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Crystal structure of the inclusion complex of the antibacterial agent triclosan with cyclomaltoheptaose and NMR study of its molecular encapsulation in positively and negatively charged cyclomaltoheptaose derivatives

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Molecular structure

ABSTRACT

The inclusion complexes of triclosan with native cyclomaltoheptaose (β-cyclodextrin, βCD) as well as with negatively and positively charged derivatives are studied. The structure of the inclusion complex βCD/triclosan in the crystalline state [P1, $a = 15.189(5)$, $b = 15.230(6)$, $c = 16.293(6)$, $\alpha = 91.07(4)$, $\beta = 91.05(3)$ $\gamma = 100.71(3)$] comprises two crystallographically independent host macrocycles A and B. The packing results in βCD dimers that align head-to-head and form infinite channels along the *c*-axis. Only one guest molecule statistically disordered over two positions, (the dichlorophenyl ring in the cavities of either A or B) corresponds to each dimer (a 2:1 host/guest complex). The enclosed dichlorophenyl ring enters the dimer through the primary side, whereas the hydrophilic chlorophenol ring extends in the space between dimers. Water molecules in five positions are also enclosed in the intradimer region, arranged on a plane perpendicular to the sevenfold axis of βCD. The NMR spectroscopic studies in aqueous solution show the presence of both 1:1 and 2:1 βCD/triclosan complexes. In the first case, two different 1:1 complexes are simultaneously present, each with either ring entering the narrow primary side of one βCD molecule. In the 2:1 complex both rings of triclosan are included in two independent βCD hosts, a precursor to the supramolecular arrangement found in the crystalline form. In the case of the negatively charged sodium heptakis[6-deoxy-6-(3-thiopropionate)]-βCD, the NMR studies at pH 7.9 show a complete inclusion of triclosan inside the host in two orientations, one for the non-ionized (phenol) and reverse for the ionized (phenolate) form. Finally, for the positively charged heptakis(6-aminoethylamino-6-deoxy)-βCD, inclusion of triclosan is possible only when the pH is raised to 10 and it is concluded that both aromatic rings are alternatively inside the cavity. However in that case also, inclusion of the entire guest in the elongated cavity is suggested.

1. Introduction

Cyclodextrins (CDs), cyclic oligosaccharides with a relatively non-polar cavity and hydrophilic outer surface, are able to form complexes with lipophilic drugs by incorporating them in the cavity, if their dimensions meet the size restrictions imposed by the latter.[1,2] The most common pharmaceutical applications of CDs are to enhance the solubility and stability of the trapped drug in aqueous solution and decrease its toxicity.[2] In solution, the molecules of the inclusion complex are in equilibrium with the free constituents. The advantage of CDs over other means of solubilization, such as co-solvents, is that the drug's solubility is related to the CD concentration by a linear relationship,[3] an important property for dilutions. A number of papers have been dedicated to reviewing not only the stabilization and solubilization of drugs, but also the in vivo applications[3,4] and safety issues associated with their use.[5]

The guest molecule of concern in the present study is the powerful antibacterial and antifungal agent triclosan [5-chloro-2-(2,4-dichlorophenoxy)phenol] or Irgasan® (Chart 1a). Triclosan is commonly used in health care for controlling infection, for example, in soaps, deodorants and toothpastes (e.g., Colgate Total)[6] in Canada, EU, USA and other countries. Recent publicity about the occurrence of triclosan in human breast milk led to investigations about its toxicity, which concluded that the quantity found in the breast milk does not pose any risk to infants.[7] Recent solubilization studies of triclosan by cyclomaltoheptaose (β-cyclodextrin or βCD)[8] have shown that the macrocycle increases the drug's solubility 80-fold and that ionization of triclosan enhances significantly the complexation efficiency. Additionally, solubility and NMR studies with hydroxypropyl βCD (HPβCD)[9] and randomly methylated βCD (RMβCD), which have higher aqueous solubility than βCD, have suggested that at CD concentrations above 5.4% both HPβCD

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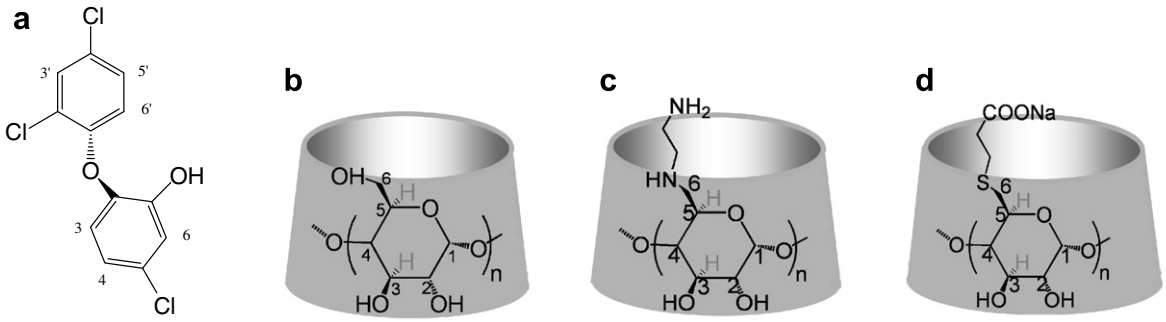

Chart 1. The structure and numbering of (a) triclosan (b) a βCD representation, (c) a **bpen** representation and (d) a **bpsp** representation.

and RMβCD form aggregates of complexes involving two to three CDs on average. Studies, on the other hand, have shown that a model system composed of a cationic polymer and the inclusion complex of triclosan with anionic sulfobutyl ether βCD (SBEβCD) could be used as a mucoadhesive sustained drug delivery vehicle under certain conditions,[10] since (a) it showed improved adhesion on the excised tissue underlying the porcine buccal mucosa surface, as compared to the neutral HPβCD and (b) this drug delivery system is much better retained on cation exchange media than on the uncharged system. Thus inclusion complexes of the drug in ionic βCD derivatives are of interest, since they could serve directly for cell or tissue attachment and improve the transport of the drug. The purpose of the present study is to investigate and compare the complexation modes of triclosan with βCD (Chart 1b), with heptakis(6-aminoethylamino-6-deoxy)-βCD (**bpen**, Chart 1c),[11] a βCD derivative positively charged at neutral pH, and with sodium heptakis[6-deoxy-6-(3-thiopropionate)]-βCD (**bpsp**, Chart 1d), a negatively charged βCD derivative. The inclusion complex structure of triclosan/βCD was determined by X-ray crystallography in the crystalline state and by NMR spectroscopy in aqueous solution. The charged CDs did not yield crystals with triclosan and the study was carried out in aqueous solution by NMR spectroscopy.

Table 1
Crystal data and structure refinement parameters

Molecular formula	$C_{98}H_{104}Cl_3O_{101.6}$
Formula weight	3013.76
Temperature (K)	100
Radiation/wavelength (Å)	0.80150
Space group	*P*1
a, α	15.189(5), 91.07(4)
b, β	15.230(6), 91.05(3)
c, γ	16.293(6), 100.71(3)
Volume (Å^3)/Z	3702(2)/1
Density (calculated) (Mg/m^3)	1.352
2θ range for data collection (°)	1.53–28.88
Index ranges	$0 \leqslant h \leqslant 18$, $-18 \leqslant k \leqslant 17$, $-19 \leqslant l \leqslant 19$
Reflections collected/observed $[F_o > 4\sigma(F_o)]$	12,369/12,083
Solution method	DIRDIF
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	12,369/29/1988
Goodness-of-fit on F^2	1.090
Final *R* indices $[F_o > 4\sigma(F_o)]$	$R_1 = 0.0658$, $wR_2 = 0.2028$
R indices (all data)	$R_1 = 0.0667$, $wR_2 = 0.2067$
Largest difference in peak and hole (e Å^{-3})	0.610 and −0.687

2. Experimental

2.1. Materials and methods

The βCD derivatives **bpen**[11] and **bpsp**[12] were prepared according to the published methods. Triclosan and βCD were obtained from Sigma–Aldrich and Jansen, respectively. All solvents were of reagent grade. Water was purified by the Purelab Plus (ELGA LABWATER) system. Buffer solutions were prepared from sodium dihydrogen phosphate–sodium hydrogen phosphate in H_2O or D_2O. The pH was measured using a MP200 Mettler Toledo pH meter. The temperature of the solutions during the experiments was maintained to ±1°.

2.2. X-ray crystallography

The complex was prepared by mixing equimolar quantities of βCD in water and triclosan in ethanol solutions. Upon mixing the two clear solutions, a suspension formed, which disappeared upon the addition of more ethanol. The clear solution was then placed in a water bath at 58 °C. Colourless crystals were obtained by slow cooling to 11 °C for a period of 11 days. Low temperature X-ray data were collected at the synchrotron radiation light source, beamline X13 of EMBL, at DESY, Hamburg, by the oscillation method using a CCD detector of 165 mm radius. A single crystal, covered with a drop of paraffin oil, was mounted on a hair fibre loop and was instantly frozen to 100 K. Crystal data and experimental details of X-ray analysis are given in Table 1. The programs DENZO and SCALEPACK[13] were used for data processing and scaling, respectively. The unit cell parameters and their esds were determined by the least square method from the high resolution frames of the collected data. The structure solution and the refinement were carried out with the programs DIRDIF[14] and SHELXL97,[15] respectively. The cyclodextrin non-hydrogen atoms were treated anisotropically. The refinement of the structure, by F^2 full matrix least squares, converged to $R_1 = 0.0658$, $wR_2 = 0.2028$ and Goodness-of-fit = 1.090, for $F_o > 4\sigma(F_o)$. Hydrogen atoms were placed at idealized positions and refined by the riding model ($U_H = 1.25U_C$). For the disordered CH_2 groups at position 6 of βCD, only H-atoms for the major orientations were used.

2.3. NMR spectroscopy

One- and two-dimensional NMR spectra were acquired at 500 MHz at 25 °C. ^{1}H NMR spectra recorded in D_2O were referenced to HOD at δ 4.79, unless otherwise stated. Continuous variation (Job) plots were constructed by mixing varying volumes of host and guest molecules to a constant final volume (0.5 mL) and total concentration of 3.45 mM. Triclosan was handled as a concentrated methanol solution (34.5 mM) that was diluted with D_2O and immediately mixed with a cyclodextrin solution at the proper ratio to avoid precipitation. In spite of that, slight to considerable cloudiness was observed when the [triclosan]/[CD] ratio exceeded 1. The resulting mixtures ranged from 90% to 10% ratio of host/guest.

NMR titrations were carried out in phosphate buffer (pH 7.9) in D_2O, where 5 mM solution of βCD or **bpsp** was titrated with triclosan, added as a concentrated (1 M) solution in $CDCl_3$ followed by purging with a stream of nitrogen. Again slight to moderate cloudiness was observed when the [triclosan]/[CD] ratio approached 1, which became significant thereafter. For ROESY experiments (298 K, with presaturation of the residual water resonance and a mixing (spin-lock) time of 350 ms at a field of ~2 kHz, using the TPPI method), D_2O solutions in phosphate buffer (pH 7.9) with equimolar quantities of the compounds were used, sonicated for 10 min and filtered (5 μm filter). Specifically, the concentrations before filtration were βCD (5.0 mM)/triclosan (5.0 mM); **bpsp** (4.0 mM)/triclosan (4.0 mM); **bpen** (4.0 mM)/ triclosan (4.0 mM) in D_2O (adjusted to pH 10.0 with NaOH).

3. Results and discussion

3.1. Crystal structure of the inclusion complex of triclosan in βCD

3.1.1. Geometry of the complex

Two crystallographically independent βCD hosts (A and B) and one guest molecule (Fig. 1a), disordered in two positions, comprise the asymmetric unit (host/guest ratio, 2:1). The numbering scheme of the host is given in Figure 1b, C(A or B)*mn* and O(A or B)*mn*, denoting the *m*th atom within the *n*th glucosidic residue (G*n*) of molecules A and B.

The stacking of the two crystallographically independent hosts A and B in the triclinic space group (Fig. 2) generates dimers (i.e., A and B′) held together by the usual seven H-bonds between the secondary hydroxyl groups.[17,18] The mean distance of the centre of mass of two consecutive dimers, as projected onto the O-4*n* mean plane, is 1.72 Å. Thus each dimer aligns almost on top of the dimer below to form an infinite channel. Each βCD dimer hosts one guest molecule, with the dichlorophenyl ring, either inside cavity A or B (otherwise some atoms of the chlorophenol ring located between the primary interface between A and B would overlap). Thus the guest is disordered in orientations **i** and **ii** (occupancies 0.5 each) occupying the dimers in a statistical fashion. The described mode of inclusion leaves one cavity in each dimer empty and it is not surprising that water molecules are located inside the dimers. The latters are found in the intradimer region (Fig. 2), arranged on a plane perpendicular to the axis of βCD and associate among themselves by H-bonding (Table 2). Their distances from the secondary hydroxyl oxygen atoms are rather long indicating very weak interactions,

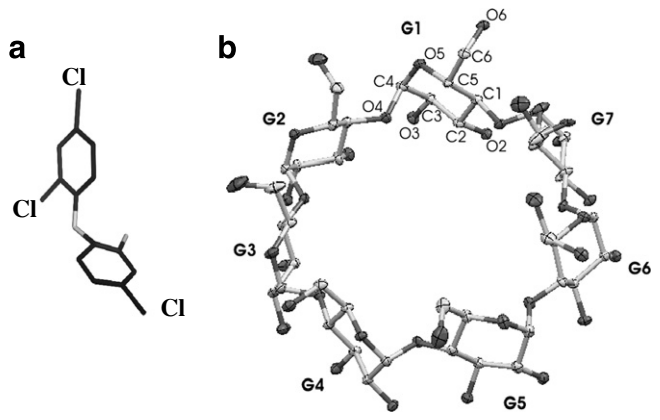

Figure 1. (a) Conformation of the triclosan molecule encapsulated in βCD (light grey colour corresponds to oxygen atoms); (b) ORTEP diagram[16] and numbering scheme of βCD (molecule A).

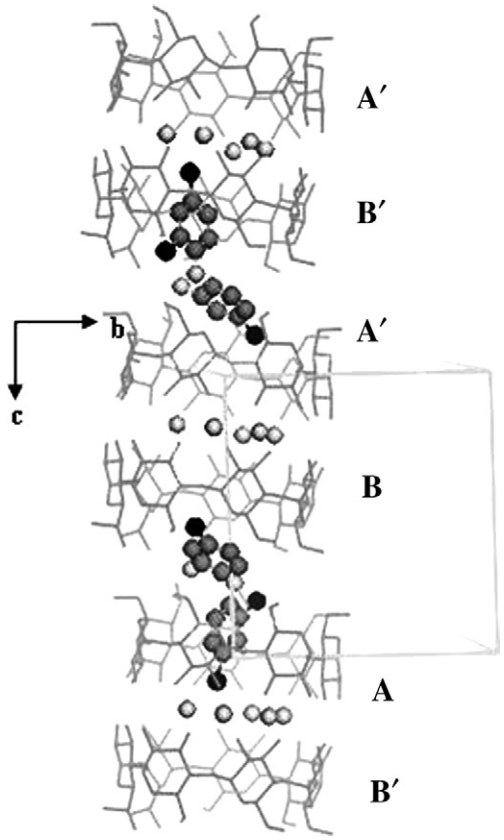

Figure 2. The crystallographically independent hosts A and B of βCD are stacked head-to-head along the *c*-axis forming an infinite channel. Shown also are the two guest orientations in different CD dimers and the water molecules inside the hydrophobic channel (colour code: grey, carbon atoms, light grey, oxygen atoms and black chlorine atoms).[19]

whereas the closest distance of two of them from the guest's chlorine atom is more than 3.40 Å.

3.1.2. Geometry of the host

The conformation of the βCD macrocycle is rather undistorted (see Supplementary data Table S1). The angles between the glucosidic oxygen atoms O4*n* do not differ significantly from 128.57°, the angle of the regular heptagon and the deviations of the O4*n* atoms from their optimum plane are very close to zero. The glucopyranose mean planes tilt very regularly towards the sevenfold axis. The glucopyranose residues adopt the regular $^4C_1$ chair conformation, as indicated by the puckering amplitude, *Q* (range of 0.557–0.586 Å for molecule A and 0.556–0.581 Å for B, respectively) and θ values (range 2.4–8.4° for molecule A and 2.6–7.9° for molecule B) calculated for the individual residues,[20] which are close to the ideal cyclohexane chair ($Q = 0.63$ Å, $\theta = 0$). As in all βCD dimeric complexes, the macrocycle conformation is stabilized through intramolecular hydrogen bonds connecting the O-3*n* and O-2($n-1$) atoms of neighbouring glucopyranose units (ranges of 2.70–2.83 Å and 2.71–2.83 Å for molecules A and B, respectively, are close to the average distance of O-3$n\cdots$O-2($n-1$) in native βCD, 2.78 Å). The dimer is formed via O-3*n*A and O-3*n*B H-bonds with distances ranging from 2.7 to 2.8 Å.[17] At the primary side, the C–OH bonds exhibit disorder and a variety of conformations as shown by the O5*n*–C5*n*–C6*n*–O6*n* torsion angles (Supplementary data Table S1): in two residues of both A and B, the O-6*n* atoms are disordered in two orientations with major occupancies 60% and 73% for G4A, G5A of molecule A and 58% and 73% for G-5B, G-6B of molecule B, respectively. In molecule A,

Table 2
Intermolecular interactions inside the cavity

(a) Hydrogen-bonds between guest's hydroxyl group and host's OHs			
O-*mn*···O-*m′n′*	Distance (Å)	C-*mn*–O-*mn*···O-*m′n′* (°)	O-*mn*···O-*m′n′*–C-*m′n′* (°)
O2C···O64a_1	2.65 (1)	133 (1)	133 (1)
O2C···O64b_2	2.62 (1)	132 (1)	132 (1)
(b) Distances of water molecules in the intradimer region with the host's secondary hydroxyl groups			
O-*mn*···O-*w* (Å)	Distance (Å)	C-*mn*–O-*mn*···O*w* (°)	
O36_2···O4W[11]	3.22 (3)	92 (1)	
O31_1···O4W[13]	3.27 (5)	101 (1)	
O37_2···O19W[10]	3.28 (2)	97 (0)	
O37_2···O1W[14]	3.22 (1)	145 (0)	
O31_2···O26W[10]	3.24 (6)	101 (1)	
O36_1···O26W[9]	3.27 (5)	92 (1)	
(c) H-bonds among water molecules in the intradimer region			
O-*w*···O-*w′* (Å)	Distance (Å)		
O4W···O19W	2.99 (4)		
O4W···O29W	2.50 (3)		
O19W···O26W	3.03 (7)		
O26W···O27W	2.55 (5)		

the two oxygen atoms, O64a and O65a of the major occupancies, point inwards [(+)-*gauche* with respect to the C5–O5 bonds], whereas all the rest point outwards [(−)-*gauche* with respect to the C5–O5 bonds]. Similarly, in molecule B, only the two disordered oxygen atoms, O-64b and O-65a with major occupancies, point inwards.

3.1.3. Geometry of guest and H-bonding interactions in the complex

The geometries of the two disordered guest molecules, **i** and **ii**, and their interactions with the hosts are very similar. The dichlorophenyl ring enters the cavity of one βCD molecule through the primary narrower side, almost parallel to the sevenfold axis of the macrocycle (dihedral angle with the O-4*n* mean plane, about 84°), whereas the quite hydrophilic chlorophenol moiety expands in the space between the primary sides between two macrocycles (Figs. 2 and 3). The dihedral angle between the two phenyl rings (Fig. 1a) is 82° and it is the same for both guests **i** and **ii**. The oxygen atom of the chlorophenol moiety forms H-bonds (Fig. 3) with two primary hydroxyl groups of the hosts pointing inwards, O64a and O64b (2.65 and 2.62 Å), respectively, Table 2.

The asymmetric unit of the βCD/triclosan complex contains 28.6 water molecules distributed over 54 sites in addition to 1 ethanol molecule, distributed over 2 sites. Of the total number of water molecules 2.8, distributed over five sites, are located inside the cavity forming a cluster, as mentioned above. The remaining are in the crystal lattice, along with the ethanol molecule, forming two water networks of H-bonds connecting the βCD channels,[21] one network linking the primary hydroxyl groups and the other linking the secondary ones (Supplementary data Table S2). There are also direct H-bonds between adjacent dimers laterally, between primary hydroxyl groups (O63···O67, 2.78 Å) and between secondary (O25···O27, 2.66 Å) connecting the host stacks and strengthening the crystal lattice. In contrast, besides the H-bonds between hosts and guest (Fig. 3), there are no close H-bonds between primary hydroxyl groups of the hosts along the channel (although disordered O64a and O65b atoms pointing inwards are close (3.18 Å), they are not in favourable position to form a mutual H-bond, because the C–O···O angles are close to 160°). Finally, some H-bonds among water molecules and glucopyranose O-5*n* atoms are observed (Supplementary data Table S2).

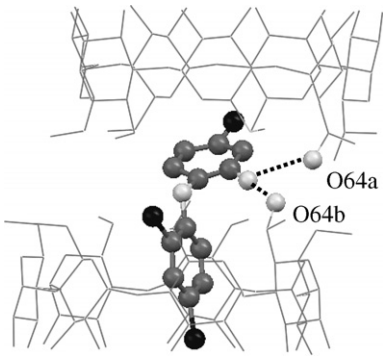

Figure 3. H-bonding between host and guest molecules in the complex.[16]

3.2. NMR studies

3.2.1. Inclusion complex of triclosan/βCD

Triclosan is practically insoluble in pure water (<1 μg/mL or 3.50 μM at room temperature),[8] however, its solubility improves upon addition of βCD. Shielding of the cavity protons H3 and H5 of βCD (Chart 1 for structure numbering) in the presence of triclosan confirmed molecular inclusion in solution. A similar result was obtained when triclosan was added to an aqueous solution of **bpsp**. In contrast, when triclosan was added to an aqueous solution of **bpen** at neutral pH, no dissolution was observed. When the pH was raised to 10, triclosan became slightly soluble (pK_a 7.9)[2] and small shielding of protons H3 and H5 to lower frequencies was observed. It is reasonable to assume that at neutral pH there is no inclusion event, whereas at alkaline pH the solubility of triclosan is increased due both to its ionization and to the formation of an inclusion complex with **bpen**. The latter, having p$Ka_1 \sim 6.4$ and p$Ka_2 \sim 9.5$[11] is mostly neutral at pH 10, which might also contribute to more efficient inclusion. Continuous variation plots were graphed from series of NMR data in order to estimate the stoichiometry of the complexes with βCD and with **bpsp**, whereas the limited solubility of the complex with **bpen** did not allow similar treatment. The maximum of the curve in the case of βCD/triclosan (Fig. 4) is not at 0.5 (for 1:1 complex) nor at 0.66 (for 2:1 complex) but at 0.6, indicating that complexes of both stoichiometries are simultaneously present in solution. A 1:1 stoichiometry is observed for **bpsp**/triclosan. In both cases at [triclosan]/[βCD] $\geqslant 1$ cloudiness was observed. Plotting of the shifts of triclosan, on the other hand, for both systems, gave peculiar curves evidently due to both poor solubility and aggregation effects of triclosan in the aqueous solution.[22] Secondary processes, for example, guest

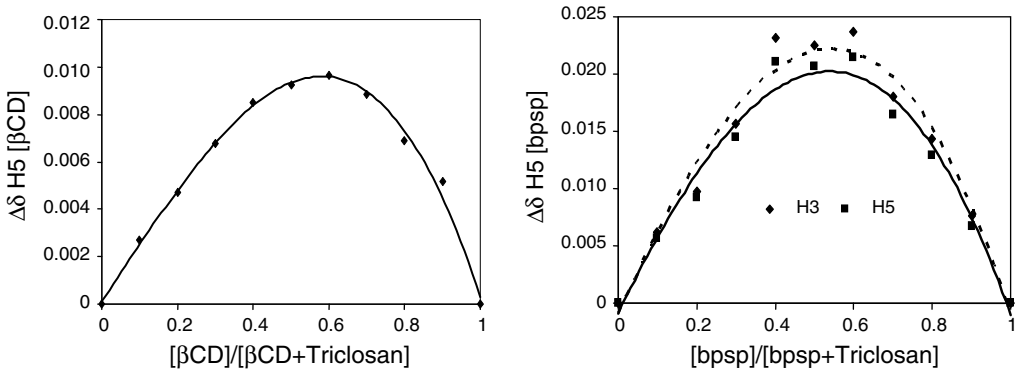

Figure 4. Continuous variation plots of triclosan with βCD (left) and **bpsp** (right). Solid and dashed lines are the results of polynomial regression for the experimental data points.

self-association,[22] are plausible as some of the signals of triclosan are shielded and some are dishielded in the presence of cyclodextrins. It is well accepted now that the observed chemical shift changes in both host and guest signals can actually arise from a combination of processes such as aggregation,[22] inclusion as well as conformational and orientational isomerization phenomena.[23] Finally additional external association may influence largely the properties of the host/guest system.[24]

Estimation of binding constants was attempted via titration experiments. For both βCD and **bpsp** (5 mM), cloudiness appeared upon titration with triclosan even before the 1:1 equivalence point, again rendering the results unreliable. The solubility of triclosan in the presence of 5 mM CDs can thus be estimated as ~3 mM. Examination of the binding modes was accomplished with 2D ROESY spectra (Fig. 5). For βCD/triclosan (Fig. 5a), it is clearly shown that dipolar interactions exist between the protons of both aromatic rings of triclosan (numbering scheme in Chart 1a) with cavity protons H3 and H5. This implies that both rings are alternatively inside the βCD cavity, thus two different 1:1 complexes are present in solution. From the strong interactions between all the protons

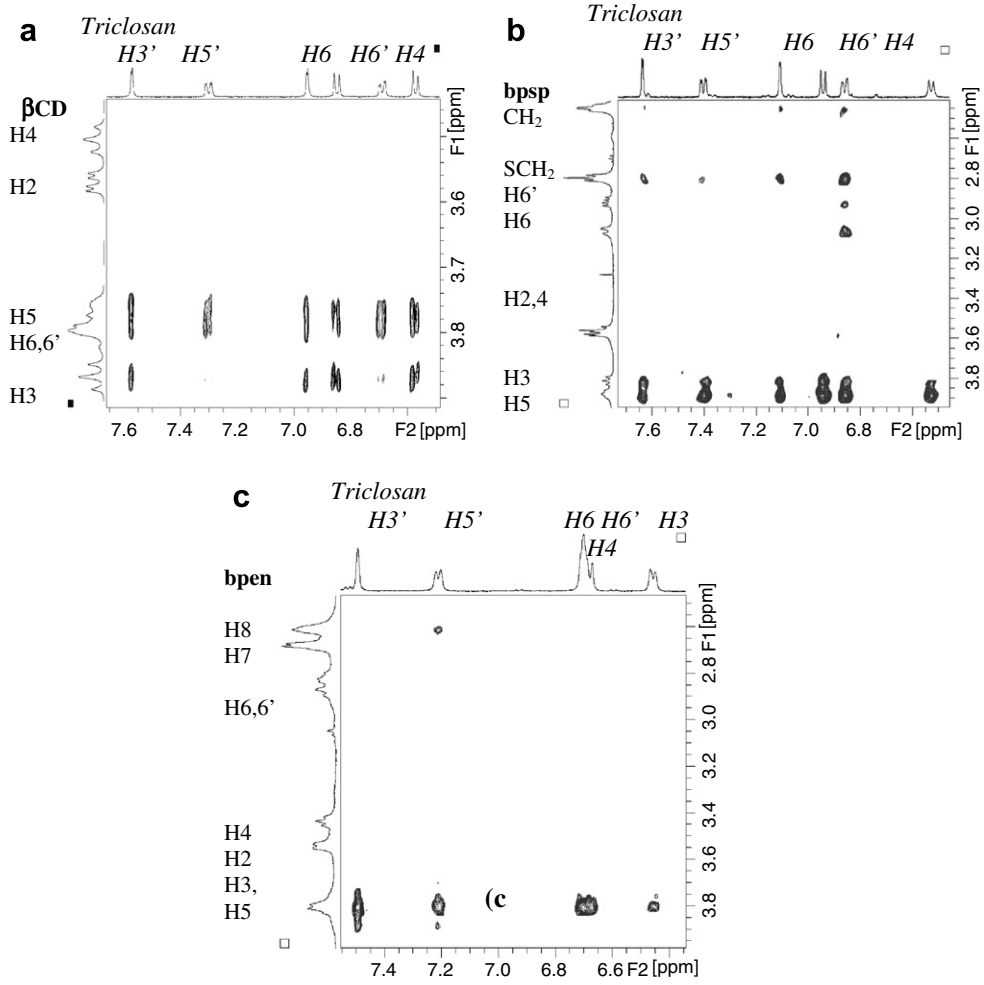

Figure 5. Partial 2D ROESY spectra of triclosan complexes in D_2O with (a) βCD at pH 7.9, (b) **bpsp** at pH 7.9 (c) **bpen** at pH 10.0.

of triclosan and H5 of βCD, and only moderate interactions of some of the triclosan protons with H3, it is fair to assume that the aromatic rings in the 1:1 complexes are closer to the smaller rim of the βCD cavity, which is in agreement with the crystal structure. In addition, presence of a 2:1 βCD/triclosan complex, in which both rings are included in different βCD macrocycles, as indicated by the crystal structure, would account for similar dipolar interactions of the two rings with H5 and H3 and indeed must be present, as indicated also from the continuous variation plot (Fig. 4a). The guest/host relative orientation in the 2:1 complex may be similar but not necessarily the same as in the crystal (Figs. 2 and 3), that is, both rings may penetrate at different depths the cavities of the two βCD hosts at the primary sides. The presence of the five water clusters in the secondary faces in the crystal may result from the solvation of the secondary side of this 2:1 complex. Therefore, it is realistic to propose that from such a supramolecular arrangement in solution the channels of crystal structure result from dimer formation by each βCD host (monomers A′, B and A, B′ in Figure 2) and subsequent guest reorganization.

The 2D ROESY spectrum of an aqueous solution of **bpsp**/triclosan at pH 7.9 (Fig. 5b) clearly indicates the presence of the entire guest inside the cavity. Both aromatic rings of triclosan are inside, since in the present case there is an elongated cavity available and all aromatic protons display dipolar interactions with both H3 and (stronger) H5. Moreover, the chlorophenol ring shows weak interactions with H6′ and the hydrogens of the –SCH_2– group on the primary side of **bpsp** as well, pointing to a structure in solution that comprises the dichlorophenyl ring inside the cavity, as in βCD, and the chlorophenol ring towards the carboxyl groups, which loosely surround it. The weak interaction, however, observed between *H3′* of the dichlorophenyl ring and –SCH_2– indicates the presence of a small amount of complex with the triclosan in reverse orientation, as well. Triclosan is 50% ionized at the working pH. It has been shown[25] that when **bpsp** includes an anionic guest, the latter is positioned with its negative charge away from the carboxylates (host–guest dipoles in opposite sense). Based on the above, it is proposed that the non-ionized triclosan enters **bpsp** with the diclorophenyl part well in the cavity and the chlorohydroxy-part in the primary side, whereas the ionized triclosan enters **bpsp** in the opposite sense.

The amino βCD, **bpen**, is soluble in water and mostly protonated at neutral pH (pK_a 6.4 and 9.5).[11] Addition of triclosan to **bpen** at neutral pH did not result in any shift of H3/H5 or in any increase of solubility of triclosan, both strongly suggesting no complexation. A slight increase of the pH did not improve the situation until the pH was raised to 10 in order to increase the triclosan solubility, even if at this pH the solubility of **bpen** is decreased, because of its reduced degree of protonation. The 2D ROESY spectrum (Fig. 5c) shows that both aromatic rings of triclosan interact with H5 and H3 which are actually overlapping, thus they are alternatively inside the CD cavity. The chlorophenol ring shows as well a weak interaction with the –NH–*CH_2*–*CH_2*–NH_2 group on the primary side of **bpen**, which indicates that at least one of the insertion modes of triclosan involves entire inclusion in the elongated cavity as in the case of **bpsp**.

4. Conclusions

The head-to-head alignment of the two crystallographically independent βCD macrocycles A and B leads to dimers that form infinite channels along the *c*-axis in the crystalline state. Only one guest molecule is included in each dimer, statistically disordered over two positions, one with the dichlorophenyl ring inside the cavity of host A and the other inside B (a 2:1 βCD/triclosan complex). In the crystal structure, both guest rings face the primary sides of hosts A and B: the dichlorophenyl ring is completely enclosed by βCD, whereas the hydrophilic chlorophenol rings extend in the space between the two hosts, its tip just entering the primary region of the second βCD. Stoichiometry studies of the βCD/triclosan complex with NMR spectroscopy in aqueous solution suggest the simultaneous presence of 1:1 and 2:1 host/guest complexes in solution. In the 1:1 case, both rings alternatively enter βCD cavity from the narrow primary side, whereas in 2:1 both rings of triclosan are included in the primary side of two βCD hosts, both arrangements in accord with that found in the crystal structure. Particularly the 2:1 host/guest complex is assumed to be a precursor of the structure in the crystals. Although the depth of penetration of the guest rings may be different in solution, it is reasonable to envisage that such a complex in aqueous solution results in the formation of the channels in the crystal lattice, via dimer formation by each βCD host and guest reorganization. The existence of the water cluster at the secondary sides of hosts A and B in the crystal structure may actually present a snapshot of the solvation at the secondary sides of the 2:1 complex in solution.

In the case of the negatively charged βCD derivative **bpsp**, which has an elongated cavity, the solution studies by NMR spectroscopy point to a complete inclusion of triclosan inside the host, with the dichlorophenyl ring inside the CD cavity and the chlorophenol ring towards the carboxyl groups for the non-ionized guest, whereas a reverse orientation is detected for the ionized one. Finally, for the positively charged βCD derivative **bpen**, inclusion is possible only when the pH is raised to 10 (where the guest is ionized, whereas the host is less protonated) and it is found that both aromatic rings of triclosan are alternatively inside the CD cavity, whereas insertion of the entire triclosan in the elongated cavity of the host is also observed.

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Supplementary data

Complete crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Center, CCDC 668566. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.06.004.

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