Volume 272, number 1,2, 171-174 FEBS 08986 October 1990

Hoogsteen versus Watson-Crick A-T basepairing in DNA complexes of a new group of 'quinomycin-like' antibiotics

Mark S. Searle[1] and Geoffrey Wickham[2]

[1]*Peter MacCallum Cancer Institute NMR Facility* and [2]*Department of Medicinal Chemistry, Victorian College of Pharmacy, 381 Royal Parade, Parkville, Victoria 3052, Australia*

Received 15 August 1990

The interaction of a new group of 'quinomycin-like' antibiotics with the DNA duplexes $d(ACGT)_2$ and $d(GACGTC)_2$ has been investigated in solution by ^1H NMR spectroscopy. By monitoring the intensity of intranucleotide base H6/H8 to deoxyribose H1'NOE cross-peaks we conclude that the terminal A-T basepairs flanking the CG bisintercalation site in the $d(ACGT)_2$ complex adopt the Hoogsteen bonding scheme, with the purine base in a *syn* conformation. By comparison in the $d(GACGTC)_2$ complex all glycosidic bond angles are *anti*, consistent with a preferred Watson-Crick basepairing scheme. Both DNA duplexes appear to be significantly unwound compared with the ligand-free DNAs. The data illustrate the influence of helical constraints on the stability of the Hoogsteen bonding scheme adjacent to the drug binding sites.

Quinomycin antibiotic; $d(ACGT)_2$; $d(GACGTC)_2$; ^1H NMR NOESY; Watson-Crick/Hoogsteen basepair

1. INTRODUCTION

The quinoxaline antitumour antibiotics are a family of cyclic depsipeptides whose biological properties result from their ability to bind to DNA in susceptible cells and interfere with nucleic acid synthesis [1,2]. Echinomycin, the best known of this class of compounds, binds to DNA through the process of bisintercalation [3] and the structure of its DNA complex has been elucidated by X-ray crystallographic analysis [4]. A remarkable feature of the $(echinomycin)_2$-$d(CGTACG)_2$ complex, in which drug molecules bracket the terminal CG basepairs, is that the antibiotics induce Hoogsteen basepairing in the two central A-T basepairs of the complex. In this conformation the purine bases are flipped into a *syn* orientation about the glycosidic bond and a different hydrogen bonding scheme is adopted between the two components of the basepair (see Fig. 1). Several studies have been directed towards determining the existence, or otherwise, of such a radical drug-induced structural change in solution. The hyper-reactivity of DNA to diethylpyrocarbonate (DEPC) in the presence of echinomycin has been interpreted by Mendel and Dervan [5] in terms of an altered DNA conformation involving Hoogsteen basepairing, while recent results have led McLean and Waring [6] to propose that the DEPC reactivity be interpreted in terms of sequence-specific unwinding of the DNA helix. To date, two solution NMR studies have been brought to bare on this issue. Gao and Patel [7] have revealed that the terminal A-T basepairs of an echinomycin complex with $d(ACGT)_2$, but not with $d(TCGA)_2$, are Hoogsteen basepaired in solution, illustrating the sequence dependent nature of the phenomenon. Gilbert et al. [8] also report that the terminal A-T basepairs of an $(echinomycin)_2$-$d(ACGTACGT)_2$ complex are stably Hoogsteen paired while the A-T basepairs at the core of the duplex are unstable but adopt the Hoogsteen pairing scheme at low temperatures where the structure is similar to that found in the echinomycin-DNA crystal structure [4].

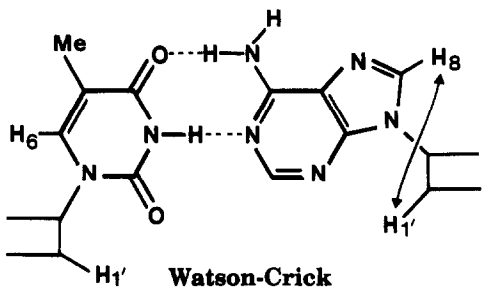
Recently, we have investigated the interaction of a new family of 'quinomycin-like' antibiotics [9] (see Fig. 2) with several defined-sequence oligonucleotide duplexes using NMR spectroscopy. These compounds have several novel structural features, such as 3-hydroxy-quinaldic acid chromophores replacing the quinoxaline rings of echinomycin, and methylcyclopropyl groups replacing the valine residues, which are capable of modifying their DNA binding characteristics compared with echinomycin. Of immediate interest is the effect of these antibiotics on the A-T basepairing scheme flanking the 5'-CG binding sites in the duplexes $d(ACGT)_2$ and $d(GACGTC)_2$.

2. MATERIALS AND METHODS

A sample of UK-65,662 (factor B) was kindly provided by Pfizer Ltd. (Sandwich, Kent, UK) and was used without further purification. Samples were prepared by dissolving two equivalents of drug in 0.5 ml of acetonitrile to which was added 0.5 ml of aqueous buffer (10 mM phosphate, 50 mM NaCl, pH 7.0) containing the appropriate oligonucleotide. The mixture was diluted to 5 ml with D_2O and the

Correspondence address: M.S. Searle, Peter MacCallum Cancer Institute NMR Facility, Victorian College of Pharmacy, 381 Royal Parade, Parkville, Victoria 3052, Australia

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50



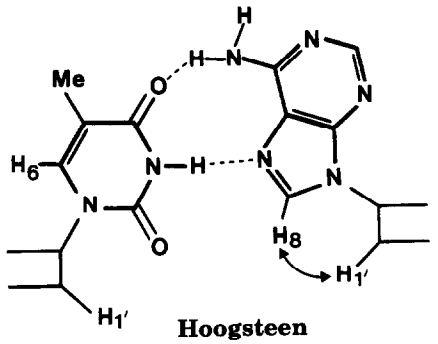


Fig. 1. A schematic representation of A-T basepairs in both the Watson-Crick and Hoogsteen pairing schemes. Arrows identify the interproton distances (H8-H1′) used to monitor the glycosidic bond conformation.

solution stirred for 48 h in a cold-room at 4°C. The solution was then lyophilized and redissolved in 0.5 ml of D_2O with the excess drug being removed by centrifugation. The QN/d(ACGT)$_2$ complex formed readily under these conditions while NMR studies were undertaken on a sample of d(GACGTC)$_2$ in which 70–80% of the duplex was ligand bound.

^{1}H NMR spectra were collected at 400 MHz on a Varian VXR400 wide-bore spectrometer. Phase-sensitive NOESY experiments were performed using the hypercomplex method of data collection. The data were recorded as 1024 points in t_2 for each of 2×320–360 t_1 values, over a spectral width of 4000 Hz, and zero-filled to a 2048×2048 data matrix prior to Fourier transformation using mild squared-guassian window functions. Data acquisition times of 24 h were typically employed.

3. RESULTS AND DISCUSSION

The glycosidic bond angles in a Watson-Crick basepaired B-DNA helical structure lie in the *anti* range [10] and this conformation is associated with a particularly weak intranucleotide NOE between base H6/H8 and the deoxyribose H1′ corresponding to a proton-proton separation of 3.5–3.7 Å. In the Hoogsteen basepairing scheme (see Fig. 1) the purine base is flipped into a *syn* orientation about the glycosidic bond in which the base H8 to deoxyribose H1′ distance decreases dramatically to 2.5–2.7 Å. The intensity of this NOE, calibrated with respect to a fixed reference distance such as that between the cytosine base H5 and H6 (2.5 Å), provides a convenient means of discriminating between the Watson-Crick and Hoogsteen basepairing schemes. The effects of UK-65,662 (QN) on the basepairing schemes in its complexes with d(ACGT)$_2$ and d(GACGTC)$_2$ have been investigated by monitoring the intensity of these cross-peaks in 100 ms NOESY spectra at 20°C. In both complexes a large number of intermolecular NOEs clearly identifies the depsipeptide ring of the antibiotic as binding in the

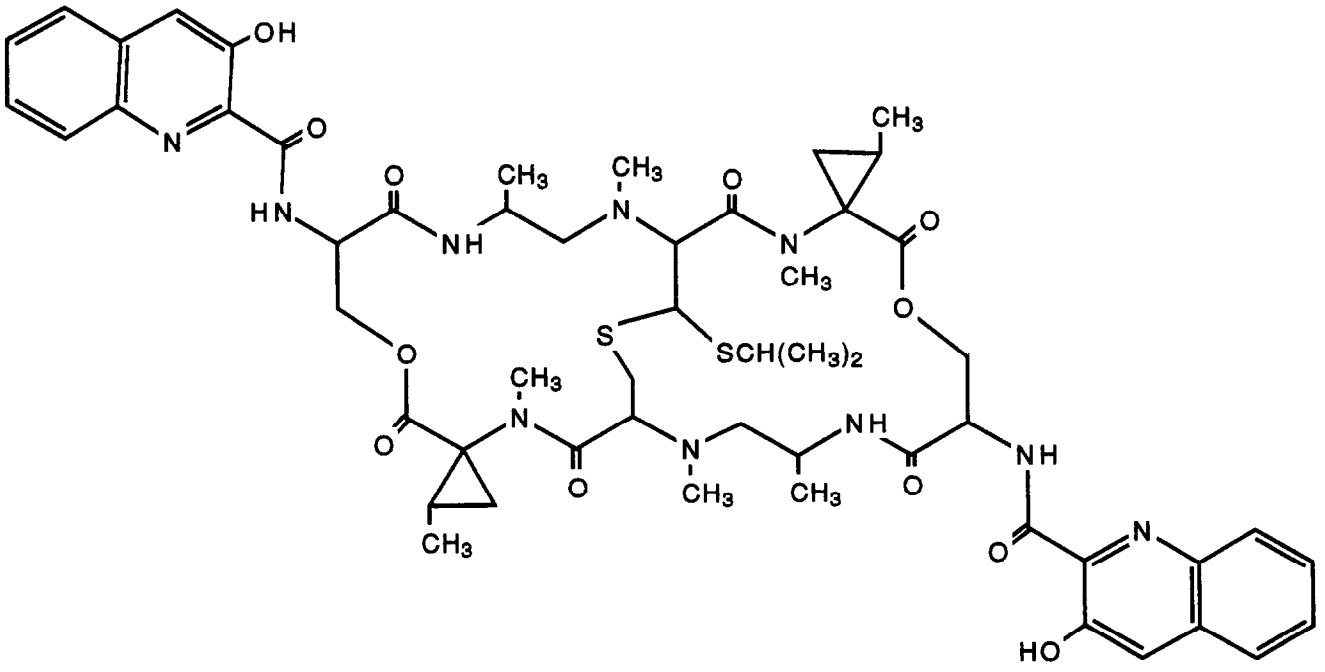


Fig. 2. Structure of UK-65,662 (factor B).

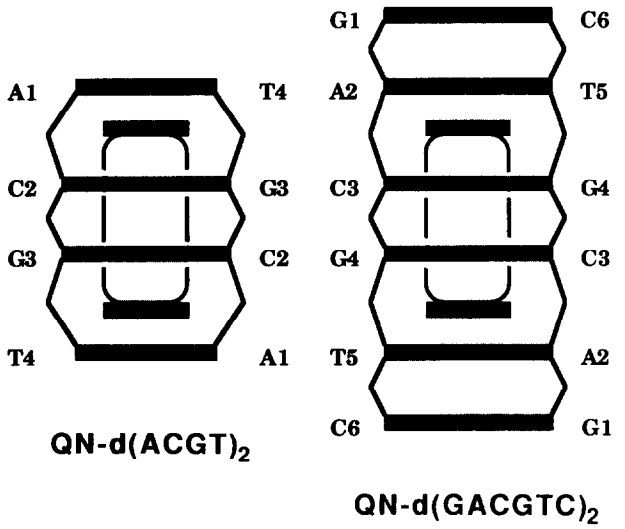


Fig. 3. Schematic representation of the QN/d(ACGT)$_2$ and QN/d(GACGTC)$_2$ complexes including the nucleotide numbering scheme adopted.

minor groove bracketing the CG basepairs at the centre of each duplex as illustrated schematically in Fig. 3. A detailed description of resonance assignments and the structure and dynamics of these complexes will be published elsewhere.

In Fig. 4 both stack plot and contour plot representations of the H6/H8-H1′ region of the NOESY spectrum of QN/d(ACGT)$_2$ are illustrated. Intranucleotide H6/H8-H1′ cross-peaks are labelled on the contour plot while the stack plot presentation provides a clearer comparison between cross-peak intensities relative to the cytosine reference peak (labelled *C2*). It is apparent that a much stronger NOE is observed for A1H8-H1′ than for any of the remaining base H6/H8 and sugar H1′ interactions. The comparable intensity of the A1 cross-peak to that of C2 H5-H6 demonstrates that A1 adopts a *syn* glycosidic orientation, consistent with the Hoogsteen basepairing scheme for A1-T4, while those of C2-G3 are Watson-Crick basepaired. Thus the an-

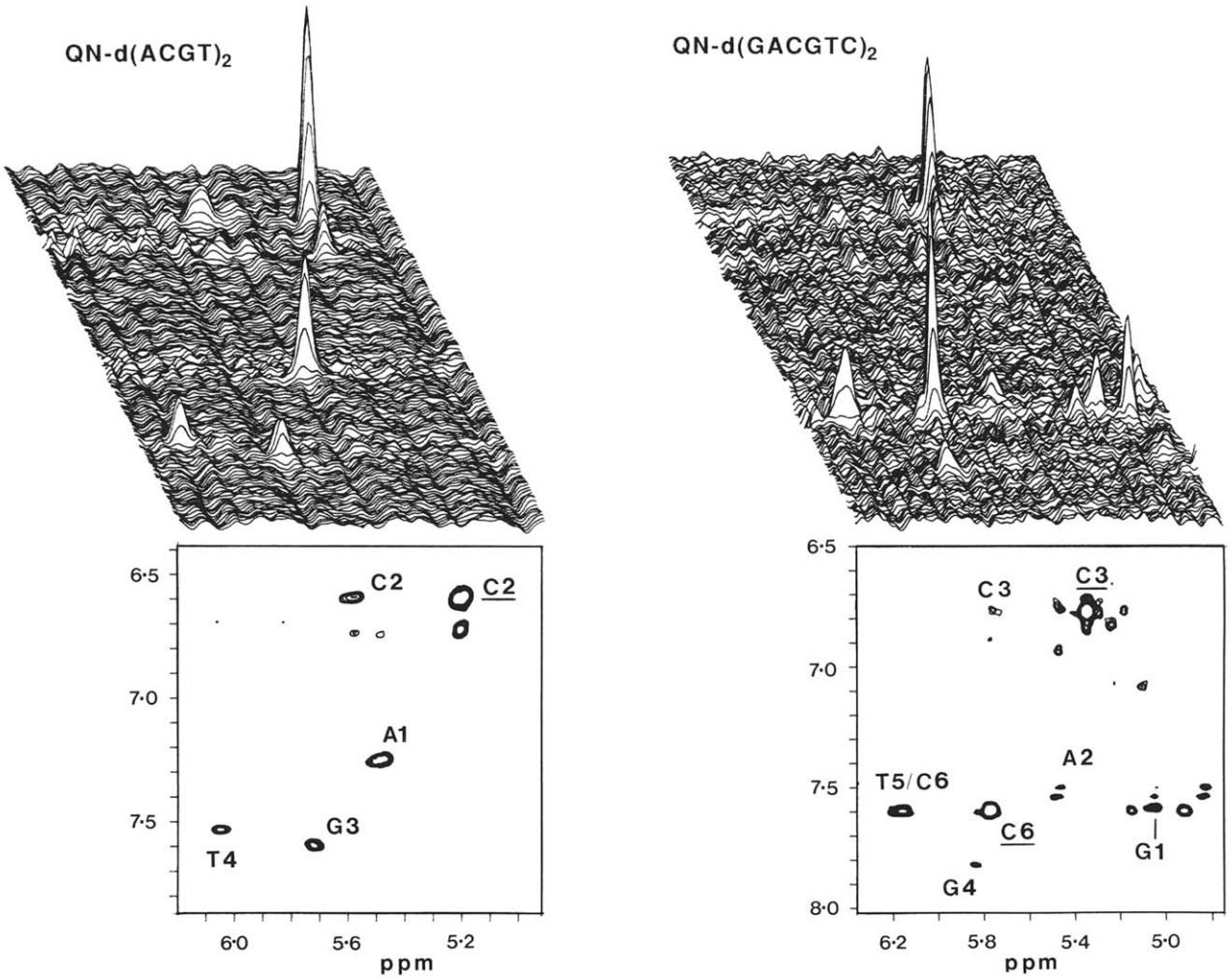


Fig. 4. Stack plot and contour plot represenations of the 100 ms NOESY spectra of QN/d(ACGT)$_2$ and QN/d(GACGTC)$_2$ recorded at 20°C. The portions of the spectra containing the H6/H8-H1′ inter- and intranucleotide NOE cross-peaks are highlighted. Intranucleotide connectivities are labelled together with the cytosine H5-H6 reference peaks (underlined) in each spectrum. The antibiotic molecule lacks a two-fold symmetry axis and partially removes the two-fold symmetry of the two duplexes under study. This is only clearly visible for the A2H8 resonances in the QN/d(GACGTC)$_2$ complex, in all other cases the chemical shift differences are too small to resolve in the NOESY spectra.

tibiotic UK-65,662 induces similar conformational changes in $d(ACGT)_2$ to those reported for echinomycin by Gao and Patel [7].

A similar region of the 100 ms NOESY spectrum of the QN/$d(GACGTC)_2$ complex is also illustrated in Fig. 4. Again the intranucleotide H6/H8-H1′ cross-peaks are labelled and all are found to have very low intensities compared with the *C3* and *C6* reference peaks, including those for A2 (equivalent to A1 in the tetramer complex; see Fig. 3). We conclude that in the complex with $d(GACGTC)_2$, under the same experimental conditions, all glycosidic bonds are in the *anti* conformation indicative of basepairs adopting the Watson-Crick pairing scheme. It is also apparent that in the spectra of both complexes internucleotide H6/H8-H1′ correlations, also found in the portions of the NOESY spectra illustrated in Fig. 4, are particularly weak compared with intranucleotide interactions. This observation, together with the pronounced downfield shifts in the ^{31}P NMR spectra of these duplexes (data not shown), leads us to conclude that there is a high degree of unwinding of the DNA helix associated with ligand binding.

The data indicate that quite different influences are at work in stabilizing the A-T basepairing scheme within these two complexes. In the absence of helical constraints, as is the case in the QN/$d(ACGT)_2$ complex, the A-T basepairs adopt the Hoogsteen pairing scheme. This suggests that the most favourable stacking interactions are those involving the quinoline chromophores and the six-membered ring of the purine base when flipped into the *syn* conformation, as reported for the crystalline complex of echinomycin [4]. In the longer sequence of DNA, the A-T basepairs flanking the CG binding site are subject to the helical constraints imposed by the terminal G-C basepairs and in this situation the Hoogsteen pairing scheme is not preferred. These results together with those of Gilbert et al. [8] illustrate the inherent instability of the Hoogsteen basepairing scheme in solution within the constraints of an extended B-DNA helix.

In conclusion, our structural interpretation of the data reported here on the QN/$d(GACGTC)_2$ complex parallels that of McLean and Waring for the observed DEPC hyper-reactivity of natural DNA in the presence of quinomycin antibiotics [6]. Their model favours sequence-specific unwinding of the DNA helix as the source of the enhanced reactivity, rather than structural changes involving Hoogsteen basepairing. Recent evidence from Fox and Kentebe [11] suggests that structural changes induced by echinomycin are not confined to regions surrounding the drug binding sites but can be cooperatively propagated over several turns of the DNA helix. The structural basis for these phenomena will be more clearly understood when further solution NMR data become available on DNA complexes of these antibiotics.

REFERENCES

[1] Ward, D., Reich, E. and Goldberg, I.H. (1965) Science 149, 1259.
[2] Waring, M.J. and Makoff, A. (1974) Pharmacology 10, 214.
[3] Waring, M.J. and Wakelin, L.P.G. (1974) Nature 252, 653–657.
[4] Ughetto, G., Wang, A.H.-J., Quigley, G.J., van der Marel, G.A., van Boom, J.H. and Rich, A. (1985) Nucleic Acids Res. 13, 2305.
[5] Mendel, D. and Dervan, P.B. (1987) Proc. Natl. Acad. Sci. USA 84, 910–914.
[6] McLean, M.J. and Waring, M.J. (1988) J. Mol. Recogn. 1, 138–151.
[7] Gao, X. and Patel, D.J. (1988) Biochemistry 27, 1744–1751.
[8] Gilbert, D.E., van der Marel, G.A., van Boom, J.H. and Feigon, J. (1989) Proc. Natl. Acad. Sci. USA 86, 3006–3010.
[9] Rance, M.J., Ruddock, J.C., Pacey, M.S., Cullen, W.P., Huang, L.H., Jefferson, M.T., Whipple, E.B., Maeda, H. and Tone, J. (1989) J. Antibiot. 42, 206–217.
[10] Saenger, W. (1984) Principles of Nucleic Acid Structure (Cantor, C.R. ed.) Springer-Verlag, New York.
[11] Fox, K.R. and Kentebe, E. (1990) Nucleic Acids Res. 18, 1957–1963.