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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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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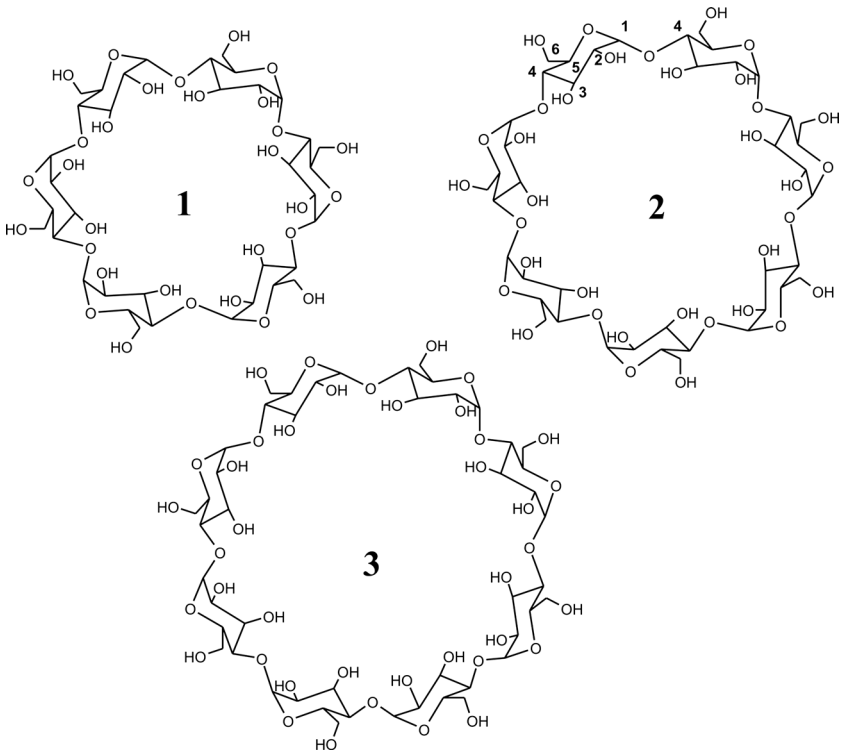

Figure 1. Chemical structures of three natural CDs: α-CD (**1**), β-CD (**2**), and γ-CD (**3**). The atom numbering shown for **2** is valid for all three CDs.

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]

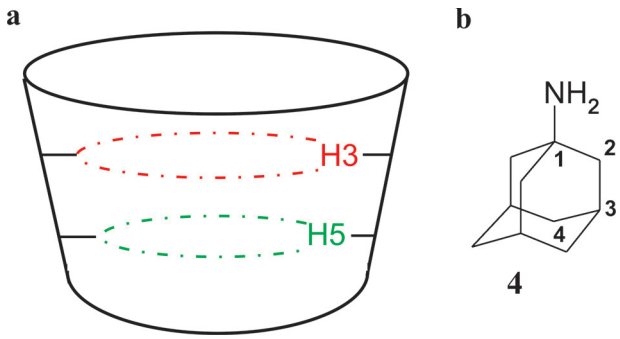

Figure 2. Schematic drawing of the macrocyclic CD host with marked internal proton positions[2] (a) and the structure of AMA (**4**) shown with atom numbering (b). The diameters of the cavities are[1]: 4.7–5.3, 6.0–6.5, and 7.5–8.3 Å for α-CD, β-CD, and γ-CD, respectively.

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.