‡ Jobin-Yvon T64000 Raman spectrometer, subtractive configuration, relevant slit widths set to 300 μm, intermediate slit between pre-monochromator and spectrograph wide open (14 mm), CCD detector, integration time 15 s. An Ar ion laser (Coherent-Innova 300-05 model with power track) provided *ca.* 100 mW at the sample position. Integrated intensities were determined using the machine software.

§ The sum $I_{OH}/I_{OH}(\text{max.}) + I_{OD}/I_{OD}(\text{max.})$ is *ca.* 1.0 at all times (deviations from unity were $< 5\%$), *i.e.* the total number of OH/D oscillators was conserved during the exchange experiments. Separate normalization of each term is required because the OH and OD stretching vibrations have different Raman activities.

¶ Owing to the necessary manipulations at the start of the experiment and at the exchange of reservoirs, the zero point of the time scale is ill defined. Therefore, only data for $t \geqslant 15$ min are used for data analysis; this affects only the intercept, but not the kinetically relevant slope in Fig. 1.

‖ In this series of experiments, each measurement had necessarily to be performed on a different sample. Although all care was used to ensure that close to identical sample conditions were provided each time, the numerical reliability is reduced compared to the data in Table 1, which was obtained from only one sample.

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