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The Respective Benefits of X-ray Crystallography and NMR for the Structural Determination of the Inclusion Complex Between Butyl-isothiocyanate and Alpha-cyclodextrin *

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Abstract

Isothiocyanates are natural products extracted from plants. These molecules which exhibit very interesting antifungal properties, are insoluble in water. To increase their solubility, we have prepared inclusion complexes with different cyclodextrins. Among all the isothiocyanates studied, we have investigated in more detail the structure of one complex: butyl-isothiocyanate and alpha-cyclodextrin, using two different techniques. Firstly, ^{1}H NMR experiments were performed and revealed the inclusion phenomenon. In parallel, crystals of butyl-isothiocyanate–alpha-cyclodextrin were grown and their crystallographic structure determined. This confirmed the inclusion of the ITC molecules and allowed us to determine the exact position of the guest. Finally, we showed that even though the complex structure was determined separately in solution and in the solid state, the structural characterisations obtained with these two techniques are complementary, enhancing the respective benefits of X-ray crystallography and NMR.

Introduction

Most of the applications of cyclodextrins (CDs) are found in chemistry, for example, for enantiomeric separation as well as in medicine and food chemistry. The use of cyclodextrins is mainly based on their high solubilizing power [1]. Inclusion complexes between cyclodextrin and a guest molecule can greatly enhance the water solubility of the guest. Proper use of these inclusion complexes for industrial applications, as for basic research, requires a complete knowledge of their structure. Several techniques, such as UV/Visible or fluorescence spectroscopy are extensively used to investigate cyclodextrin complexes, but they do not provide the structure of the complex. During the last few years, NMR spectroscopy has become one of the most important methods for structural elucidation of cyclodextrin complexes [2]. The interest in such a technique is based on its ability to allow precise characterization of non-covalent binding between the cyclodextrin and a guest molecule. However, a limitation of such a technique is that mainly proton and carbon NMR signals are exploited to characterize the structures of complexes. Therefore, the characterization of the inclusion of inorganic molecules requires another technique such as crystallography [3]. Determination of solid state structures is generally limited by the requirement of obtaining single crystals suitable for analysis by X-ray crystallography. In this study, we present the structure of a complex of alpha-cyclodextrin and butyl-isothiocyanate (butyl-ITC). Isothiocyanates, which can be represented as R-NCS, R being an alkyl, phenoxy, sulfoxyl group for example, exhibit very interesting antifungal properties but are insoluble in water [4]. To overcome this insolubility problem, inclusion complexes of isothiocyanates with different cyclodextrins were studied [5]. Herein we report on the structure of butyl-ITC and its α-CD complex in solution as determined by ^{1}H NMR spectroscopy, while the solid state structure of the same inclusion complex was determined by X-ray diffraction analysis. Using NMR, it was possible to confirm the inclusion phenomenon, analysis of the crystal structure led to a more precise characterization of the same complex.

Experimental

Preparation of butyl-ITC-α-CD complex in solution and analysis using ^{1}H NMR

A soluble complex was prepared by mixing 10 mg of α-CD previously lyophilised and resuspended in 400 μL of D_2O, with 2 μL of liquid butyl-ITC obtained from Aldrich.

The ^{1}H NMR spectra were recorded at 305 K in D_2O on a Bruker Advance DRX spectrometer equipped with a 5 mm TXI probe operating at 500 MHz.

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Preparation of the crystalline complex, data collection, and structure refinement

12 μL of butyl-ITC were added to a solution of α-CD (200 mg in 10 mL) in water at 50 °C. After mixing of the two components, the solution was left to cool slowly at room temperature. Small transparent crystals of the above complex were obtained. To perform crystallographic analysis, a single crystal was sealed in a thin glass capillary, in the presence of the mother liquor. It was then mounted on an Enraf-Nonius CAD4 diffractometer (measurement details are listed in Table 1). During data collection, intensities of three standard reflexions were checked every hour and recentering was carried out every 200 measured reflexions. Intensities were corrected for Lorentz and polarisation factors. The structure refinement was carried out using the positional parameters of α-CD in the structure described by Nicolis *et al.* [6] as the starting model. Alternate consecutive cycles of least-squares refinement and difference Fourier calculations were then done using SHELX-97 [7]. These revealed atomic positions of the 2 α-CD molecules and of 9 water molecules per asymmetric unit. Five glucose subunits, among the 12 present, exhibiting positional disorder were given anisotropic displacement parameters. The seven other glucose subunits were refined isotropically. Most hydrogen atom positions of the skeletons of the two CDs were determined from difference maps. For the missing ones, ideal positions were calculated. The positions of all these hydrogens were not refined. At this stage, residual electronic density within the cavity of the CDs was analysed using the TURBO-FRODO program [8]. This electronic density corresponds to the ITC molecule expected to be included in the CDs. The guest molecule was then introduced as a rigid body, as a best fit to the electron density peaks. The final R factor ($I > 2\sigma$) is 0.072. Without the contribution of the guest molecule, this R value is 0.094.

Results and discussion

^{1}H NMR structure

To characterize the structure of the complex made with butyl-ITC and α-CD, ^{1}H NMR analysis was performed. Figure 1 shows the comparison of the ^{1}H NMR spectra of the complex (Figure 1A) recorded in D_2O at 305 K, with that of α-CD alone (Figure 1B). The expected signals of the external protons of the α-CD cavity are found for α-CD alone; H-1 is centered at 5.25 ppm (not shown) , H-6, 6′ at 4.05 ppm, and H-2 at 3.81 ppm and H-4 at 3.76 ppm. Signals of the protons of the inside of the cavity, are found at ca. 4.16 ppm and 4.05 ppm for H-3 and H-5, respectively (Figure 1B). In the presence of butyl-isothiocyanate, most of these ^{1}H NMR signals are shifted. The most perturbed signals are those of H-3 and H-5, which are indistinguisable as they are superimposed at 4.10 ppm (Figure 1A). Such a shift in frequency for the protons inside the cavity of cyclodextrin suggests the formation of an inclusion complex. ^{1}H NMR signals of the alkyl chain of butyl-ITC (not shown)

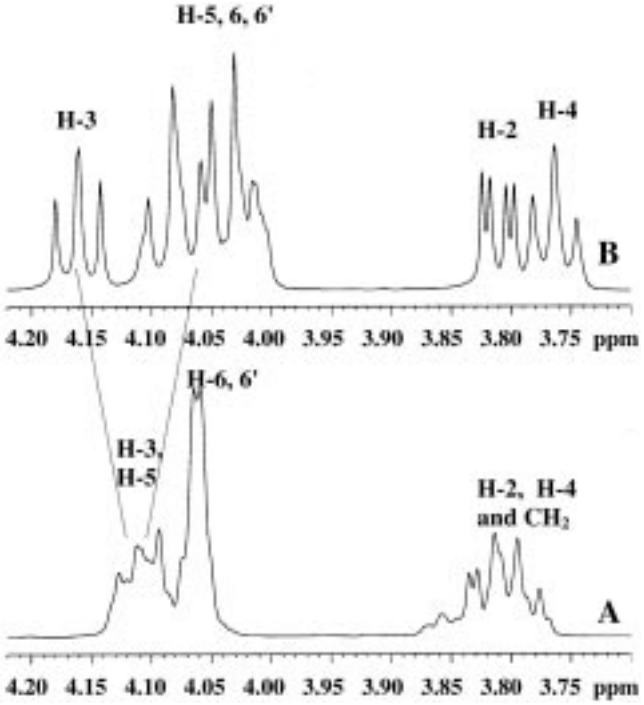

Figure 1. Partial ^{1}H NMR spectra recorded at 305 K in deuterium oxide, of alpha-cyclodextrin and butyl-isothiocyanate, **A**, α-cyclodextrin in deuterium oxide, **B**.

are identified at 1.20 ppm for the methyl group, 1.75 ppm, 2.00 ppm and 3.84 ppm for the three CH_2 groups, going in order from the methyl group to the NCS group. This last peak signal is very broad (from 3.76 to 3.87 ppm) and is only partly distinguishable from the signals of H-2 and H-4 of α-CD. The presence of the proton signals of the ITC molecule shows that at least part of it is solubilized in water in the presence of α-CD, which corroborates the fact that the guest molecule is included in the CD cavity. Integration of the different signals of the ITC and the α-CD indicates the formation of a 1:1 complex. To confirm the formation of the complex, two-dimensional ROESY experiments were performed. ROESY experiments are expected to show the dipolar interactions of the protons of the inside of the cavity of the α-CD, with those of any ITC included. Figure 2 shows the partial 2D-ROESY spectrum of the complex. Three cross-peaks are found at ca. 4.10 ppm, arising from dipolar correlation between H-3 and/or H-5 of α-CD and two CH_2 groups and the methyl group of the guest molecule. Interpretation of this 2D-ROESY spectrum indicates that the ITC protons and the H-3 and H-5 α-CD protons are within 4 Å of one another. Thus, it clearly confirms the formation of an inclusion complex between the two species.

Crystallographic structure

The analysis of the ^{1}H NMR data demonstrated the formation of an inclusion complex between butyl-ITC and α-CD in solution. Nevertheless, it was not possible to determine if the NCS group was located on the side of the primary or the secondary hydroxyls. Therefore, to better characterize the whole structure of this complex, crystals of the butyl-ITC-

Table 1. X-ray diffraction experimental details

Formula	2α-CD-C_4H_9NCS, $9H_2O$
Formula weight	2223 g.mol^{-1}
Cell setting	Triclinic
Space group	P1
a (Å)	13.830 (6)
b (Å)	13.882 (4)
c (Å)	15.727 (7)
α (°)	93.06 (3)
β (°)	91.72 (4)
γ (°)	119.58 (4)
V (Å^3)	2615 (2)
Dc (g.cm^{-3})	1.41
Crystal size (mm^3)	0.25*0.25*0.5
Diffractometer	CAD4
Radiation	MoK$_\alpha$ (λ = 0.71069 Å)
μ	0.15 mm^{-1}
F (000)	1212
Temperature	Room temperature
θ Range (°)	1–25
Scan type	$\omega/2\theta$
Number of measured reflections	6648
Number of independant reflections	6366
Number of reflections observed $I > 2\sigma I$	4930
Number of parameters used	903
Weighting scheme	$w = 1/[\sigma^2(F_0)^2 + (0.1157P)^2 + 3.4611P]$ Where $P = (\mathrm{Max}(F_0^2, 0) + 2F_c^2)/3$
Final R ($I > 2\sigma I$)	0.072
wR	0.177
Max. height in difference Fourier map (e. Å^{-3})	0.51
Min. height in difference Fourier map (e. Å^{-3})	−0.39
$(\Delta/\sigma)_{\max}$	0.03

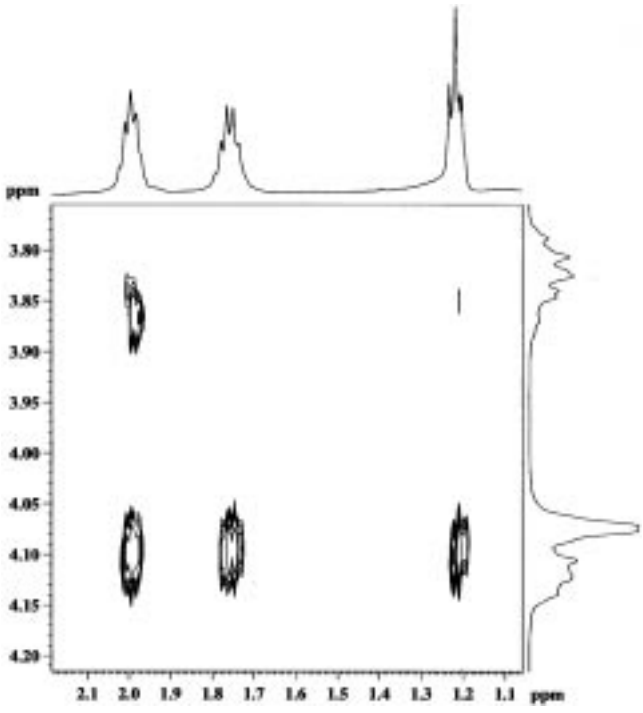

Figure 2. Partial 2D-ROESY spectrum of the butyl-ITC – α-CD complex recorded at 305 K in deuterium oxide.

α-CD complex were grown. The crystal structure analysis showed the global structure of the cyclodextrin molecules and the position of the guest molecules. According to crystallographic data, the α-CD molecules are stacked along the crystallographic c-axis to form columns with infinite internal channels. The α-CD molecules are alternatively head-to-head and tail-to-tail (i.e. the primary hydroxyls facing each other). In our case, the asymmetric subunit is made of two tail-to-tail α-CD molecules (A and B) which form a cavity that contains one guest molecule. As already found for different α-CD structures of triclinic form [9, 10], the 2 α-CD molecules are almost parallel and are slightly inclined from the c-axis. The O(4) least squares planes of molecules A and B form dihedral angles of 9.2° and 8.1° respectively with the (a,b) plane, and an angle of 3.7° with respect to each other. Moreover, these two molecules are laterally shifted by ca. 1.6 Å. Comparing our structure with the triclinic forms cited above, the conformation of the two α-CD molecules and their relative positions are slightly different, although, in our case CD molecule A and B positions fit well with those described by Nicolis *et al.* [6]. The tilt angles of the different glucose rings having a $^4C_1$ chair conformation, are listed in Table 2. As in other similar structures, all the primary hydroxyl groups (C(6)–O(6) bond) were found to

Table 2. Tilt angles (°) of A and B α-CD molecules with e.s.d's in parentheses

	Cyclodextrin A	Cyclodextrin B
Glucose 1	19.9 (3)	11.1 (4)
Glucose 2	8.2 (2)	13.7 (3)
Glucose 3	10.8 (2)	7.9 (1)
Glucose 4	9.9 (3)	13.1 (2)
Glucose 5	12.9 (4)	8.0 (2)
Glucose 6	12.8 (3)	11.1 (4)

point away from the center of the α-CD. Surprisingly, some disorder occurs in the secondary hydroxyl positions. Two oxygen atoms of the secondary hydroxyls of molecule B are disordered; O(3) is disordered in glucose number 2 and O(2) in glucose 6.

In the crystal packing, α-CD molecules are maintained by strong hydrogen bonds. α-CD molecules belonging to the same column appear to be linked to each other by direct hydrogen bonds between oxygens O(3)–O(3) and O(2)–O(3) of secondary hydroxyls, according to the hydrogen positions found. The primary hydroxyls of two adjacent cyclodextrins of the same column are hydrogen bonded not directly but mediated by one or two water molecules as already described [6, 10]. In addition, several hydrogen bonds are found between adjacent columns. Due to the inclination of the CD molecules with respect to their lateral neighbors, these hydrogen bonds can also link cyclodextrins A and B of different columns. There are also some direct hydrogen bonds between secondary hydroxyls of different columns, in particular between the disordered secondary hydroxyls of the two adjacent molecules. Eight secondary hydroxyl groups are involved in these intercolumn hydrogen bonds (Table 3). Interestingly, these oxygen atoms are hydrogen bonded as well to a water molecule that also exhibits high disorder. This is illustrated in Table 3, which summarizes the hydrogen bond distances between O(9) and the secondary hydroxyls. For the primary hydroxyls, one or two water molecules are involved in these hydrogen bonds between different columns. All these inter-column hydrogen bonds seem to be as important as those found in a same column for the global 'stability' of the crystal. This illustrates the fact that, in that case, the CD molecules cannot be considered as dimers, as it is often described for β-CD. Effectively, comparing the several structures of α-CD complexes with isomorphous packing, the flexibility of the packing seems to be greater in the region of the secondary hydroxyls compared to the primary hydroxyl region. This flexibility is also indicated by the observed disorder of the secondary hydroxyls in the crystal structure reported here. Another example of packing flexibility of similar crystal structures of α-CD complexes was described in the case of a large guest, whose inclusion leads to an 'opening of the molecular capsule' between the rims of the secondary hydroxyl groups [11].

Table 3. Selection of short contacts indicating possible hydrogen bonds

Secondary hydroxyl	Interdimer distances
O22B′ ... O36B′$^{(i)}$	2.90 (5) Å
O25B ... O26A$^{(ii)}$	2.76 (1) Å
O31A ... O23A$^{(i)}$	2.89 (1) Å
O31A ... O36B′$^{(i)}$	3.02 (2) Å
O21A ... O24B$^{(iii)}$	2.86 (1) Å
Short contacts water involving disordered molecule (9)	
O31A ... O9′$^{(i)}$	3.04 (4) Å
O21A ... O9′$^{(i)}$	2.78 (4) Å
O34B ... O9′$^{(iv)}$	2.81 (4) Å
O36B' ... O9′$^{(v)}$	2.60 (4) Å
O36B ... O9$^{(v)}$	2.70 (2) Å
O26B ... O9$^{(v)}$	2.75 (1) Å
O33A ... O9$^{(v)}$	2.68 (2) Å
O25A ... O9$^{(iv)}$	2.71 (1) Å

Symmetry codes: (i) $x+1$, $y+1$, z; (ii) $x-1$, $y-1$, z; (iii) $x+1$, y, z; (iv) x, $y+1$, z; (v) x, y, z.

As already indicated above, the two α-CD units of the asymmetric subunit contain one whole guest molecule. The stoichiometry of the complex is one butyl-ITC for two cyclodextrins. The ITC molecule was built as a rigid body, the C–N–C angle was fixed at 165°, as in the structure determined by Hardgrove *et al.* [12]. To determine its position in the α-CD cavity, residual electron density inside the CD cavity was analyzed using the TURBO-FRODO program. This electron density was calculated using the refined positions of cyclodextrin and water molecules, without the contribution of ITC. It revealed an electron density area for which the ITC block fits two positions. These two positions, which stand out because of the high electron density of the sulfur atom compared to other atoms, are related by a pseudo twofold axis which is also found for the two CD molecules. The structure of the complex could thus be deduced. When the alkyl chain of the butyl-ITC molecule is completely included in the cavity of molecule A, the NCS group is located near the primary hydroxyls of molecule B. In addition, the structure shows that the sulfur atom is located at the same level as the H-5 hydrogen of molecule B (see Figure 3). Due to the presence of a pseudo-binary axis, a symmetrical location for the ITC molecule can be found, the alkyl being then in molecule B. After refinement, the occupancy factors for these two positions were found to be identical. Residual electronic density found near the secondary hydroxyls inside the cavity, may be interpreted as a disordered water molecule.

Conclusion

In this paper, we studied the inclusion of butyl- isothiocyanate in α-cyclodextrin. Using ^{1}H NMR, we showed that a 1:1 complex was formed in solution. As only hydrogen atoms are detected, only the CH_2 of the alkyl chain of

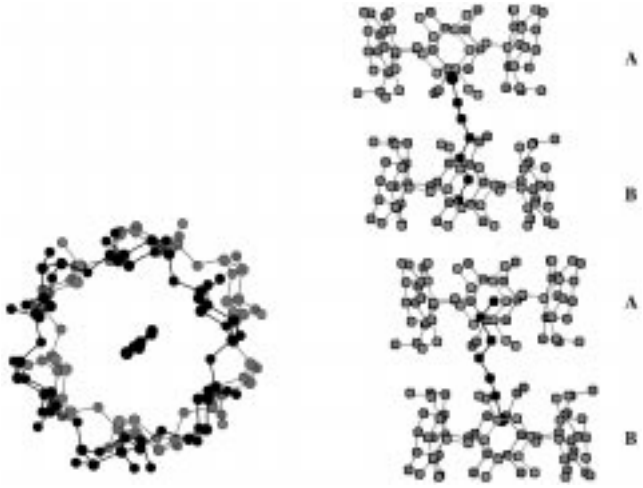

Figure 3. Description of the α-$(CD)_2$–C_4H_9NCS. 9 H_2O complex. Left: View along the molecular axis of the α-CD showing two α-CDs containing one ITC. Right: View of two unit cells of the complex, illustrating the two orientations of the ITC molecules. Water molecules are not shown for the sake of clarity.

butyl-ITC is found in the cyclodextrin cavity. The structure determined using crystallographic analysis is more detailed as most atomic positions of the CD and the ITC could be clearly determined. It shows that the complex is made of two cyclodextrins and one butyl-isothiocyanate. The isothiocyanate molecule possesses two different orientations in the cavity, probably due to its linearity. The NCS group was found on the side of the primary hydroxyls, with the butyl end being near the secondary hydroxyls. It appeared that the proportions of the complex obtained with the NMR experiments and the X-ray data are different. The proportion 1:2 found in the solid state can be explained by the packing of the cyclodextrins and the length of the ITC molecule. In solution, no steric interaction is expected to prevent full complexation, i.e. a 1:1 complex. In addition, the size of the guest molecule can influence the stoichiometry in the crystal, as was already described by Klingert and Rihs [11]. Nevertheless, both techniques show similar inclusion of the alkyl chain in the cavity, which enhances the high complementarity of NMR and X-ray diffraction.

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