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Cavity Hydration and Competitive Binding in Methylated *β*-Cyclodextrin

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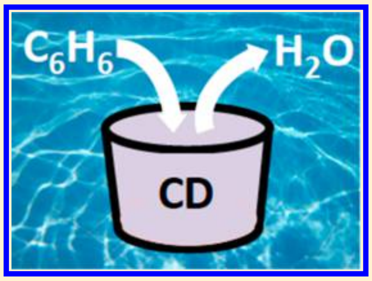
Supporting Information

ABSTRACT: Raman multivariate curve resolution (Raman-MCR) spectroscopy is used to measure the vibrational spectrum of water molecules in the cavity of an aqueous methylated *β*-cyclodextrin (Me-*β*-CD), as well as to quantify the competitive expulsion of those waters by benzene. The Me-*β*-CD cavity is found to contain 5−6 water molecules whose structure is remarkably similar to that of bulk water, although slightly more tetrahedral and with fewer weak hydrogen bonds. The binding constant of benzene to Me-*β*-CD, obtained using Raman-MCR, is found to be similar to that of benzene to *β*-CD (previously determined by other means). The competitive displacement of water by benzene in Me-*β*-CD is quantified by explicitly including the release of cavity-bound water molecules in the measured equilibrium constant.



Displacement of "high-energy" water from a nonpolar host cavity has been proposed as a driving force for supramolecular host−guest binding processes.[1,2] For instance, it has been suggested that liberation of water from cyclodextrin (CD) cavities leads to an enthalpy gain because the expelled water molecules can form better hydrogen bonds in the bulk medium than inside the nonpolar cavity.[3] In addition, calorimetric measurements and molecular-dynamics (MD) simulations of binding in cucurbiturils have found a correlation between the number of water molecules in the unbound host cavity and their binding constants.[4,5] However, no previous studies have reported direct experimental observations of the structure of water in such nonpolar host cavities in aqueous solution. Here, we do so by combining Raman spectroscopy and multivariate curve resolution (Raman-MCR) to measure the hydration-shell vibrational spectra of water-filled and benzene-filled methylated *β*-cyclodextrin (Me-*β*-CD) host molecules. Our results reveal that there are 5−6 water molecules in the unbound Me-*β*-CD host, in reasonable agreement with values inferred from previously reported MD simulations[1,6−8] as well as the crystal structure[9] of non-methylated *β*-CD. Moreover, our results show that the cavity-bound water has a structure that is quite similar (but not identical) to that of bulk water. In addition, we demonstrate that Raman-MCR may be used to determine the binding constant of benzene to Me-*β*-CD and find that it is comparable to that for benzene binding to nonmethylated *β*-CD. We discuss the implications of the results regarding the competitive displacement of cavity-bound waters by benzene.

Previous MD simulations of various aqueous cavities predict that stable hydrogen-bonded water clusters can be formed inside cavities of diameters larger than ∼1 nm, while smaller nonpolar cavities, which could accommodate up to 4 water molecules, may remain dry in an equilibrated aqueous solution.[10] These predictions are consistent with MD simulations of a synthetic host whose hydrophobic cavity was found to fluctuate between completely empty and water-filled states with an average of 4.5 bound water molecules.[11] These predictions are also supported by experimental NMR[12] and X-ray[13] structure measurements of hydrated proteins with dry nonpolar cavities. Another experimental X-ray and neutron diffraction study of a self-assembled framework with very large (∼27 nm^3) nonpolar (aromatic) cavities revealed that the cavities are filled by an ice-like water structure.[14] Other studies have suggested that the "high-energy" waters inside nonpolar cavities resemble a supercritical fluid[1,15] or may undergo vapor−liquid (drying) fluctuations.[16] Different MD simulation studies of aqueous *β*-CD have predicted an average of as few as ∼4 or as many as ∼7 water molecules inside the cavity, while an experimental neutron diffraction study found ∼6 cavity-bound waters in crystalline *β*-CD, whose structure is more ordered than the interstitial waters.[9] Another simulation study has predicted that alkylation of *β*-CD does not significantly change its overall cavity size and water occupancy.[7]

Here we probe the structure of the cavity-bound water molecules in Me-*β*-CD by measuring the change in its hydration-shell spectrum upon displacing the cavity-bound waters by benzene-d_6. Previous Raman-MCR studies of aqueous benzene solutions have found that its hydration-shell spectrum consists of only a relatively small and narrow high-frequency peak at ∼3610 cm^{-1} assigned to a *π*-hydrogen bond between water and benzene[17] (shown in the lower inset panel of Figure 1c). Previous UV spectroscopy, vapor pressure, and calorimetric studies found that benzene binds to all cyclodextrins with binding constants ranging from $\log K = 0.96$ in *γ*-cyclodextrin[18] to $\log K = 1.93−2.23$ in *β*-cyclodextrin[18,19] at ambient conditions, whereas the reported *α*-cyclodextrin/

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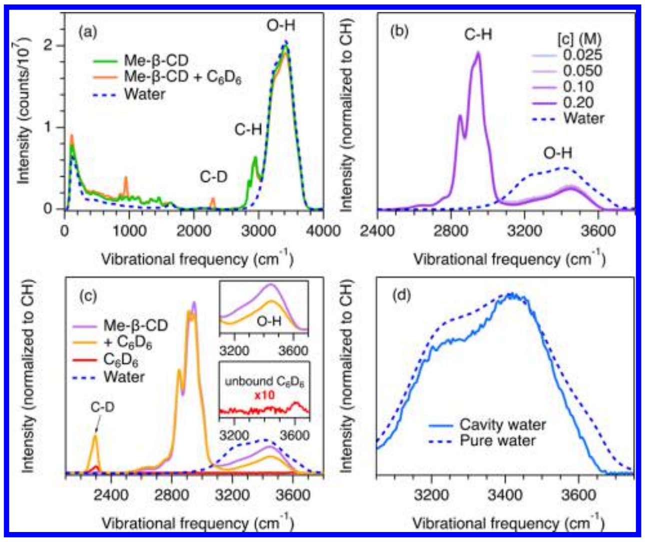

Figure 1. (a) Raman spectra of pure water, 0.1 M Me-*β*-CD, and 0.1 M Me-*β*-CD + benzene-d_6. (b) Hydration shell spectra of Me-*β*-CD at different concentrations (normalized to the CH peak area) and pure water (arbitrarily scaled). (c) Hydration-shell spectra of Me-*β*-CD with and without bound benzene-d_6. The inset panels in (c) show expanded views of the O−H stretch region. (d) Comparison of the O−H stretch bands of cavity-bound and pure liquid water molecules (scaled to the same peak height). The cavity-bound O−H band in (d) is obtained from the difference between the purple and orange O−H bands in (c).

benzene binding constant is between these limits.[18] Thus, the displacement of cavity-bound water molecules by benzene should make it possible to obtain the Raman spectrum of the displaced water molecules. The following is a more detailed description of how we used this strategy to determine the number and structure of cavity-bound waters, and to quantify the competitive displacement of water by benzene in the Me-*β*-CD cavity.

Self-modeling curve resolution (SMCR)[20−23] is used to obtain Raman-MCR solute-correlated (SC) spectra of the unbound and bound aqueous host and guest molecules. The resulting SC spectra contain spectral features arising from intramolecular vibrational modes of the solute as well as from solvent molecules whose vibrations are perturbed by the solute. The pure water component is constrained to the experimentally measured water spectrum while the SC component is equivalent to the minimum-area (non-negative) difference between the solution and pure water spectra (see the previous Raman-MCR studies[20−23] and the Supporting Information for further details).

Figure 1a shows the measured Raman spectra of pure water and an aqueous 0.1 M Me-*β*-CD solution with and without deuterated benzene. Benzene-d_6 was used as the guest in order to distinguish its C−D stretch band from the C−H band of the host (as shown in Figure 1a). The binding of benzene to the Me-*β*-CD leads to a change in both the average frequency and intensity of the benzene-d_6 C−D stretch band near 2300 cm^{-1} as well as changes in the Me-*β*-CD C−H stretch band near 2900 cm^{-1}.

Figure 1b shows Raman-MCR SC spectra obtained from aqueous Me-*β*-CD with concentrations ranging from 0.025 to 0.2 M, as well as the Raman spectrum of pure water. All the SC spectra are normalized to the Me-*β*-CD C−H stretch band area. The fact that the resulting Me-*β*-CD SC spectra are essentially concentration independent implies that there is no significant aggregation of Me-*β*-CD over this concentration range.

Figure 1c compares the SC spectra of the unbound and benzene-complexed Me-*β*-CD and benzene-d_6, and the inset panels show expanded views of the associated SC O−H stretch bands. The Me-*β*-CD SC O−H stretch bands arise from perturbation of the vibrational structure of the entire hydration-shell of Me-*β*-CD (as well as from OH groups of Me-*β*-CD itself). Unbound benzene has essentially no hydration-shell (on this scale), as indicated by the red curve in Figure 1c (and the expanded view in the lower inset panel). This small unbound benzene contribution has been subtracted from the SC spectrum of the aqueous Me-*β*-CD + C_6D_6 solution (orange curve). The SC spectrum of the unbound Me-*β*-CD host (purple curve) in Figure 1c is the same as the 0.1 M SC spectrum in Figure 1b. The difference between the purple and orange curves in Figure 1c represents the spectrum of the water molecules that were present in the Me-*β*-CD cavity before they were displaced by benzene. The area of this difference band implies that five to six water molecules were present in the Me-*β*-CD cavity before binding benzene. This estimate is obtained assuming that the Raman cross section of the bound waters is the same as that in liquid water.

Figure 1d compares the resulting O−H stretch bands of the cavity-bound water (solid blue curve) and pure water (dashed blue curve). The similarity of the two O−H stretch bands in Figure 1d indicates that the structure of the cavity-bound water is similar to that of liquid water. The slightly lower average O−H band frequency (by ∼9 cm^{-1}) of the cavity-bound water suggests that these waters are slightly more tetrahedrally ordered than bulk water, as previous studies have demonstrated a strong correlation between average O−H frequency and tetrahedral order.[23] Moreover, the high frequency edge of the cavity-bound water O−H band begins near 3650 cm^{-1}, while that for pure water begins at a higher frequency of ∼3700 cm^{-1}, thus implying that there are fewer weak H-bonds in the cavity-bound water than in bulk water.

In order to quantify the number of water molecules in the Me-*β*-CD cavity, we need to know the number of host molecules that contain a bound benzene, which requires determining the equilibrium constant for this host/guest binding process. Although the binding constant of benzene to (nonmethylated) *β*-CD has previously been measured, there are no previous reports of the binding constant of benzene to Me-*β*-CD. However, some *β*-CD derivatives (e.g., hydroxypropyl-*β*-CD) have been found to have benzene binding constants that are similar to those for *β*-CD while others (e.g., *β*-CD-thioethers) were found to differ significantly.[24] Here we quantify the binding constant of benzene to Me-*β*-CD using Raman-MCR. To the best of our knowledge, this is the first such Raman-based determination of any host−guest binding constant.

Given the low aqueous solubility of benzene, we found it advantageous to perform the binding constant measurement in a way that differs somewhat from previously employed spectrometric titration procedures.[25] Our strategy relied on first obtaining Raman-MCR spectra from solutions that were equilibrated with a liquid benzene phase that remained continuously in contact with either ∼1 mL of pure water or ∼1 mL of 0.1 M Me-*β*-CD. Thus, both equilibrated solutions necessarily have approximately the same saturated concentration (∼23 mM)[26,27] of unbound benzene. The SC spectrum

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of unbound benzene in water (red curve in Figure 1c) was obtained from an SMCR decomposition of the spectra of a saturated aqueous benzene solution and pure water, as previously reported.[17] The SC spectrum of the cavity-bound benzene host−guest complex was obtained from an SMCR decomposition of the 0.1 M Me-β-CD solution in equilibrium with benzene and a separate saturated aqueous solution of benzene (treated as the solvent). Thus, the benzene-d_6 features in the resulting SC spectrum should pertain only to the host−guest complex (with no contribution from the unbound benzene-d_6). Our results reveal that the benzene-d_6 C−D band frequency decreases by ∼7 cm^{-1} (and increases in intensity by ∼40%) upon binding to the Me-β-CD cavity (as further described below and in the Supporting Information).

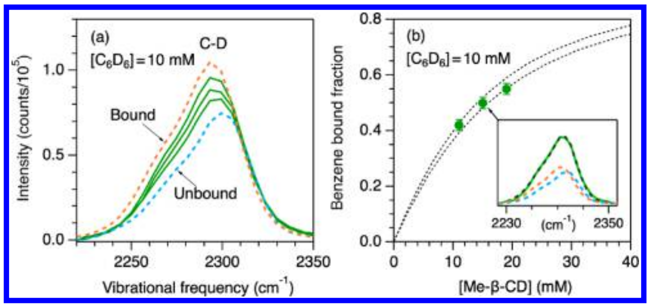

Figure 2. Spectroscopic determination of the host−guest binding equilibrium constant and Raman cross section ratio of bound and unbound benzene-d_6 (guest), in solutions with a total benzene-d_6 concentration of 10 mM. (a) The C−D stretch bands of the unbound (dashed blue) and bound (dashed orange) benzene-d_6 are compared with those obtained from mixtures containing 10 mM benzene-d_6 and either 10, 15, or 20 mM Me-β-CD. (b) The fraction of bound benzene-d_6 (points) is plotted as a function of total Me-β-CD concentration and compared with binding curves pertaining to $K = 92$ and 110. The inset panel in (b) shows the TLS reconstruction (dashed curves) of the measured (solid green curve) guest C−D stretch band pertaining to the middle point.

Figure 2a shows the C−D stretch band spectra of the unbound (dashed blue) and bound (dashed orange) benzene-d_6, along with spectra obtained from solutions consisting of a mixture of unbound and bound benzene-d_6 (solid green) in solutions with a total Me-β-CD concentration of 10, 15, and 20 mM. All the C−D stretch bands in Figure 2a pertain to a total benzene-d_6 concentration of 10 mM. The difference in intensity between the bound and unbound benzene-d_6 bands indicates that the Raman cross section of benzene-d_6 increases by a factor of 1.4 ± 0.1 upon binding to Me-β-CD, as obtained directly from the measured spectra of the above solution, as well as the solutions saturated with benzene-d_6 (as described in the Supporting Information).

Figure 2b shows the fraction of bound benzene-d_6 as a function of total Me-β-CD concentration, obtained by performing a total-least-squares (TLS) fit of each of the solid green bands in Figure 2a to a linear combination of the corresponding unbound and bound component bands (as further described in the Supporting Information). The inset panel in Figure 2b shows the resulting unbound and bound component intensities obtained from such a TLS fit to the spectrum obtained with a total benzene concentration of 10 mM and a total Me-β-CD concentration of 15 mM. The good agreement between the reconstructed (dashed black) band and the measured (solid green) band confirms the accuracy of the total-least-squares fit. The two dotted black curves in Figure 2b are binding curves pertaining to binding constants of $K = 92$ (1/M) and 110 (1/M), respectively, whose average is $K = 101 \pm 10$ (1/M) (or $\log K = 2.00 \pm 0.05$). Note that this binding constant is quite similar to the $1.93 < \log K < 2.23$ binding constants previously obtained (by other means) for benzene binding to nonmethylated β-CD.[18,19]

In summary, Raman-MCR has been used to obtain the spectrum of cavity-bound water molecules in Me-β-CD, as well as the binding constant associated with the displacement of those water molecules by benzene-d_6. The results indicate that the cavity-bound waters have a structure that is quite similar to that of liquid water, although with slightly greater tetrahedrality and fewer weak H-bonds. The benzene binding constant of $\log K \sim 2$ implicitly pertains to a process in which benzene displaces cavity-bound waters, although those waters are not included in defining the equilibrium as $H + G \rightleftharpoons HG$, and thus $K = [HG]/[H][G]$. To quantify the relative binding affinities of benzene and water, it is necessary to express the equilibrium constant in a form that explicitly includes the cavity-bound (and displaced) water molecules, $K' = [HG][H_2O]^n/[H(H_2O)_n][G]$, where $5 \le n \le 6$ is the number of cavity-bound waters, and $K' = K[H_2O]^n$ (obtained as further described in the Supporting Information). The K' equilibrium constant implies that, if the unbound water and benzene were (hypothetically) scaled to the same 1 M concentration, then the fraction of benzene-filled to water-filled Me-β-CD cavities would be on the order of $K' \sim 10^{12}$, which corresponds to a partial molar Gibbs energy difference of $\sim RT\ln[10^{12}] \sim 70$ kJ/mol between the water-filled and benzene-filled Me-β-CD cavity.

With regard to the influence of "high energy" waters in host−guest binding, it is important to note that the cavity-bound waters in an equilibrium aqueous solution necessarily have the same chemical potential (partial molar Gibbs energy) as the surrounding liquid water. Since the same is true for any aqueous host−guest binding process, the differences between any such competitive water-displacement processes are necessarily dictated by differences between the partial molar free energies of the unbound and bound host and guest molecules rather than by that of the cavity-bound water molecules in the unbound host. More specifically, host−guest binding equilibria can only be influenced by binding-induced changes in host−guest and solute−water interactions, and cannot be affected by binding-induced changes in water−water energy and entropy, which are necessarily precisely compensating.[2,28]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.9b00939.

> Experimental details, TLS spectral decomposition, determinations of Raman cross section, binding constant, and number of water molecules in the Me-β-CD cavity (PDF)

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Author Contributions

Both authors contributed to the design, performance, and description of these experiments.

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Notes

The authors declare no competing financial interest.

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