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Charge Determines Guest Orientation: A Combined NMR and Molecular Dynamics Study of β-Cyclodextrins and Adamantane Derivatives

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Abstract

The strong binding of the adamantyl moiety to the cavity of β-cyclodextrin makes it a common binding motif in supramolecular chemistry and a common model system. Despite the attention, there are still unresolved questions regarding the orientation of the adamantane derivatives in the inclusion complexes - do they protrude from the wide or narrow opening of the cyclodextrin hosts? A combined analysis of ROESY NMR and molecular dynamics simulations allows the conclusion that positively charged adamantane derivatives are oriented with the hydrophilic group protruding from the wider opening of the cyclodextrin, while negatively charged adamantane derivatives form two co-existing types of complexes where the hydrophilic group respectively protrudes from the wide and narrow opening. Interestingly, structural modifications of the cyclodextrin host only has a slight impact on the guest orientation.

Introduction

The spherical hydrocarbon, adamantane (Ad), constitutes an almost perfect size-match to the hydrophobic cavity of the circular oligo-saccharide, β-cyclodextrin (βCD) (Figure 1), and adamantyl-carrying guest molecules generally form very stable inclusion complexes with βCDs in aqueous solution. Indeed, the world record for binding to the natural βCD is being held by an adamantyl-carrying anti-malarial drug candidate, having a binding constant of $2.4 \cdot 10^6$ M^{-1}.[1] The Ad:βCD binding motif is therefore exploited in various forms of supra-molecular structures and materials, including supra-molecular polymers[2] and self-healing gels[3,4]. A new type of atomically precise gold nanoparticle has been synthesized using adamantanethiolate as ligands,[5] which have been shown to bind βCD,[6] thereby expanding the potential applications of the gold nanoparticle. In addition to its relevance in supra-molecular chemistry, the Ad moiety is found in a range of drugs where it is a common lipophilic "add-on".[7] Cyclodextrins, and in particular βCD, are often used to enhance the aqueous solubility of drugs *via* the formation of water-soluble inclusion complexes, and this adds to the relevance of studying this particular host-guest interaction. Ad:CD inclusion complexes are also commonly used model systems for fundamental studies of intermolecular interactions.[8–11]

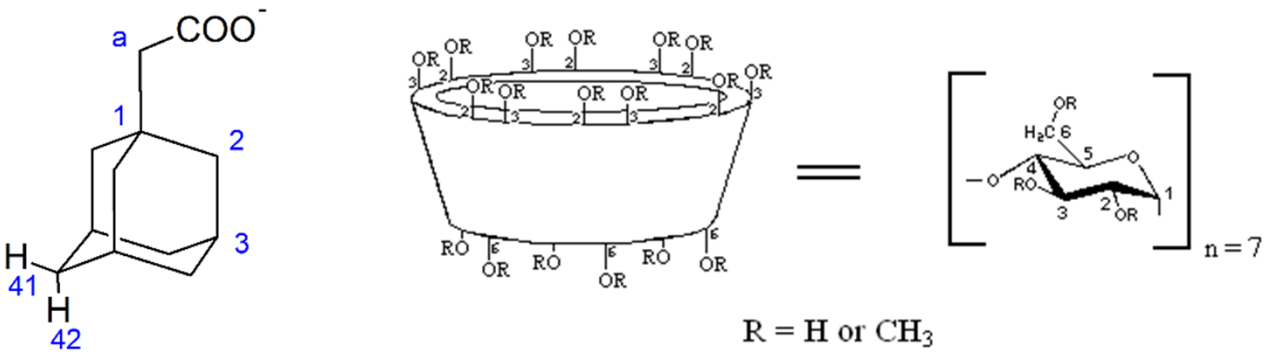

Figure 1 Structures of the almost spherical adamantane molecule functionalized with an acetate group and the circular cyclodextrin molecule. Complexes of three Ad derivatives and three cyclodextrins are studied. The Ad derivatives differ in the functional group at the 1 position while the cyclodextrins differ in the site of methylation. The rims of the natural βCD are lined with hydroxyl groups, DMβCD is methylated at the 2 and 6 positions, while TMβCD is methylated at the 2,3 and 6 positions.

Complexes between Ad derivatives and βCD have been studied for at least 50 years,[12] and crystal structures confirm the (complete) inclusion of the adamantyl moiety into the CD cavity.[13] Most of the studied Ad derivatives are mono-functionalized, typically containing a single carboxylate[14] or ammonium group[15] attached to the adamantyl sphere, but the binding properties of many other derivatives have also been studied.[16–21] Despite numerous studies of Ad:βCD complexes not much is known about the orientation of the included Ad derivatives. Since the attached functional groups are typically hydrophilic and often charged they are not included in the hydrophobic CD cavity, but it is not clear whether they protrude from the wide or narrow rim of the CD (hereafter termed type I and type II complexes, respectively, see Figure 2). The guest orientation may very well depend on the characteristics of the functional group and on the modification of the CD. Understanding the relations between the molecular structures and the orientation of the guest molecules is important for the rational design of new supra-molecular structures and interpretation of experimental results. Unfortunately, the literature is sparse and ambiguous on this topic. Ad derivatives with positively charged ammonium groups seem to form type I complexes with natural βCD[22,23] and with βCDs modified at the narrow rim[22,24]. Ad derivatives with negatively charged carboxylates have also been reported to form type I complexes with natural βCD[25] and with a βCD modified at the narrow rim[24], but the carboxylate group reportedly protrudes from the narrow rim in ternary complexes with natural βCD[23]. Glyco-conjugated Ad derivatives form type I complexes with natural βCD,[20] while there are indications that 1-bromoadamantane form type II complexes in addition to type I complexes[19]. All of the abovementioned orientations are for complexes in solution, and the conclusions are primarily based on ROESY NMR. ROESY signals arise from hydrogen nuclei (protons) that are close in space. Based on the relative magnitudes of ROESY signals arising from interactions between hydrogens on the host and guest molecules, the geometry of the complex

might be deduced. However, for this particular type of complexes the ROESY signals can be difficult to interpret as the round adamantyl moiety enjoys some freedom to tilt from side to side inside the CD cavity, leading to numerous ROESY interactions with the CD protons. In the solid state, X-ray crystallography reveals that 1-adamantanol forms both type I and type II complexes with a methylated βCD.[13] In contrast, most of the abovementioned studies of complexes in solution report the formation of a single type of complexes, most of them type I and only a few type II complexes[16,17,23]. It is possible, however, that many of the investigated complexes in solution form both type I and type II complexes. The ROESY signals have in most cases been interpreted in a qualitative (or semi-quantitative) fashion and it is possible that the conclusions regarding the orientation of the guests are incomplete. As the present study shows, negatively charged Ad derivatives do not have a strongly preferred orientation and forms both type I and type II complexes with natural βCD, 2,6-dimethyl-βCD (DMβCD), and permethylated βCD (TMβCD). To arrive at this conclusion, it was necessary to perform a quantitative analysis of NMR ROESY spectra combined with structural information from Molecular Dynamics simulations. By studying the complexes of two negatively charged and one positively charged Ad derivative and their complexes with three βCDs we seek to establish general relationships between the molecular structures and the orientation of the Ad guests. The obtained knowledge is useful for the design of supramolecular structures that employ the Ad:βCD binding motif as well as for the interpretation of structural and thermodynamic data for similar inclusion complexes. Further, identification of NMR "fingerprints" characteristic of type I and type II complexes will ease the structural characterization of similar complexes.

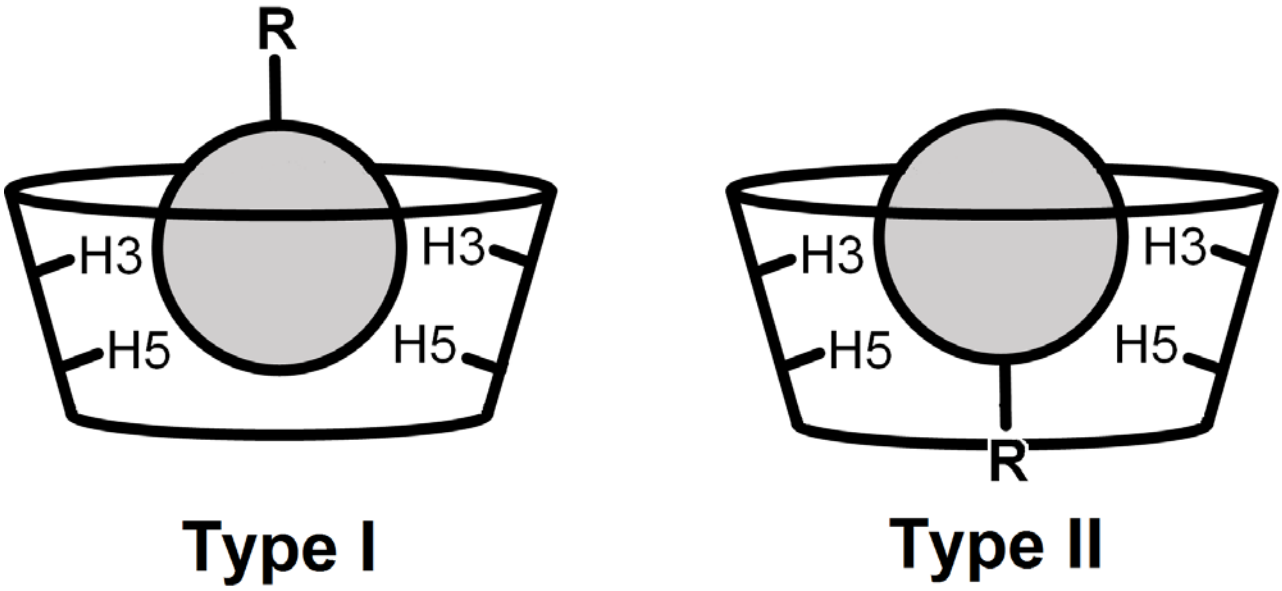

Figure 2 In type I complexes the charged group, R, of the spherical adamantane guest protrudes from the wider opening of the circular CD host molecule. In type II complexes it protrudes from the narrow opening.

Experimental and Theoretical Methods

Materials

1-Adamantylamine and β-cyclodextrin were purchased from Adamas-Beta, Shanghai, China. Adamantane-1-carboxylic acid and 2-(1-adamantyl)acetic acid were purchased from J&K Chemical, Shanghai, China. Heptakis(2,6-di-O-methyl)-β-cyclodextrin and heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin were from Sigma-Aldrich.

Nuclear magnetic resonance spectroscopy

Spectra of the complexes were recorded on 10-15 mM equimolar mixtures of Ad derivatives and cyclodextrins in 50 mM D_2O phosphate buffer, adjusted to pH 7.2. Protons were assigned based on 1D ^{1}H-NMR and 2D HSQC spectra. 2D ROESY NMR spectra were recorded with 16 scans and 256 and 2048 data points in the F1 and F2 direction, respectively. All spectra were recorded on a Bruker Avance-600 NMR spectrometer equipped with a cryogenically cooled probe.

Molecular dynamics simulations

Molecular dynamics simulations were carried out in NAMD,[26] using the CHARMM carbohydrate force field[27,28] for β-cyclodextrin. For the Ad derivatives, bond, angle and dihedral parameters were generated with the CGenFF program[29,30] (version 1.0.0) while atomic charges were taken from the CHARMM General Force Field[31] (version 3.0.1) and slightly adjusted to ensure correct total charge, as described in the Supporting Information. Complexes were solvated with TIP3P water in cubic boxes with a side-length of 34Å. Charge neutrality was obtained by adding a sodium or

chloride ion to the solvation boxes. Simulations were run for 30 ns with periodic boundary conditions using 2 fs time steps, the first 10 ns were equilibration runs. The trajectories from the remaining 20 ns were analyzed in VMD[32] to yield the interatomic distances.

Results and Discussion

The inclusion modes of three Ad derivatives, adamantane-1-carboxylate (AdCOO), 1-adamantyl ammonium ($AdNH_3$), and 1-adamantane acetate ($AdCH_2COO$), in complexes with three CDs, natural βCD, 2,6-dimethyl-βCD (DMβCD), and permethylated βCD (TMβCD), in total 9 complexes, were studied. At the experimental pH AdCOO and $AdCH_2COO$ carry one negative charge and $AdNH_3$ one positive charge. For each complex, a ROESY spectrum was recorded and two MD simulations were run, one for each of the two guest orientations.

For all complexes, the ROESY spectra showed strong interactions between most guest protons and the interior protons of the CDs, H3 and H5 (see Figure 2), thereby proving the formation of inclusion complexes. The ROESY spectrum of the βCD:$AdNH_3$ complex is shown in Figure 3 as an example. In this spectrum, there is a strong cross peak between P2 on the guest and H3 on the CD but hardly any cross peak between P2 and H5. Considering the locations of these protons on the host and guest molecules, a missing or weak interaction between P2 and H5 indicates the formation of type I complexes.[19,20,22] This pattern was also observed for the complexes of $AdNH_3$ with the other two CDs, DMβCD and TMβCD, suggesting a general tendency of $AdNH_3$ to form type I complexes with βCDs.

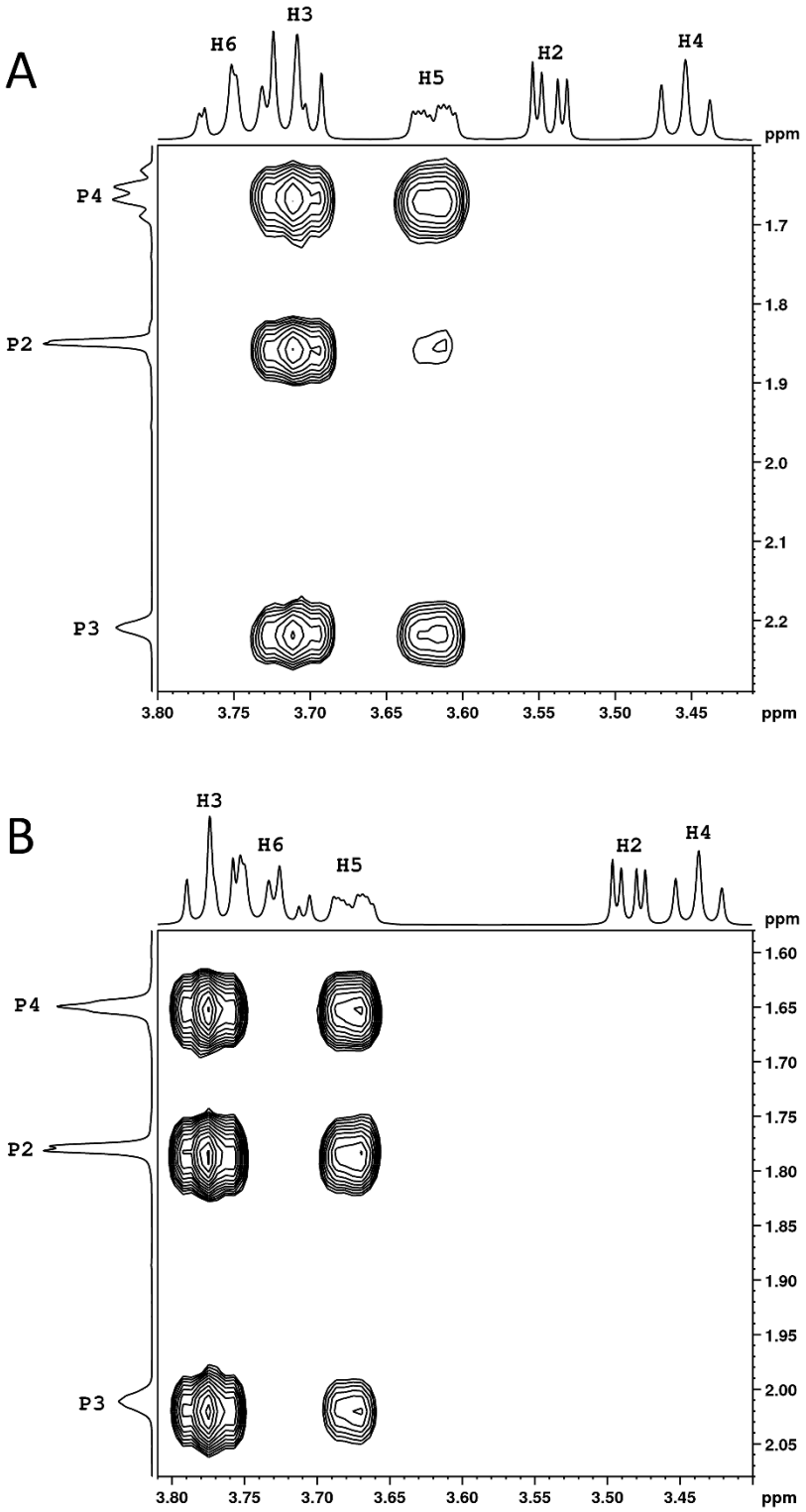

Figure 3 2D ROESY NMR spectra of the βCD:AdNH$_3$ complex (A) and the βCD:AdCOO complex (B). Protons on the host and guest are labelled "H" and "P", respectively.

For each of the investigated complexes, MD simulations of both type I and type II type complexes were carried out. For the complexes with $AdNH_3$, only the type I complexes were stable. Despite several attempts using different initial structures the type II complexes fell apart within a few nanoseconds. The $AdNH_3$ guest fell out of the narrow opening of the CD and the complexes did not re-form during the 30 ns of simulation. It seems that $AdNH_3$ exclusively forms type I complexes with the three investigated CDs.

The ROESY spectra of the complexes with the two negatively charged guests were more ambiguous. As seen on Figure 3, there is now a strong cross peak between P2 and H5 but also an equally strong cross peak between P2 and H3. The presence of the P2-H5 cross peak indicates the presence of type II complexes where the charged group and the P2 protons protrude from the primary rim, but this orientation does not seem consistent with the strong P2-H3 interactions. Is it possible that the Ad guest in a type II complex can tilt to such an extent that P2 gets sufficiently close to H3 to result in these strong interactions? Or maybe the structural fluctuations are so large that the charged group is sometimes sucked into the CD cavity. This, however, would be in contradiction to the conventional wisdom that hydrophilic groups are not included in the hydrophobic cavity of CDs. Answering these questions and explaining the ROESY pattern of the complexes with the negatively charged guests requires a closer look at the MD simulations.

Structures of the simulated complexes

The MD simulations showed that both type I and type II complexes of βCD with AdCOO were stable within the 30 ns simulations, and the nonpolar adamantyl moiety remained inside the CD cavity while the charged carboxylate group stayed outside the cavity. The structural fluctuations were generally small, as shown in Figure 4. The CD structure was rather rigid with an averaged

Root Mean Square Deviation (RMSD) of 0.58 Å and 0.49 Å in the type I and type II complexes, respectively. The AdCOO guest was a little more mobile, having and RMSD of 1.75 Å and 1.14 Å in the type I and type II complexes, respectively. The motion of the guest was mainly related to the rotation of the spherical adamantyl moiety inside the CD cavity and the concurrent movement of the carboxylate group, which formed hydrogen bonds with the hydroxyl groups at the rims of the CD. The fluctuations in inclusion depths were very small, and apart from rotation the adamantyl sphere hardly moved. Very similar structures were observed with $AdCH_2COO$.

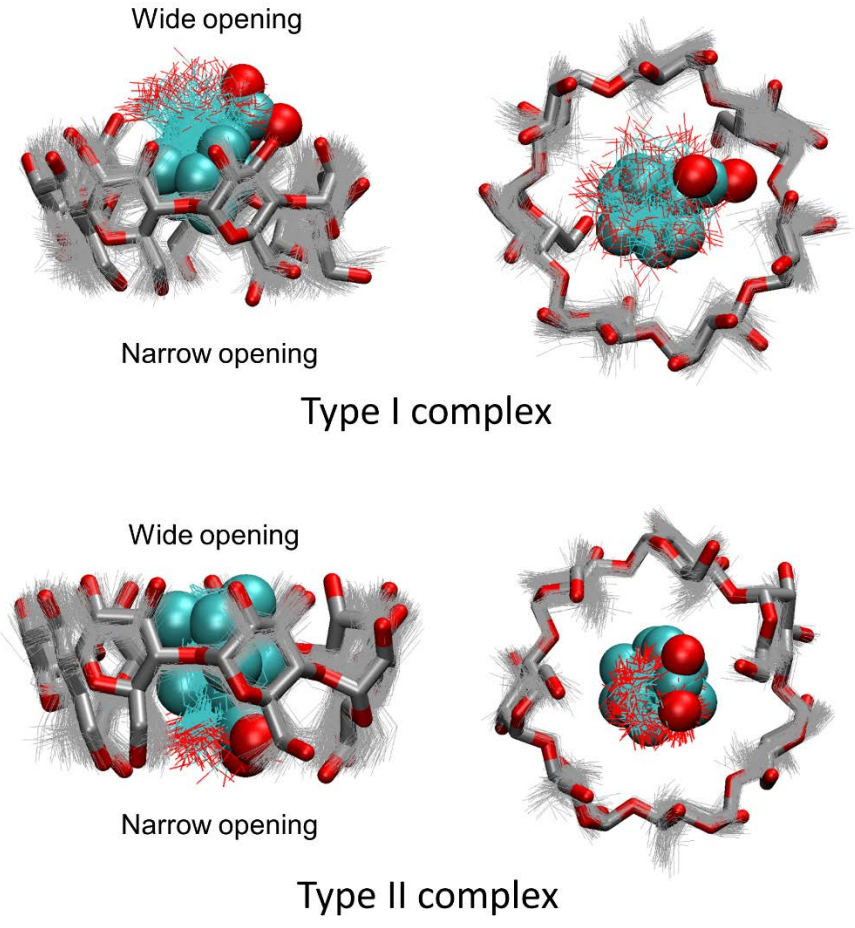

Figure 4 Structures of the βCD:AdCOO type I (top) and type II (below) complexes obtained from the MD simulations. The lines show the structural fluctuations. Hydrogens have been omitted for clarity.

According to the simulations, the methylated CDs formed type I and type II complexes with AdCOO and $AdCH_2COO$ with an overall structure similar to the complexes with natural βCD but with some minor but interesting differences. While these guests formed stable type I complexes with DMβCD, the guests occasionally left the cavity of the type II complexes through the narrow opening. But unlike the case for $AdNH_3$, the complexes never completely dissociated, and the negatively charged guests moved back into the CD after a short time. With TMβCD, these guests formed stable complexes but the CD in the type I complexes adopted a somewhat flattened conical structure with significant tilts of the glucose units around the glycosidic bonds, resulting in an almost complete closing of the narrow opening. This was not the case for the type II complexes where the CD structure resembled that of natural βCD.

Quantitative analysis of ROESY spectra

From the discussion so far, it seems that a qualitative analysis of the ROESY spectra cannot provide a firm conclusion regarding the orientation of the guests. The two "probes" on the CD, H3 and H5, are too close to each other, and the guests tilt to an extent that most of the protons on the guest may interact substantially with both H3 and H5, as observed in the experimental ROESY spectra (Figure 3). A quantitative analysis of the ROESY intensities must be conducted and compared to the inter proton distances that can be obtained from the MD simulations. The volumes of the ROESY cross peaks, ROE, are roughly proportional to the distance raised to the power of minus six. In a dynamic system, the distance should be averaged over all molecular conformations such that $ROE \propto \langle r^{-6} \rangle$, where r is the inter proton distance. Further, due to the many chemically equivalent protons in the guest and host molecules, many pairs of host and guest protons contribute to each of the observed ROESY cross peaks. For example, there are 7 H3 protons in each CD and 6 P2

protons in each Ad derivative, leading to 42 inter proton distances whose contributions must be summed up to give the total P2-H3 interaction. Even when protons are chemically non-equivalent their ROESY peaks may overlap, as is often the case for P41 and P42 whose overlapping peaks are denoted by P4 in Figure 3. In such cases, all of the distance contributions are summed up and compared to the experimental ROESY volume.

Combining experimental ROESY signals with distances obtained from various molecular modeling methods have previously been used to elucidate the structures of CD complexes.[19,33–36] In principle, each ROE signal can be converted to a distance provided that the constant of proportionality is known, but this requires a reference signal between two protons at a known distance. Alternatively, the ratios of the experimental ROEs can be compared to the ratios of the distances from the MD simulations to see whether the modeled complex is in accordance with the observed ROEs. Since most of the guest protons interact with both H3 and H5 of the CD, this ratio has previously been used.[33,35] Thus, if the structures of the modeled complexes are correct, the experimentally determined ratio on the left-hand side of equation 1 should be equal to the theoretically obtained ratio on the right-hand side:

$$\frac{ROE_{H5-Pi}}{ROE_{H3-Pi}} = \frac{\langle r_{H5-Pi}^{-6} \rangle}{\langle r_{H3-Pi}^{-6} \rangle} \qquad (1)$$

where ROE_{H5-Pi} is the volume of the ROESY cross peak between H5 and a proton on the Ad guest, Pi, and $\langle r_{H5-Pi}^{-6} \rangle$ is the time-averaged interproton distance as determined from the MD simulations. The experimental ROE ratios for the complexes with the $AdNH_3$ guest are shown in Table 1 together with the distance ratios obtained from the MD simulations of the type I complexes.

Table 1 Comparison of ROESY Cross Peak Volumes to Distance Data from MD Simulations.

Complex	Interaction with guest proton: P2	P3	P4
βCD:$AdNH_3$			
$<r^{-6}>$ H5/H3 (Type I)[a]	0.13	0.68	1.08
ROE H5/H3[b]	0.14	0.66	1.10
DMβCD:$AdNH_3$			
$<r^{-6}>$ H5/H3 (Type I)	0.17	0.69	1.20
ROE H5/H3	0.17	0.69	1.16
TMβCD:$AdNH_3$			
$<r^{-6}>$ H5/H3 (Type I)	0.17	0.72	1.14
ROE H5/H3	0.16	0.69	1.38

a) *Ratio of the time average of the r^{-6} distances between a given guest proton and the H3 and H5 host protons.*
b) Ratio of ROESY volumes for the cross peaks of a given guest proton, Pi, with the cavity protons, H5 and H3, of the cyclodextrin host.

For each of the complexes of $AdNH_3$ with the three CDs, Table 1 reveals a good agreement between the ROESY volumes and the distances obtained from the simulations of the type I complexes. It is now quite evident that $AdNH_3$ exclusively forms type I complexes with natural βCD, DMβCD and TMβCD. The data also confirm that an absent or very weak P2-H5 ROESY cross peak is a good indication of the formation of type I complexes, in accordance with previous interpretations.[19,20,22]

As mentioned above, the ROESY spectra of the complexes with AdCOO and $AdCH_2COO$ seemed ambiguous to whether the complexes are of type I or type II. The MD simulations of both type I and II complexes were stable, in contrast to $AdNH_3$ where the type II complexes dissociated. This allowed for a comparison of the ROESY volumes to both types of simulated complexes. Using the same approach as for the $AdNH_3$ complexes, the H5/H3 ROESY ratios were compared to the simulations, as shown in Table 2 and Table S1 for the AdCOO and the $AdCH_2COO$ complexes, respectively. For all of these complexes, the ROESY data do not seem to agree with the distance data for neither the type I nor the type II complexes. Only in one case, for the TMβCD:AdCOO complex, the ROESY data seem to be in reasonable agreement with the simulated type II complex,

but for the rest of the complexes the discrepancies are large and it is certainly not possible to conclude whether type I or type II complexes are formed. Obviously, the situation is more complicated than for the $AdNH_3$ complexes. It is possible that the failure is due to the presence of both type I and type II complexes. These complexes may co-exist in solution and both contribute to the observed ROESY intensities. In the following, this hypothesis will be pursued.

Table 2 Comparison of ROESY Cross Peak Volumes to Distance Data from MD Simulations.

	Interaction with guest proton:		
Complex	P2	P3	P4
βCD:AdCOO			
$\langle r^{-6} \rangle$ H5/H3 (Type I)	0.20	0.64	1.10
$\langle r^{-6} \rangle$ H5/H3 (Type II)	5.30	0.40	0.54
ROE H5/H3	0.56	0.44	0.77
DMβCD:AdCOO			
$\langle r^{-6} \rangle$ H5/H3 (Type I)	0.23	0.85	1.39
$\langle r^{-6} \rangle$ H5/H3 (Type II)	4.36	0.43	0.57
ROE H5/H3	0.82	0.34	0.45
TMβCD:AdCOO			
$\langle r^{-6} \rangle$ H5/H3 (Type I)	0.14	0.76	1.12
$\langle r^{-6} \rangle$ H5/H3 (Type II)	5.72	0.43	0.70
ROE H5/H3	2.47	0.41	0.67

a) *Ratio of the time average of the r^{-6} distances between a given guest proton and the H3 and H5 host protons.*
b) Ratio of ROESY volumes for the cross peaks of a given guest proton, Pi, with the cavity protons, H5 and H3, of the cyclodextrin host

Each of the complexes in solution contributes to the observed ROESY intensities. For a given cross peak, the ROESY signal is a weighted average of the individual contributions. When type I and II complexes are present the measured ROE volume is:

$$ROE = f \times ROE_I + (1 - f) \times ROE_{II} \qquad (2)$$

where f is the fraction of complexes present as type I complexes, and ROE_1 and ROE_{II} are the contributions from type I and II complexes, respectively. Inserting the expected r^{-6} dependency of the ROESY intensities yields:

$$ROE = k \times [f \times \langle r^{-6} \rangle_I + (1 - f) \times \langle r^{-6} \rangle_{II}] \quad (3)$$

where *k* is a proportionality constant and the *r*'s are the inter nuclear distances obtained from the MD simulations. Equation 3 applies to each pair of interacting nuclei so ROE is an array of the experimental ROE volumes while $\langle r^{-6} \rangle_I$ and $\langle r^{-6} \rangle_{II}$ are arrays of the corresponding r^{-6} distances. Now we wish to determine the values of *f* that best reproduce the experimental ROE volumes. This was achieved by least squares regression of equation 3 to the experimental ROE's, yielding *f* and *k* as regression coefficients.

Figure 5 shows that a mixture of 52% type I complexes and 48% type II complexes to a large extent can account for the experimental ROESY volumes in the spectrum of the TMβCD:$AdCH_2COO$ complex. The standard deviation on *f* is low, only 7%, and strengthens the conclusion that TMβCD:$AdCH_2COO$ exists as both type I and type II complexes in approximately equal amounts. Table 3 shows that for most of the complexes with negatively charged guests type I and type II complexes co-exist, at least if one standard deviation on *f* is considered statistically sufficient. In the case of DMβCD:$AdCH_2COO$ the peaks of P2 and P4 overlap and their ROESY interactions cannot be separated. As P2 and P4 are found in opposite ends of the adamantyl moiety their overlap makes it difficult to determine the inclusion direction, thus the inconclusive result for this particular complex.

Figure 5 When 52% of the TMβCD:AdCH_2COO complexes are of type I and 48% are of type II the experimental ROESY volumes are nicely reproduced from the inter nuclear distances. Each circle represents a ROESY peak, a few of them are labelled. "3Me" refers to the 3-O-methyl group on TMβCD.

Table 3 Contribution of Type I and Type II Complexes to Observed ROESY Volumes.

Complex	*f*, fraction of type I complexes in % ± standard deviation	Free energy difference between type I and type II complexes (kJ/mol)
βCD:AdCOO	74 ± 12	-2.6 ± 1.6
DMβCD:AdCOO	50 ± 13	0.0 ± 1.3
TMβCD:AdCOO	30 ± 17	2.1 ± 2.2
βCD:AdCH_2COO	72 ± 16	-2.3 ± 2.2
DMβCD:AdCH_2COO	111 ± 21	NA
TMβCD:AdCH_2COO	52 ± 7	-0.2 ± 0.7

Summarizing discussion

The combined ROESY and MD approach showed that the positively charged $AdNH_3$ guest exclusively forms type I complexes while both types of complexes are formed with the negatively charged AdCOO and $AdCH_2COO$ guests. The preferred orientation of charged guests has previously been rationalized based on the dipole moments of host and guest.[37] Natural CDs have a small dipole moment with the positive end at the narrow opening and the negative end at the wider opening.[38] It is then favourable for the dipole moment of the guest molecule to align antiparallel to the dipole moment of the CD, meaning that cationic guests form type I complexes and anionic guests type II complexes, as observed for *p*-substituted derivatives of tert-butylbenzene.[37] Anionic guests such as dibenzofuran carboxylate,[35] aromatic carboxylates,[39] and several bile acids[40–42] all seem to form type II complexes, where the negative charge protrudes from the narrow opening. However, the present study shows that negatively charged Ad derivatives form both type I and type II complexes. Most of the abovementioned conclusions were drawn on the basis of a qualitative analysis of ROESY spectra. Is it possible that a quantitative analysis, as in the present case, would have revealed the presence of both orientations? It is often anticipated that only one orientation is present, but maybe it is a common phenomenon that significant populations of both types of complexes are present.

It is remarkable that the type of host hardly influences the preferred direction of the guests, despite the significant structural differences between the natural βCD, the partially methylated DMβCD, and the per-methyleated TMβCD. Especially, the relative populations of type I and II complexes with anionic guests are not much affected by the host, meaning that the investigated structural modifications of the host only has a small impact on the relative free energies of the two orientations, as seen in Table 3. It is surprising that methylation of all hydroxyl group on the host CDs does not have a bigger impact on the free energy difference. After all, the carboxylate groups

are expected to interact quite differently with hydroxyl groups than methoxy groups. This supports the idea that specific interactions, such as hydrogen bonds between host and guest, are generally not very important for the stability of CD inclusion complexes in water. Rather, it is non-specific interactions, such as the classical hydrophobic effect[43] and the release of cavity waters[44], that drives the complexation.

Conclusion

The present study has demonstrated that a combined quantitative analysis of ROESY NMR and molecular dynamics simulations is a strong tool for the structural characterization of cyclodextrin inclusion complexes. It is shown that cationic adamantane derivatives are oriented in the cyclodextrin cavity with the positively charged group protruding from the wider opening. Anionic adamantane derivatives, on the other hand, do not have a strong preference for either of the two possible orientations, and complexes of both orientations co-exist. The quantitative analysis is in most cases able to estimate the relative populations of complexes where the negatively charged group protrudes from the wider and narrow opening, respectively. The degree of methylation of the cyclodextrin does not have a large impact on the guest orientation, although there seems to be a tendency that methylation makes it more favorable for the negatively charged group to protrude from the narrow opening.

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Supporting Information. Atom types and atomic charges of adamantane derivatives. Comparison of ROESY and $\langle r^{-6} \rangle$ ratios.

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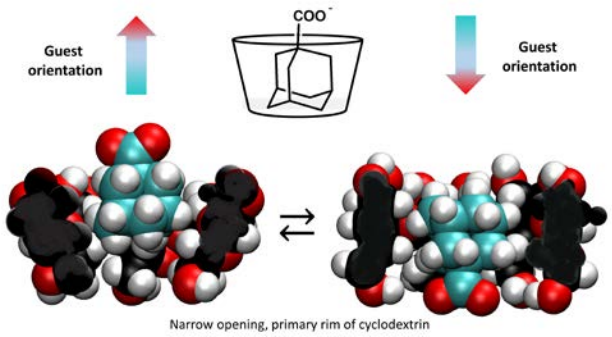
TOC Graphic


Guest orientation
COO⁻
Guest orientation
Narrow opening, primary rim of cyclodextrin