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Folding of a β-cyclodextrin monosubstituted at its secondary face, revealed by NMR studies of local structural variations

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Abstract

The ^{1}H NMR study of a CD mono-functionalized at its secondary face is presented. It is shown that mono-(3-Tyr-3-deoxy-*altro*)-β-CD undergoes a solvent-dependent conformational change. Passing from organic medium to water, a stable self-inclusion complex is formed. Such a folding is accompanied by a local structural modification of the substituted altrose ring, which can be easily detected from the important variation of its H1–H2 coupling constant.

1. Introduction

Among the different kinds of natural or artificial receptors, cyclodextrins (CDs) attract increasing attention due to their ability to encapsulate a wide range of guest molecules in aqueous solution.[1] To improve their properties, chemical modifications of CDs have been envisaged, mostly on their primary face. In particular, grafting peptides or other bioactive markers to CDs is an attractive approach for drug vectorization.[2–4] However, NMR studies revealed that precautions should be taken when such new carriers are designed, since the presence of tyrosine (Tyr) or phenylalanine (Phe) at the C-terminal position of a peptide grafted at the primary face of β-CD, results in occupation of the cavity by the aromatic side-chain, thus preventing the formation of intermolecular inclusion complexes.[5,6]

With the aim of facilitating the design of site specific molecular carriers we decided to extend these studies in the case of monofunctionalized CD at position 3, using **1** as a model compound.

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2. Results and discussion

Chemical modification of CDs at their secondary face is more difficult than at the primary one for reasons of lower reactivity and higher steric hindrance.[7] Compound **1** was synthesized via D'Souza's protocol.[8] Mono-(2-tosyl-2-deoxy)-β-CD was prepared with the aid of NaH in dry DMF and purified by flash chromatography using the NH_4OH (6%):EtOH:*n*ButOH (5:5:1 v/v) system. Treatment with aqueous ammonia leads to the formation of a 2,3-*manno*-epoxide, the diaxial opening of which results in mono-(3-amino-3-deoxy-*altro*)-β-CD. Coupling of the latter with BOC-Tyr via the DICP/HOBt method, followed by the acidic cleavage of the BOC amino protecting group (TFA, no scavenger) afforded compound **1** (charged intermediates and final product were purified with ion exchange chromatography).

Mono-*altro*-CDs lack the C_7 symmetry of the parent natural CD molecules. This, in addition to the limited range of the sugar proton resonances, results in complicated NMR spectra. Almost complete assignment was made by means of semisoft and classical two-dimensional experiments (Fig. 1), using the well-resolved (at 800 MHz) anomeric protons as starting point. The one amino-modified altrose and the six glucose units were named **A**, **B**, **C**, etc., according to IUPAC rules. Scalar couplings and dipolar interactions were then exploited to obtain the intra-residue attribution and the sequencing of the sugar units (Table 1 and Fig. 2).

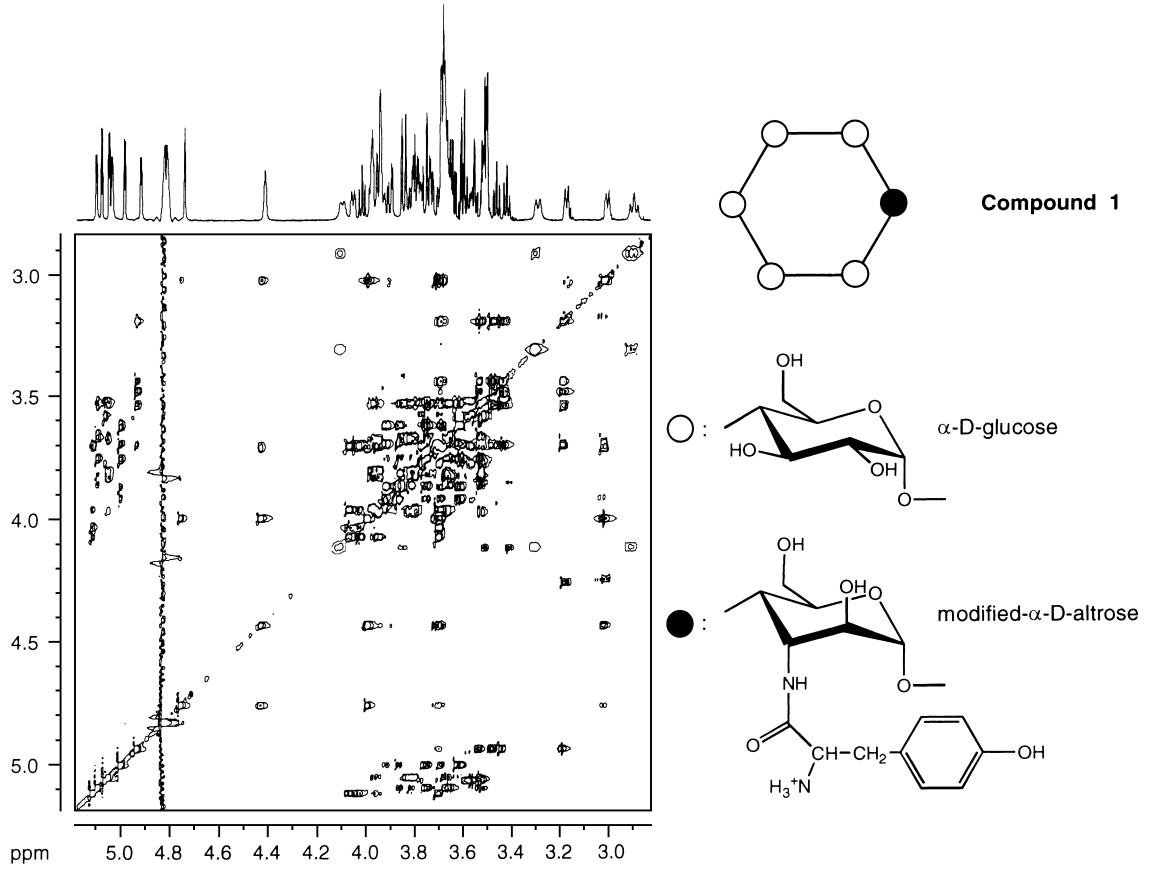

Figure 1. Part of the 800 MHz TOCSY map of compound **1** (shown at the right) in D_2O, at 291 K, used for spectral assignment. A 90 ms DIPSI-2 composite spin–lock sequence was used for isotropic mixing

Table 1
Chemical shifts (in ppm) of compound **1** protons in D_2O, at 291K

Pyranoside Unit	H1	H2	H3	H4	H5	H6,6'
A	4.746	3.982	4.420	3.985	3.011	3.691
B	5.052	3.555	3.580	3.509		
C	5.103	3.692	4.023	3.674	4.061	3.949
D	4.989	3.611	3.745	3.589	3.683	3.880
E	4.924	3.527	3.468	3.430	3.180	3.689
F	5.082	3.645	3.741	3.517	3.668	3.826
G	5.040	3.517	3.817	3.661	3.790	3.957

Tyr	Hα	Hβ	Hβ'	Hδ	Hε
	4.104	3.297	2.901	7.175	6.764

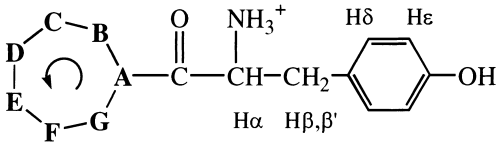

Figure 2. Graphical representation of the assigned sugar units of compound **1**. The cavity is seen from the primary side and the arrow indicates the direction of the α 1→4 glucosidic bond

The study of the homonuclear dipolar cross relaxation is essential not only for sequential assignment but also for the extraction of structural information. Inspection of the off-resonance ROESY[9] spectrum (Fig. 3) reveals the presence of through-space proximities between the Tyr aromatic and some CD non-anomeric protons, essentially H3 and H5. Since these protons are pointed towards the interior of the cavity, we can safely deduce that an inclusion complex is formed. Similar to mono-(6-Tyr-6-deoxy)-β-CD,[5] the aromatic side chain of compound **1** penetrates deeply into the CD cavity as it is indicated by the crosspeaks between Tyr aromatics and CD H5 protons, located close to the smaller rim of the host.

Further sample dilution or temperature increase (up to 80°C), did not substantially change the spectral characteristics suggesting that the inclusion complex should be an intramolecular one and quite stable. This was further confirmed with displacement experiments where up to 7 equivalents of phenol, a molecule imitating Tyr side chain, were progressively added to an aqueous solution of compound **1**. Again, the spectral characteristics of the latter remained essentially unchanged and off-resonance ROESY experiments showed that only Tyr aromatic protons are close to CD internal protons, indicating that compound **1** has a different behavior than the pendant-modified CDs.[10,11]

Comparison of the spectra recorded in aqueous solution and in DMSO (Fig. 4), a solvent which is known to expel most of the guests from CD cavities, shows a larger dispersion of the former one. This may be partially attributed to the ring current shifts provoked by the entrance of the aromatic ring in the CD cavity in a rather fixed position. Another marked difference is the splitting of altrose anomeric proton (unit **a**); from a well-resolved doublet (≥6.7 Hz) in organic solvent, we pass to a quasi singlet (1.4 Hz) in water.

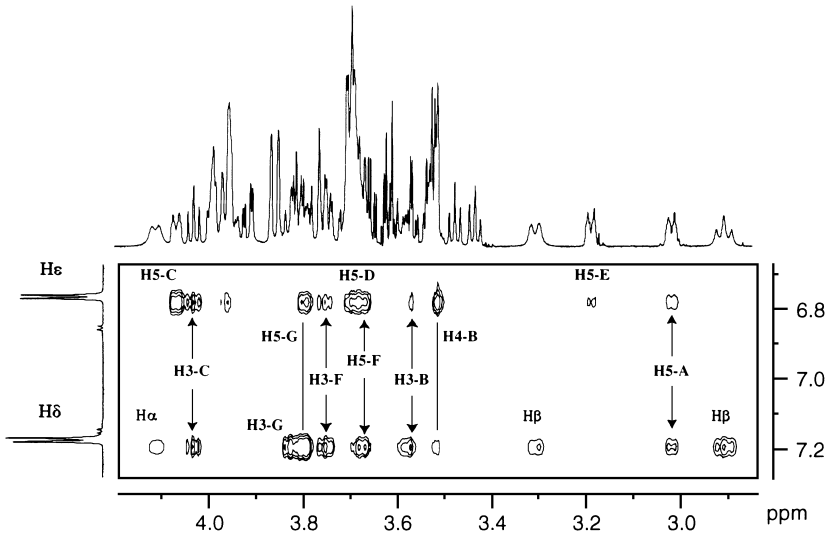

Figure 3. Aromatic–aliphatic region of the off-resonance ROESY map of compound **1** (the spectrum was recorded with a 150 ms spin-lock pulse) showing the through space dipolar interactions between Tyr aromatic and CD non-anomeric protons

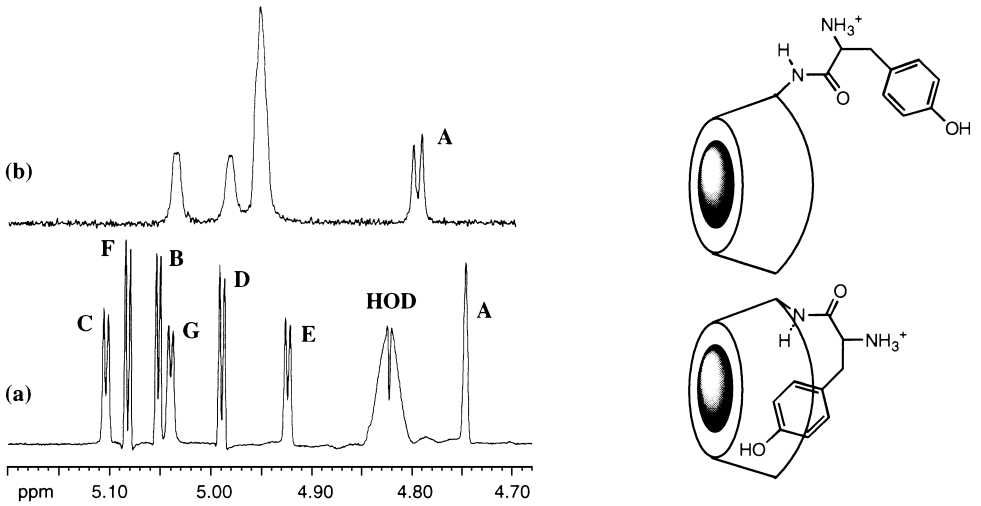

Figure 4. 800 MHz ^{1}H NMR spectra showing the anomeric region of compound **1** in water, at 291 K (a), and in DMSO, at 298 K (b). The corresponding structures are shown on the right

Searching the literature, we found that in other similar analogs[12–14] bearing a polar substituent at the position 3, the altrose unit exists essentially in the $^1C_4$ conformation. This should reflect a stabilization due to the existence of extensive intramolecular hydrogen bond pattern but also to the favorable equatorial disposition of the polar substituent, which puts it out of the hydrophobic cavity. Moreover, in our previous studies on CD homo- and heterodimers connected through their secondary side[15] we observed that when the linker is short or rigid or when DMSO is used as solvent, the altrose gives rise to $J_{12} = 7.6$ Hz corresponding to the $^1C_4$. In all these cases the CD cavities are empty. Contrary, when the linker of the dimers was flexible and long enough (i.e. a C8

chain) it was found to be included in one of the two CDs.[15] The altrose unit of the occupied cavity was moved to the 'less stable' $^4C_1$ conformation, characterized by a small $J_{12} = 1.7$ Hz. The other altrose coupling constants of compound **1** are compatible with the two chair conformations ($J_{23} = 10.5$ Hz in DMSO and $J_{23} = 3.1$ Hz in water), contrary to the case of α-cycloaltrin[16] where NMR studies in water have shown that a skew (twist-boat) $^0S_2$ form should not be neglected.

The reason of the transition of the altrose unit in compound **1** (from DMSO to water), is that $^4C_1$ conformation makes the substituent at position 3 axial and pointed to the interior of the cavity, thus facilitating the entrance of the Tyr side chain in it. From the other part, the free energy gain due to the self-complexation process serves to compensate for the conversion of the altrose to the otherwise 'less stable' $^1C_4$ conformation. It should be noticed that now the cavity is more distorted and this results in an additional increase of the spectral dispersion in water, relative to what is observed in DMSO.

Having determined the different structural behavior of compound **1** in water and in DMSO, we thought that an intermediate situation might occur with a coexistence of 'occupied' and 'empty' cavities. Titration experiments of the [D_6]DMSO sample with D_2O revealed that, at a certain mixture composition, doubling of many of the resonances occurs. Temperature dependence (Fig. 5) and ROESY experiments confirmed the two-site slow-exchange character of this phenomenon. Altrose H1 proton exist both as a singlet and a doublet, implying the equilibrium of $^4C_1$ and $^1C_4$ conformations. The different behavior of Tyr aromatic protons can be explained from the fact that self-inclusion provokes minor changes of Hδ environment since it is probably situated closer to the secondary rim of the cavity; contrary Hε protons can penetrate deeper into the cavity where the environment differs a lot of that of the bulk solvent.

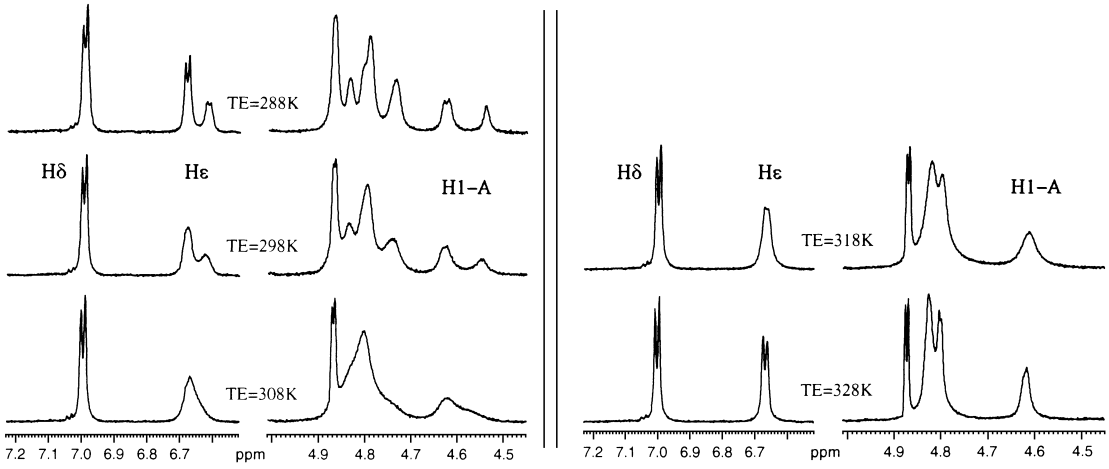

Figure 5. 600 MHz spectra of compound **1** in a [D_6]DMSO:D_2O mixture (40:15 v/v) at different temperatures

We should like to notice that these results and their explanation are in good agreement to those recently reported on the study of mono-*altro*-β-CD, free and as an intermolecular complex with adamantane-1-carboxylate.[17] The authors proposed an induced fit binding mechanism, since in their case the change of altrose conformation (less pronounced than here) can be safely attributed to an improved geometry of the cavity for a better fitting. In our case, due to the intramolecular character of complexation, we cannot quantify the influence of the new global geometry of the

cavity (more distorted than that of the natural β-CD) on the binding of a more planar guest, although we feel that it should contribute to the high stability of this complex.

3. Conclusion

The above-presented NMR studies are evidence of the self-complexation process that mono-(3-Tyr-3-deoxy-*altro*)-β-CD undergoes in aqueous solution as well as the important flexibility of its cavity. The easily measured altrose H1–H2 coupling constant can be used as a reliable indicator, capable of monitoring not only the local conformation of the altrose unit but also the global structure of substituted CDs at position 3. Moreover, we would like to emphasize the need for incorporating adequate spacers when peptides would be grafted at position 3 of CDs, for the construction of new target directed carriers.

4. Experimental

^{1}H NMR spectra were acquired on Bruker Avance DRX-800 and DRX-600 spectrometers equipped with triple resonance H/C/N inverse probes and three axes pulsed field gradient modules. Initial sample concentration was approximately 5 mM. In the semisoft experiments, H1 proton selection in the indirect dimension (F1) was achieved via Gaussian 90° or 270° and Gaussian cascade Q3 pulses, while non-anomeric protons were detected separately in F2 thanks to the digital filtering.

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