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MOLECULAR MODELS FOR THE INTERACTION OF THE ANTI-TUMOUR DRUG NOGALAMYCIN WITH DNA

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Abstract—Computer graphics and non-bonded energy calculations have been used to model the intercalative binding of the anti-tumour antibiotic nogalamycin, to double-stranded DNA. The drug is predicted to bind preferentially to A–T rich sites. Specific hydrogen bonds to the exocyclic amino group of adenine bases on the 3′ side of an intercalative site have been found by the modelling procedure.

The anthracycline antibiotic nogalamycin (I) has activity, both *in vitro* and *in vivo* against several leukemia cell lines [1–3]. Several related compounds, such as the 10-descarbomethyl *dis*-nogamycin, have superior activity; in many cases these are paralleled by enhancements of the DNA-binding properties shown by nogalamycin itself.

Studies on the *in vitro* interaction of nogalamycin with double-stranded DNA [4–6] have demonstrated classical DNA intercalative behaviour. Thus, the drug progressively removes and reverses the supercoiling of closed-circular duplex DNA, with an unwinding angle of 18° [7], relative to one of 26° for ethidium. DNA viscosity is enhanced upon binding, sedimentation rate decreased, and the temperature at which the helix-coil transition occurs (T_m), is raised. The binding constant is of the order of $10^6 M^{-1}$. There have been several reports that nogalamycin exhibits A–T preference [8–10] although no definitive study on this issue has as yet been performed. However, kinetic studies on the rate of drug dissociation from complexes with different polydeoxynucleotides have recently shown a markedly enhanced rate for the poly(dA–dT).poly(dA–dT) complex [11]. In general, removal of the nogalose ring group, as in 7-deoxy nogalarol (II), results in significant lowering of DNA affinity, as measured by affinity constants and T_m values.

The present study provides a detailed molecular model for the intercalative complex between nogalamycin and DNA using a combined computer graphics and empirical energy calculation approach [12–14]. The recent crystal structure determination of nogalamycin [15] has established relative and absolute stereochemistry, as well as many conformational details. We have used this molecular structure as our starting point.

METHODS

The DNA intercalation site geometry used was taken from the crystal-structure analysis of a dinucleoside phosphate d(CpG)–proflavine complex [16]. Accurate structural data on intercalation is only available at the dinucleoside level. Comparative analysis of this and other structures has shown that many backbone conformational features are invariant regardless of the nature of the intercalated drug, and thus the overall geometry can be taken as a first-order representation of intercalation complexes in general.

The sequence in this model, d(CpG), was altered to d(TpA) by modification of appropriate atoms on the bases. The base turn angle, equivalent to an unwound inter-base pair angle in DNA, was 17°; this was taken to correspond with the observed 18° value for nogalamycin and DNA (i.e. 36° for B-DNA minus 18° unwinding angle).

The dinucleoside geometry was held fixed throughout the study; the drug was positioned using the computer graphics display such that the anthracyclic chromophore was parallel to and mid-way between the two base pairs of the intercalation site, i.e. at 3.4 Å from each. Translational and rotational movements were then performed on the drug molecule, subject to the restriction of remaining in the intercalation plane. The intermolecular energy at each position was approximated by a non-bonded potential energy term

$$V_{N-B} = \frac{-A}{r^6} + \frac{B}{r^{12}}$$

where the parameters A and B were taken from Ref. 17. These parameters have a degree of "softness" and are designed to in part take account of bond angle flexibility. Electrostatic contributions to the energy were not calculated since (i) in this study we are primarily interested in possible molecular arrangements, which would be largely governed by non-bonded forces, (ii) calculation of partial atomic charges for a molecule of the complexity of nogalamycin would be difficult and liable to very considerable uncertainties.

All computer graphics manipulations and associated calculations were performed on a Gresham-Lion

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Supervisor 214 system linked to a PDP 11/34A computer, using the MOLEC molecular graphics software package [18]. Atomic coordinates for the postulated models are available upon request.

RESULTS AND DISCUSSION

Preliminary attempts at fitting nogalamycin into the model DNA binding site, immediately showed that the drug could not slide into the 6.8 Å base-pair separation cavity. This problem is due to the drug molecule having a bulky sugar group at each end, with a minimum width of over 12 Å. It is not possible to open an intercalation site beyond ~ 10 Å, even with maximum staggering of the nucleotide backbone torsion angles. Thus, nogalamycin can only form an intercalative complex by interacting with, and hence stabilising, non-base-paired regions of DNA, in such a manner that the resulting complex would be a truly intercalative one. An alternative, with the drug bound to a single-stranded site, is not easily feasible since the bulky sugars tend to exclude extensive chromophore–base overlap (see below). The stabilisation of pre-melted regions of DNA indicates a preference for interaction with A–T regions, in accord with previous observations [8–11]. Furthermore, it might be expected that nogalamycin would prefer an A–T rich sequence rather than an isolated A–T site embedded in a G–C rich region, since the former would be more likely to be transiently non-base-paired. Overall then, nogalamycin has inbuilt sequence-preference intercalative behaviour, in marked contrast to intercalators such as daunomycin or proflavine, which are able to slide into a standard unwound DNA site.

Detailed examination of the nogalamycin intercalative modelling showed that it was necessary to alter the conformation of the methoxycarbonyl side chain substituent on the drug molecule from that found in the crystal structure [15], in order to obtain optimal interaction (Fig. 1). These alterations did not significantly alter the drug's intramolecular energy, and merely reflect the fact that the crystal structure represents a "frozen-out" low-energy state.

It was found that two alternative structures for the nogalamycin–dinucleoside complex could be constructed.

(i) The nogalose sugar snugly fits in the DNA minor groove (Figs. 2–5). The non-bonded energy of interaction is -74.9 kcal mole^{-1}, and the drug chromophore lies at approximately right-angles to the long axis of the base-pairs, with relatively little chromophore–base overlap. Steric hindrance from the sugars forces the chromophore to lie in this position approximately mid-way between each nucleotide strand, with there being little possibility of lateral movements before the non-bonded energy

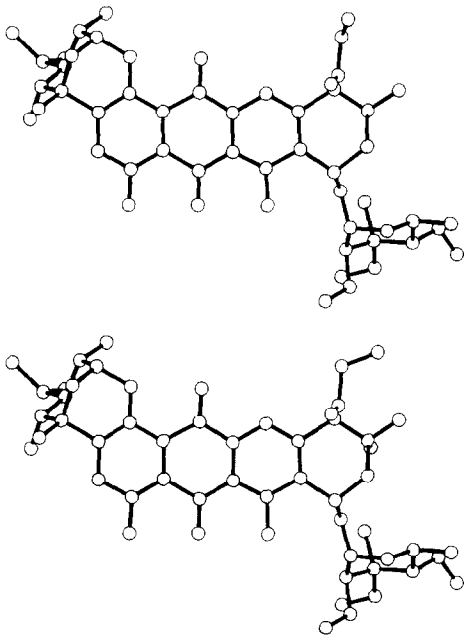

Fig. 1. Computer-drawn representations of the conformation of nogalamycin (a) as found in the crystal structure [15], and (b) as altered for optimum intercalation.

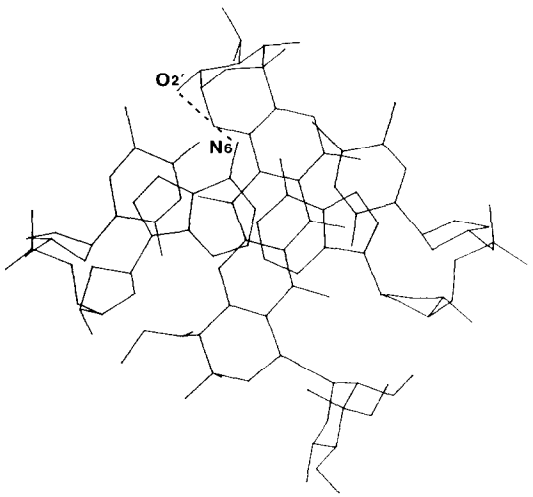

Fig. 2. Computer-drawn representation of the minimum-energy intercalation model (i), viewed looking down onto the base pairs.

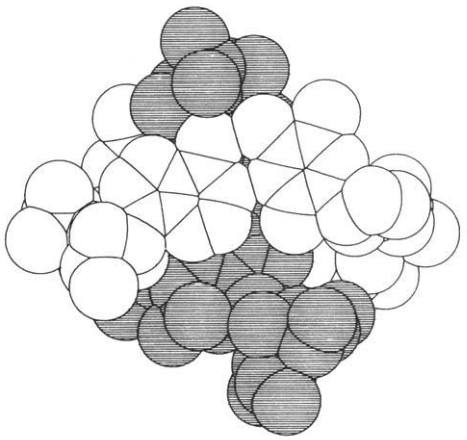

Fig. 3. Space-filling representation of complex (i), viewed looking down onto the base pairs.

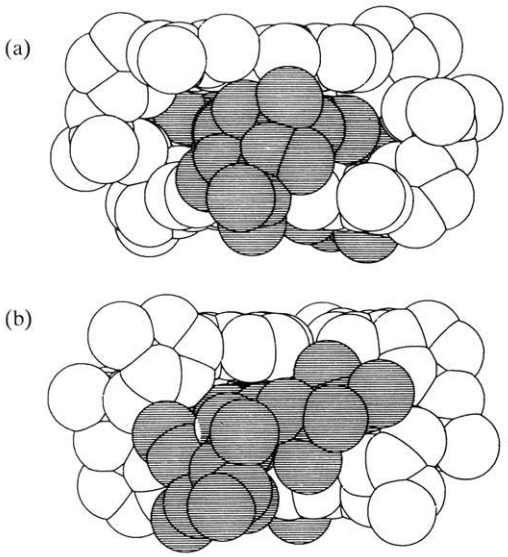

Fig. 4. Space-filling representation of complex (i), (a) showing the major groove binding, and (b) minor groove binding.

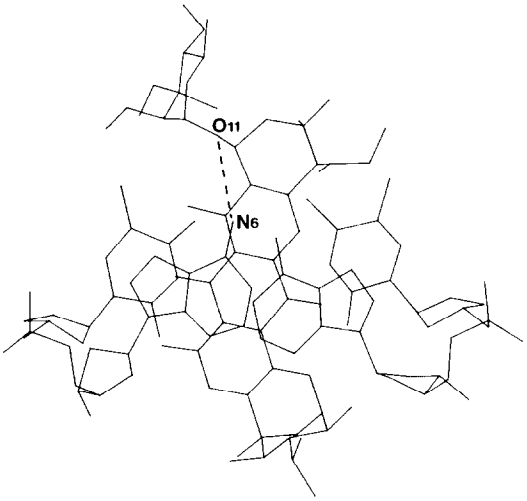

Fig. 6. Computer-drawn representation of the minimum-energy intercalation model (ii), viewed looking down onto the base pairs.

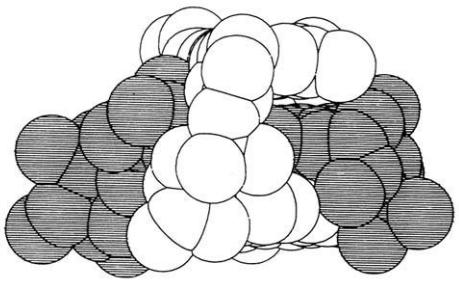

Fig. 5. Space-filling representation of complex (i), viewed along the base-pair plane, and having one nucleotide strand behind the other.

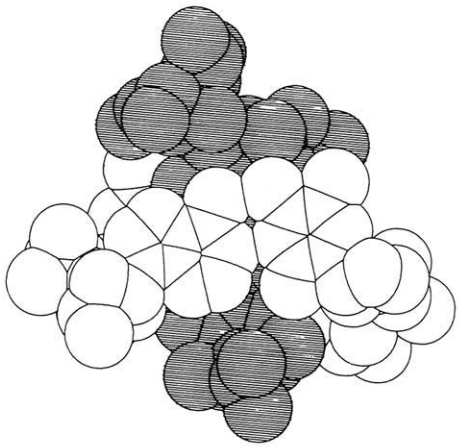

Fig. 7. Space-filling representation of complex (ii), viewed looking down onto the base pairs.

becomes unacceptably high. The overall chromophore–base pair arrangement resembles that found in the crystal structure of a daunomycin–hexanucleotide complex [19]. The interactive graphics system located a specific hydrogen bond between drug and dinucleoside; this is between the adenine N6 amino group on strand 2 and the 02′ hydroxyl oxygen atom attached to the major groove amino sugar. This oxygen acts as a hydrogen-bond acceptor. The N6.....02′ distance is 2.97 Å, the H6.....02′ distance is 2.30 Å, and the N6–H6.....02′ angle is 156°, thus ensuring a standard hydrogen-bond geometry. This interaction imparts a 3′-end adenine specificity to the drug binding in addition to the A–T rich preference.

(ii) The alternative arrangement, with the drug rotated by ~ 180° and thus the nogalose sugar in the major groove, has a non-bonded interaction energy of −70.7 kcal mole^{-1}. This is only slightly higher than state (i), and may not be a significant difference. The structure (Figs. 6–8) has a very similar chromophore disposition with respect to the base pairs. A specific (though weak) hydrogen bond has been located

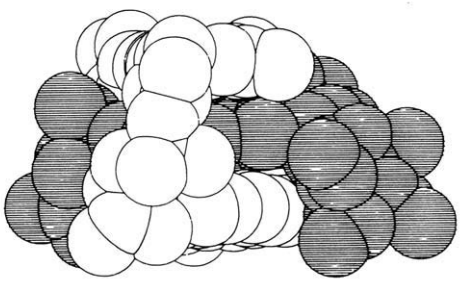

Fig. 8. Space-filling representation of complex (ii), viewed along the base-pair, plane, and having one nucleotide strand behind the other.

between the amino N6 group of strand 1 adenine, and the carbonyl oxygen atom 011 of the methoxycarbonyl side chain. The N6.....011 and H6.....011 distances are 3.17 Å and 2.30 Å respectively, and the N6–H6.....011 angle is 145°. Thus, this arrangement for the complex concurs with model (i) in having a specific hydrogen bond to a 3′ adenine at the intercalation site, though here it is on the opposite strand. There are then in principle three types of intercalation sites for nogalamycin that allow adenine-specific intercalation: (a) a self-complementary T(3′,5′)A one, (b) a general *X*(3′,5′)A one, and (c) a general T(3′,5′)*X* one, where *X* may be any of the four DNA bases. Site (a) can accommodate model (i) or (ii), and is undoubtedly preferred over the other two because of its statistically greater propensity for base-pair melting. Sites (b) and (c), which can in principle have a C–G base pair at the non-specific site of the intercalation site, would have the specific nogalamycin interactions with models (ii) and (i) respectively.

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REFERENCES

1. J. R. Brown, in *Molecular Aspects of Anti-cancer Drug Action* (Eds. S. Neidle and M. J. Waring), pp. 57–92. Macmillan, London (1983).
2. P. F. Wiley, J. L. Johnson and D. J. Houser, *J. Antibiotic* **30**, 628 (1977).
3. L. H. Li, S. L. Kuentzel, L. I. Murch, L. M. Pschigoda and W. C. Krueger, *Cancer Res.* **39**, 4816 (1979).
4. W. Kersten, H. Kersten and W. Szybalski, *Biochemistry* **5**, 276 (1966).
5. R. K. Sinha, P. Talapatra, A. Mitra and S. Maxunder, *Biochim. biophys. Acta* **474**, 199 (1977).
6. T. W. Plumbridge and J. R. Brown, *Biochem. Pharmac.* **28**, 3231 (1979).
7. E. F. Gale, E. Cundcliffe, P. E. Reynolds, M. H. Richmond and M. J. Waring, *The Molecular Basis of Antibiotic Action*, 2nd Edn. John Wiley, London (1981).
8. B. K. Bhuyan and C. G. Smith, *Proc. natn. Acad. Sci. USA.* **54**, 566 (1965).
9. S. Marciana, M. Terbojevich, D. Vedaldi and G. Rodighiero, *Il. Farmaco Ed. Sci.* **32**, 248 (1977).
10. K. Olson, D. Luk and C. L. Harvey, *Biochim. biophys. Acta* **277**, 269 (1972).
11. K. R. Fox and M. J. Waring, personal communication.
12. S. A. Islam, S. Neidle, B. M. Gandecha and J. R. Brown, *Biochem. Pharmac.* **32**, 2801 (1983).
13. S. A. Islam and S. Neidle, *Acta crystallogr.* **B39**, 114 (1983).
14. A Subbiah, S. A. Islam and S. Neidle, *Carcinogenesis* **4**, 211 (1983).
15. S. K. Arora, *J. Am. chem. Soc.* **105**, 1328 (1983).
16. H.-S. Shieh, H. M. Berman, M. Dabrow and S. Neidle, *Nucleic Acids Res.* **8**, 85 (1980).
17. A. J. Stuper, T. M. Dyott and G. S. Zander, in *Computer-Assisted Drug Design* (Eds. E. C. Olsen and R. E. Christofferden), pp. 383–414. American Chemical Society, Washington (1979).
18. S. A. Islam and S. Neidle, *The MOLEC Molecular Graphics Package* (1983).
19. G. J. Quigley, A. H.-J. Wang, G. Ughetto, G. van der Marel, J. H. van Boom and A. Rich, *Proc. natn. Acad. Sci. USA* **77**, 7204 (1980).