COMMUNICATION

Structure of the nogalamycin–d(ATGCAT)$_2$ complex in solution: DNA recognition at an isolated TpG site

Huw E. Williams and Mark S. Searle *

Department of Chemistry, University of Nottingham, University Park, Nottingham, UK NG7 2RD

The solution structure of the 1:1 nogalamycin–d(ATGCAT)$_2$ complex, determined from high resolution NMR data and refined using restrained molecular dynamics, reveals details of the interaction and preferred orientation of the antibiotic at its high affinity 5′-TpG intercalation site.

The anthracycline antibiotics are amongst some of the most widely used and most effective chemotherapeutic agents available. The molecular basis for their mode of action lies in their ability to inhibit transcription factor binding or interfere with topoisomerase II activity largely through DNA recognition and the formation of a highly stable drug–DNA complex.[1] The unique structure of nogalamycin [Fig. 1(*a*)], which permits the antibiotic to thread through the DNA helix and interact with both the major and minor grooves simultaneously,[2] results in a high binding affinity and slow binding kinetics[3] that have been linked to its potent biological activity[4,5] but also its higher level of cytotoxicity than observed for other members of the anthracycline family.[3]

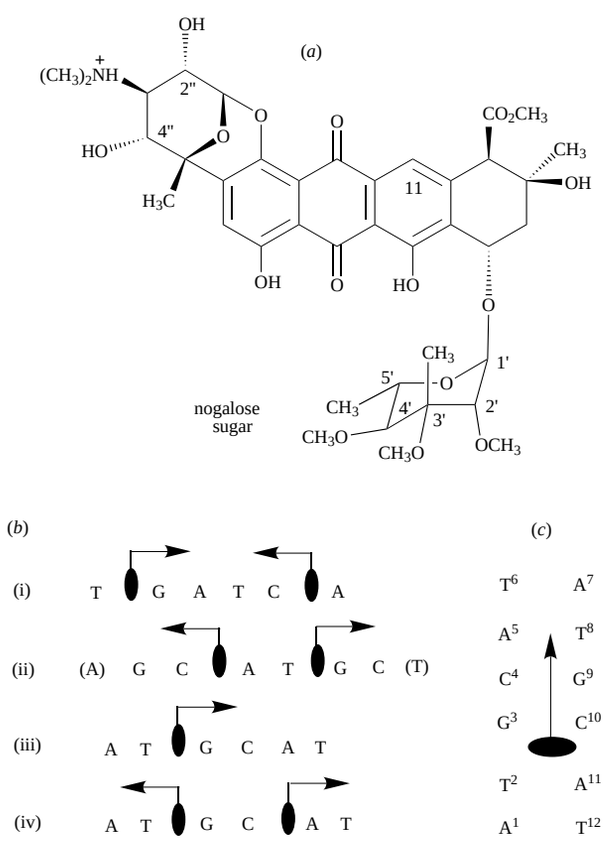

Fig. 1 (*a*) Structure of nogalamycin showing atom labelling relevant to the NOE data highlighted in the text. (*b*) Schematic illustration of the 2:1 complex of nogalamycin studied by X-ray analysis (i), 2:1 complexes studies by NMR (ii), and the hexamer sequence studied in this work (iii) and (iv). Nucleotide numbering scheme is shown in (*c*). The head of the arrow represents the alignment of the nogalose sugar with respect to the DNA sequence. The intercalated aglycone of the antibiotic is represented by a small oval.

The DNA sequence selectivity of nogalamycin has been studied and shown to involve intercalation preferentially at the 5′-pyrimidine–purine steps 5′-TpG (and equivalent 5′-CpA) and 5′-CpG.[6] The molecular basis for high affinity binding to these sites has been investigated by X-ray[7–13] and NMR studies[2,14–16] of a number of complexes. An analysis of the preferred binding orientation of nogalamycin at the asymmetric 5′-TpG site has been complicated by two factors, end-effects and the stoichiometry of the complexes studied, as summarised in Fig. 1(*b*). Nogalamycin intercalates at the TpG step and the nogalose sugar lies in the minor groove, its position and orientation indicated by the arrow head shown. In all X-ray structures reported to date, antibiotic molecules are bound at the terminal intercalation site [for example, Fig. 1(*b*) (i)], leading to the suggestion that the bound orientation is largely dictated by end-effects,[11] with the nogalose preferring to lie in the groove rather than overhang the ends of the duplex and interfere with crystal packing. In contrast, the NMR structures[2,14] [represented in Fig. 1(*b*) (ii)] accommodate two bound drug molecules in close proximity such as to suggest that steric interactions between nogalamycin molecules dictate their bound orientation.[11] These authors concluded that in longer sequences of DNA, with intercalation sites situated further apart, the alternative (180° related) orientation may be observed for the drug bound at the 5′-TpG site.

To address the question of the orientational preference of the antibiotic and probe its molecular basis we have studied by NMR the complex of nogalamycin with the hexamer duplex d(ATGCAT)$_2$ containing adjacent 5′-TpG and 5′-CpA high affinity sites. The two sites are in non-terminal positions so as to avoid the complications of end effects. The two possible modes of complexation are shown in Fig. 1(*b*) (iii) and (iv). The formation of a 1:1 nogalamycin–d(ATGCAT)$_2$ complex, with the bound orientation as shown in (iii), precludes binding at the second site on steric grounds. Alternatively, drug molecules can be accommodated at both the 5′-TpG and 5′-CpA sites in a 2:1 (nogalamycin)$_2$–d(ATGCAT)$_2$ complex provided the bound orientation is that shown in (iv), where steric repulsions between drug molecules largely dictate the orientation. We have examined these possibilities through NMR titration studies and detailed structural analysis of the resulting complex.

Titration of the antibiotic into a 3 mM solution of d(ATGCAT)$_2$ (100 mM NaCl, 10 mM phosphate buffer at pH 7.0 and 288 K) leads to the lifting of the dyad symmetry of the duplex with the number of resonances in the ^{1}H NMR spectrum consistent with the formation of a single complex with one antibiotic molecule bound per duplex. We see no evidence for multiply bound species. A full assignment of the complex

* E-Mail: mark.searle@nottingham.ac.uk

from an analysis of 2D homonuclear NOESY, DQF–COSY, TOCSY and heteronuclear ^{1}H–^{13}C HMQC data sets has enabled a large number of intermolecular NOEs to be identified. The DNA sequential assignment pathway involving deoxyribose H1′ and base H6/H8 helps identify the TpG intercalation site [Fig. 2; for nucleotide numbering scheme see Fig. 1(*c*)]. The NOE connectivities are contiguous along strand 7–12, however, in strand 1–6 the T2H1′→G3H8 NOE is undetected implying an interproton separation significantly larger than for the standard B-DNA conformation alluding to DNA unwinding and intercalation at this site. This is confirmed by strong NOEs from the antibiotic aglycone H11 to deoxyribose protons T2H1′ and G3H1′ that can only be satisfied by intercalation at the TpG step with the orientation of the antibiotic as represented by Fig. 1(*b*) (iii). Many NOEs from the methyl and methoxy groups of the nogalose sugar to deoxyribose protons located in the minor groove determine with some precision the position and orientation of the nogalose sugar with respect to the intercalation site.[2,14–16] For example, NOEs from 3′-OCH_3 to C4H1′ and A5H1′, and from 2′-OCH_3 to C10H1′ and A11H1′ unambiguously establish that the nogalose lies along the groove pointing towards the adjacent CpA site. A full set of NOE restraints have been determined for the complex and structures calculated using restrained molecular dynamics† using the AMBER suite of programs.[17] We have employed an explicit solvation model using the particle mesh Ewald summation method to treat electrostatic interactions.[18] A family of 10 overlayed structures is shown in Fig. 3, which satisfies all NOE restraints extremely well (RMS deviation from input restraints 0.18 Å). The nogalose sugar forms extensive hydrophobic interactions with the minor groove, as illustrated by the many intermolecular interactions involving drug methyl or methoxy groups. However, the molecular basis for the

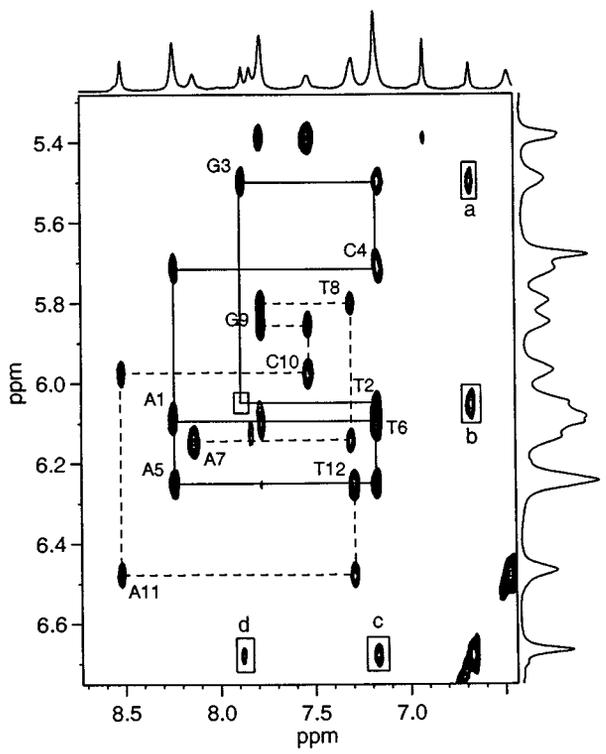

Fig. 2 Portion of the 500 MHz 200 ms NOESY spectrum of the nogalamycin–d(ATGCAT)$_2$ complex recorded at 288 K, pH 7.0. Sequential connectivities between deoxyribose H1′→base H6/H8 are highlighted for the two strands (solid lines *versus* broken lines). The empty box represents the expected position of the internucleotide G3H8–T2H1′ NOE that is absent from the spectrum. Several additional drug–DNA NOEs are highlighted that define the 5′-TpG intercalation site: (a) G3H1′–H11, (b) T2H1′–H11, (c) T2H6–H11 and (d) G3H8–H11.

† A set of 198 NOE restraints (20 drug–DNA restraints) were determined from NOE cross-peak integration in 500 MHz NOESY spectra collected at 288 K with mixing times between 50 and 300 ms. Cross-peak volumes were normalised to several fixed reference distances within the DNA structure including deoxyribose H2′–H2″, thymine Me–H6 and cytosine H5–H6 cross-peaks, according to the NOEs being calibrated.[20–22] Distances were estimated from data at a number of mixing times using linear regression to extrapolate to 0 ms mixing time to minimise the effects of spin diffusion on distance estimates.[23] A 20% error bound was applied to all distance restraints. Structure calculations were carried out on an R10000SC Silicon Graphics work station using AMBER 4.1.[17] The drug was parameterised and point charges determined by a semi-empirical approach within Spartan 3.1 using the AM1 method.[24] The starting structure for the drug–DNA complex was modelled using the Leap module within AMBER by sandwiching the drug between two fragments of canonical B-DNA generated using NUCGEN. Counterions were added to neutralise phosphate charges and the system solvated to a minimum distance of 5 Å around the solute using boxes of 216 TIP3P water molecules.[17] To equilibrate the system, a 5000 step conjugate gradient minimisation was applied to first the water, then water and counterions and finally the whole system. With the coordinates of the DNA, counterions and drug frozen, the water was subjected to 10 ps molecular dynamics at 100 K, followed by 10 ps in which the counterions were released. The system was heated to 300 K over 5 ps and held there for a further 5 ps with the particle mesh Ewald summation algorithm activated.[17,18] Coordinate restraints on the system were gradually released over 30 ps. At this stage the RMS deviation from the experimentally determined distance restraints was 0.71 Å. The NOE restraints were then introduced gradually over 2.5 ps at 300 K by increasing the restraint force constant from 0 to 32 kcal mol^{-1} Å^{-2}, followed by 100 ps constant temperature dynamics at the end of which the system was cooled to 1 K over 2 ps, and held at 1 K for 5 ps, to generate a low energy structure. The RMS deviation from the input restraints averaged over the last 5 ps of the dynamics run was 0.18 Å. In Fig. 3, 10 snap-shots taken over 10 ps of the dynamics simulation at 300 K are illustrated having a pairwise RMSD over all heavy atoms of 0.37 Å.

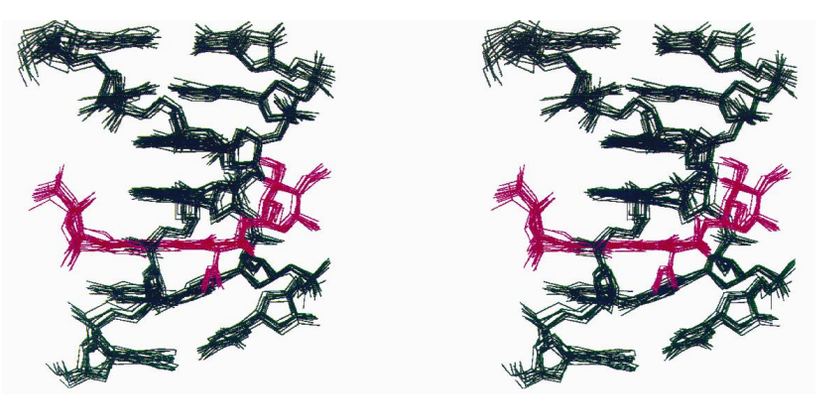

Fig. 3 Stereoview of 10 overlayed structures taken over 10 ps of constant temperature dynamics (300 K) of the nogalamycin–d(ATGCAT)$_2$ complex (displayed using MOLMOL[19]). View showing the threading of the antibiotic (magenta) through the DNA helix illustrating the position and orientation of the nogalose sugar in the minor groove (right) and bicyclic sugar in the major groove.

sequence specificity of nogalamycin appears to lie in the interactions in the major groove where the 4″-OH of the bicyclic sugar, in particular, forms a specific hydrogen bond with the N7 of guanine at the intercalation site.[2,7–16]

We have shown that nogalamycin forms a 1 : 1 complex with $d(ATGCAT)_2$ in which only one of the 5′-TpG sites is occupied. It is evident from a detailed determination of the structure of the complex that in its preferred orientation the nogalose sugar occludes the minor groove presenting a steric block to binding at the second 5′-CpA (5′-TpG) site. We are led to conclude that the end effects and steric factors, alluded to in earlier work,[11] do not compromise the binding interaction of the drug at its high affinity 5′-TpG binding site.

Acknowledgements

We gratefully acknowledge the support of an EPSRC studentship to H. E. W. and financial contributions from the Nuffield Foundation, Royal Society and the Department of Chemistry at the University of Nottingham. We thank John Keyte (Department of Biochemistry) for oligonucleotide synthesis, and Dr Charles Laughton for helpful discussions.

References

1 J. F. Fisher and P. A. Aristoff, *Prog. Drug. Res.*, 1988, **32**, 411.
2 M. S. Searle, J. G. Hall, W. A. Denny and L. P. G. Wakelin, *Biochemistry*, 1988, **27**, 4340.
3 K. R. Fox, C. Brasset and M. J. Waring, *Biochem. Biophys. Acta*, 1985, **840**, 383.
4 B. K. Bhuyan and A. Dietz, *Antimicrob. Agents Chemother.*, 1965, 836; B. K. Bhuyan and F. Reusser, *Cancer Res.*, 1970, **30**, 948; L. H. Li, S. L. Kuentzel, L. L. Murch, L. M. Pschigoda and W. C. Krueger, *Cancer Res.*, 1979, **39**, 4816.
5 H. Ennis, *Antimicrob. Agents Chemother.*, 1981, **19**, 657.
6 K. R. Fox and M. J. Waring, *Biochemistry*, 1986, **25**, 4349.
7 Y.-C. Liaw, Y.-G. Gao, H. Robinson, G. A. van der Marel, J. H. van Boom and A. H.-J. Wang, *Biochemistry*, 1989, **28**, 9913.
8 L. D. Williams, M. Egli, Q. Gao, P. Bash, G. A. van der Marel, J. H. van Boom, A. Rich and C. A. Frederick, *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 2225.
9 Y.-G. Gao, Y.-C. Liaw, H. Robinson and A. H.-J. Wang, *Biochemistry*, 1990, **29**, 10 307.
10 M. Egli, L. D. Williams, C. A. Frederick and A. Rich, *Biochemistry*, 1991, **30**, 1364.
11 C. K. Smith, G. J. Davies, E. J. Dodson and M. H. Moore, *Biochemistry*, 1995, **34**, 415.
12 C. K. Smith, J. A. Brannigan and M. H. Moore, *J. Mol. Biol.*, 1996, **263**, 237.
13 G. S. Schuerman, C. K. Smith, J. P. Turkenburg, A. N. Dettmar, L. Van Meervelt and M. H. Moore, *Acta Crystallogr.*, *Sect. D*, 1996, **52**, 299.
14 X. Zhang and D. J. Patel, *Biochemistry*, 1990, **29**, 9451.
15 M. S. Searle and W. Bicknell, *Eur. J. Biochem.*, 1992, **205**, 45.
16 H. Robinson, Y.-C. Liaw, G. A. van der Marel, J. H. van Boom and A. H.-J. Wang, *Nucleic Acids Res.*, 1990, **18**, 4851.
17 D. A. Pearlman, D. A. Case, J. W. Caldwell, W. S. Ross, T. E. Cheatham, D. M. Fergusson, G. L. Seibel, U. C. Singh, P. K. Weiner and P. A. Kollman, AMBER 4.1, 1995, University of California, San Francisco, 1995.
18 T. E. Cheatham, J. L. Millar, T. Fox, T. A. Darden and P. A. Kollman, *J. Am. Chem. Soc.*, 1995, **117**, 4193.
19 R. Koradi, M. Billeter and K. Wuthrich, *J. Mol. Graphics*, 1996, **14**, 51.
20 M. S. Searle, *Prog. Nucl. Magn. Reson. Spect.*, 1993, **25**, 403.
21 D. J. Patel, L. Shapiro and D. Hare, *Quat. Rev. Biophys.*, 1987, **20**, 35.
22 R. V. Hosur, G. Govil and H. T. Miles, *Magn. Reson. Chem.*, 1988, **26**, 927.
23 J. D. Beleja, J. Moult and B. D. Sykes, *J. Magn. Reson.*, 1990, **87**, 375.
24 SPARTAN 3.1, 1994–1995, Wavefunction Inc., USA.

Paper 7/07093D
Received 1*st October* 1997
Accepted 18*th November* 1997