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ACS OMEGA

Article

Cite This: ACS Omega 2018, 3, 1014−1021

Effect of Dimethyl Sulfoxide on the Binding of 1-Adamantane Carboxylic Acid to β- and γ-Cyclodextrins

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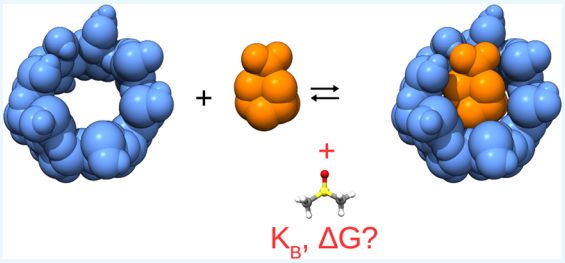
Supporting Information

ABSTRACT: Most therapeutic targets are proteins whose binding sites are hydrophobic cavities. For this reason, the majority of drugs under development are hydrophobic molecules exhibiting low solubility in water. To tackle this issue, a few percent of cosolvent, such as dimethyl sulfoxide (DMSO), is usually employed to increase drug solubility during the drug screening process. However, the few published studies dealing with the effect of adding DMSO showed that the affinity of hydrophobic ligands is systematically underestimated. To better understand the effect of DMSO, there is a need of studying its effect on a large range of systems. In this work, we used β- and γ-cyclodextrins (made of 6 and 7 α-D-glucopyranoside units, respectively) as models of hydrophobic cavities to investigate the effect of the addition 5% DMSO on the affinity of 1-adamantane carboxylic acid (ADA) to these cyclodextrins. The two systems differ by the size of the cyclodextrin cavity. The evaluation of binding constants was performed using ultrasound velocimetry, nuclear magnetic resonance spectroscopy, and molecular simulations. All techniques show that the presence of 5% DMSO does not significantly modify the affinity of ADA for γ-cyclodextrin, while the affinity is dramatically reduced for β-cyclodextrin. The bias induced by the presence of DMSO is thus more important when the ligand volume better fits the cyclodextrin cavity. Our work also suggests that free energy calculations provide a sound alternative to experimental techniques when dealing with poorly water-soluble drugs.



INTRODUCTION

The majority of drugs are small ligands whose purpose is to bind therapeutic targets, mostly proteins.[1,2] Because most of the newly discovered targeted binding sites are small hydrophobic pockets located in proteins,[3,4] drugs under development are also mainly hydrophobic.[5] To deal with this increasing number of poorly water-soluble drugs,[5] new strategies have to be developed for drug discovery and delivery.[6] Most of them rely on the use of specific molecules that help solubilize the insoluble drugs. Among these molecules, dimethyl sulfoxide (DMSO) is widely used as a cosolvent due to its known ability to readily dissolve a wide range of compounds, including hydrophobic molecules. DMSO is particularly used during the drug discovery process in screening techniques such as cell-based assays or high-throughput screening.[7] These techniques allow rapid identification of active compounds, antibodies, or genes of therapeutic interest. Once a therapeutic target is identified, the goal consists in selecting the ligands with the highest affinity for the designated target. Hydrophobic drugs are first solubilized in a 100% DMSO solution, then titrated to an aqueous solution containing the therapeutic protein target so that the volume fraction of DMSO is on the order of a few percent. Indeed, exceeding 10% of DMSO volume concentration is known to unfold proteins[8−10] as well as to induce cell death.[11] Thus, the ligand affinity is systematically evaluated in the presence of less than 10% of DMSO. Surprisingly, the literature dealing with the effect of a small addition of DMSO on binding constant measurements is sparse. It may be because measuring the binding constant of poorly water-soluble or insoluble drugs in the absence of a cosolvent is challenging, as

Received: August 18, 2017
Accepted: January 5, 2018
Published: January 25, 2018

most techniques require a drug concentration significantly higher than the solubility of the drug. The few experimental articles[12−15] systematically measured binding constants that are weakened in the presence of DMSO compared to values performed without it. Hence, screening techniques that use DMSO most likely underestimate the efficiency of hydrophobic drug candidates. Consequently, there is an urgent need either better quantifying the effect of DMSO to be able to correct its effect on a given system, or using new approaches to evaluate binding constants without the need for a cosolvent. In this context, cyclodextrins represent an attractive model to investigate the effect of a cosolvent in binding events at a hydrophobic site. They are small molecules made of six to eight glucopyranose units assembled into a truncated cone shape, the inner hydrophobic cavity of which can accommodate various small hydrophobic molecules. Their inner cavity can be considered as a simplistic model of a hydrophobic pocket found in proteins of pharmaceutical interest. Moreover, cyclodextrins are widely used in commercial formulations to encapsulate hydrophobic drugs.[5,16,17] Similarly to drug screening techniques, the production of cyclodextrin based formulations requires the use of a cosolvent to form the hydrophobic drug−cyclodextrin complex. The choice of the cosolvent is a balance between improving the solubility of the hydrophobic drug and decreasing its affinity for cyclodextrins. Because this balance is difficult to predict, most medicinal products contain a majority of empty cyclodextrins, hence leading to less efficient formulations. The understanding of the effect of cosolvents on the binding of cyclodextrins should at the same time help optimize cyclodextrin based formulations as well as predict the affinity of hydrophobic ligands with more accuracy during the drug discovery process. In this article, we have investigated using experimental and numerical techniques the difference in binding constant of a hydrophobic ligand for β- and γ-cyclodextrins performed with or without 5% DMSO, which is a typical concentration employed in screening techniques (i.e., in the range of 1−10%). To the best of our knowledge, the effect of a few percent of DMSO on the binding of chemically unmodified cyclodextrins has never been investigated. All previous studies dealt with large concentrations, ranging from 25% to 100% DMSO.[18−20]

RESULTS

Sound Velocity Measurements. The effect of 5% DMSO (v/v) on the binding of 1-adamantane carboxylic acid (ADA) to β- and γ-cyclodextrin (β- and γ-CD) was investigated at 25 °C. This system was previously studied in aqueous solution[21,22] as well as in the presence of various osmolytes,[23] except DMSO. Many studies demonstrated a 1:1 stoichiometry for the complexes of ADA with β- and γ-CD.[23−25] In particular, the number of water molecules leaving the hydration shell of ADA and β-CD after their binding were determined to be 20−25 water molecules from sound velocity measurements,[21] which are known to be extremely sensitive to changes in hydration.[26] Indeed, the strength of a ligand affinity is proportional to the number of solvent molecules released from the solvation shell of the binding sites and to the energetic frustration experienced by these molecules, which would prefer going to the bulk than staying in the solvation shell of hydrophobic solutes. This frustration is reduced when the physical properties between solvent molecules in the solvation shell and in the bulk are closer. Thereby, we first measured the sound velocities, U and U_o, of an ADA solution and of its solvent (either made of an aqueous buffer with or without 5% DMSO), respectively. Both velocities were measured each time a titration was performed using the same CD solution. For each titration, we calculated the increment of sound velocity $[U] = (U_o - U)/(U_o[\mathrm{ADA}])$, where [ADA] is the ADA molar concentration. The values of $\Delta[U](r) = [U](r) - [U](0)$ are plotted in Figure 1 as a function of the molar ratio r = [CD]/[ADA], where [CD] is the molar concentration of added CD. The variation of $\Delta[U](r)$ is mainly due to a change in the properties of solvent molecules (water or DMSO) that are released from the binding sites due to the ADA binding to CD. The Newton−Laplace equation, $\beta_S = 1/(\rho U^2)$ relates the sound velocity U to the coefficient of adiabatic compressibility β_S and the density ρ. Since changes in compressibility are much larger than that of density,[21,22] we can consider that $\Delta[U](r)$ is mainly a reflection of compressibility changes in solvent molecules that no longer hydrate the binding sites. It can be fitted to the following equation:

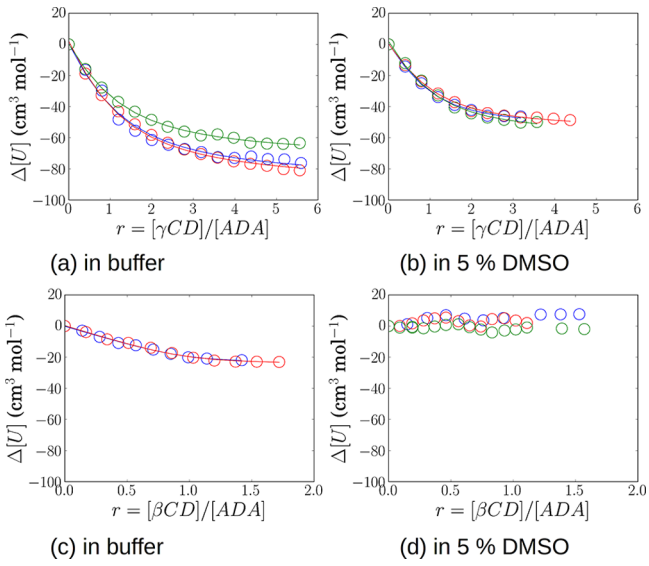


Figure 1. Variation of the sound velocity increment, $[U]$, of a solution of adamantane carboxylic acid upon addition of β (c, d) or γ-cyclodextrin (a, b). The left (a, c) and right columns (b, d) correspond to measurements performed in buffer and in 5% DMSO buffer, respectively. The various colors correspond to different titration measurements.

$$\Delta[U](r) = \alpha(r)[U](\infty) \quad (1)$$

where $\alpha(r)$ is the molar fraction of bound ADA and $[U](\infty)$ is the value at $r = \infty$. Knowing that β- and γ-CD bind only one ADA, we can easily relate $\alpha(r)$ to the binding constant K_b (see eq 3). A fit of the γ-CD data in buffer leads to a binding constant K_b = (340 ± 25) M^{-1}, in good agreement with a published value, (324 ± 11) M^{-1}, also measured using sound velocity.[22] The binding constant slightly increased to (509 ± 50) M^{-1} in the presence of 5% DMSO. For β-cyclodextrin, we derived in buffer a value K_b = (5700 ± 3000) M^{-1}, whereas we could not observe a significant variation for $[U](r)$ in the presence of 5% DMSO. The K_b values can be easily converted into a change of Gibbs free energy $\Delta G = -RT \ln(K_b)$ (where R is the gas constant and T is the temperature, that is, 298 K) accompanying the binding event (see Table 1). As K_b varies exponentially, small variations in entropy (ΔS) or enthalpy (ΔH) can lead to huge variations in K_b, whereas $\Delta G = \Delta H -$

DOI: 10.1021/acsomega.7b01212
ACS Omega 2018, 3, 1014−1021

Table 1. Values of the Binding Constant of the Association of 1-Adamantane Carboxylic Acid with β- and γ-Cylcodextrin Evaluated by Sound Velocity, NMR, and Simulation in a 50 mM Phosphate Buffer at pH 7.5, without or with 5% DMSO

		K_b in M^{-1} (ΔG in kJ/mol)		
	solvent	from sound velocity	from NMR	from simulation
γ-CD	buffer	340 ± 25 (−14.4 ± 0.2)	H3, 236 ± 10 (−13.5 ± 0.1) H5, 173 ± 21 (−12.8 ± 0.3)	348 ± 141 (−14.4 ± 0.9)
	5% DMSO	509 ± 50 (−15.4 ± 0.2)	H3, 148 ± 7 (−12.4 ± 0.1) H5, 174 ± 7 (−12.8 ± 0.1)	109 ± 59 (−11.4 ± 1.3)
β-CD	buffer	5700 ± 3000 (−21.4 ± 1.3)	H3, 3200 ± 150 (−20.0 ± 0.1) H5, 710 ± 38 (−16.3 ± 0.1)	882 ± 180 (−16.8 ± 0.5)
	5% DMSO		H3, 172 ± 18 (−12.8 ± 0.3) H5, 44 ± 8 (−9.4 ± 0.3)	47 ± 57 (−8.1 ± 3.2)

$T\Delta S$ does not overexpress these energetic variations. Since K_b and ΔG are equally employed and given in the literature, we will provide both values. When considering the binding free energy ΔG, the ADA to γ-CD binding is only (1 ± 0.4) kJ mol^{-1} more stable in 5% DMSO than in buffer (see Table 1). For β-CD, the lack of significant velocity variation in the presence of DMSO can be due, according to eq 1, to a negligible value of K_b or of $|[U](\infty)|$ or both. The latter scenario is most probable since for γ-CD we observe that the addition of DMSO reduces the difference $[U](0) - [U](\infty)$ (see Figure 1), that is, the difference between compressibilities of solvents in the bulk and in the solvation shell of ADA and CD binding sites. So if the same phenomenon occurred for β-CD, it would have led to a different value, $[U](0) - [U](\infty)$, smaller than the detection threshold of the velocimetry technique (which can be estimated around 8 cm^3 mol^{-1}).

Another technique is needed to confirm the above scenario. In binding studies, the technique of choice is isothermal titration calorimetry (ITC). Unfortunately, this technique provided unreproducible results when performing titration measurements on solution containing 5% DMSO. In this respect, Fotiadou et al.[27] also could not get reproducible results on cyclodextrin derivatives when using 80% DMSO "due to ground noise coming from a strong DMSO−water exothermic association which is very much dependent on the exact composition of the mixture". For this reason, the authors performed only measurements in pure DMSO. Thus, we used a more adapted method, that is, nuclear magnetic resonance (NMR) technique.

Nuclear Magnetic Resonance Measurements. We performed NMR measurements in which water and DMSO were deuterated. Stock solutions of cyclodextrins were prepared in deuterated buffers with or without the presence of 5% DMSO-d_6. ADA stock solution was prepared by solubilizing ADA in a volume of CD stock solution. Then, samples were prepared by mixing a volume of CD stock solution with another volume of ADA stock solution, so that the CD concentration remains constant through all samples, while AD concentration is diluted. For each sample, we monitored the chemical shift of the peak signal ascribed to hydrogen atoms of cyclodextrin (see Figure S1). In good agreement with other publications,[28,29] the H3 and H5 protons, located inside the CD cavity, are the most affected by the ADA inclusion, while the H6 proton, which is located at the edge of the smaller opening face is weakly shifted. In contrast, the chemical shifts of protons located outside the cavity (i.e., H1, H2, and H4) weakly change upon complexation (data not shown). In this context, we only considered the H3 and H5 protons in our NMR analysis. The variations of the chemical shifts were

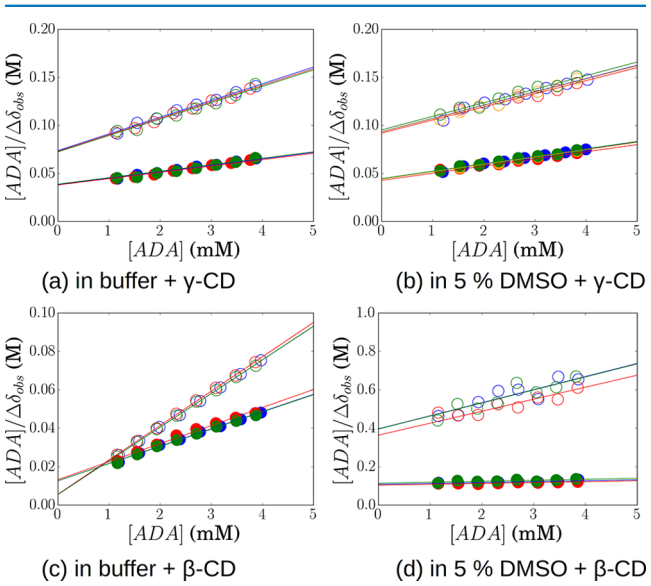


Figure 2. Variations of chemical shift as a function of [ADA]. In each panel, the various colored symbols correspond to different titration measurements where the values of $[ADA]/\Delta\delta_{obs}$ were obtained from H3 (○) and H5 (●). The top panels (a, b) were performed with γ-cylcodextrin and the bottom panels (c, d) with β-cylcodextrin, while the left column (a, c) deals with experiments in buffer and the right column (b, d) in the presence of 5% DMSO.

plotted as a function of [ADA] (see Figure 2). In a one-to-one stoichiometry as expected in our systems, these variations must behave linearly according to Scott's equation:[28,30]

$$\frac{[\mathrm{ADA}]}{\Delta\delta_{obs}} = \frac{[\mathrm{ADA}]}{\Delta\delta_{max}} + \frac{1}{K_b\Delta\delta_{max}} \quad (2)$$

where $\Delta\delta_{obs}$ represents the observed chemical shift difference of CD proton H3 or H5 between free CD and CD + ADA complexes and $\Delta\delta_{max}$ is the chemical shift difference at saturation. Since a linear behavior was indeed observed in all our figures, we could extract the apparent binding constant K_b by fitting the NMR data to eq 2. For γ-CD, the data based on H3 protons shows that K_b slightly decreases from (236 ± 10) M^{-1} to (148 ± 7) M^{-1} when 5% DMSO is added, while K_b is almost constant when considering H5 protons (see Table 1). For data derived from H3 protons, this corresponds to a slight increase in free energy of about 1.1 ± 0.3 kJ mol^{-1}, from (−13.5 ± 0.1) to (−12.4 ± 0.1) kJ mol^{-1}, while this change is

DOI: 10.1021/acsomega.7b01212
ACS Omega 2018, 3, 1014−1021

null for H5 protons. These variations are different from what has been measured by sound velocity (a slight decrease in free energy), but we can consider that both NMR and velocity techniques measured only small changes upon the addition of DMSO. Note also that the two techniques do not probe the same observables: for sound velocity the change in properties of solvents released from the solvation shell of binding sites is monitored, whereas for NMR the change in the chemical environment of two cyclodextrin proton types is monitored. Moreover, it is noteworthy that the H5 proton of β-CD is the most sensitive to ADA complexation. The latter protons are located in the interior cavity of CD close to the narrower rim. As there is no chemical shift assigned to H6 protons, this result suggests that ADA inclusion reaches the H5 protons but not the H6 ones. However, the K_b estimation is obviously more precise for H3 protons than for H5 protons, for which broadened and shape-changing NMR signal over the range of low concentration conditions (data not shown) can hamper an accurate estimation of the chemical shift. For β-CD, we succeeded to measure a binding constant in the solution containing 5% DMSO; this value is equal to (172 ± 18) M^{-1} for H3 protons and (44 ± 8) M^{-1} for H5 protons, while in buffer we measured, respectively, (3200 ± 150) M^{-1} for H3 protons and (710 ± 38) M^{-1} for H5 protons. We observe for both proton types a 17 times decrease in K_b values upon the addition of DMSO, while the value for H3 protons is closest to the value derived from sound velocity. Although K_b is reduced by the presence of DMSO, it still exhibits a significant value that should have been detectable by previous velocity measurements, which demonstrates that the value of $[U](0) - [U](\infty)$ has to be very small in the presence of DMSO. The observed variation in K_b corresponds to a significant difference in free energy ΔG of about 7 kJ mol^{-1}, with values increasing from (−20.0 ± 0.1) kJ mol^{-1} in buffer to (−12.8 ± 0.3) kJ mol^{-1} in DMSO based on H3 proton and from (−16.3 ± 0.1) kJ mol^{-1} to (−9.4 ± 0.3) kJ mol^{-1} based on H5 proton.

Molecular Simulations and Free Energy Calculations. Molecular simulations allow the evaluation of the binding free energy using so-called "alchemical" approaches.[31] It consists in calculating the free energy of the simulated complex (see Figure S2) via a suitable thermodynamic cycle involving alchemical transformations (i.e., by decoupling nonbonded interactions of the ligand from its environment). Using this method, we obtained the change in free energy associated with the binding of ADA to CD. However, one has to bear in mind that the accuracy of such calculations relies on the force field. It is especially challenging when it comes to carbohydrate molecules such as cyclodextrins. In the literature, some simulation studies assessing the effects of solvents on the deformation of cyclodextrins were published recently.[32,33] The one from Zhang et al.[32] used the GLYCAM06[34] (derived from AMBER) while the other from Khuntawee et al.[33] used the GROMOS 53A6 for carbohydrates.[35] Both studies revealed that the flexibility of the cyclodextrin depends not only on the solvent used but also on the ligand.

Here we used the GROMOS $53A6_{GLYC}$ force field[36] which is an update of GROMOS 53A6. The systems we simulated consisted of a box containing either a β or γ-cyclodextrin bound to an adamantane carboxylic acid and solubilized either in water or in water with 5% DMSO (together with a sodium ion to keep the complex neutral). Of note, all free energy values ΔG presented here were corrected for finite-size and discrete solvent effects due to the net charge of ADA (i.e., −1) (see SI). In all cases, the corrections were quite small (about 1 kJ mol^{-1} or lower). When DMSO molecules are present, they solvate the cavities of both β- and γ-CD before their complexation with ADA. For γ-CD, we calculated a change in free energy, ΔG, associated with the binding equal to (−14.4 ± 0.9) kJ mol^{-1} in buffer and (−11.4 ± 1.3) kJ mol^{-1} in 5% DMSO. This gives a difference of 3 kJ mol^{-1}, which is closer to the variation observed for H3 atoms in our NMR experimental data (1.1 kJ mol^{-1}) and confirms the fact that DMSO does not significantly modify the affinity of ADA for γ-CD. In terms of binding constants, it means that $K_b = \exp -(\Delta G/(RT))$ is equal to (348 ± 141) M^{-1} in water and (109 ± 59) M^{-1} in the presence of 5% DMSO. For β-cyclodextrin, ΔG equals (−16.8 ± 0.5) kJ mol^{-1} in water and (−8.1 ± 3.2) kJ mol^{-1} in DMSO. This increase of 8.7 kJ mol^{-1} in free energy upon DMSO addition also agrees very well with NMR measurements. From these free energy values, we conclude that the binding constant is reduced from (882 ± 180) to (47 ± 57) M^{-1} upon the addition of 5% DMSO. We also performed the same calculations without restraining the movement of ADA. Similar free energy values were obtained, that is, (−17 ± 2) kJ mol^{-1} in buffer and (−7 ± 3) kJ mol^{-1} in 5% DMSO for γ-CD, while for β-CD we have (−14 ± 3) kJ mol^{-1} in buffer and (−9 ± 1) kJ mol^{-1} in 5% DMSO.

DISCUSSION

From our experimental and simulation results, we conclude that the presence of 5% DMSO significantly decreases the affinity of ADA for β-CD, while its effect is marginal on ADA affinity for γ-CD. The decrease in ADA affinity for β-CD is consistent with published works performed using other ligands at higher volume concentration of DMSO. For instance, Siegel and Breslow[18] showed that the binding of β-CD with *m-tert*-butylphenyl acetate becomes weaker when increasing DMSO concentration, with K_B values equal to 10^4, 500, and 67 M^{-1} at 0, 50%, and 99% DMSO, respectively. Similar observations were made by Tachibana et al.[37] for the complexation of carbozole with β-CD when the DMSO addition varies from 105 to 60% and by Eftink and Harrison[38] for the complexation of *p*-nitrophenol with α-CD with 10% to 50% of DMSO. In some cases, a high concentration of DMSO can prevent a complexation from taking place, like for the binding of puerarin with β-CD, which does not occur at 100% DMSO.[20] Finally, Bernad-Bernad et al.[19] showed that the binding constant of albendazole and mebendazole to α, β-, and γ-CD drastically decreases when 25%, 50%, or 75% DMSO is added, but at 100% DMSO, if the binding constant is smaller than that in water it is larger than that in a mixture of water and DMSO. However, the behavior is different for thianedazole: while additions of DMSO continuously increase the binding constant for β-CD, it first increases it for α- and γ-CD at 36% and 50% DMSO and then decreases it for 75% and 100%. Thianedazole has also a much weaker affinity for cyclodextrins in water (K_b = 24, 38, and 100 M^{-1} for α-, β-, and γ-CD, respectively) than the two other drugs (K_b = 300−315, 1200−1500, and 1000−900 M^{-1} for α-, β-, and γ-CD, respectively). These results show that the addition of DMSO cannot be systematically ascribed to a decrease in binding constant and depends both on the type of cyclodextrin and on the ligand. Most studies point out the importance of the size ratio between the ligand and the cyclodextrin cavity. In our experiments, it is indeed the only difference between the two AD/CD complexes. ADA's volume fits very well within the β-CD cavity, while the bigger γ-CD

DOI: 10.1021/acsomega.7b01212
ACS Omega 2018, 3, 1014−1021

cavity gives room for ADA to move. We expect a smaller number of hydrogen bonds between the frustrated solvent molecules inside the cavity as its size decreases, which strongly modifies the entropy. This would induce a larger difference in solvent properties between the molecules in the bulk and those in the solvation shell for β-CD compared to γ-CD, which is consistent with sound velocity measurements (as given by $[U](\infty)$). The difference contributes to make the binding of ADA to β-CD in water much stronger than that to γ-CD. However, it is still difficult to assess the exact contribution of DMSO. To gain further insight on this question, future work should focus on the decomposition of the free energy into its enthalpic and entropic contributions.

CONCLUSION

The above results suggest that the effect of DMSO is stronger for hydrophobic ligands whose volume better fits the cavity of a hydrophobic binding site. For these complexes, the ligand affinity determined in the presence of DMSO may not reflect its affinity in the absence of DMSO, such as in vivo conditions. Ideally, binding properties should be determined in the absence of any cosolvent, which is likely impossible using experimental techniques for poorly water-soluble ligands. However, our free energy calculations using computer simulations could reproduce accurately the experimental variations of binding free energies. This suggests that such a numerical approach represents a valuable alternative to experimental techniques to determine binding constants without the need of a cosolvent.

METHODS

Materials. β-Cylcodextrin (purity >97%), γ-cylcodextrin (purity >98%), 1-adamantane carboxylic acid (purity >99%), and dimethyl sulfoxide (DMSO) (purity of >99.9%) were purchased from Sigma-Aldrich. The solubilities of β-cylcodextrin and γ-cylcodextrin in water at 25 °C are 18.5 and 232 g/L, respectively,[39] while the solubility of 1-adamantane carboxylic acid in buffer was about 0.75 g/L. We evaluated the latter by sound velocity measurements as the value of sound velocity is very sensitive to both aggregation and solute concentration.[40] All samples were prepared with solute concentrations below their solubility values. Water was purified using a PURELAB Option-Q unit (ELGA LabWater). D_2O and deuterated dimethyl sulfoxide (DMSO-d_6) were purchased from Eurisotop (Saint-Aubin, France).

Sample Preparation. We prepared two kinds of pH 7.5 buffers. The first one is a 50 mM phosphate buffer, prepared by dissolving the appropriate amounts of sodium phosphate dibasic and sodium phosphate monobasic in a required volume of water (H_2O). Then the pH was adjusted to 7.5 when necessary. The second one followed the same preparation except for the addition of 5% (v/v) DMSO during the buffer preparation. For sound velocity measurements, H_2O was used, whereas for NMR D_2O was used along with deuterated DMSO.

Ultrasound Velocity. Sound velocities of solutions were measured at 25 °C using a previously described differential resonator method,[41−44] at a frequency of 7.5 MHz with a precision of 0.15 cm/s in buffers. A solution of 1-adamantane carboxylic acid (0.76 mL) filled a first thermostatted cell (with an initial concentration of 0.5−0.7 g/L depending on the sample), while the second cell contained the same volume of solvent (either a 50 mM phosphate buffer at pH 7.5 or the same buffer with the addition of 5% (v/v) of DMSO). In each cell, two lithium niobate piezotransducers face each other. The analysis of the frequency−amplitude characteristic of the resonator was performed with an Agilent (model E5100A) network/spectrum analyzer. Titration was performed in both cells by adding an identical volume of a solution of cyclodextrin solubilized in the same buffer as for ADA. For each titration, we derived the sound velocity increment $[U] = (U - U_o)-(U_o[\mathrm{ADA}])$, where U and U_o are the sound velocities in the solution and in its solvent, respectively, and [ADA] is the molar concentration of 1-adamantane carboxylic acid. The variation in $[U]$ values is eventually plotted as a function of the molar ratio, $r = [\mathrm{CD}]/[\mathrm{ADA}]$, of cyclodextrin to 1-adamantane carboxylic acid and can be fitted by the following equation $[U](r) = [U](r = 0) + \alpha(r)[U](r = \infty)$, where $\alpha(r)$ is the fraction of bound 1-adamantane carboxylic acid. Taking $Y = 2K_b[\mathrm{ADA}]$, it can be easily demonstrated that $\alpha(r)$ is a solution of the equation:

$$\alpha^2 - \left(r + 1 + \frac{2}{Y}\right)\alpha + r = 0 \tag{3}$$

It should be stressed that the above technique probes all the sample volume (i.e., all solvent molecules also contribute to sound velocity). Any discrepancy between the concentration of titrant or of solvent between the titrated solution (i.e., solute solubilized in the solvent) and the titrated solvent would lead to a wrong estimation of sound velocity increment. Thus, extra care should be exerted when using DMSO due to absorption/desorption phenomena on cell and transducer surfaces. In particularly, cells should always be washed with a 5% DMSO buffer and be soaked for hours with it before starting measurements.

NMR. All solutions were freshly prepared either in D_2O or in D_2O with 5% DMSO-d_6 and buffered with 10 mM phosphate buffer at a pH of 7.5. Two stocks solutions were prepared, the first one with 0.1 mM of either β- or γ-CD and the second one containing 0.1 mM of either β- or γ-CD supplemented with 0.1 mM of adamantane. A volume v_1 of the first stock solution, that is, without ADA, was added to and mixed with a volume v_2 of the second stock solution, that is, with ADA, so that the total volume was always 500 μL. By varying v_1 and v_2, we prepared solutions with a resulting [CD]/[ADA] ratio ranging from 0.4 to 4, while the concentration of β or γ-CD was kept constant. Each experiment was repeated three times. 1D ^{1}H NMR spectra were recorded on an Avance II Bruker AC-400 MHz Bruker NMR spectrometer. Each spectrum was acquired with 128 scans, and the temperature was maintained at 25 ± 0.1 °C. Spectral acquisition and processing and chemical shift determination were performed using TopSpin 2.1 software. Chemical shifts are given in ppm (δ) and calibrated to the residual solvent HDO peak at 4.79 ppm. The complexation-induced chemical shift difference is given as the difference between the chemical shift of the free cyclodextrin molecule and the chemical shift of the bound CD molecule: $\Delta\delta = \delta_{free} - \delta_{complex}$.

Data Fitting. For velocity and NMR experiments, the data were fitted using the least-squares method accessible from the scipy module of python (www.scipy.org). For several series of titration corresponding to the same type of experiment (i.e., same solvent, same cyclodextrin, and NMR based on same proton), all the points were fitted once using an identical unknown binding constant, K_b, while other unknown parameters, $[U](\infty)$ for sound velocity and $\Delta\delta_{max}$ for NMR, were different depending on the series. Indeed, a global fitting allows us to achieve better accuracy on the K_b value.[45] The

DOI: 10.1021/acsomega.7b01212
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error associated with each parameter was obtained from the autocorrelation matrix.

Free Energy Calculations. All molecular dynamics (MD) simulations were performed with GROMACS 5.0.7.[46] The GROMOS 53A6$_{GLYC}$ force field[36] was used for β- and γ-CD, the GROMOS 54A7[47] force field for ADA, along with the SPC model for water.[48] For all MD simulations involved in free energy calculations, we used the Langevin integrator with a 2 fs time step, a friction coefficient of 0.1 ps^{-1} and a temperature of 298 K. All bonds were constrained with the P-LINCS algorithm,[49] and the full rigidity of the water molecules was enforced by the SETTLE algorithm.[50] For NPT simulations, pressure was maintained at 1 bar using the Parrinello−Rahman barostat[51] with a relaxation time of 4 ps and an isothermal compressibility of 4.5 × 10^{-5} bar^{-1}. Lennard-Jones interactions were computed using a twin-range scheme set to 8 and 14 Å, and the neighbor list was updated every 10 steps. Electrostatic interactions were computed using the particle-mesh Ewald (PME) method[52,53] with a real-space cutoff distance of 14 Å, a grid spacing of 1.2 Å, and an interpolation scheme of order 4. The structures of β-CD, γ-CD, and ADA were obtained from CSD Cambridge Structural Database (CDDC 648855),[54] Protein Data Bank (PDB 1D3C),[55] and PubChem (CID 13235) respectively. To construct the complex CD−ADA, we first performed some self-assembly MD simulations in the NPT ensemble using the leapfrog integrator. The ligand spontaneously bound to the CD within a few tens of nanoseconds. For all simulations involving the complex or the ADA ligand in water, we added one sodium ion to neutralize the ADA carboxylic group. Neutrality was maintained to fit our experimental conditions performed at pH 7.5. All the systems were obtained from self-assembly simulations and then used for free energy calculations. The composition of each system is described in Table 2. Four and three replicas were performed

Table 2. Composition of the Systems Used for Free Energy Calculations

systems	CD	ADA	Na^+	SPC	DMSO
β-CD	1	1	1	2120	0
β-CD	1	1	1	2120	29
γ-CD	1	1	1	3105	0
γ-CD	1	1	1	3105	42

for a system containing β- and γ-CD, respectively. To assess the affinity of ADA for both types of CD we used a free energy perturbation method. ADA was decoupled from CD or solvent via a linear alchemical pathway. The distance between the centers of mass of CD and ADA was restrained[56] by a harmonic potential with a force constant of 1000 kJ mol^{-1} nm^{-2}. Ten windows were used for distance restraints, 5 for electrostatic decoupling, and 15 for van der Waals interaction decoupling. Each window was simulated for 50 ns. The last 40 ns were used for analysis. Results were analyzed with the python package pymbar[57] which implements the multiple Bennett acceptance ratio.[58] The evaluation of the binding free energy of a charged species such as carboxylic ADA (net charge of −1) using explicit-solvent simulations and lattice-sum methods induces some artifacts that need to be corrected. We used the analytical scheme suggested by Rocklin et al.[59] for correcting the free energy evaluated from our simulations:

$$\Delta G_{MD,NBC} = \Delta G_{MD,PBC}(L) + \Delta\Delta G_{ANA}(L) + \Delta\Delta G_{DSC}(L) \quad (4)$$

where $\Delta G_{MD,PBC}(L)$ is the raw value calculated from our simulations using periodic boundary conditions, $\Delta\Delta G_{ANA}(L)$ is a correction term for finite-size effects, $\Delta\Delta G_{DSC}(L)$ is a correction term for discrete solvent effects, and $\Delta G_{MD,NBC}$ is the binding free energy corrected for all these effects. Reported in SI is the description of how $\Delta\Delta G_{ANA}(L)$ and $\Delta\Delta G_{DSC}(L)$ were calculated. All reported values in this manuscript and in Table 1 are the corrected free energies $\Delta G_{MD,NBC}$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01212.

Detailed description of the correction made to the free energy of binding, figure detailing the H atom locations in the cyclodextrins, and figure exhibiting the complexes ADA−β-CD and ADA−γ-CD (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was performed through access to the HPC resources of the institute for computing and data sciences (ISCD) at Université Pierre et Marie Curie and support from GENCI-CINES (Grant No. 2016-A0010706720).

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