International Journal of Pharmaceutics, 90 (1993) 35–41

IJP 02947

Molecular modelling and NMR NOE experiments: Complementary tools for the investigation of complex ibuproxam-β-cyclodextrin topology

Nadia Mulinacci, Fabrizio Melani, Giovanni Mazzi and Franco F. Vincieri

Università degli Studi di Firenze, Dipartimento di Scienze Farmaceutiche via G. Capponi 9, 50121 Firenze (Italy)

(Received 31 March 1992)
(Accepted 14 June 1992)

Key words: Ibuproxam; β-Cyclodextrin; Molecular modeling; NMR; Nuclear Overhauser effect

Summary

The aim of this paper is to study the use of computer molecular modelling and ^{1}H-NMR NOE data for gaining insight into the mode of inclusion of the ibuproxam (IBX) molecule in the hydrophobic cavity of β-cyclodextrin (βCyD) in solution. In the IBX-βCyD complex, the guest molecule shows very favourable structural features for a spectroscopic investigation of this type. The interatomic distances relative to some atoms of the IBX-βCyD complex, were calculated with molecular graphic systems. Through the comparison between the experimental values of nuclear Overhauser enhancements and the theoretical data obtained with molecular modelling studies, it was possible to establish the real structure of the IBX-βCyD complex in solution.

Introduction

In a previous study, we evaluated the capacity of the cyclodextrins (α, β, γ) for forming inclusion complexes with (*RS*)-2-(4-isobutylphenyl) propiohydroxamic acid (ibuproxam, IBX) to increase the solubility, dissolution rate and, presumably, also the bioavailability of this drug (Mazzi et al., 1988).

This study showed that βCyD was the most suitable for complexing with IBX in an aqueous medium; we thus considered it interesting to conduct further investigations into the conformational structure of the IBX-βCyD complex.

Important information on the solid-phase structure of these complex systems may be obtained via X-ray analyses when a suitable crystal of the product to be tested is available (Saenger, 1976, 1984). X-ray diffractograms of lyophilized IBX-βCyD complex have been reported in a previous paper (Mazzi et al., 1988). These spectra demonstrated the complete amorphousness of the product obtained, thus making it impossible to gain more complete information from them. The use of two techniques, computer-aided molecular modelling and NMR NOE studies, has proved to hold promise for such purposes, especially for systems or molecules of biological interest which 'act' in an aqueous medium.

Correspondence to: N. Mulinacci, Università degli Studi di Firenze, Dipartimento di Scienze Farmaceutiche via G. Capponi 9, 50121 Firenze, Italy.

The aim of the present investigation was focussed on the use of computer molecular modelling and NMR NOE data for gaining insight into the mode of inclusion of the IBX molecule in the hydrophobic cavity of βCyD in solution.

Materials and Methods

Materials

(*RS*)-2-(4-Isobutylphenyl)propiohydroxamic acid (IBX) was obtained by courtesy of L. Manetti Roberts & Co. (Firenze, Italy), β-cyclodextrin (βCyD) was purchased from Sigma Chemicals Co. (St. Louis, U.S.A.) and used without further purification. The IBX-βCyD inclusion complex was obtained as previously described (Mazzi et al., 1988). All other materials and solvents were of analytical reagent grade.

Methods

^{1}H-NMR

Chemical shifts were measured relative to the peak of the solvent D_2O (4.74 ppm) with a Bruker AMX 600 at 600 MHz in Fourier transform mode. The NOE experiments were performed on a Varian Gemini 200 at 200 MHz with a Varian NOE DIFF program, version 6.3 A. All spectra were recorded with a 5 mm tube at the probe temperature (25°C) in D_2O.

For the NOE experiments the same solutions were used as for ^{1}H-NMR, without degassing. Other NOE experiments were performed using $DMSOd_6$, again without degassing.

To assess whether the calculated NOE were independent of the concentration effect, the experiments were carried out at different concentrations of the IBX-βCyD complex (5, 10 and 15 mg/ml). The results were constant at the different tested concentrations. The NOE measurements were made during steady-state experiments.

Computer graphics

All molecules were constructed using the Discover program, version 2.7.0 and Insight II program version 2.0.0. from Biosym Technologies (10065 Barnes Canyon Road, San Diego Ca. 92121), run on the Personal Iris from Silicon Graphics. The method for calculation of force fields was CVFF without any simulation of molecular dynamics.

Results and Discussion

It has been shown that computer-aided molecular modelling can be used to predict the conformation and interaction energy of β-cyclodextrin complexes (Cabral Marques et al., 1990; Dauchez and Vergoten, 1990; Van Helden et al., 1990; Konstense et al., 1990; Lejo Yeux et al., 1990; Myles et al., 1990).

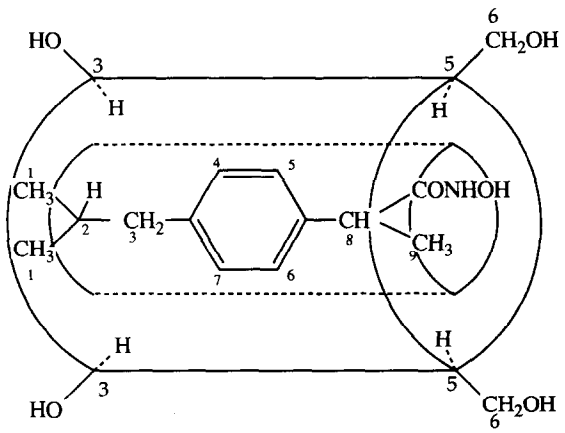
In this study, the molecular graphics studies also represent an important tool for investigating the conformation of the most favoured complexes and to obtain a better knowledge of the geometry of the system and of the topology of the interactions between IBX and β-CyD.

It is well known that β-CyD is a toroidal molecule with a truncated cone shape, having secondary hydroxyl groups on the C-2 and C-3 atoms which are located on the wider side of the truncated cone, while the primary hydroxyl groups on C-6 are positioned on the opposite narrower side. It might well be supposed, in view of size of the IBX, that two types of complex exist: one in which the two IBX methyl groups (H1-IBX) are nearer the wider side of the β-CyD toroidal structure (complex A, Fig. 1) and the other where the two IBX methyls are instead located on the opposite side, i.e., near the narrower opening of the body of the cone (complex B, Fig. 1).

Thus, the conformations at minimum energy levels of the two possible complexes were calculated (see Figs. 2a, b and 3a, b), and for each the interatomic distances and docking energies of the *R* and *S* enantiomers, respectively, were measured (Table 1). With this type of calculation the theoretical findings are strictly related to the complexes in a vacuum and not in solution.

It is not possible, from analysis of the findings in Table 1 alone, to establish which of the two complexes, A or B, actually exists in aqueous

COMPLEX A



COMPLEX B

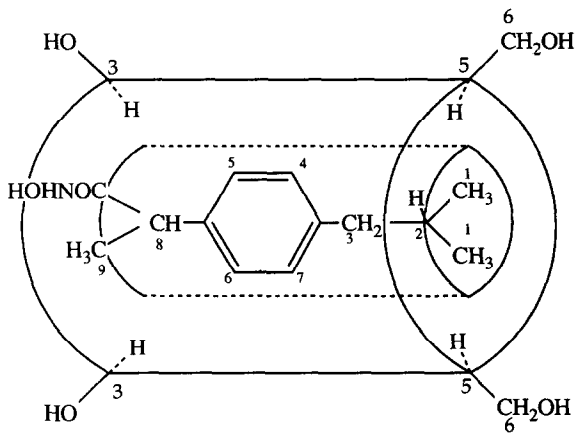


Fig. 1. Scheme of the two possible complexes between IBX and βCyD.

solution, since all four of the hypothesized structures show approximately the same values for both the formation energy and docking energy. This result clearly indicates that complexes A and B (Fig. 1) have the same probability of existence, at least theoretically. It should also be pointed out that in this specific case β-CyD is probably unable to discriminate between the two enantiomers, *R* and *S*.

Table 2 lists the chemical shift values of IBX, β-CyD and the IBX-β-CyD complex, respectively, as well as the differences between the signals of the two molecules alone and complexed. It may be noted that the more significant values are recorded for the IBX aromatic protons, for which the multiplicity of the signal also changes, passing from a doublet for IBX alone, to a double doublet for complexed IBX.

The same change in the multiplicity of the signal of the aromatics, with very similar chemical shifts, is recorded for IBX in MeOH, DMSO and $CHCl_3$. It may thus be supposed that the protons of the aromatic ring, both in the complexed IBX and in MeOH, DMSO and $CHCl_3$ solutions, are 'immersed' in a hydrophobic medium which certainly has very different polarity features from those of H_2O. This different chemical environ-

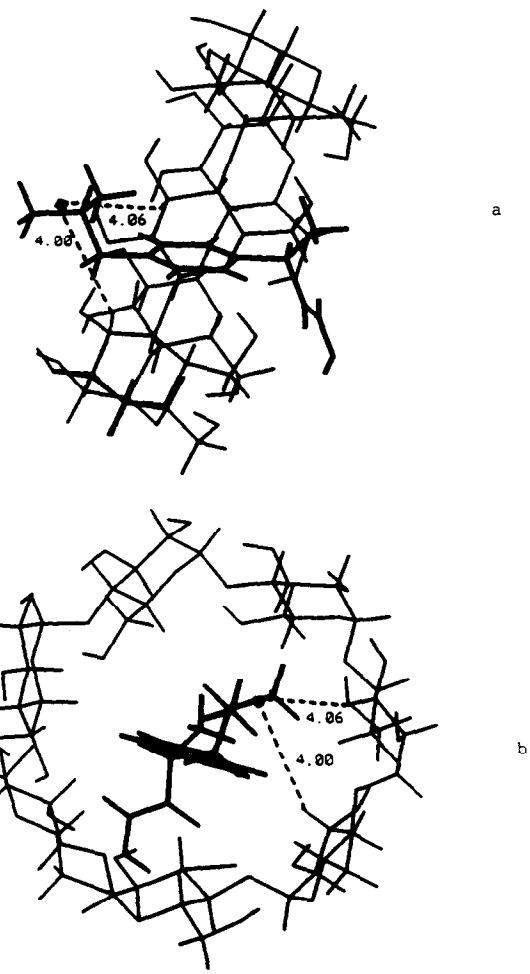


Fig. 2. (a,b) Relative position of IBX and βCyD in complex A; minimized structure with Discover program (see experimental section).

TABLE 1

Energy values (kcal / mol)

	Complex A (*R*)	Complex A (*S*)	Complex B (*R*)	Complex B (*S*)
Formation energy of complex	209.7	210.5	213.2	207.5
Docking energy	−30.6	−30.5	−26.3	−35.0

ment thus allows a change in multiplicity of the aromatic signals to occur. Hence, the ^{1}H-NMR findings only indicate that the aromatic ring of IBX is located inside the hydrophobic cavity of β-CyD, but gives no clear indications concerning the positioning of the whole molecule relative to β-CyD. On the other hand, it is possible with NOE experiments alone to identify complex A as that which is more favoured in an aqueous medium. Indirect proof of the existence of the complex in aqueous solution and hence of the validity of NOE as an investigation tool was obtained from the observation that in $DMSOd_6$ no nuclear Overhauser effect is recorded between the IBX and β-CyD protons and thus no complex exists in this organic solvent. For this test DMSO was found to be the most suitable organic solvent in that it allowed preparation of solutions at the desired concentrations.

TABLE 2

Chemical shifts (ppm) of the IBX and β-CyD protons in free and complex states in D_2O solution

Protons	Free state (ppm)	Complex state (ppm)	$\Delta\delta_{\text{free-complex}}$
IBX			
H1	0.909	0.964	−0.055
H2	1.876	1.906	−0.030
H3	2.524	2.545	−0.021
H4,7	7.300	7.126	0.174
H5,6	7.315	7.275	0.040
H8	3.660	–	–
H9	1.491	1.506	−0.015
β-CyD			
H1	5.115	5.093	0.022
H2	3.693	3.681	0.012
H3	3.996	3.899	0.097
H4	3.630	–	–
H5	–	–	–
H6	–	3.834	–

Table 3 lists the interatomic distances in Å relative to some atoms of the IBX-βCyD complex, calculated with molecular graphic systems and the increments of some proton signals calculated with NOE experiments in D_2O solution. Because our available IBX sample is a racemate we also considered it to be useful to report in Table 3 the average data relative to the *R* and *S* enantiomers of both complexes A and B.

A significant nuclear Overhauser enhancement is observed (Table 3) between the H1-IBX and the H3-βCyD protons with a signal increase of 0.8% (see Fig. 4a). Another intense nuclear Overhauser effect was recorded between the H9-IBX and H6-βCyD protons with an increase in the signal of 0.25% (see Fig. 4b). By presaturating the signal relative to the H1-IBX protons, as well as the NOE effect on the H3-βCyD protons, we also recorded an increase of 0.16% of the signal with a chemical shift value of 3.54 ppm. The signals of the protons at positions 2, 4 and 5 of βCyD fall around this value. (Fig. 4). It is well known that the protons at position 5 are the only ones, together with those at position 3, which are positioned towards the inside of the βCyD hydrophobic cavity (Fig. 1). Moreover, it was calculated by molecular modelling that for complex A there is an interatomic distance between the H5-βCyD and H1-IBX of 6.0 Å (Table 3). It appears to be reasonable to attribute this slight NOE effect exclusively to the protons at position 5 of βCyD, ruling out any interaction with the protons at positions 2 and 4 which are both positioned towards the outside of the hydrophobic cavity and are hence unfavourably placed for interaction with the H1-IBX protons.

Comparing the values of the interatomic distances obtained with the method of force fields in a vacuum with the increments of the NOE sig-

TABLE 3

The shortest distances (Å) between different atoms of IBX-β-CyD complex evaluated with molecular modelling and the increments (%) of the signals calculated via NOE experiments

Distances between H-CyD and H-IBX	Complex A (*R*)	Complex A (*S*)	Complex B (*R*)	Complex B (*S*)	Complex A [average (*R*; *S*)]	Complex B [average (*R*; *S*)]	^{1}H-NMR NOE/D_2O (increments of the signals %)
H3-βCyD-H1-IBX	4.0	3.8	5.6	6.3	3.9	6.0	0.8
H6-βCyD-H9-IBX	4.6	4.2	7.5	7.9	4.4	7.7	0.25
H5-βCyD-H1-IBX	6.1	5.9	3.6	4.0	6.0	3.8	0.16

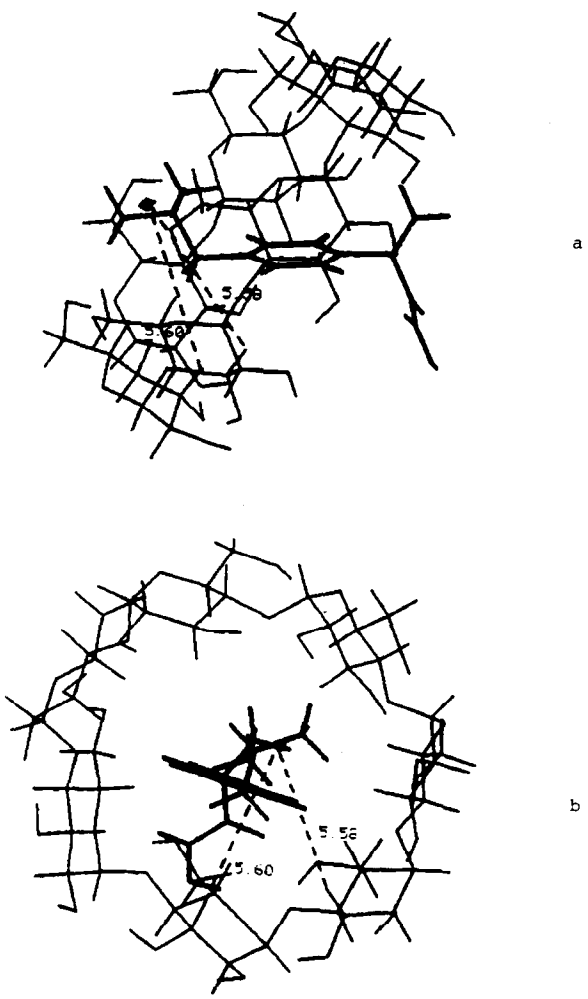


Fig. 3. (a,b) Relative position of IBX and βCyD in complex B; minimized structure with discover program (see Materials and Methods).

nals, it is reasonable to suppose that the complex present in solution is that of type A (Fig. 1).

In this complex, the guest molecule shows very favourable structural features for a spectroscopic investigation of this type, since two chemically very different groups are arranged at the *para* position on the benzene ring. In our NOE experiments the strongest nuclear Overhauser effects were seen just between these two terminal groups of IBX and the protons at position 3 and 6 of βCyD. This happy coincidence thus enabled us to establish the positioning of the guest molecule in the hydrophobic cavity of the βCyD toroidal structure. The NOE findings from the study of the IBX aromatic protons, even if significant, are nevertheless insufficient alone to diagnose the structure of the complex. They merely show how the benzene ring is completely inserted inside the hydrophobic cavity, and do not indicate the positioning of the whole IBX molecule in relation to βCyD.

Conclusion

In the case of the IBX-βCyD complex, it was seen that investigation with molecular modelling alone, while allowing the construction and three-dimensional manipulation of the molecular complex, is insufficient for describing the real structure of this complex in solution. This method must therefore be combined with other investigative techniques such as ^{1}H-NMR and, particularly, NMR NOE.

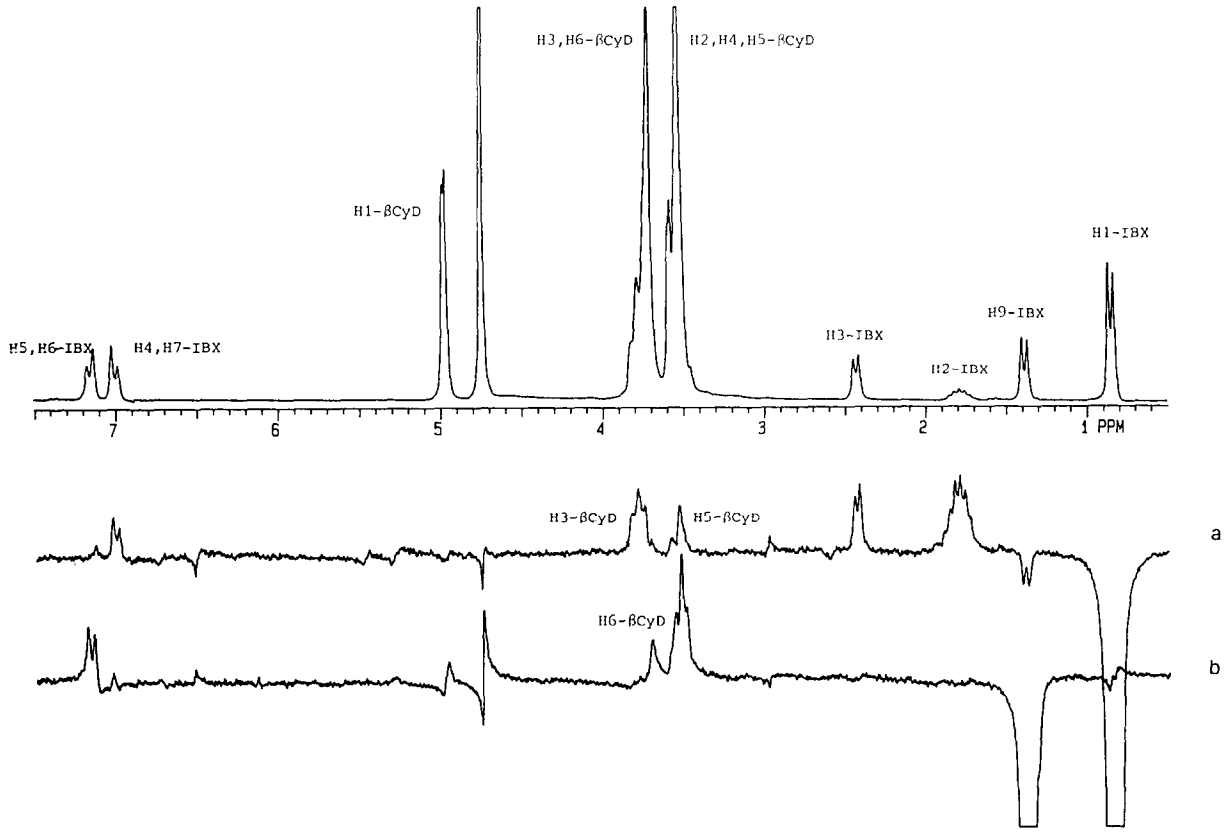


Fig. 4. ^{1}H-NMR spectra of IBX-βCyD complex in D_2O solution and NOE experiments a and b. (a) Nuclear Overhauser effects when the protons H1-IBX had been presaturated. (b) Nuclear Overhauser effects when the protons H9-IBX had been saturated.

The interactions between these two techniques allow a comparison and an integration of the theoretical findings obtained in a vacuum (computer graphics) with experimental findings related to the complex in solution, and certainly lead to more complete results which correspond more closely to reality. More complete structural information can also be obtained by combining the two techniques with X-ray measurements which unfortunately are often difficult to carry out for many inclusion complexes.

Acknowledgements

This research was supported by grants from M.U.R.S.T. The authors wish to acknowledge Mrs Sandra Gallori for technical support and Servizio Nazionale di Risonanza Magnetica Nucleare, Dipartimento di Chimica Università degli Studi di Firenze for making the Bruker AMX 600 available.

References

Cabral Marques, H.M., Hadgraf, J., Kellaway, I.W. and Pugh, W.J., Studies of cyclodextrin inclusion complexes. II Molecular modelling and ^{1}H-NMR evidence for salbutamol-β-cyclodextrin complex. *Int. J. Pharm.*, 63 (1990) 267–274.

Dauchez, M. and Vergoten, G., Determination of a force field for carbohydrates, application to the molecular modelling of cyclodextrins. *Minutes of the 5th International Symposium on Cyclodextrins*, Editions De Santé, Paris, 1990, pp. 101–106.

Kostense, A.S., Van Helden, S.P. and Janssen, L.H.M., Modelling and conformation analysis of β-cyclodextrin complexes. *Minutes of the 5th International Symposium on Cyclodextrins*, Edition De Santé, Paris, 1990, pp. 161–166.

Lejo Yeux, P., Wouessidjewe, D. and Duchéne, P., Improvements in the water solubility and stability of melphalan by use of β-cyclodextrin and hydroxypropyl β-cyclodextrin. *Minutes of the 5th International Symposium on Cyclodextrins*, Editions De Santé, Paris, 1990, pp. 348–352.

Mazzi, G., Vincieri, F.F., Forni, F., Mulinacci, N. and Celli S., Formation of inclusion complex between the non steroidal anti-inflammatory drug (RS)-2-(4-isobutylphenyl-propiohidroxamic acid and β-cyclodextrin. *Acta Pharm. Technol.*, 34 (1988) 17–21.

Myles, A.M.C., Lee, P.K.Y., France, G., Barlow, D.J. and Lawrence, M.J., Structural studies of indomethacin-cyclodextin complexes. *Minutes of the 5th International Symposium on Cyclodextrins*, Editions De Santé, Paris, 1990, pp. 173–179.

Saenger, W., Mechanism of adduct formation and intermolecular interactions In Pullman, B. (Ed.), *Cyclodextrin Inclusion Complexes*, Reidel, Dordrecht, 1976, p. 265.

Saenger, W., Structural aspects of cyclodextrins and their inclusion complexes. In Atwood, J.L., Davies, J.E. and MacNicol, D.D. (Ed.), *Inclusion compounds*, Vol. 2, Academic Press, London, 1984, p. 231.

Van Helden, S.P., Hilbers, H.W., Van Drooge, M.J., Jansen, A.C.A. and Janssen, L.H.M., A molecular modelling study on the complex formation of p-substituted phenols in α-cyclodextrin, and a comparison with thermodynamic data. *Minutes of the 5th International Symposium on Cyclodextrins*, Editions De Santé, Paris, 1990, pp. 243–248.