Carbohydrate Research, 235 (1992) C1–C4
Elsevier Science Publishers B.V., Amsterdam

Preliminary communication

One- and two-dimensional ^{1}H NMR investigations of the inclusion of the anti-cancer drug mitoxantrone in cyclomaltooligosaccharides

Nicolaos Birlirakis and Bruno Perly

Service de Chimie Moléculaire, Centre d'Etudes Nucléaires de Saclay, F-91191 Gif sur Yvette (France)

(Received June 1st, 1992; accepted July 24th, 1992)

The anthracenedione derivative mitoxantrone (1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione, DHAQ, Novantrone®) has undergone extensive clinical screening and displays significant efficiency in the treatment of breast cancer, leukemia, and lymphoma. Mitoxantrone binds strongly to DNA through intercalation as well as inter- and intra-strand crosslinking, and inhibits DNA and RNA synthesis[1]. We report herein the use of high field NMR techniques to demonstrate the formation of inclusion complexes of mitoxantrone with cyclomaltooligosaccharides, a process which can be of value in controlling the bio-availability of such bio-active compounds in physiological media[2].

The complex formation is expected to induce shifts of the resonance of the cyclomaltooligosaccharide protons, especially of H-3 and H-5, which are directed towards the interior of the cavity[3]. The variation of the ^{1}H NMR spectra of cyclomaltoheptaose and cyclomaltooctaose in the absence and in the presence of variable amounts of mitoxantrone hydrochloride is shown in Fig. 1. Shifts of the H-3 protons are clearly observed, whereas those of the H-5 protons are partially obscured by signals from the drug. Conversely, signals from the H-2 and H-4 protons are weakly affected. This suggests interactions of the drug with both cyclic oligosaccharides from the secondary hydroxyl side. In the case of the H-3 protons of cyclomaltoheptaose, the observed shifts are smaller than expected from a simple consideration of ring current effects. This observation will be clarified by the ROESY experiments described herein.

The aromatic nature of mitoxantrone makes it prone to self-associate in aqueous solution. This process will compete with inclusion and has to be taken into account to derive the effective association constant with cyclomaltooligosaccha-

Correspondence to: Dr. B. Perly, Service de Chimie Moléculaire, Centre d'Etudes Nucléaires de Saclay, F-91191 Gif sur Yvette, France.

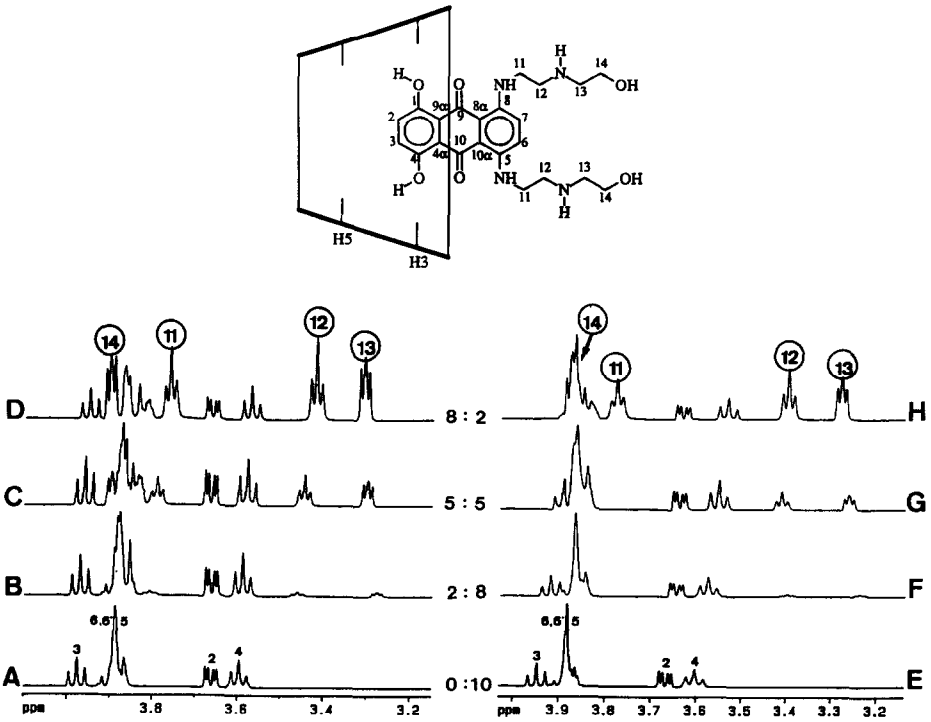

Fig. 1. Partial 500 MHz ^{1}H NMR spectra of cyclomaltoheptaose alone (A) and in the presence of mitoxantrone (B–D), cyclomaltooctaose alone (E) and in the presence of mitoxantrone (F–H). Guest-to-host ratios are given (mM) between the two sets of spectra. Protons of mitoxantrone are indicated by circled numbers. All experiments were performed at 298 K in ^2H_2O.

rides. Concentration dependent experiments indeed revealed a downfield shift of the proton resonances upon dilution, attributable to self-association due to π–π stacking. Moreover, 1D NOE difference experiments (not shown) have evidenced a proximity (intermolecular) between the H-2,3 and H-6,7 protons, indicative of a head-to-tail dimer formation. The dimerization constant can be determined from the variation with concentration of the chemical shifts of the aromatic protons. The data obtained were used as input values in an iterative program using the SIMPLEX algorithm to fit the experimental data[4]. A value of $k_{dim} = 22\ M^{-1}$ was obtained. Taking self-association of mitoxantrone into account, the inclusion constants (using the H-3 proton of the host) were calculated by a computer-assisted iteration of the experimental data[4]. The derived inclusion constants were $k_{incl} = 180$ and $300\ M^{-1}$ for cyclomaltoheptaose and cyclomaltooctaose, respectively.

In order to obtain further information about the spatial proximities of protons between the guest and the host molecules, 2D ROESY experiments[5] were performed. This was preferred to the more classical NOESY[6], which showed poor sensitivity in the present case because of unfavorable correlation times. The H-6

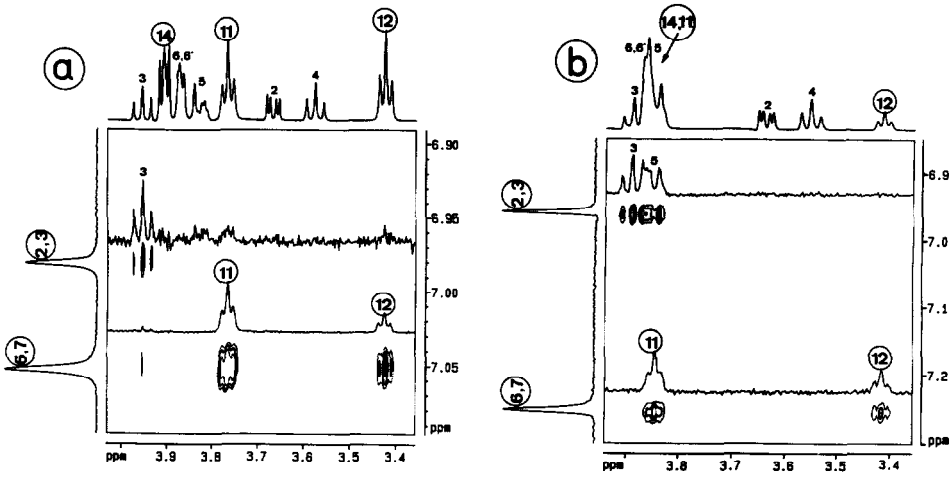

Fig. 2. Partial contour maps from 2D ROESY spectra of mitoxantrone with cyclomaltooligosaccharides recorded in ^2H_2O at 500 MHz and 298 K with 500 ms mixing time in a 2G spin-lock field. (a) Interaction with cyclomaltoheptaose (sample D of Fig. 1). (b) Interaction with cyclomaltooctaose (sample G of Fig. 1). Solid lines are sections taken at the level of crosspeaks.

and H-7 protons of mitoxantrone show strong crosspeaks with the H-11 and H-12 protons of each of its side chains, confirming the assignment. The presence of intermolecular contacts (distance ≤ 5 Å) between the aromatic protons (H-2 and H-3) of mitoxantrone and the H-3 protons of cyclomaltooligosaccharides (Figs. 2a and b) confirms the formation of inclusion complexes. It is also noteworthy that the crosspeak correlating the resonances of the H-2 and H-3 protons of mitoxantrone, and H-5 of cyclomaltooctaose (Fig. 2b), is missing in the ROESY spectrum of mitoxantrone and cyclomaltoheptaose. This implies that the guest molecule enters into cyclomaltoheptaose and cyclomaltooctaose from the large hole, but the penetration is deeper in the latter. Inclusion of the drug from the narrower primary hydroxyl hole of cyclomaltooctaose can be ruled out, since no crosspeak between the resonances of the H-2 and H-3 protons of the guest, and H-6 of the host was observed. The unexpected weak shifts of the H-3 protons of cyclomaltoheptaose upon inclusion of the drug can be explained by compensation effects of different anisotropic groups (aromatic and carbonyl groups). This indicates that analysis of induced shifts to derive geometrical considerations[7] can be misleading and should be completed by the more relevant determination of dipolar contacts, as performed in the NOESY or ROESY experiments.

One of the factors affecting the stability of the inclusion complexes is hydrogen bonding between guest polar groups and primary or secondary hydroxyl groups of cyclomaltooligosaccharides. In order to estimate the contribution of this type of interaction, we carried out ^1H NMR studies of mitoxantrone with octakis(2,3,6-tri-*O*-methyl)cyclomaltooctaose, which lacks free hydroxyl groups. Comparison of the ^1H NMR spectra in the absence and in the presence of one molar equivalent of

the drug showed no shift variation in the protons of the potential host. This may indicate that no inclusion is taking place and that the hydroxyl protons of the host play a key role in the stabilization of the inclusion complex.

The capability of ^1H NMR spectroscopy for monitoring inclusion processes between drugs and cyclomaltooligosaccharides is thus clearly evidenced. Mitoxantrone is encapsulated in both cyclomaltoheptaose and cyclomaltooctaose and this could be used for adjusting its release rate. Moreover, it can be predicted that this process should increase the solubility of other analogs belonging to the mitoxantrone family of anticancer drugs, which are sparingly soluble in water and physiological fluids.

ACKNOWLEDGMENTS

The authors are greatly indebted to Dr. P. Poitou (Laboratoires Lederle, France) for providing the mitoxantrone sample and to Dr. H. Galons (Université Paris V, France) for the kind gift of permethylated cyclomaltooctaose.

REFERENCES

1 D.S. Albert, *Invest. New Drugs*, 3 (1985) 101–107.
2 J. Blanz, K. Mewes, M. Beth, K.P. Zeller, and G. Ehninger, *Magn. Res. Chem.*, 29 (1991) 1072–1074.
3 F. Djedaïni and B. Perly, *Magn. Res. Chem.*, 28 (1991) 372–374.
4 F. Djedaïni and B. Perly, in D. Duchêne (Ed.), *New Trends in Cyclodextrins and Derivatives*, Editions de Santé, Paris, 1991, pp 217–246.
5 A.A. Bothner-By, R.L. Stephens, and J.M. Lee, *J. Am. Chem. Soc.*, 106 (1984) 811–813.
6 S. Macura, K. Wüthrich, and R.R. Ernst, *J. Magn. Reson.*, 47 (1982) 351–357.
7 D.J. Wood, F.E. Hruska, and W. Saenger, *J. Am. Chem. Soc.*, 99 (1977) 1735–1740.