

Wepster, 1980). A more recent MM2 force field found the symmetrical geometry ($\omega = 0^\circ$) to be the more stable one (Burkert & Allinger, 1982), and it would appear that the twisted *tert*-butyl group is an artefact of the earlier force fields.

Packing

The packing of TRANS in the crystal is illustrated in Fig. 6. Two carboxyl groups form the well-known eight-membered ring around a centre of symmetry. The geometry of the hydrogen-bonding scheme is added in Table 2.

The authors are indebted to Professors B. M. Wepster and H. van Bekkum, Dr J. M. A. Baas and Dr A. J. van den Berg for their critical reading and discussion of the manuscript.

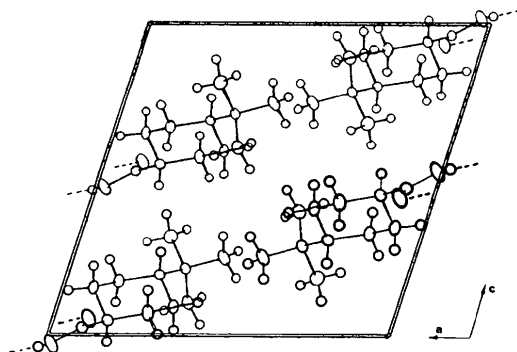


Fig. 6. Drawing of the unit-cell contents viewed down *b*. Hydrogen bonds are indicated by broken lines.

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Nucleic Acid Binding Drugs. X.* A Theoretical Study of Proflavine Intercalation into RNA and DNA Fragments: Comparison with Crystallographic Results

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Abstract

The minimum-energy structure for the interactions of the intercalation drug proflavine with the dinucleoside phosphates cytidylyl-3',5'-guanosine and deoxycytidylyl-3',5'-deoxyguanosine have been found by means of a combination of computer graphics and

empirical energy calculations. The minimum-energy positions for the drug, given the crystallographically observed nucleotide backbone conformations as starting points, are very close to the positions in the crystal structures of the complexes, with the intercalated proflavine molecule inserted from the major-groove direction in each case. Alternative orientations for the drug were found to be much less stable. NMR studies in solution [Patel (1979)]. *Biopolymers*, **16**,

* Part IX: Aggarwal, Neidle & Sainsbury (1983).

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2739–2754; Patel & Canuel (1977). *Proc. Natl Acad. Sci. USA*, **74**, 2624–2628] are fully consistent with these observations. The finding in this study of close correspondence between observed and calculated structures for the drug complexes implies that the ‘soft’ parameterization of the force field used here, with its considerable advantages in computational speed over a more rigorous approach, may be especially helpful in combined molecular graphics and energetic approaches to drug-receptor binding.

Introduction

The interactions of the frameshift mutagen proflavine (Fig. 1) with double-stranded nucleic acids have been the subject of much study (reviewed, for example, by Gale, Cundliffe, Reynolds, Richmond & Waring, 1981; Neidle, 1979). At a detailed structural level, X-ray crystallographic analyses have elucidated the intercalative binding between proflavine and (i) the ribonucleoside phosphates CpG* (Neidle *et al.*, 1977; Berman *et al.*, 1979), 5-iodo-CpG (Reddy, Seshadri, Sakore & Sobell, 1979) and the ternary complex with CpA and UpG (Aggarwal, Islam, Kuroda & Neidle, 1984) and (ii) the deoxyribodinucleoside phosphate dCpG† (Shieh, Berman, Dabrow & Neidle, 1980; Neidle, Berman & Shieh, 1980). All of these complexes have two antiparallel strands Watson–Crick hydrogen-bonded together. NMR studies have in general not been able to define the structures of these complexes in solution to a level of detail equivalent to that of these X-ray structures; however, a number of theoretical studies have been performed which examine drug intercalation into dinucleoside dimers (Pack & Loew, 1978; Ornstein & Rein, 1979; Miller, Brodzinsky & Hall, 1980; Malhotra & Hopfinger, 1980; Pack, Hashimoto & Loew, 1981; Dearing, Weiner & Kollman, 1981). These studies have concentrated on questions of backbone conformation, sugar puckers and flexibility.

It has now been shown that all the experimentally observed drug–dimer duplex structures have a close correspondence in dinucleoside backbone torsion angles (Sheih *et al.*, 1980; Berman, Neidle & Stodola, 1978) and thus all these structures may be said to fall into a single conformational class, regardless of the nature of the intercalated drug.

The present study is concerned with the question: given an opened-up duplex dimer of this generalized

conformation, what are the low-energy positions that proflavine, an archetypal intercalating drug, would adopt? Furthermore, the correspondence between these results and the crystallographically derived structures has been analysed in an attempt to define how such structures actually relate to theoretical predictions. The two test structures for this study are the proflavine complexes with CpG and dCpG, which are among the best determined crystallographically in these series.

Methods

All calculations and computer-graphics manipulations were performed on a PDP 11/34A computer linked to an interactive computer-graphics system (Gresham-Lion Supervisor 214).

The energy of a particular state was calculated as the sum of a number of intercalation terms representing non-bonded, torsional, electrostatic and hydrogen-bond energies:

$$V_{\text{TOT}} = V_{\text{N-B}} + V_{\text{TORS}} + V_{\text{E-S}} + V_{\text{H-B}}$$

The non-bonded parameters used were the ‘soft’ parameter set *B* given elsewhere (Islam & Neidle, 1983). Hydrogen-bond parameters were from Hagler, Huler & Lifson (1974).

Partial charges for the proflavine cation were calculated using the *GAUSSIAN78 ab initio* computer programs; these were close to those reported (Dearing *et al.*, 1981). Those for the dinucleosides were taken from Pearlstein, Dreno, Pensak & Hopfinger (1981). Torsion barrier heights were from Hopfinger (1973). Total minimization of conformational energy with respect to torsion angles was performed using the method of conjugate gradients (Fletcher & Reeves, 1965). Convergence was judged to have been achieved when consecutive cycles of minimization resulted in no more than a 1° change of any torsion angle, and a gradient of less than 0.4 kJ mol⁻¹ deg⁻¹. Gradient is defined as $dE/d\tau$, with τ being a torsion angle. The ‘soft’ non-bonded parameter set allows for low-energy structures with slightly closer intermolecular non-bonded contacts than ‘harder’ ones and, accordingly, at least in part compensates for the use of fixed bond lengths and angles. It is well known that small changes in particularly the latter parameters can help to stabilize otherwise higher-energy structures. The minimization of the >300 bond lengths and angles in the structures studied in this paper is computationally expensive and so the use of ‘soft’ parameters is a test of their ability to relate to experimental structures.

When solvent contributions were not explicitly modelled, either a distance-dependent formalism for the dielectric constant ϵ such that: $\epsilon = 1$ for $r_{ij} < 3 \text{ \AA}$, $\epsilon = 0.75r_{ij} - 1.25$ for $3 \text{ \AA} < r_{ij} < 7 \text{ \AA}$ and $\epsilon = 4$ for $r_{ij} < 7 \text{ \AA}$, or an $\epsilon = r_{ij}$ model was used. Water-molecule

* Cytidylyl-3',5'-guanosine.

† Deoxycytidylyl-3',5'-deoxyguanosine.

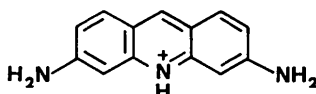


Fig. 1. Proflavine.

coordinates were taken directly from the crystallographic studies.

H-atom coordinates have been published for the proflavine-CpG structure (Berman *et al.*, 1979); these were not obtained directly from the X-ray data, but were derived from standard geometric considerations. Idealized H-atom positions were calculated for the proflavine-dCpG structure.

In a typical analysis, a proflavine molecule was manipulated by rotational and translational movements of 0.1 Å steps on a grid in the plane mid-way between the base pairs of the dinucleosides. This procedure was performed on the computer-graphics display whilst the intermolecular interaction energy was being calculated at each step. Inter- and intramolecular-energy minimization was then performed with such positions as starting-points, and all dinucleoside backbone and glycosidic torsion angles taken as variables. Total minimization was not performed at each grid point, as the combined graphics-energy calculations were found to be readily capable of locating low-energy regions. A limited series of minimizations (not detailed below) showed the justification of this approach.

Results and discussion

(a) Intercalation in CpG from the major-groove direction

Interaction from the major-groove side of CpG, that is with the drug's exocyclic N atoms oriented into the major groove and its long axis roughly parallel to the long axis of the base pairs, is shown in Fig. 2. The minimum-energy position (Table 1 and Fig. 3) is close to the crystallographic one, and only differs by being some 0.5 Å deeper into the major groove than the latter. This is due to increased drug-

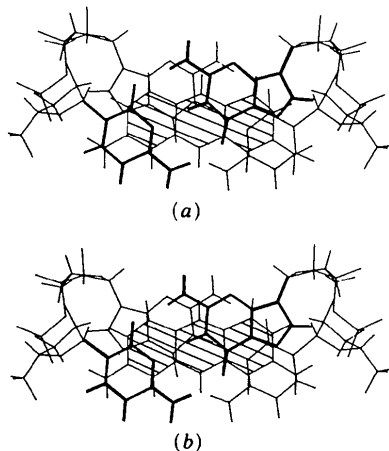


Fig. 2. The structure of the proflavine complexes of (a) CpG and (b) dCpG, as found in their crystal structures. The drug chromophore is shaded for clarity. View is onto the base-pair plane.

Table 1. *Intermolecular energies* (kJ mol⁻¹) of proflavine intercalation

	van der Waals	Electrostatic	Hydrogen-bonding	Total	Fig.
CpG ^M (cryst.)	-247.8	-200.6	-59.4	-507.8	2
CpG ^M (min.)	-280.5	-201.1	-63.5	-545.1	3
CpG ^P (min.)*	-209.4	-240.8	0	-450.2	4
	-186.0	-205.6	0	-391.6	4
CpG ^m (min.)	-113.3	-231.2	0	-344.4	5
dCpG ^M (cryst.)	-245.8	-265.8	0	-511.6	2
dCpG ^M (min.)	-258.3	-262.5	0	-520.8	3
dCpG ^P (min.)*	-185.5	-278.8	0	-464.4	4
	-173.1	-201.1	0	-374.2	4
dCpG ^m (min.)	-95.3	-242.4	0	-337.7	5

Abbreviations: *M* = major-groove intercalation; *m* = minor-groove intercalation; *p* = perpendicular intercalation.

* First line: diagonal; second line: true perpendicular.

Table 2. *Geometry of the hydrogen bonding between the exocyclic N atom of proflavine and a phosphate O atom for CpG*

Note that since the complex has exact twofold symmetry, the hydrogen bonding is identical for both dinucleoside strands.

	N...O	H...O	∠N-H...O
Crystal structure	3.00 Å	2.00 Å	173°
Minimum-energy position	2.89	1.91	165

base overlap, resulting in enhanced van der Waals intercalation stabilization. Note that only about half of the total energy is due to this factor. The difference in energy between observed and calculated structures is 37 kJ mol⁻¹. The flexibility [as judged by positional changes of the proflavine without producing significant changes in energy (<2 kJ mol⁻¹)] of this proflavine within the major-groove minimum-energy position was restricted by the development of unfavourable close contacts between the proflavine and the atoms of ribose groups (close contacts refer to a situation where the distance between a given pair of atoms is closer than the sum of the van der Waals

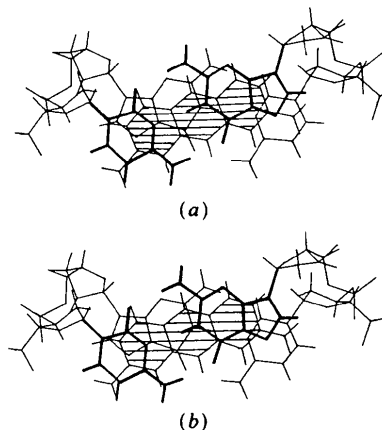


Fig. 3. The minimum-energy conformations for major-groove intercalation into (a) CpG and (b) dCpG.

Table 3. Changes in backbone torsion angle ($^{\circ}$) produced by total-energy minimization for the major-groove intercalation structures

	Torsion angle about bond					
	C(3')C-O(3')C	O(3')C-P	P-O(5')G	O(5')G-C(5')G	C(5')G-C(4')G	
CpG: strand 1	0.0	1.7	-2.3	-2.9	-1.1	
strand 2	-0.1	-2.6	-1.9	-0.3	-0.1	
dCpG: strand 1	-0.7	1.6	1.5	-2.7	-1.3	
strand 2	-0.4	-1.6	-0.6	0.7	0.0	

radii of the atoms). The rotational flexibility of the proflavine molecule is limited to about 5° when in the intercalation site. The differences between the crystallographic and minimum-energy conformations will to some extent depend upon the types of energy formalism being employed (Islam & Neidle, 1983).

An important factor restricting drug movement was found to be hydrogen bonding between the proflavine and the dinucleoside. In the crystallographic structure hydrogen bonds exist between the phosphate groups of the dinucleoside and the exocyclic groups of the proflavine (Berman *et al.*, 1979).

The computational model used here for hydrogen bonding (Hagler *et al.*, 1974) has no explicit angular dependence, and thus it was of interest to compare the minimum-energy conformation with the crystallographic structure. The hydrogen-bonding parameters are given in Table 2. Table 3 lists the changes in dinucleoside backbone torsion angles produced by energy minimization. These changes are small, and do not significantly alter the twofold symmetry of the complex.

(b) Perpendicular modes of binding to CpG

Possible binding modes for this proflavine with its long axis perpendicular to the phosphate-phosphate interstrand vector (Fig. 4) were investigated. It should be noted that in the perpendicular mode of binding the proflavine molecule could be manipulated into a

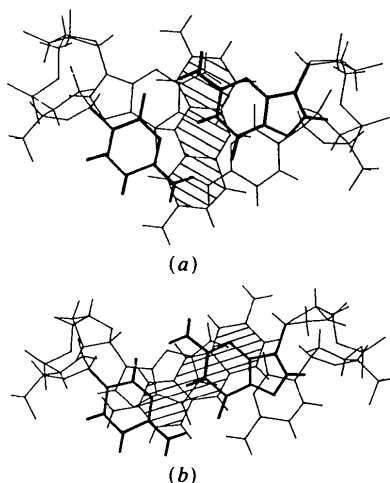


Fig. 4. (a) Perpendicular binding mode for CpG and (b) diagonal mode for dCpG.

low-energy position from both the major- and the minor-groove direction. The flexibility of the molecule in this perpendicular mode of binding was investigated; the minimum-energy position is shown in Fig. 4 and its energy given in Table 1. The possibilities of hydrogen-bond formation between the proflavine and the dinucleoside backbone were investigated but were limited by the development of unfavourable close contacts between the ribose sugars and proflavine atoms, thus not enabling the drug's N atoms to approach sufficiently close to the phosphate groups. It was found that in the minimum-energy position the long axis of the proflavine is inclined at about 90° to the phosphate-phosphate vector. It is noteworthy that this angle is close to that found between the drug daunomycin and a hexanucleotide (Quigley, Wang, Ughetto, van der Marel, van Boom & Rich, 1980). Stabilization was somewhat enhanced by orienting the drug diagonally, at an angle of $\sim 45^{\circ}$ (Fig. 4); this increased both electrostatic and van der Waals energy stabilization compared to the perpendicular situation, with the former actually being greater than for major-groove intercalation (Table 1). However, the relative lack of base-drug overlap in either the diagonal or perpendicular bases makes them overall of higher energy than the major-groove state.

(c) Minor-groove binding to CpG

In this situation, the drug was docked from the minor-groove direction, with the chromophore oriented parallel to the P...P interstrand vector. A low-energy state analogous to the major-groove one was not possible; attempts were made with the exocyclic N atoms both facing away and towards the minor groove. This was ascribable to unfavourable close contacts developing between the drug and the ribose sugars. Fig. 5 shows the low-energy structure found, which does not have any drug-base overlap, and hence is some 200 kJ mol^{-1} less favourable than major-groove intercalation. The observed base-turn angle of 33° for the CpG-proflavine structure [defined as the angle subtended by the two interstrand C(1')...C(1') vectors], has been suggested (Neidle, Kuroda, Aggarwal & Islam, 1983) to correlate with interstrand P...P separation. Thus dinucleoside structures with smaller base-turn angles will have the two backbones further apart, which could result in some improvement in minor-groove intercalation.

(d) Major-groove intercalation in dCpG

The crystallographic and minimum-energy conformations are shown (Figs. 2 and 3 respectively). It is notable that for the crystallographic conformation the intermolecular energy of binding is almost equal to that of the CpG–proflavine crystal structure, although no proflavine–dinucleoside hydrogen bonds exist in the deoxy structure. The minimum-energy position was again located by translation into the major groove. The changes in torsion angles after minimization (Table 3) were small. A prominent feature of the proflavine–dCpG crystal structure is the existence of a highly structured water network. The procedure for locating the approximate minimum-energy drug position was repeated with the water molecules which constituted the first hydration shell. There are hydrogen bonds in the crystal structure between several water molecules and the proflavine N atoms. It was not possible to analyse fully the effects of these water molecules. Preliminary results, however, indicated that the calculated position of the proflavine was ~ 0.4 Å closer to the crystallographic position, upon minimization with solvent molecules present. These had the effect of making the drug molecule ~ 0.4 Å less buried in the major groove.

(e) Perpendicular binding to dCpG

Results were again very similar to the CpG structure. In the perpendicular mode of binding the structure is more stable than that for the CpG complex (Table 1), although the resulting structures are similar and the proflavine molecule lies (long axis of the proflavine) at about 90° to the $P \cdots P$ vector. Diagonal binding, as in the CpG case, results in enhanced electrostatic contributions and an overall

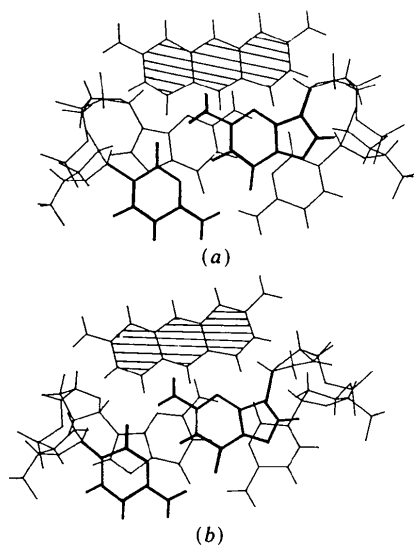


Fig. 5. Minor-groove interactions of (a) CpG and (b) dCpG with proflavine.

stabilization compared to the fully perpendicular case.

(f) Minor-groove binding to dCpG

Interaction is analogous to the CpG case, with steric hindrance to effective intercalation. The binding is thus largely a surface one, with the drug protruding far into the minor groove (Fig. 6).

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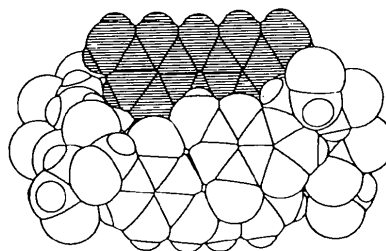


Fig. 6. Space-filling representation of the minor-groove minimum-energy dCpG–proflavine structure. The drug molecule is shaded for clarity.

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Acta Cryst. (1984). **B40**, 429–434

The Structure of Pilocarpine Hydrochloride, $C_{11}H_{17}N_2O_2^+ \cdot Cl^-$: A Muscarinic Alkaloid

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Abstract

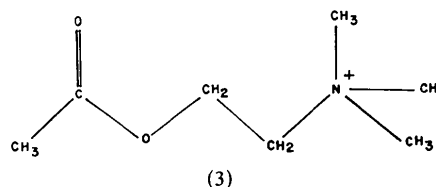
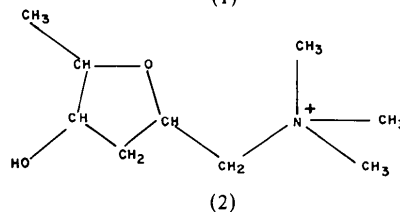
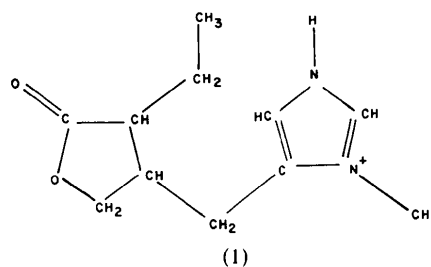
$M_r = 244.72$, at ambient room temperature (~ 295 K) and at 77 K, monoclinic, $P2_1$, $Z = 2$, $a = 11.057$ (1) [10.965 (5)] Å, $b = 9.212$ (1) [9.177 (3)] Å, $c = 6.697$ (1) [6.507 (3)] Å and $\beta = 110.05$ (2) [109.19 (4)]°, $V = 637.8$ (2) [618.4 (5)] Å³ (the values in brackets for the 77 K determination). $D_x = 1.276$ g cm⁻³, $F(000) = 260$, $\lambda(\text{Mo } K\alpha) = 0.71069$ Å, $\mu(\text{Mo } K\alpha) = 2.92$ cm⁻¹, $R = 0.043$ [0.031] for 2751 [3864] contributing reflections. The *N*-methylimidazole ring is protonated at the position of the secondary N atom and the ring is planar. The NMR spectrum of the compound was studied over the pH range 2.6–10.6; these spectra show that there is a delocalization of π electrons over the bonds between the two N atoms of the protonated imidazole ring. The Cl^- ion in the crystal structure is hydrogen-bonded to the protonated N atom at a distance of 3.035 (2) [3.030 (2)] Å. The conformation of the molecule differs significantly from that found in pilocarpine trichlorogermanate and from models of muscarinic agents.

Introduction

Pilocarpine (1) is one of several alkaloids that mimic the effect of acetylcholine on autonomic effector cells that control smooth muscle contraction. The compound binds to the muscarinic class of acetylcholine receptors (Birdsall, Burgen & Hulme, 1978) and exhibits partial agonist activity.

Muscarine (2), pilocarpine (1), and acetylcholine (3) have considerable differences in their chemical

structures yet all bind to the muscarinic receptor while only acetylcholine has activity at the nicotinic receptor. An explanation for the affinity of muscarinic agents for their receptor has been sought in terms of a specific binding conformation. Three distinct models appear in the literature: in the first, Kier (1967, 1968) predicted on the basis of extended Hückel theory (EHT) that there were two preferred low-energy conformations of acetylcholine. One, the folded conformation with a hydrogen-bond acceptor



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