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# Stereochemical model for proflavine intercalation in A-DNA

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ABSTRACT

Linked-atom molecular modelling was employed to determine the steric and torsional requirements for intercalation of proflavine into a double-stranded region of DNA compatible with adjacent regions of cohelical A-DNA. The optimum intercalation conformation is characterized by the dihedral angles $\xi$ and $\psi$ becoming *trans*, with all sugars retaining the characteristic C3'-*endo* pucker. This extended conformation results in virtually no helical unwinding, suggesting it may be an appropriate model for an intercalative intermediary in mutagenesis by virtue of its similarity to standard helical DNA.

## INTRODUCTION

Drugs containing planar aromatic groups have long been recognized as potent genetic marking and mutagenic agents, and their effects upon nucleic acids have been reviewed extensively (1-3). The strongest mode of binding between such chromophores and double-stranded polynucleotides is well established as intercalation, the insertion of the aromatic group between adjacent base pairs of the duplex, accompanied by an extension of the nucleotide backbone at the intercalated residue (4,5). Polynucleotide intercalation often entails pronounced structural alterations over larger regions of the molecule, most notably in covalently closed DNA duplexes where local helical unwindings due to intercalation often induce significant helical supercoiling (6,7).

Many crystallographic studies of complexes between intercalative drugs and small nucleotides have been reported (8-13). For several of these complexes where the bases participate in standard Watson-Crick pairing, a persistent feature of the nucleotide backbone has been claimed to be a sugar puckering pattern of the form C3'-*endo* (3' → 5') C2'-*endo* (14). However, in recently solved 2:3 complex of CpG with proflavine, intercalation was accommodated with all sugars maintaining the C3'-*endo* form, with the necessary nucleotide extension achieved through conformational transitions about backbone single bonds and adoption of the high *anti* form by the purine nucleosides (13). For none of these structures are the environments about the

 

intercalating dinucleoside identical to those encountered in polynucleotides: for dinucleoside-drug complexes, each nucleoside has but one ester linkage and is flanked by two drugs rather than by adjacent, stacked bases. Attempts to extrapolate polymeric conformations from such dinucleoside structures necessarily result in bent or dislocated polymers (i.e., adjacent helical regions noncoaxial) (14), and hence their applicability to mutagenic events which mimic normal transcription or replication is not clear.

Previously, we have described the application of a linked-atom modelling system to determine the steric and conformational requirements for intercalation of proflavine into otherwise helical B-DNA (15). For that system, the optimum intercalative conformation was deduced to entail a transition of the backbone dihedral angles $\xi$ = $\theta$[C3-C4-C5-O4] and $\psi$ = $\theta$[C5-O4-P-O1] of the intercalated residue to values in the *trans* range, possibly abetted by a transition of one sugar to the C3'-*endo* puckered form. The helical unwinding per intercalation associated with this conformation was calculated to be $-18^{\circ}$, a value confirmed by anisotropy fluorescence decay measurements of ethidium binding to B-DNA (16). Subsequently, analysis of nmr measurements of tetranucleotide complexes with proflavine have resulted in improved positioning for the intercalated proflavine, using the nucleotide geometry proposed (17). Here we examine the conformational requirements for proflavine intercalation into helical A-DNA.

## EXPERIMENTAL PROCEDURE

The model system employed in this study is essentially identical to that described in our modelling of intercalation in B-DNA (15). By use of a linked-atom least-squares computer program (18), two complementary oligonucleotides, each of $2N$ bases, and a proflavine molecule were generated from idealized steric parameters (fixed bond lengths and bond angles, standard base and sugar ring shapes). Four positioning parameters associated with the 3' terminal base of each strand were fixed at values corresponding to A-DNA (19), with an axial separation of $2.56N$Å between the roots. These conditions enforce compatability with adjacent regions of A-DNA at each end of the duplex. For economy, a conformational identity was imposed between the two nucleotide chains, creating a diad axis through the intercalation site. The only variables during iterations of conformation refinement were $12N$-7 single-bond dihedral angles, a turn angle $T$ relating the roots of the two nucleotide chains, and three positioning parameters for the drug. The variable torsion angles were tied elastically to average staggered values for each conformation examined. During refinement of the nucleotide conformation

$7N+5$ constraints were imposed upon the system: $5N$ to ensure standard Watson-Crick pairing of all bases, $2N-1$ to ensure that all bases maintain the same inclination to the helix axis as in A-DNA, and six to enforce cohelicity of the terminal sugars. (Since these last constraints are redundant, the system actually had $5(N-1)$ degrees of freedom.) In addition to these exact constraints, the program further simulated the energetic demands of the molecules by alleviating overly short nonbonded contacts and including an attractive term for stacking interactions between the drug and its neighboring bases.

RESULTS

Candidate conformations tested by this modelling system were selected from a survey of dinucleoside monophosphate conformations (20) which included all sterically feasible combinations of mononucleotide average values for the dihedral angles $\xi$, $\psi$ and $\theta$ with C3'-*endo* sugars. Only one conformation provided satisfactory fulfilment of constraints upon the $N=2$ (tetranucleotide) model system; this conformation is characterized by the torsion angles $\xi$ and $\psi$ adopting values in the *trans* range at the intercalated residue, with all other dihedrals falling into their normal polymeric ranges. Table 1 lists the conformation angles defining the nucleotide structure for the model case $N=2$, and Figure 1 depicts the optimum symmetric intercalation conformation for a hexanucleotide duplex (model case $N=3$) consistent with adjacent regions of A-DNA. The turn angle $T$ over three residues in $96.6^{\circ}$, corresponding to a helical unwinding of only $-1.6^{\circ}$ from the A-DNA conformation. All torsion angles fall into staggered ranges observed in monomers and polymers, and the root-mean-square difference of these angles from their appropriate standards is 1.23 standard deviations, indicating an unstrained conformation. All imposed constraints are satisfied to within 0.03Å, and the shortest nonbonded contact is 2.60Å between O5' and O1' of the intercalated residue.

One phosphate oxygen from each strand points directly inward toward the drug, introducing the possibility of additional nucleotide-drug interactions. With the nucleotides fixed and the proflavine free to move within the intercalation pocket, it was observed that reasonable hydrogen bonds could be

Table 1: Conformation Angles for Intercalated A-DNA

| | $\xi$ | $\theta$ | $\psi$ | $\phi$ | $\omega$ | $\chi$ | Sugar Pucker |
|---|---|---|---|---|---|---|---|
| Residue 1 | 43.6 | -179.8 | - 64.8 | -72.5 | -148.7 | - | C3'-*endo* |
| Residue 2 | 149.0 | -148.1 | -159.4 | -99.1 | -148.5 | 83.4 | C3'-*endo* |
| Residue 3 | 59.5 | -172.3 | - 87.1 | -60.3 | -172.6 | 73.5 | C3'-*endo* |

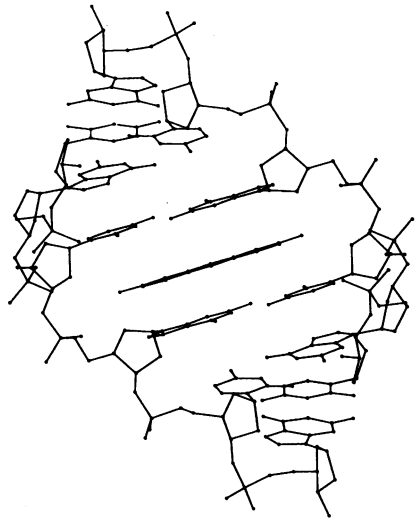

Figure 1: The least strained conformation for a fragment of DNA with proflavine symmetrically intercalated, consistent with adjacent regions of DNA in the A helical form, viewed facing the DNA major groove along the diad axis of the complex. The characteristic nucleotide conformation is extended in a manner similar to that employed for B-DNA intercalation (15), except in this case sugars are puckered C3'-*endo*.

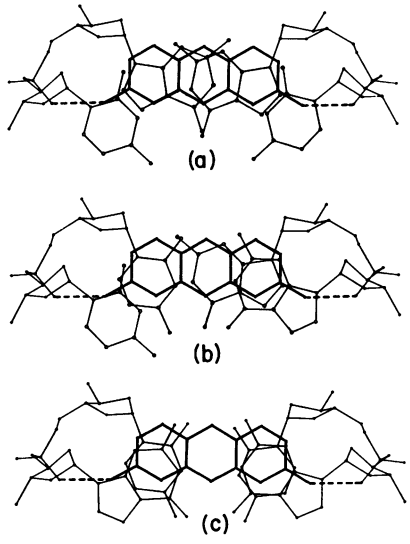


Figure 2: Stacking interactions between proflavine (bold lines) intercalated in the A mode between various base pairs of DNA, with the proflavine positioned to form hydrogen bonds (dashed lines) with backbone phosphate oxygens. For the base sequences a) (CpG)•(CpG), b) (GpG)•(CpC), and c) (GpC)•(GpC) flanking the drug, there are respectively two, one and zero full purine-proflavine stacks.

formed between the proflavine amino groups and phosphate oxygens on *both* strands simultaneously. The length of these hydrogen bonds is 2.95Å and the angle N(amino)--O--P is 116.9°. These hydrogen bonds and the resultant base-drug stacking interactions are shown in Figure 2.

Introduction of ribose hydroxyl groups to this structure entailed no additional steric difficulties, indicating a similar conformation may also be adopted by RNA.

DISCUSSION

The mode of intercalation described here appears structurally and energetically feasible and may be utilized by double-stranded DNA as well as RNA in binding aromatic molecules. The nucleotide backbone at the intercalated residue is extended by adoption of *trans* values for the conformation angles $\xi = \theta$[C3-C4-C5-O4] and $\psi = \theta$[C5-O4-P-O1], a conformational transition similar to that noted previously in our modelling of B-DNA intercalation. All sugars adopt the C3'-*endo* puckered form characteristic of A-family polynucleotides, and here a mixture of sugar puckerings at the intercalated residue entails a significant worsening of nonbonded interatomic contacts in the residues adjacent to the intercalation site. Overall, the principal features of this polymer model (all sugars C3'-*endo*, no helical unwinding, and phosphate-amino hydrogen bonds) bear a striking similarity to those of the 2:3 dinucleoside-proflavine crystal structure (13).

In this model there is no difference in the puckering of successive sugar rings at the intercalation site, and little difference in the orientation of these rings with respect to the bases. It is unlikely, therefore, that a second intercalant would be excluded from an immediately adjacent site for purely steric reasons. If the neighbor exclusion principle is found to apply also to intercalation in A-type structures, then one should look to factors such as preferential drug binding to particular base sequences (21-23) to explain this effect.

Our model implies also that intercalation may in some circumstances be undetectable by certain techniques. For example, intercalative bindings within a region of closed circular DNA in the A conformation would not cause any change in superhelical density. Thus proton magnetic resonance studies (24) which suggest that aromatic amino acids intercalate in polynucleotides are not necessarily incompatible with density gradient studies (25) which indicate no change in the superhelical density of such complexes.

It is tempting to speculate that various intercalative drugs which selectively inhibit DNA replication or RNA transcription do so by preferen-

tially binding to DNA in the B or A mode, respectively. The distinctly different circular dichroism profiles exhibited for complexes of acridine orange with DNA and with RNA (26) may be attributable to adoption of different intercalative modes by the two polymers. It should be emphasized that the precise details of any intercalative binding are likely dependent upon the electronic configuration of the drug itself (21); nonetheless, it is noteworthy that DNA may have more than one general family of intercalatable conformations.

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