COMPLEXATION OF ACTIVE ANTIBACTERIAL ADAMANTANOL DERIVATIVES WITH βCD. STRUCTURE DETERMINATION BY X-RAY CRYSTALLOGRAPHY AND NMR

PERRAKIS , A.[1], TSITSA, P.[2], HAMODRAKAS, S.J[3], ANTONIADOU-VYZA, E.[2]
[1]*Netherlands Cancer Institute, Plesmanlaan 121, Dept. H2 1066 CX Amsterdam, The Netherlands*
[2]*Dept. of Pharmaceutical Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece*
[3]*Dept. of Biochemistry, University of Athens, Panepistimiopolis, Athens 15701, Greece*

1. INTRODUCTION

The decyl-quaternary ammonium salt of adamantanol (I-1-10) is a very active antibacterial, prepared in our laboratory, as a typical analog of the widely used cationic antiseptics. It is active against Gram positive and at higher concentration, against Gram negative microorganisms. The effectiveness of this class of compounds appears to be a result of disturbance caused to the microbial cell membrane permeability leading to leakage of intracellular compounds.
On the course of our invastigation of the pharmacological properties of adamantane ring bearing compounds, their interaction with β-cyclodextrins (βCD) and the structure-properties relationship of the complexes was investigated by means of NMR and X-ray crystallography. Here we report the X-ray structure of the complex of b-cyclodextrin with I-1-10 determined at atomic resolution and a study of the complex by NMR in solution.

2. MATERIALS AND METHODS

2.1. Crystallization

The ligand I-1-10 was complexed with β-cyclodextrin and crystalllized by slow evaporation from distilled water. The crystals used for data collection were short needles of approximate dimensions 0.05 x 0.05 x 0.1 mm. They belong to the orthorhombic space group $P2_12_12_1$ with cell dimensions 15.0 x 17.0 x 32.5 A.

2.2. Data collection

For room temperature data collection the crystals were glued at the end of a glass fibre. Diffraction data were collected using synchrotron radiation on the X31 beamline of EMBL at DESY, Hamburg and a MAR image plate area detector. The wavelength for data collection was set to 0.75 A in order to maximize the anomalous scattering of the Br atom. Data were collected to a maximum resolution of 1.15 A, within 3 hrs. All diffraction data were indexed and scaled with the programs DENZO and Scalepack. The reliability index R_{sym} was 8.3 %.
For low temperature data collection the crystals were transferred in a drop of oil and picked up with a small loop of a wool fibre, with approximate diameter 1 mm. Then they were exposed to a stream of evaporating nitrogen at 150 K.

Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **25**: 157–160, 1996.

Data were collected on the BW7 beamline of EMBL. Usage of a bigger image plate in comparison with the room temperature data collection allowed to collection of data to a maximum resolution of 0.9 A. R_{sym} for this dataset was 5.8 %.

2.3 NMR Spectroscopy

The structure of the complex in aqueous solution was also studied by NMR Spectroscopy. Spectra were recorded at 200 MHz, using D_2O as solvent, at room temperature. Gaussian enchancement was used.

3. RESULTS AND DISCUSSION

3.1. Structure solution

The structure was solved using the room temperature data. The position of the Br atom was located from both the Patterson synthesis and the anomalous difference Patterson synthesis, utilizing the program SHELXS86. Attempts to get a good quality map using either Patteron expansion methods or direct methods were unsuccesfull.

A novel protocole was then applied to obtain an interpretable map. The program suite ARP, primarly used in protein crystallography for improvement and completion of models, was used. Using only the Br atom as a starting model, 40 cycles of sparse matrix unrestrained least squares refinement were applied. After each cycle 2 oxygen atoms were added to the model automatically on the basis of the density in the difference Fourier synthesis map. Another 60 cycles followed, in which the model was updated after each cycle more extensively, 6 atoms were allowed to be added and 6 to be removed in accordance with density and geometrical criteria. Atoms were added where density above 4 rms in the difference Fourier synthesis was present and if an atom already existed in a distance between 1.1 and 3.0 A, which covers covalent and hydrogen bonding interactions. After that, the model, consisting at that stage of only oxygen atoms, showed clearly all atoms of the cyclodextrin, the ligand and 10 water molecules.

Only 4 additional atoms did not make chemical sense (two in each side of the Br atom to compensate apparently for the high anisotropy of that atom along that direction and two to compensate for well defined hydrogen atoms of the structure) and thus were removed.

It must be noted, that as it could be judged after complete refinement of the structure, the initial phase error which was 72°, dropped to only 14° with respect to the final model, just by applying this protocole.

3.2. Refinement

After identifying and assigning the correct atom types the model was subjected to 10 cycles of full matrix least squares refinement using SHELXL-93. Hydrogens were added and an additional 20 cycles of refinement were performed in which each non-hydrogen atom was assigned an anisotropic temperature factor. After adjustment of the weighting scheme the final R factor converged to 7.9 % indicating a structure of very good quality.

For the low temperature data, the hydrogens and anisotropic temperature factors of the room temperature model were removed and the model was subjected to 10 cycles of refinement.

A similar protocole like for room temperature data was then applied. The final R factor converged to 6.4 %, which is exceptionally low for structures of cyclodextrin complexes.

3.3. Description of the structure

The hydrophobic adamantane group is burried within the hydrophobic cavity of one cyclodextrin molecule. The cyclodextrin lies at an angle of 20° with respect to the plane formed by the crystallographic a and b axes. The aliphatic tail extends away from the cyclodextrin in a direction almost parallel to the a axis of the cell. After the tertiary nitrogen, the direction of the chain is changed by 90° and becomes effectively parallel to the long c axis, getting gradually disordered. Towards its end it regains order and comes to the proximity of the cyclodextrin ring of the molecule related by the 2_1 symmetry along the long c axis. Thus, the end of the chain interacts from the opposite side of the cyclodextrin ring than the side where the adamantane group lies.
The Br atom is coordinated by the O10 atom of the adamantane moiety and three more water molecules. All three water molecules form additional hydrogen bonds both to other waters and cyclodextrin sugar ring oxygens.
A rather complex hydrogen bonding network between the water molecules and the cyclodextrin and ligand is present. Every water molecule is involved in at least one hydrogen bond with mainly the O2, O3 and O6 atoms of the seven sugars comprising the cyclodextrin. All O2, O3 and O6 atoms are involved in a hydrogen bond with at least one water from the same or another asymmetric unit.
Only one water molecule forms a hydrogen bond to the oxygen atached to the adamantane moiety. This water in turns interacts with another water attached to the O3 atom of one of the sugar rings. This is the only hydrogen bonding interaction between the ligand and the cyclodextrin molecule in which the hydrophobic part of the ligand is burried in. Interestingly this oxygen atom is hydrogen bonded to the O3 atom of the sugar ring of a cyclodextrin molecule related by the 2_1 symmetry along the a axis.

3.4 ^{1}H NMR Spectroscopy

The complex formation induces chemical shift changes in the resonance of the CD protons, especially of protons directed towards the interior of the βCD cavity (H-C3,H-C5) and of the drug protons. (Tale I). The proton NMR spectrum of the pure molecule in D_2O consists of from six different groups of peaks.
The largest $\Delta\delta$ values were observed for the 4 axial and 9 axial protons of adamantane ring in the range of 0.16 ppm. A slight modification was observed to the signals corresponding to the 4 equatorial and 9 equatorial protons of the ring. The significant displacement of the signals corresponding to the rest of adamantane ring protons were expected because of the existing slope of ring axis in the βCD cavity.

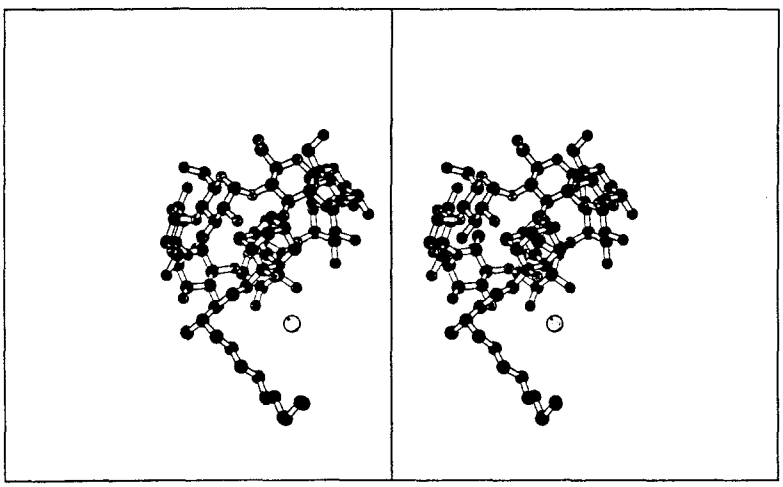
4. CONCLUSION

In the prepared I-1-10 βCD complex the hydrophobic adamantane group is burried within the hydrophobic cavity of one βCD molecule. The aliphatic tail is extended away from the βCD and becames gradually more disordered. Towards its end the tail becames more ordered and interacts with the βCD of a neighboring assymetric unit. The Br ion comes to 3.2 A distance with the O atom attached to the C10 of the adamantane ring.
The observed resonance modifications of both molecules revealed interactions between CD and the adamantane moiety of the active molecule, which shields this part from the solvent. Therefore it appears that the structure of the complex is quit similar both in solution and in the solid state.

TABLE I Chemical Shifts δ(ppm) of ADM-10 and β-CD in the Free and Complex State.

Proton*	δ_o (free)	δ_c (complex)	$\Delta\delta$ (δ_c-δ_o)
-$(CH_2)_9\underline{CH_3}$(t,2H)			
-$(CH_2)_7$-(m,14H)	1,2768	1,2802	+0,003
	1,3537	1,3696	+0,002
Ad(d,2H,4eq,9eq)	1,5668	1,6266	+0,071
	1,6128	1,6840	+0,071
Ad(m,12H)	1,7374	1,7923	+0,055
	1,7772	1,8561	+0,079
Ad(d,2H,4ax,9ax)	2,0860	2,2477	+0,162
	2,1462	2,3075	+0,163
$N(CH_3)_2$(s,6H)	3,0581	3,0726	+0,014
β-CD			
H3	4,043	3,926	-0,117
	3,878	3,987	-0,102
	3,949	3,800	-0,147
H5	3,540	3,855	-0,287
	3,586	3,873	-0,315
Anomeric	5,0800	5,0652	-0,015
	5,0976	5,0482	-0,050

*Only the protons that show chemical shift changes



Stereo-pair showing the structure of the complex of I-1-10 with βCD

REFERENCES

[1]Antoniadou-Vyza, E., Tsitsa, P., Tsantili-Kakoulidou, A., Hitiroglou, E.: New adamantan-2-ol and adamantan-1-methanol derivatives as potent antibacterials. Synthesis, antibacterial activity and lipohpilicity studies. *J. Eur. Med. Chem.* 31, 105-110 (1996)
[2]. Krishnamurthy, V., Pradeep,S., Olah, A. : Study of Substituent Effects on One-Bond NMR Coupling Constants in adamantane Derivatives. *J. Org. Chem.* 48, 3373-78 (1983).
[3]. Arndt, U.W.; Wonacott, A.J. : *The rotation method in crystallography*, North Holland Publishing company 75-103 (1977)
[4]. Cosier, J.Glazer, A.M. : A nitrogen-gas-stream cryostat for general Xray diffraction studies. *J. Appl. Cryst.* 105-107 (1986)
[5]. Otwinowski, *Z. Denzo: An oscillation data processing program for macromolecular crystallography*, Yale University, New Haven, USA.(1993)
[6]Sheldrick, G.M. :SHELXL-93, *Program for crystal structure refinement.* University of Gottingen, Germany(1993)
[7]Lamzin, V. : Automated refinement of protein models. *Acta Crystal.* D 49, 129-147 (1993)