Comparative Proton Nuclear Magnetic Resonance Studies of Amantadine Complexes Formed in Aqueous Solutions with Three Major Cyclodextrins

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ABSTRACT: Host–guest complexes of alpha-, beta-, and gamma-cyclodextrins (α-CD, β-CD, and γ-CD, respectively) with amantadine (1-aminoadamantane, AMA; an antiviral agent) were characterized in aqueous solutions using proton nuclear magnetic resonance (NMR) spectroscopy. Host–guest molecular interactions were manifested by changes in the chemical shifts of AMA protons. NMR Job's plots showed that the stoichiometry of all the studied complexes was 1:1. Two-dimensional T-ROESY experiments demonstrated that the complexes were formed by different degrees of incorporation of the adamantyl group of AMA into the CD cavity. The mode of AMA binding was proposed. The AMA molecule came into the α-CD cavity (the smallest size) or β-CD cavity (the intermediate size) through its wide entrance to become shallowly or deeply accommodated, respectively. In the complex of AMA with γ-CD (the largest cavity size), the adamantyl group was also quite deeply inserted into the CD cavity, but it arrived there through the narrow cavity entrance. It was found that the adamantyl group of AMA was best accommodated by the β-CD cavity. The binding constants K_{aa} of the studied complexes (in M^{-1}), determined from DOSY NMR, were fairly high; their values in an ascending order were: α-CD (183) < γ-CD (306) ≪ β-CD (5150).
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Keywords: cyclodextrins; amantadine; complexation; inclusion compounds; NMR spectroscopy; ROESY; DOSY; diffusion; binding constants

INTRODUCTION

Alpha-cyclodextrin (α-CD) **1**, beta-cyclodextrin (β-CD) **2**, and gamma-cyclodextrin (γ-CD) **3** are natural macrocyclic oligosaccharides built of 6, 7, and 8 glucopyranose units, respectively (Fig. 1). These CDs are capable of forming host–guest inclusion complexes by entrapping small hydrophobic molecules (guests) in the hydrophobic cavity of the macrocyclic sugar (host).[1] The inside surface of the CD cavity is lined with glucose hydrogens H3 and H5, which protrude into this cavity forming two rings (Fig. 2a).[1] Complexation with CDs significantly modifies drug solubility, bioavailability, and stability.[2,3] Therefore, CDs have many practical applications in pharmaceutical formulations, especially as drug carrier systems[2,4,5] and solubilizing agents.[3,6]

A number of adamantane-based compounds show significant biological activity[7] and are used as active pharmaceutical ingredients (API).[8] The large, lipophilic adamantyl group of API can fit hydrophobic-binding sites on cell receptors and facilitate drug passage across the blood–brain barrier, a crucial route in the treatment of neurological diseases.[9] Various compounds containing the adamantyl group exhibit antiviral,[10] antimicrobial,[11] antiparkinsonian,[12] and neuroprotective properties.[13] 1-Aminoadamantane **4** (Fig. 2b), better known as amantadine or 1-aminoadamantane (AMA), is an antiviral agent used for symptomatic and prophylactic treatment of influenza type A.[14] AMA is an inhibitor of the proton-selective channel of the M2 protein of the influenza type A virus,[15] so it deteriorates viral replication. AMA is also an antiparkinsonian agent for treating extrapyramidal reactions.[13]

The inclusion complexes of CDs with adamantane[16] and various adamantane derivatives[17–19] have been studied using proton nuclear magnetic resonance (NMR) spectroscopy in aqueous solutions. Referring to the adamantane derivatives used in therapy, only the rimantadine complex with β-CD was precisely described using NMR.[20] Complexes of α- and β-CD with AMA,[21,22] and those of β-CD with memantine,[22] protonated 1-aminoadamantane,[23] and 2-aminoadamantane[23] have only been characterized using UV/Vis spectrophotometry. The binding constants K_a of CD–AMA complexes were estimated at 25°C using the UV/Vis method to give the following values: 1.1×10^5 or 7.9×10^3 for β-CD[21,22] and 271 ± 8 for α-CD.[21]

So far, the CD–AMA complexes have not been studied using solution NMR. They should be well characterized, because AMA is the simplest drug with the adamantyl group. For this reason, those complexes can serve as practical and convenient reference species in the complexation studies of other adamantyl-containing drugs. For us, the solution NMR data on the CD–AMA complexes are needed for comparisons with ongoing solid-state NMR structural studies.

Nuclear magnetic resonance spectroscopy is very useful in pharmaceutical analysis[24] and in drug design.[25] Several NMR techniques are well suited to investigating the structure, stoichiometry, and thermodynamics of CD complexes. They can provide essential information on host–guest complexation. CDs and their complexes with various molecules have been

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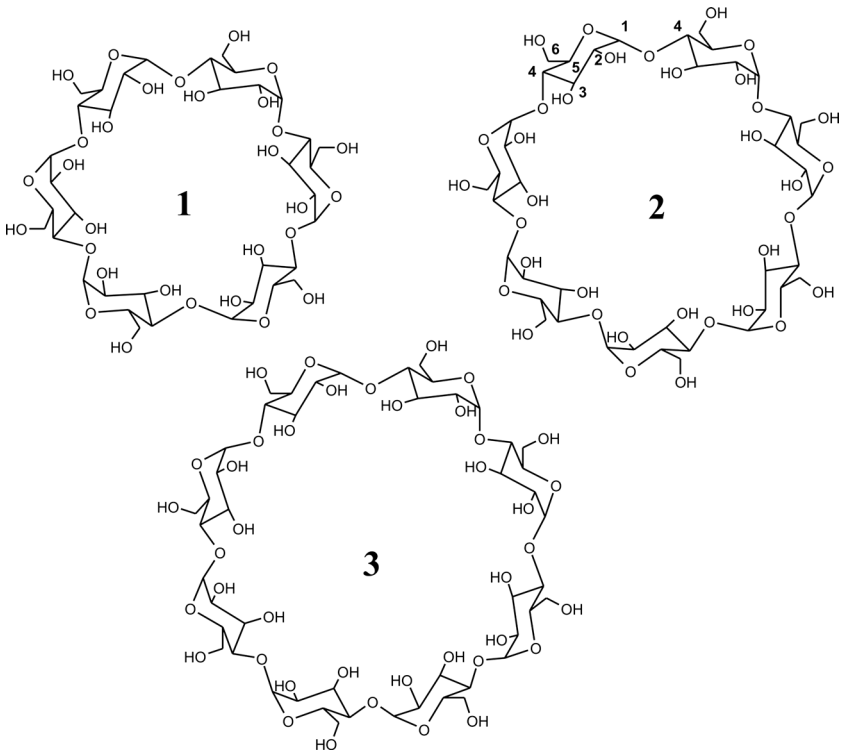

Figure 1. Chemical structures of three natural CDs: α-CD (**1**), β-CD (**2**), and γ-CD (**3**). The atom numbering shown for **2** is valid for all three CDs.

comprehensively investigated using solution and solid-state NMR.[26,27] As CD aggregation may prevent the expected inclusion of guest molecules into cavities, the occurrence of such inclusion should be definitively verified. The structures of true inclusion complexes, formed by adamantyl groups placed inside CD cavities, were confirmed by the observation of cross-peaks between the H-3 and H-5 of the CD and the protons of the guest molecule in ^{1}H–^{1}H NOESY and ROESY spectra.[19,20,28,29]

To date, CDs have not been used in pharmaceutical formulations of AMA. As AMA is poorly soluble in water, it has been applied as a hydrochloride or sulfate. Currently, its typical dosage forms are capsules or syrups for oral administration. The required solubility of AMA can also be achieved by complexation with CDs. Furthermore, such delivery might change the release kinetics of the drug, increase its bioavailability, improve therapeutic efficacy, and restrict side effects.[30] CDs can also enable other-than-oral routes of drug administration, for example, transdermal,[31] which is worth exploring for AMA. Obviously, adequate pharmacokinetic studies are needed, but the structural characterization of the CD–AMA complexes reported in this work is the first step. Generally, these complexes may become attractive alternatives to AMA salts.

The DOSY method to determine K_a is rather rarely used, so our methodology may be advantageous in other analytical studies of host–guest complexes of pharmaceutical interest. Furthermore, the NMR DOSY method is much simpler than the previously used UV/Vis method. Neither AMA nor its complex with CD has characteristic absorption bands, so the spectrophotometric determination should be carried out indirectly using an indicator such as methyl orange, which competes with AMA for the CD, thereby interfering in the complexation.[21]

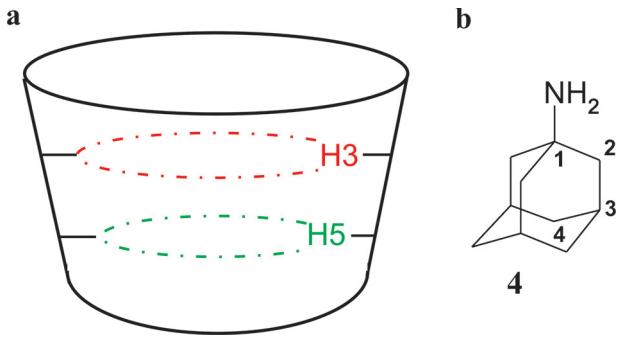

Figure 2. Schematic drawing of the macrocyclic CD host with marked internal proton positions[2] (a) and the structure of AMA (**4**) shown with atom numbering (b). The diameters of the cavities are[1]: 4.7–5.3, 6.0–6.5, and 7.5–8.3 Å for α-CD, β-CD, and γ-CD, respectively.

In this paper, inclusion complexes of AMA with three natural CDs **1–3** were characterized in water solutions using proton NMR. Our work was focused on the specificity and effectiveness of AMA binding to CDs with various cavity sizes. The free amine was chosen instead of the protonated amine form (AMA hydrochloride or sulfate), because the former guest is less hydrated and thus gives stronger complexes with CD hosts.[21] The stoichiometry of the three CD complexes with AMA was determined on the basis of chemical shifts changes (Job's method of continuous variation). Two-dimensional (2D) ^{1}H–^{1}H T-ROESY experiments allowed us to determine the mode of AMA complexation by the hydrophobic cavity of the CD host. The binding constants of the CD–AMA complexes were calculated using diffusion coefficients derived from DOSY measurements, providing information on complexation strength.

EXPERIMENTAL

Materials

Pure, solid AMA was obtained in our laboratory from AMA hydrochloride, purchased from Erba Lachema s.r.o. (Brno, Czech Republic). AMA–HCl was dissolved in water to give 1.4 M solution and then a slight excess of NaOH (pure p.a.) in 1.1 M solution was added under stirring. A white precipitate of the free amine was formed. This mixture was extracted twice with chloroform (pure p.a.). The organic solution was dried with solid KOH, filtered, and then the solvent was evaporated. The solid white precipitate of AMA was dried over 13 h at 45°C in vacuum; m.p. 180°C–181°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (broad s, 2H, NH_2), 1.53–1.69 (m, 12H, H2, and H4), 2.01–2.09 (m, 3H, H3); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 30.0 (C-3, CH), 36.5 (C-4, CH_2), 46.4 (C-2, CH_2), 47.5 (C-1, *C*-NH_2); ^{13}C CP/MAS NMR δ 30.6 (C-3, CH), 37.1 (C-4, CH_2), 47.1 (C-2, CH_2), 47.6 (C-1, *C*-NH_2) (see Supp. Information).

Cyclodextrins **1–3** of pharmaceutical grade were purchased from CTD, Inc. (Alachua, Florida) and used without further purification.

Deuterium oxide for NMR measurements (99.8 atom % D) was purchased from ARMAR Chemicals (Döttingen, Switzerland).

Conventional and T-ROESY Proton NMR Experiments

Conventional proton 1D NMR experiments for Job's plots were carried out at 298 K using a Varian VNMRS spectrometer (Varian, Inc., NMR Systems, Palo Alto, California) operating at 300 MHz for protons, using the standard Varian VnmrJ software library (VnmrJ version 2.1B Software from Varian, Inc., NMR Systems, Palo Alto, California). One-dimensional proton spectra were acquired with 64 scans and the relaxation delay of 1 s. A typical Job's plot analysis was carried out to determine the stoichiometry of the complexes.[26,32] For each CD, nine solutions for NMR experiments were prepared by mixing the AMA stock solution (2.51 mM) with the CD stock solution (2.51 mM) at the following volumetric ratios: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. Thus, the total concentration of AMA and CD was kept constant. The samples were prepared directly in NMR tubes. The pH level was monitored potentiometrically and remained quite stable within the range of 10.4–10.9.

Correlation 2D T-ROESY[33] measurements were carried out at 298 K on a Varian VNMRS spectrometer operating at 500 MHz for protons, using the Varian VnmrJ software. Samples for T-ROESY experiments were prepared by mixing the AMA and CD stock solutions (both 2.51 mM, see above) at a volumetric ratio of 1:1. The NMR experimental conditions were as follows: spectral width of 4000 Hz, acquisition time of 0.25 s, 64–128 transients per increment for 256 increments, a 5.2 kHz spin lock field centered at the water resonance, 300 ms mixing time, and 1024 complex data points in the F2 domain. The spectra were processed with Gaussian window functions in both dimensions.

All samples were dissolved in D_2O. As NMR standards (e.g., 4,4-dimethyl-4-silapentane-1-sulfonic acid, DSS) can form inclusion complexes with CDs,[34] internal referencing was ruled out for 2D spectra. Proton chemical shifts were measured relative to the water HOD signal, treated as a secondary standard (4.65 ppm from DSS). 3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt was used as an external reference to precisely determine proton chemical shifts for Job's plots.

Diffusion Proton NMR Measurements

Pulsed field gradient spin echo (PFGSE) measurements were performed at 298 K without spinning on a Varian VNMRS spectrometer, operating at 500 MHz for protons. A 5-mm Z7#x02010;SPEC Nalorac IDG500-5HT probe (Nalorac Corp., Martinez, California) equipped with an actively shielded z-gradient coil was used to give a maximum gradient strength of 61.1 G/cm.

^{1}H DOSY spectra were acquired using the Oneshot (stimulated echo sequence incorporating bipolar gradients) pulse sequence with 64–128 transients, 16 dummy scans, and a 20% imbalance factor.[35] The diffusion time (Δ) and the total diffusion-encoding gradient duration (δ) were chosen according to the value of D (Δ = 80 ms for AMA and 100 ms for all the complexes; δ = 2 ms). Sixteen values of the diffusion-encoding gradient were used, incremented from 1.9 to 50.4 G/cm in such steps that the strength of the next gradient was equal to the previous gradient squared. The spectral width was 8000 Hz (16,384 complex points) and the relaxation delay was 2 s. Processing was carried out using the Varian VnmrJ software with the option of correction for spatially nonuniform pulsed field gradients (DOSY presentation). Diffusion coefficients were determined by one component nonuniform gradient fitting using the SCORE[36] procedure from the DOSY Toolbox package (the free, open source software, version 0.54; 15 Mar 08) of M. Nillson.[37]

Determination of Binding Constants of the Studied Complexes

Cyclodextrins–AMA complexes undergo rapid equilibrium in solution, involving a free CD host and an AMA guest. In the case of 1:1 stoichiometry, this equilibrium can be expressed as follows:

$$\mathrm{CD} + \mathrm{AMA} \rightleftarrows \mathrm{CD-AMA} \tag{1}$$

In dilute aqueous solution, in which the activity coefficients are close to unity, the binding constant K_a is defined by the equation[38]:

$$K_a = \frac{[\mathrm{CD-AMA}]}{[\mathrm{CD}][\mathrm{AMA}]} \tag{2}$$

where [CD–AMA], [CD], and [AMA] are the molar concentrations of the complex, CD and AMA, respectively, in the equilibrium state. Therefore:

$$K_a = \frac{[\mathrm{CD-AMA}]}{([\mathrm{CD}]_0 - [\mathrm{CD-AMA}]) \times ([\mathrm{AMA}]_0 - [\mathrm{CD-AMA}])} \tag{3}$$

where $[CD]_0$ and $[AMA]_0$ are the initial concentrations of CD and AMA.

The most popular method for the determination of K_a of host–guest complexes, especially in supramolecular chemistry, is NMR titration.[39] Another approach applied in this work makes use of diffusion coefficients D (m^2/s) measured using DOSY NMR under a PFGSE.[39] The diffusion coefficients of uncomplexed guest molecules (D_{AMA}) and uncomplexed host molecules (D_{CD}) can be measured directly from appropriate aqueous solutions of the guest and of the host. However, the diffusion

coefficients of host–guest complexes ($D_{CD–AMA}$) cannot be determined that way. As the exchange rate between the free and complexed species is fast on the time scale of the NMR experiment, the observed diffusion coefficients of CD and AMA ($D_{OBS–CD}$ and $D_{OBS–AMA}$, respectively) have average values weighted by mole fractions of their bound and free molecules. It is also expected that in the case of a small guest molecule binding to a large host molecule, the D value of the latter species is only insignificantly affected by the complexation. Therefore, the diffusion coefficient of the CD–AMA complex and the observed diffusion coefficient of the CD host in the equilibrium state are assumed to be equal: $D_{CD–AMA} \cong D_{OBS–CD}$.[39,40] This simplification allows one to skip NMR titration and determine K_a from a single DOSY experiment.

Consequently, the observed diffusion coefficient $D_{OBS–AMA}$ of AMA in a solution containing CD can be expressed by the following equations[40,41]:

$$D_{OBS–AMA} = x_{AMA}D_{AMA} + x_{CD–AMA}D_{CD–AMA} \quad (4)$$

$$D_{OBS–AMA} = x_{AMA}D_{AMA} + (1 - x_{AMA})D_{OBS–CD} \quad (5)$$

where x_{AMA} and $x_{CD–AMA}$ are the mole fractions of uncomplexed AMA and AMA complexed by CD, respectively. Then, x_{AMA} can be derived from 5:

$$x_{AMA} = (D_{OBS–AMA} - D_{OBS–CD})/(D_{AMA} - D_{OBS–CD}) \quad (6)$$

to relate [CD–AMA] to $[AMA]_0$:

$$[CD - AMA] = (1 - x_{AMA})[AMA]_0 \quad (7)$$

by means of those diffusion coefficients. The substitution of Eq. 6 into Eq. 7, and then the insertion of the resultant equation into Eq. 3 leads to the final expression for the binding constant:

$$K_a = \frac{1 - \frac{D_{OBS–AMA} - D_{OBS–CD}}{D_{AMA} - D_{OBS–CD}}}{\frac{D_{OBS–AMA} - D_{OBS–CD}}{D_{AMA} - D_{OBS–CD}} \times \left([CD]_0 - [AMA]_0 + \frac{D_{OBS–AMA} - D_{OBS–CD}}{D_{AMA} - D_{OBS–CD}} \times [AMA]_0\right)} \quad (8)$$

RESULTS AND DISCUSSION

The Effect of Cavity Size on Complexation

Host–guest molecular interactions are manifested by changes in the chemical shifts of the protons involved in complex formation. Particularly large effects are usually observed for that part of the guest molecule, which is immersed in the CD cavity. For AMA, it is the adamantyl group that is expected to enter the CD cavity,[21,22] so the chemical shifts of H2, H3, and H4 were monitored (Fig. 2b). In the host molecules, complexation especially affects the positions of H3 and H5 signals from the protons located inside CD cavities (Fig. 2a).

According to Job's procedure, we recorded and analyzed ^{1}H NMR spectra of aqueous solutions containing AMA mixed with one of the three natural CDs. For each CD, the sum of CD and AMA concentrations was kept constant throughout a series of samples, whereas their molar fractions MF varied from 0.1 to 0.9. For comparison, corresponding solutions containing only uncomplexed AMA ($MF_{CD} = 0$) or uncomplexed CDs ($MF_{CD} = 1$) were also studied. With increasing MF_{CD} regular

Table 1. The Increments of the Proton Chemical Shifts of AMA, Observed for 0.9 mol Fractions of CDs

	Δδ (Hz)		
AMA Protons	α-CD	β-CD	γ-CD
H2	56.6	87.3	53.1
H3	33.1	83.2	27.7
H4	19.2	52.2	13.6

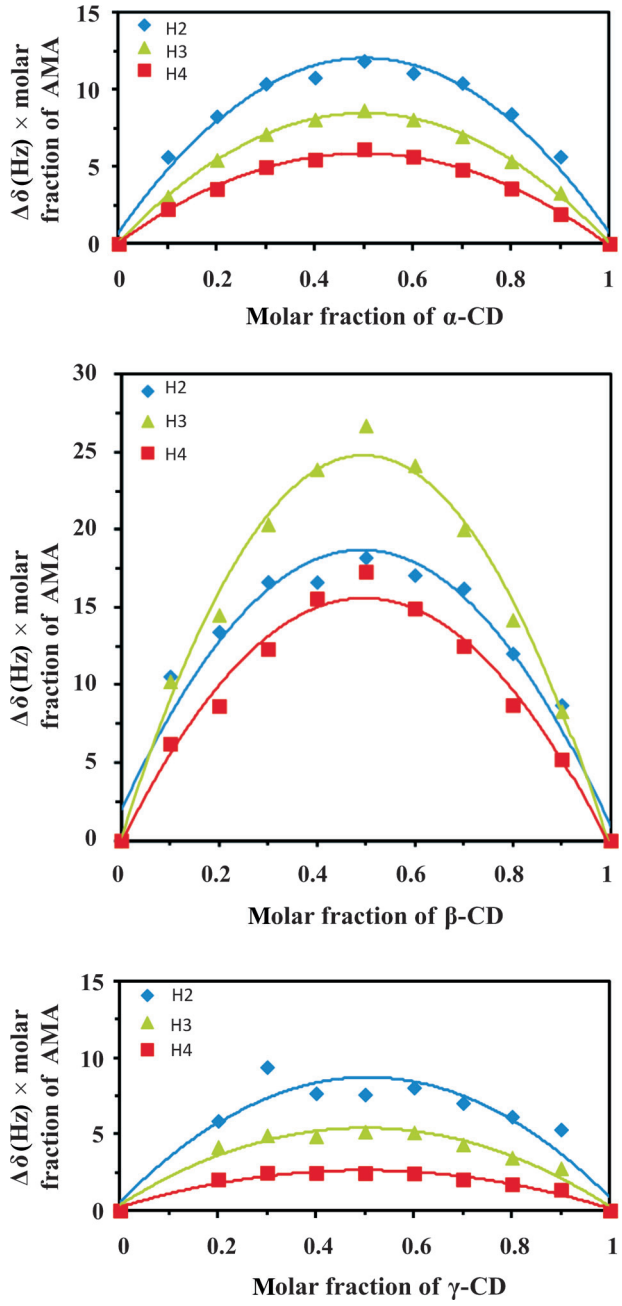

Figure 3. Job's NMR plots for H2 (♦), H3(▲), and H4(■) of AMA in the presence of α-CD (a), β-CD (b), and γ-CD (c).

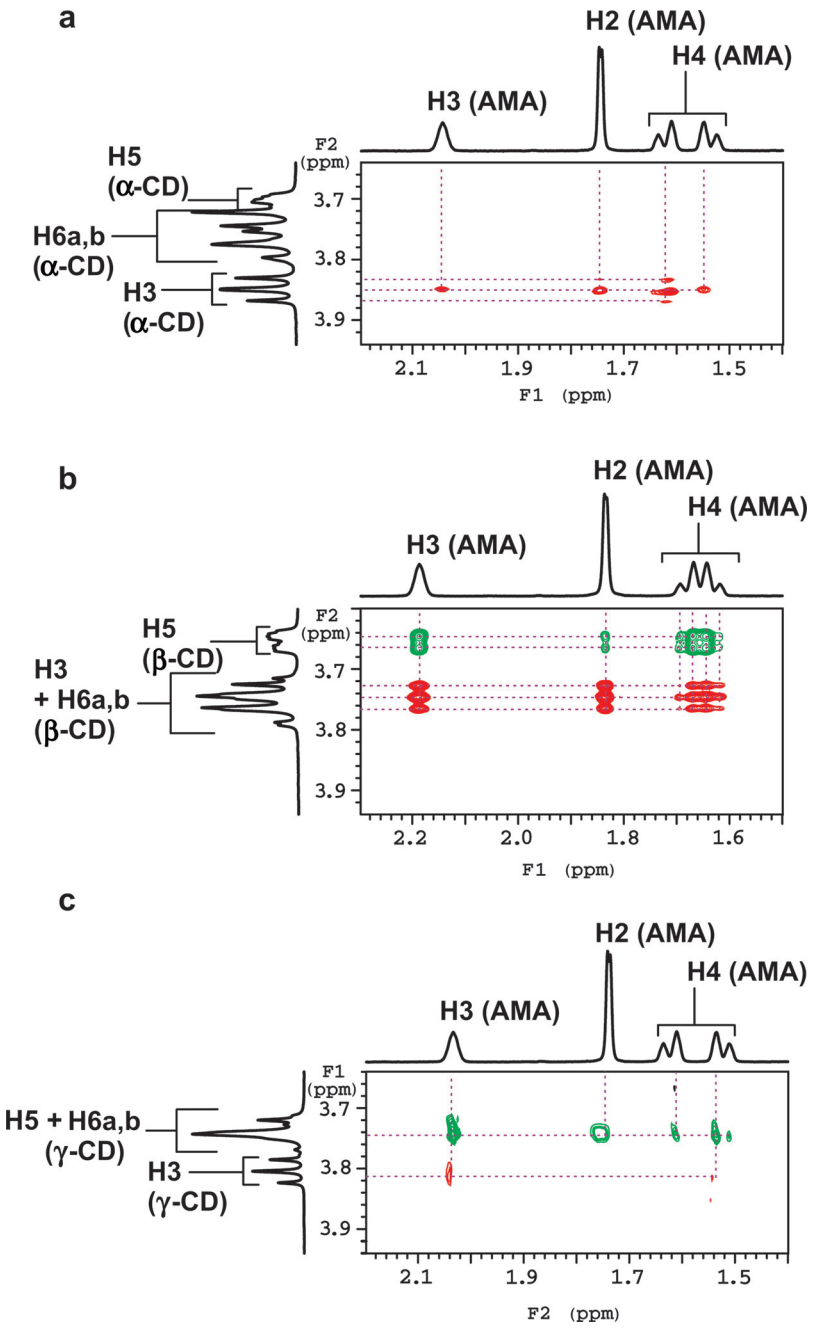

Figure 4. Selected regions of T-ROESY spectra of the studied complexes in D_2O: α-CD–AMA (a), β-CD–AMA (b), and γ-CD–AMA (c). The plots show ROE cross-peaks between the CD cavity peaks (H3 in red and H5 in green) and the adamantyl peaks of AMA.

changes Δδ:

$$\Delta\delta = \delta^{\mathrm{AMA}}_{\mathrm{observed}} - \delta^{\mathrm{AMA}}_{\mathrm{uncomplexed}} \tag{9}$$

were observed in the chemical shifts of AMA protons, which were caused by complexation (see Supp. Information). A positive Δδ value corresponds to a high-frequency shift. The determined Δδ values are indicative of adamantyl group insertion into the cavities of all three CDs.

The largest high-frequency shifts were found for H2, H3, and H4 of AMA complexed by β-CD at $MF_{\mathrm{CD}} = 0.9$ (Table 1). In contrast, the corresponding Δδ values were significantly smaller for complexes with α-CD and γ-CD, amounting to about half of those for β-CD. Therefore, it can be concluded that the strongest interaction occurs between AMA and β-CD. Thus, of the three studied hosts, the adamantyl group best fits the cavity of the intermediate-sized CD.

Amantadine complexation by CDs was also reflected by H3 and H5 chemical shifts of β-CD and γ-CD protons. The proton signals moved to a lower frequency upon complexation. However, analysis was hampered by an overlap of some resonances. For α-CD, chemical shift changes were too small to be observed with a 300-MHz spectrometer.

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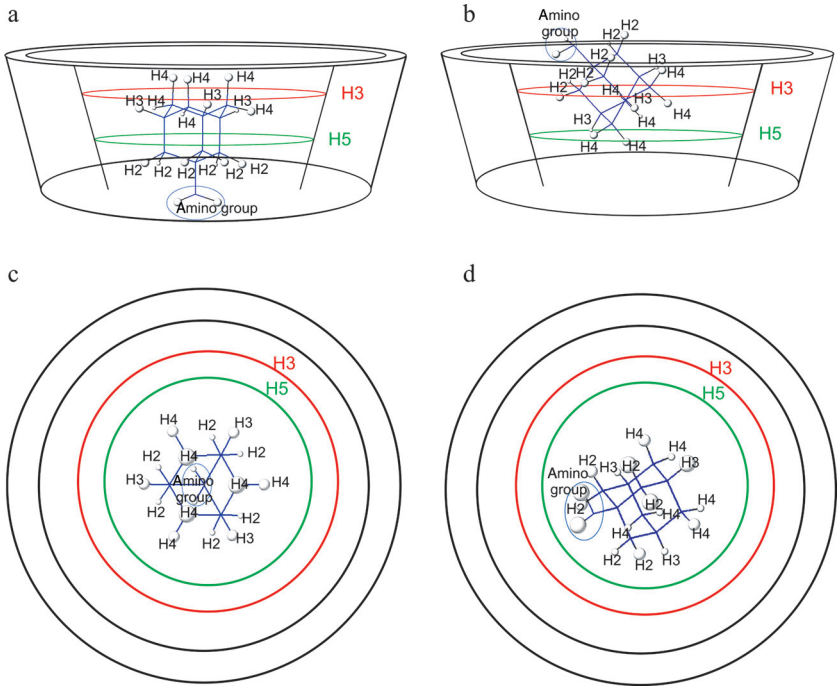

Figure 5. HyperChem-optimized structures of two possible γ-CD–AMA complexes: the adamantyl group enters the CD cavity through its narrow (a and c) or broad (b and d) entrance. The pictures present side views (a and b) and planar projections observed from the top along the cavity axis (c and d). For the sake of clarity, the structure of γ-CD has been simplified to a truncated cone, whereas the positions of the internal H3 and H5 protons are accurately represented by red and green circles, respectively.

Stoichiometry of the Studied Complexes Inferred from Standard Proton NMR

Cyclodextrins usually form 1:1 complexes with guests in aqueous solutions. However, other molecular ratios (2:1, 2:2, or more complicated configurations) are also possible, and such complexes can coexist with those falling under the simple 1:1 stoichiometry.[42] To find the stoichiometry of AMA–CD complexes, we have inspected the Job's NMR plots constructed using Δδ increments of AMA chemical shifts caused by the presence of CDs in aqueous solutions (Fig. 3). For each CD, a maximum was found for $MF_{CD} = 0.5$, which unequivocally confirmed the 1:1 stoichiometry of their complexes with AMA.

Structure of the Studied Complexes Inferred from Proton T-ROESY NMR

The arrangement of CD and AMA components in their host–guest complexes was studied using through-space NMR correlations. ^{1}H–^{1}H T-ROESY NMR experiments were carried out to determine how the AMA molecule can enter α-CD, β-CD, and γ-CD cavities and how deep inside it can settle. ROESY is a technique of choice for medium-sized molecules with a molecular weight of around 1000 D (such as CDs), for which NOE's are often close to zero and the resulting cross-peaks cannot be observed using NOESY.[43] In fact, a useful NOESY spectrum was only acquired for the strongest complex, that between AMA and β-CD (see Supp. Information). Figure 4 shows selected regions of the T-ROESY spectra recorded for equimolar solutions of AMA with α-CD (a), β-CD (b), and γ-CD (c). The 1D ^{1}H NMR spectra of those CDs are well known and have already been assigned.[26,44] Therefore, the assignment of ROE cross-peaks was straightforward.

Because of steric reasons, in the case of smaller CDs (α-CD and β-CD), the adamantyl group can only get into the CD cavity from the broader side of its truncated cone. For AMA complexed by β-CD, there are prominent ROE cross-peaks between the H3 and H5 peaks from the protons placed in the host cavity and the H2, H3, and H4 peaks from the adamantyl protons of the guest. In particular, the H5(CD)–H4(AMA) cross-peaks are indicative of deep incorporation of the adamantyl group of AMA into the β-CD cavity. Thus, this T-ROESY spectrum indicates that the adamantyl group best fits the cavity of β-CD.

For AMA complexed by α-CD (the smallest cavity size), there are only ROE cross-peaks between the H3 peak from the cavity protons of CD and all the adamantyl peaks of AMA. It can be inferred that in this case the AMA molecule is shallowly inserted into the CD cavity through its wider entrance and that complexation is weaker than with β-CD.

The T-ROESY spectrum of γ-CD–AMA solution (Fig. 4c) indicates that a complex has been formed and that the adamantyl group of AMA has been placed in the CD cavity. It is clear that adamantyl protons are involved in a dipolar interaction with the H5 protons of CD, as evidenced by their cross-peaks. H3(CD)–H3(AMA) cross-peaks are discernible, H3(CD)–H4(AMA) cross-peaks are hardly seen, and H3(CD)–H2(AMA) cross-peaks are absent. Such a cross-peak pattern is rather consistent with complex formation by the adamantyl group entering the CD cavity through its narrow entrance (Figs. 5a and 5c). The AMA molecule is probably deeply inserted in the CD cavity because the ring of H5 protons is wide enough to accommodate the adamantyl group. We infer that the complex of AMA with γ-CD is stronger than that with α-CD (see below). This unexpected effect can be caused by a different mode of complexation.

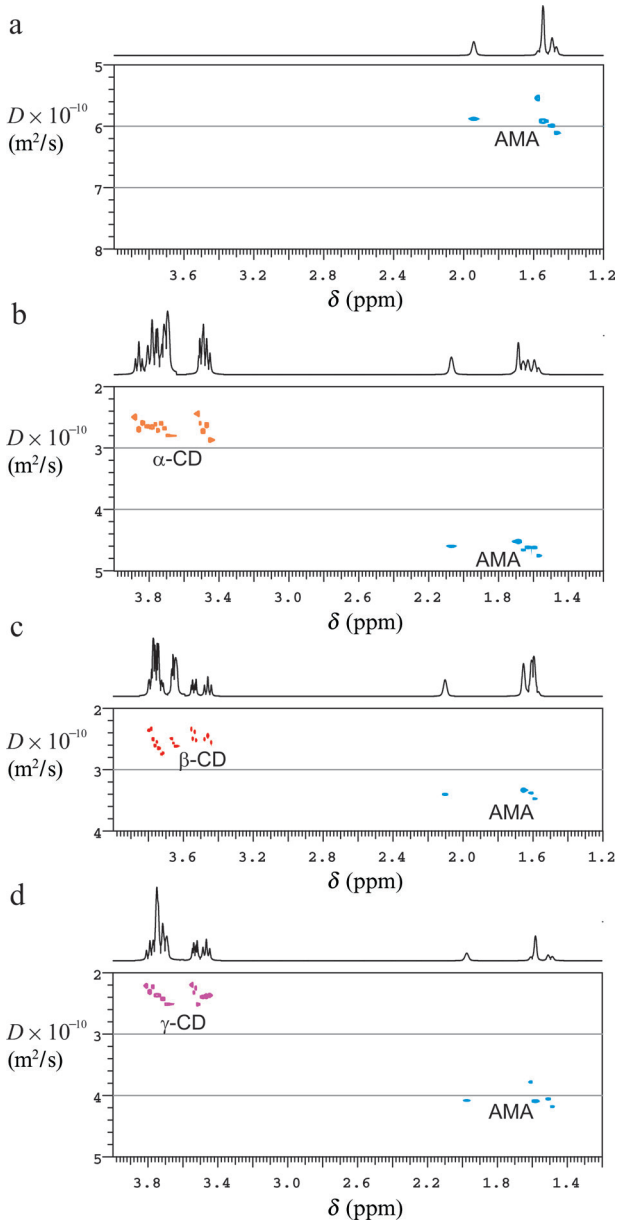

Figure 6. ^{1}H DOSY spectra of free AMA (a), AMA complexed by α-CD (b), AMA complexed by β-CD (c), and AMA complexed by γ-CD (d).

Computational studies of the interaction of drugs with CDs are rather fallible. Therefore, we carried out only simplified computations to visualize possible γ-CD–AMA complexes. To obtain a dependable graphical representation of those complexes, and compare their relative interproton distances, we prepared Figure 5 with the help of the HyperChem 8.0 program from Hypercube, Inc., Gainesville, Florida, (molecular mechanics package, AMBER force field). Previously optimized fixed geometries of γ-CD and AMA were used to verify their possible complexes. Water molecules were not taken into account. Two potential cases were considered. The first structure was created for the adamantyl group coming through the narrower entrance to the γ-CD cavity. In this case, the AMA molecule adopted a central position in the cavity with its long axis aligned with the CD axis and its amine group sticking out of the cavity (Figs. 5a and 5c). The second structure was created for the adamantyl group coming through the broader entrance to the γ-CD cavity. In this case, the AMA molecule adopted a tilted noncentral position inside the CD cavity with its amine group close to glucose hydroxyl groups (Figs. 5b and 5d). As it was stated before, only the first structure is consistent with the cross-peak pattern observed in the T-ROESY NMR experiment.

Binding Constants of the Studied Complexes Inferred from Proton DOSY NMR

The binding constants of AMA complexes with α-CD, β-CD, and γ-CDs were evaluated using NMR PFGSE.

Each DOSY spectrum displays PFGSE results in a 2D diagram with a conventional chemical shift spectrum in the first dimension and diffusion coefficients of the involved species in the second dimension. The DOSY spectra of uncomplexed AMA and its complexes with CDs in aqueous solutions are presented in Figure 6.

The diffusion coefficients inferred from the DOSY NMR measurements and the binding constants K_a of the studied complexes calculated from Eq. 8 are collected in Table 2. The K_a values are high and given here in the ascending order: α-CD < γ-CD ≪ β-CD. The results indicate strong complexation of AMA by CD, especially in the case of β-CD. Again, it was confirmed that the β-CD cavity can best accommodate the adamantyl group of AMA. It is noteworthy that the binding constant significantly decreases for the slightly larger γ-CD. The α-CD cavity is too small to enclose the adamantyl group, and so the corresponding binding constant is the lowest.

CONCLUSIONS

Our NMR findings clearly indicate that AMA can be efficiently complexed with all three natural CDs in aqueous solutions. The host–guest complexes exhibit 1:1 stoichiometry and are formed by means of the adamantyl group of AMA. The adamantyl group enters the CD cavity either through its broad (α-CD and β-CD) or narrow (γ-CD) entrance. In the former case, it is more

Table 2. Diffusion Measurements Data and the Determined Binding Constants K_a for AMA Complexes with α-CD, β-CD, and γ-CD

	Initial Concentration (M)				
	$[CD]_0$	$[AMA]_0$	D_{OBS-CD}[a] ($\times 10^{-10}$m^2/s)	$D_{OBS-AMA}$[b] ($\times 10^{-10}$m^2/s)	K_a (M^{-1})
α-CD	0.0050	0.0025	2.73 ± 0.03	4.61 ± 0.05	183 ± 26
β-CD	0.0025	0.0025	2.59 ± 0.03	3.41 ± 0.03	5150 ± 980
γ-CD	0.0050	0.0025	2.42 ± 0.02	4.09 ± 0.04	306 ± 32

[a] D_{OBS-CD} is the diffusion coefficient for appropriate CD in the presence of AMA.
[b] $D_{OBS-AMA}$ is the diffusion coefficient for AMA in the presence of appropriate CD.
The diffusion coefficient for uncomplexed AMA (D_{AMA}) was (5.97 ± 0.06) × 10^{-10}m^2/s (measured at the concentration of 0.0025 M).

(β-CD) or less (α-CD) deeply incorporated into the CD cavity. The binding strength is governed by cavity size with the β-CD cavity best accommodating the adamantyl group of AMA. The binding constants K_a (M^{-1}) of the studied complexes are relatively high; in the ascending order these are: $\alpha - CD(183) < \gamma - CD(306) \ll \beta - CD(5150)$.

Considering the above-mentioned conclusions, β-CD could be applied in pharmacy for encapsulation of AMA and other drugs containing the adamantyl group to improve their pharmaceutical performance.

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