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β-Cyclodextrin and 5-Methoxytryptammonium Ion Host-Guest Association *in vacuo*: Simulation of Non-Covalent Inclusion by Molecular Dynamics

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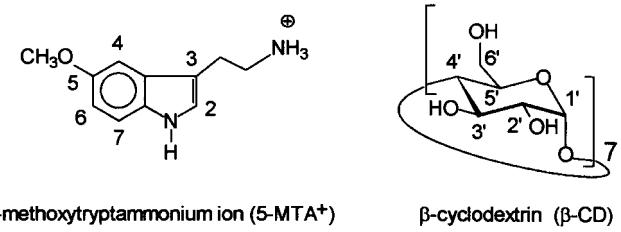
Abstract

The structure of gaseous charged host-guest adducts of cyclodextrins (CDs) generated by mass spectrometry (MS) still requires to be addressed correctly, as these adducts could be true inclusion complexes or non-specific proton bound heterodimers (PBHs). The present theoretical study of inclusion complex vs. PBH of 5-methoxytryptammonium ion (5-MTA$^+$) with β-CD *in vacuo* by energy minimisation and molecular dynamics proved that the most stable forms of β-CD/5-MTA$^+$ non-covalent association are inclusion complexes. PBHs evolved to genuine inclusion complexes with two different host-guest arrangements during MD runs.

Introduction

The major amount of structural studies of host-guest (h-g) complexes of cyclodextrins (CDs) [1] is focused on the solid state, via thermal [2] and X-ray diffraction methods [3], and in solution, via NMR spectroscopy [4]. In the last decade the investigation was extended to the gas phase, mainly by mass spectrometry (MS) [5] and molecular dynamics simulation [6]. The MS experiments using 'soft' ion evaporation processes allowed detecting gaseous charged species of h-g CDs complexes and provided molecular and/or chiral recognition evidence [7, 8]. However, these MS findings were regarded cautiously because of possible 'false positive' results [8, 10] as MS artefacts due to the formation of proton-bound heterodimers (PBHs), rather than true charged inclusion complexes. In hydrophilic solvents the formation of h-g complexes is essentially driven by the 'hydrophobic effect'. The basic question was then whether the same complexes could also exist as bare desolvated and charged gaseous associations in MS experiments. The charging of the 'neutral' h-g systems by electrospray ionisation (ESI) could indeed perturb the intrinsically weak h-g binding interactions and the following desolvation step could be too hard a process for the survival of the gaseous inclusion complexes [9]. Recently, some indirect evidence of the surviving capability of intact charged CDs h-g complexes in gas phase [8, 10–12] was reported. Stronger evidence was provided by two studies of the exchange reactions of the included guests with neutrals or hydrogen/deuterium with D_2O within the cell of Fourier transform ion cyclotron resonance mass spectrometers (FT-ICR-MS) [13, 14].



Scheme 1. Molecular formulae and atom numbering of guest and host.

The present paper reports energy minimisations and molecular dynamics (MD) simulations of the 5-methoxytriptammonium ion (5-MTA$^+$)/β-CD complex in the gas phase (Scheme 1). The complex was chosen as suitable model system for simulations as it was well characterised both in water solution by NMR [15] and in the gas phase by MS and tandem MS [12]. The simulations are aimed at providing information for interpreting the MS behaviour of CD h-g complexes by investigating the structural features and stability of two different types of 1:1 associations *in vacuo*: inclusion complex vs proton-bound heterodimer.

Methodology

All simulations were carried out with the package InsightII/Discover 2000, Molecular Simulations Inc. (San Diego, USA), using the consistent valence force field, CVFF. The initial geometries of both the β-CD and of the 5-MTA$^+$ ion were generated using available library fragments, and thoroughly minimised before studying their interaction. The minimisation of the initial geometries of the adduct was

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carried out adopting at first a harmonic, and then a Morse potential for bond stretching. The optimised geometries were in turn used as input for MD simulations for 500 ps ($T = 300$ K) with time steps of 1 fs, after the initial thermalisation of 30 ps. The coordinates obtained during the trajectories were saved every ps (a frame, henceforth), and minimised. Most dynamic runs showed significant conformational rearrangements of the host-guest adducts. Therefore, we carried out much longer dynamic simulations lasting 5 ns of the representative cases. All MD runs showed unambiguously that no other change took place after the initial 500 ps, thus indicating that the final geometries were indeed robust minima.

The geometries were analysed through the radial distribution function (RDF) plots using the frames saved during the MD runs. The RDF $g_{ij}(r)$ gives the probability density of finding atoms j at a distance r from atoms i. In the present case, $g_{ij}(r)$ is defined as

$$g_{ij}(r) = \frac{d\langle N_{ij}(r)\rangle}{dV(r)}$$

where $d\langle N_{ij}(r)\rangle$ is the average number of times atoms j are comprised in a spherical shell of thickness dr at a distance r from atom i. In this case, $g_{ij}(r)$ is also the average local density of atoms j in the shell volume $dV(r)$ comprised at a distance between r and $r + dr$ from atoms i.

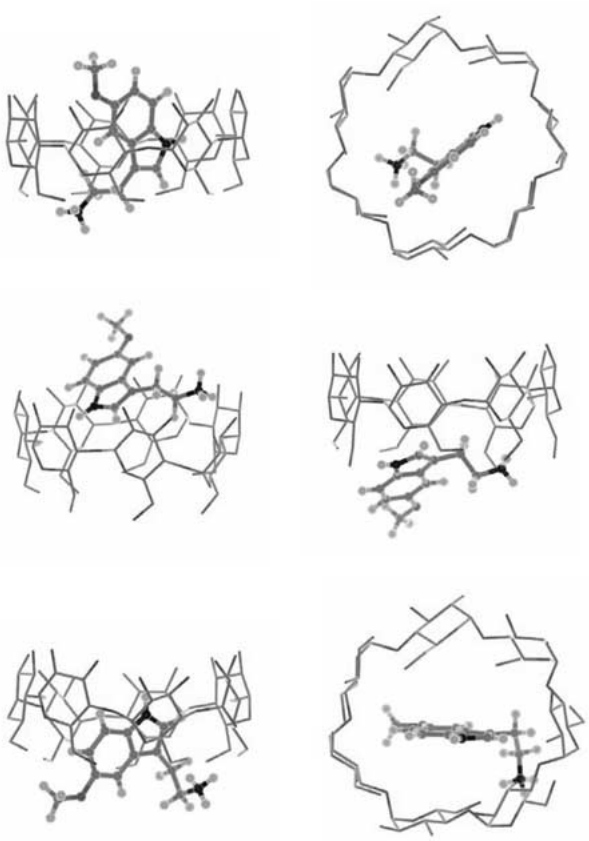


Figure 1. Top: geometry (∥ arrangement) of the absolute minimum after MD and energy minimisations (side and top view). A similar geometry was shown by the lowest-energy complex found after the initial minimisations. Middle: Examples of the external adducts corresponding to local energy minima having the guest ion above either the secondary (left) or the primary OHs rim (right). Bottom: geometry (⊥ arrangement) of the local minimum after MD and energy minimisations (side and top view). The colour codes are the same as in Scheme 2.

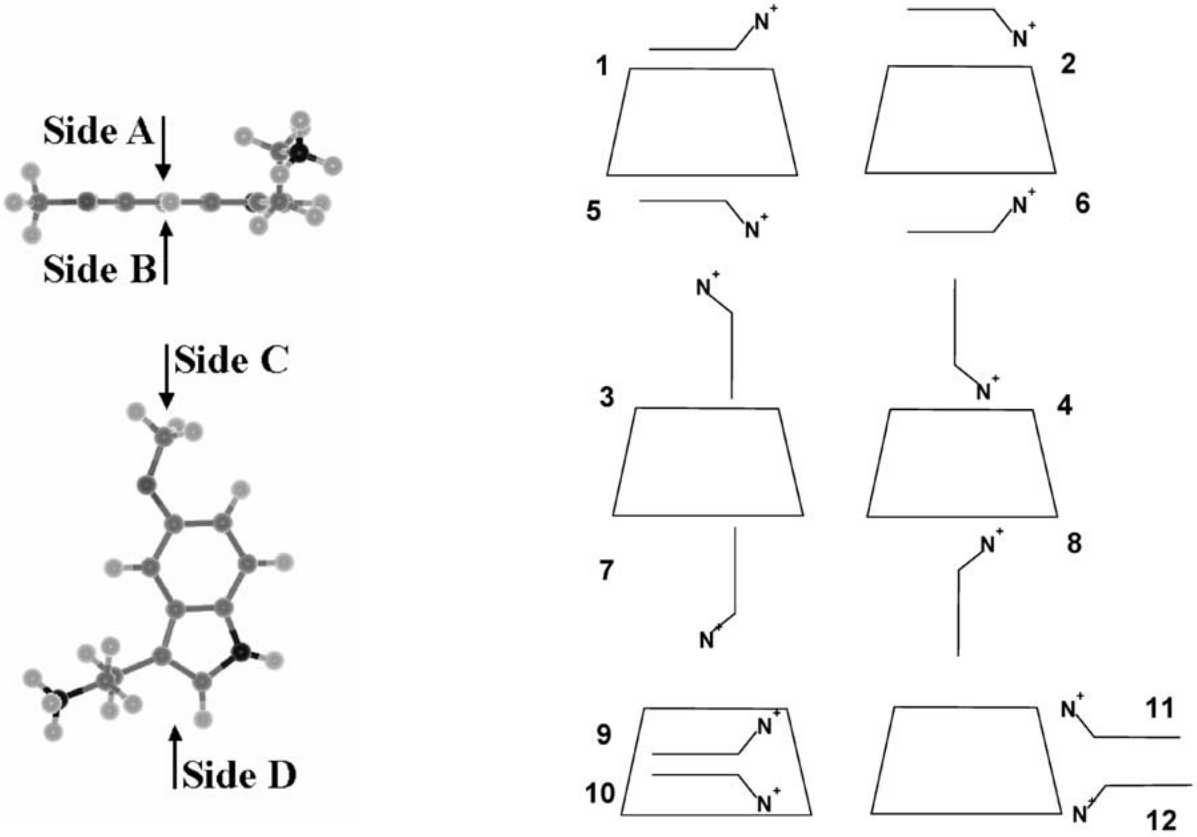
Results and discussion

Docking and preliminary energy minimisations

Twelve starting geometries of the host-guest adduct were generated according to Scheme 2. The results of the energy calculations and MD runs are summarised in Table 1. The lowest energy minimum in the initial optimisations was found by placing the 5-MTA$^+$ ion within the cavity with the ammonium group pointing towards the primary rim of β-CD. The energy minimised structure (entry 9 of Table 1) shows the guest ion aligned with the C_7 symmetry axis of the macrocycle and forming three hydrogen bonds involving the ammonium ion and the indole NH proton. This structural motif is also present in the global minimum (see next section and Figure 1 at top). On the other hand, the opposite orientation of the NH_3^+ group (position 10 of Scheme 2), leads to expulsion of the guest from the cavity and to a final geometry similar to what found when the guest ion is placed above the secondary OHs rim (middle of Figure 2).

When the guest is placed outside the β-CD above either rim, it enters the guest cavity if the methoxy group points toward the host molecule and the ammonium ion is far from the two rims. In this way, the aromatic rings are pulled inside the cavity, while the ammonium ion remains outside, close the either rim where it may form strong hydrogen bonds. In the resulting h-g compounds, a lower energy is achieved if the ammonium ion is close to the primary OHs rim, similar to the geometry discussed before. The opposite guest orientation with the ammonium ion close to the secondary OHs rim has a larger energy by almost 100 kJ mol^{-1}, and a correspondingly smaller interaction energy. The lower stability of the latter inclusion complex should be attributed to the formation of weaker H-bonds at the larger CD rim (by about 63 kJ mol^{-1}), and to the large deformation of the macrocycle imposed by such H bonds (a larger strain energy of about 34 kJ mol^{-1}), unlike what happens at the smaller rim.

Conversely, the initial geometries having the NH_3^+ group close to either rim do not lead to inclusion, and two sets of similar energy minima are found, with the 5-MTA$^+$ ion sitting above either rim, or showing at best a very loose inclusion (positions 1, 2, 4 and 5, 6, 8 in Scheme 2 and Figure 1 at middle). Note that in both geometries the adducts lack the van der Waals interactions within the cavity. Moreover, when the guest is located above the secondary OHs rim (entries 5, 6, 8 in Table 1) it forms fewer and/or weaker H-bonds. Finally, when the guest ion is initially located close to the lateral surface of β-CD, so as to mimic the arrangement of PBH, it does form a single H-bond with a hydroxyl group of the closer rim, either by shifting above the primary rim, or remaining at the side surface. In both cases, and in particular in the latter one, the total energy is quite large.



Scheme 2. Left: lateral and top view of the 5-MTA$^+$ ion, showing the four sides that can approach the host molecule. Colour codes: C, green; O, red; N, blue; H, grey. Right: sketch and numbering of the 12 starting geometries of the host-guest complexes for energy calculations and MD. The same numbering is used in the first column of Table 1.

Table 1. Summary of energy calculations (kJ mol^{-1}). The starting geometries are sketched in Scheme 2

Starting geometry	E_{tot} (In.)[a]	E_{int} (In.)[b]	E_{tot} (Fin.)[c]	$E_{int.}$ (Fin.)[d]	Final Final geometry
1	931.1	183.8	891.3	223.6	∥
2	915.5	199.4	881.7	233.2	⊥
3	888.5	226.4	857.9	257.0	∥
4	926.5	188.4	880.4	234.5	⊥
5	1014.6	100.3	862.6	252.3	∥
6	1014.0	100.9	857.5	257.4	∥
7	981.4	133.6	901.7	213.3	⊥
8	1020.3	94.6	862.2	252.7	∥
9	882.5	232.4	853.4	261.5	∥
10	1004.7	110.2	866.2	248.7	⊥
11	1014.8	100.1	889.8	225.1	⊥
12	1114.9	0.0	859.9	255.1	∥

[a] Initial total energy: energy of the complex after guest-to-host docking and minimisation of the assembly.
[b] Initial interaction energy: E (isolated host) + E (isolated guest) − E (complex). Calculated reference values: E (isolated host) = 760.7 kJ mol^{-1}; E (isolated guest) = 354.2 kJ mol^{-1}. Positive E_{int} values yield the energy required to separate host and guest to infinite distance.
[c] Final total energy: deepest energy minimum of the complex after MD and minimisation.
[d] Final Interaction energy: E (isolated host) + E (isolated guest) − E (complex deepest minimum).

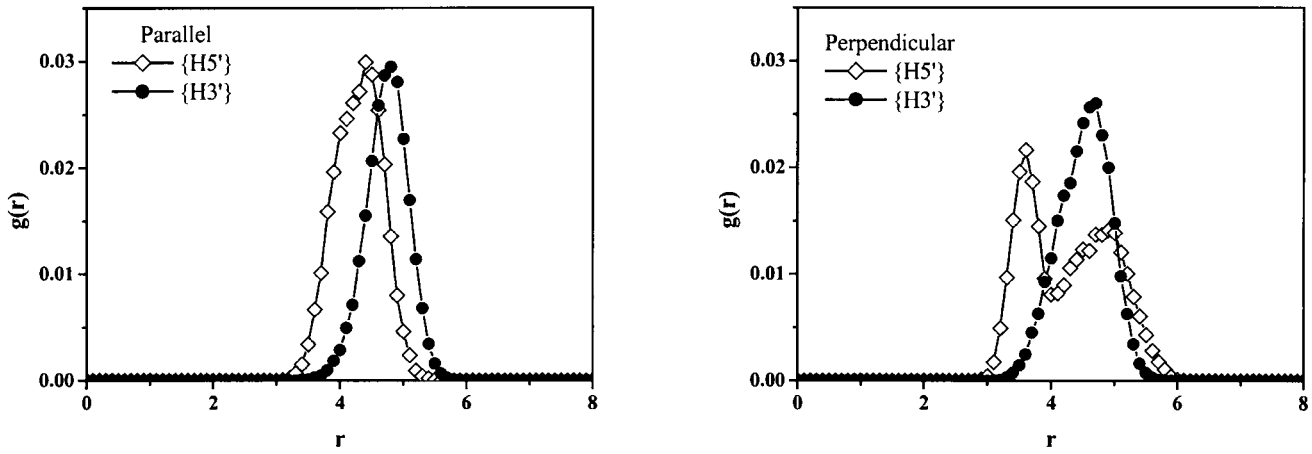


Figure 2. The radial distribution function $g(r)$ of the set of β-CD inner hydrogen atoms $\{H3'\}$ and $\{H5'\}$ as a function of the distance r from the centre of mass of β-CD for the parallel ($\parallel$, left) and the perpendicular ($\perp$, right) guest orientation (see text).

These preliminary energy minimisations suggest that we may have either true inclusion complexes, or simple electrostatic adducts with the 5-MTA$^+$ ion outside the cavity on either rim of the β-CD. The interaction energies of these conformations span a relatively large interval, but are significant in most cases.

MD simulations and final energy minimisations

All the optimised geometries were used as starting points for the MD simulations and subsequent minimisations. In this way, two types of inclusion complexes were found, where the guest ion is allocated with the main axis of the indole ring system either parallel (type $\parallel$) or perpendicular (type $\perp$) to the C_7 symmetry axis of β-CD. The lowest energy minima of each type proved to be very stable in 5 ns MD runs, thus showing to correspond to robust energy minima.

The most stable inclusion complex (Table 1, entry 9) belongs to type $\parallel$. Its structural features are similar to those of the lowest-energy complex obtained before the MD runs, though slight readjustments of the host-guest geometry allow the guest to better penetrate into the cavity and interact with the host. To within 13 kJ mol^{-1}, energy minima of the same type ($\parallel$) are also reached by all the complexes having the guest placed above the secondary rim as input for the MD runs (Table 1, entries 3, 5, 6, 8). This result shows that a genuine inclusion complex is also achieved by time evolution of a host-guest adduct initially lacking true inclusion. In the global energy minimum, the β-CD retains its average C_7 symmetry, and does not undergo significant distortions compared to its free state. This result can be shown through the single maximum of RDF plots of the sets of the inner CD hydrogen atoms $\{H5'\}$ and $\{H3'\}$ from the centre of mass of the macrocycle reported in Figure 2 (left). The interaction energy is due to a combination of van der Waals forces within the cavity, of dipolar interactions and of three H-bonds involving two hydrogen atoms of the ammonium ion and the indole NH.

A different host-guest arrangement is obtained in the MD runs having the guest ion in the perpendicular ($\perp$) orientation, with the aromatic rings inside the cavity, and both the ammonium ion and the methoxy group just outside the primary OHs rim (Figure 1, bottom). Such geometry is arrived at starting both with the guest above the primary rim and hosted within the cavity parallel to the C_7 axis with the ammonium ion close to the secondary OHs rim of the β-CD. This final arrangement in kept unchanged for more than 4.5 ns in long MD runs lasting 5 ns. The latter result indicates that this local energy minimum is again robust. A few quasi-degenerate h-g complexes are present in the perpendicular arrangement, with a total energy within 20 kJ mol^{-1}. The most stable among these local minima is larger than the absolute one by 27 kJ mol^{-1}, a difference mostly due to the strain associated with the ellipsoidal deformation of the β-CD at the primary rim, as displayed in Figure 1 (bottom). Correspondingly, the RDF plot of hydrogen atoms $\{H5'\}$ close to the primary OHs rim shows a twin peak (Figure 2, right), related to the shorter and to the longer semiaxis of the ellipse, unlike what shown by hydrogen atoms $\{H3'\}$.

Conclusions

A general feature of the present simulations *in vacuo* is that in both arrangements ($\parallel$ and $\perp$) the driving force for the host-guest interaction is due to the combination of van der Waals interaction of the aromatic system with the non-polar cavity and hydrogen bonds between the ammonium ion and the primary OHs of β-CD. Any adduct not showing inclusion of the aromatic rings has a larger energy, mostly due to the lack of relevant van der Waals interactions within the cavity. These results rule out a significant contribution of PBHs to the population of gaseous charged h-g adducts of CD detected by MS in favour of true inclusion complexes.

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