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Visualization of planar drug intercalations in B-DNA

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ABSTRACT

A computerized linked-atom modeling system was developed to examine the stereochemical requirements for intercalation of planar drugs into DNA. All classes of conformational possibilities for extending the polynucleotide backbone were examined for their ability to accommodate insertion of a drug into a base-paired region of DNA compatible with adjacent regions of *B*-DNA while stacking interactions, steric strain and non-bonded interatomic contacts were optimised. One conformation was found which proved superior to all others in ability to satisfy these criteria: an extension of the backbone by characteristic changes in two torsion angles to *trans* values, plus a change in one sugar puckering to C3'-*endo* to relieve strain in an adjacent residue. The turn angle distributed over three polynucleotide residues for this most general mode of intercalation is 90°, equivalent to a helical unwinding of -18° for *B*-DNA.

INTRODUCTION

The mode of binding to polynucleotides by a variety of drugs and dyes containing fused aromatic rings has long been envisaged as intercalation: insertion of the drug chromophore between adjacent base pairs of a double helix, accommodated by an unwinding and extension of the polynucleotide in the vicinity of the intercalation site. Such an interaction was first proposed on the basis of X-ray diffraction, flow birefringence and low-angle X-ray scattering of proflavine-DNA complexes.[1] X-ray diffraction from DNA-ethidium bromide complexes[2] yielded a similar conclusion: the retention of a strong 3.4Å meridional reflexion coupled with a loss of well-defined layer lines indicated alignment of the drug chromophores with the base pairs and a disordering of the helical pitch, particularly if the drug molecules were intercalated at irregular intervals. Fuller and Waring[2] produced a model for this interaction; however, it was not well-defined

conformationally and was based on an assumption of one drug intercalation per base pair, i.e. every residue being extended.

Several drugs have been co-crystallized with mononucleotides or dinucleoside phosphates and the structures of the complexes determined by X-ray crystallography. Notable among these are the structures of actinomycin D with deoxyguanosine,[3] 9-aminoacridine with a dinucleoside phosphate (ApU),[4] and ethidium bromide with a dinucleoside phosphate (iodoUpA).[5] Sobell and Jain have described a model for the actinomycin-DNA interaction that uses intercalation and peptide-guanine hydrogen bonding. The intercalation site has been characterized as possessing a mixed sugar puckering of the type deoxyguanosine (C3'-*endo*) linked 3' to 5' to deoxycytidine (C2'-*endo*) and the additional separation of the DNA bases is contrived by distortions of the sugar rings and backbone bond and conformation angles from standard values.

From the crystal structure of a 1:1 complex of ethidium with the dinucleoside monophosphate iodoUpA a mixed pattern of sugar puckering, similar to that proposed for the actinomycin-DNA interaction, was reported,[5] and this feature has been proposed as a general mechanism for intercalation of drugs into polynucleotides. However, since the chemical structures and the environments for these complexes are somewhat different from those encountered in polymers, their applicability to DNA is still indeterminate. (E.g., in the ethidium-dinucleoside complex there is only one phosphodiester linkage to each sugar, and each base interacts with two drugs, only one of which is intercalated. Similarly in the actinomycin-deoxyguanosine complex, there are no ester linkages to the sugars.) The structure of the complex containing 9-aminoacridine,[4] showing successive bases parallel but unstacked and pointing away from each other and hydrogen bonded in a non-Watson-Crick fashion, more clearly demonstrates that not every drug-dinucleoside structure may be appropriate for extrapolation to interactions involving polynucleotides. We therefore examined models for the intercalation of simple planar chromophores into DNA in search of a general intercalation mechanism compatible with an extended DNA duplex with unstrained conformations and with base stacking as the principal binding force (i.e. with no implicit assumptions about special interactions between the drug and DNA.) Our objectives were to

determine the optimum conformation in the immediate site of intercalation *and* in the adjacent region perturbed by the presence of the drug, and to assess the helical unwinding produced by such a conformation. The latter parameter is of some practical significance as measurements of the amount of supercoiling in closed circular DNA and of the steric effects of repressors and operators binding to DNA are frequently calibrated from measurements of helical unwindings due to intercalating drugs. Estimates[1,2,6-9] of this parameter have ranged from -45° to +12°. CsCl titration studies of ethidium bound to closed circular DNA indicate an unwinding of -26°,[8] and recent fluorescence anisotropy decay studies of ethidium bound to DNA yield a value for helical unwinding from the *B*-DNA conformation of -17° per intercalation.[9]

EXPERIMENTAL PROCEDURE

The model system for intercalation studies consists of three molecules constructed in the linked-atom fashion:[10] a drug chromophore and two nucleotide segments of complementary sequences composed of 2N bases each. These three molecules comprise the intercalation region in an otherwise standard, double-helical DNA. Associated with the base on the 3' terminus of each strand are four positioning parameters (a radius and three Eulerian angles) fixed at values corresponding to *B*-DNA[11] to ensure that the adjacent regions are coaxial helices of this type. An orienting angle T about the helix axis between the two strands is variable, allowing for determination of the helical unwinding ΔT due to intercalation. Here $\Delta T = T-(2N-1)t$, where t is the turn angle per residue in the standard helix. The two terminal base pairs have a fixed axial separation of $2Nh$, where h is the rise per residue of the standard helix, i.e. we assume that intercalation results in an extension h of the intercalation region. For *B*-DNA, $t = 36.0°$ and $h = 3.38$Å.

In linked-atom modelbuilding, bond lengths, bond angles and sugar ring shapes are kept fixed at standard values.[12,13] In addition, the conformation angles (χ) of the first and last sugar rings with respect to their attached bases are kept fixed at *B*-DNA values to fulfill the requirement of adjacent cohelical *B*-DNA regions. Thus the only conformational variables are the 12N-7 conformation angles for each strand. These are depicted in Fig. 1

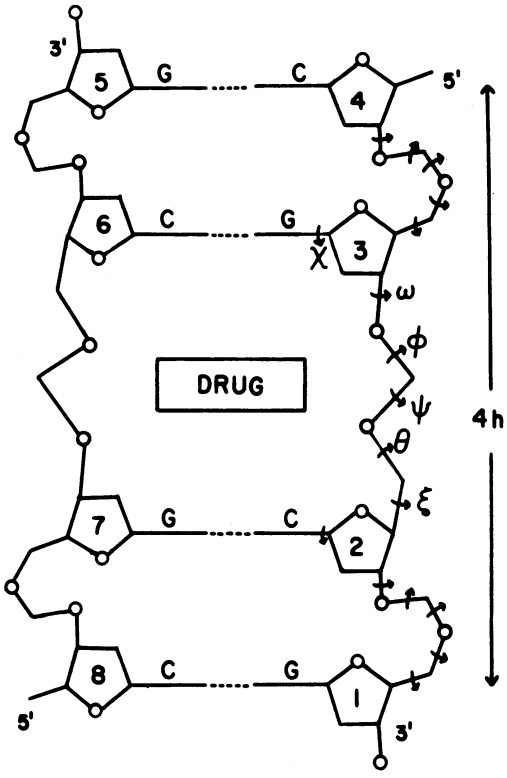

Figure 1: A schematic diagram of the basic intercalation modeling unit, consisting of a drug chromophore intruded between the middle base pairs of a complementary duplex of 2N base pairs (here N=2). The two end pairs are constrained to adopt orientations to the helix axis as in *B*-DNA, and all bases are constrained to form standard Watson-Crick pairs and to adopt the same inclination to the helix axis. The modeling unit is described in cartesian coordinates with the diad in the (bottom) terminal base pair as the x-axis and the helix axis as the z-axis. The variable torsion angles for one strand are denoted by arrows.

for the case N=2.

Upon this system we impose the further condition that all the bases be paired in the Watson-Crick fashion with 6 fixed relationships *between* the two bases of a pair; these include three hydrogen-bond distances plus two angle constraints to create a twist axis coplanar with both bases and to form a local diad axis in the pair relating the glycosylic bonds. The sixth relation between the bases involves the angle γ that the normal to a base makes with the helix axis. Empirically we know this angle to be related to h,[14] so constraining each base to adopt the same γ as *B*-DNA completes a set of self-consistent constraints forming standard base pairs and also encouraging favorable base stacking. Since the values of γ for the root bases are already fixed, 2N-1 additional γ constraints are required for each strand. Coupled with the 10N distance and angle constraints, the total number of constraints imposed upon the nucleotide system is 14N-2. To ful-

fill these requirements there are a total of 24N-13 variables in the system (torsion angles plus T), yielding 10N-11 degrees of freedom in the most general modeling system.

For the case of a symmetric chromophore intercalated into two self-complementary strands, we choose to impose a conformational identity between the two strands, relating them by a diadic axis midway between base pairs N and N+1 (i.e. through the intercalation site). Operationally this is achieved by generating the second chain from the first by rotating the root 180° about the x-axis (see Fig. 1), translating it $2Nh$ along the z-axis and rotating it by T about the z-axis. The formation of the central diad ensures that making base pairs 1 through N will result in identical pairs from 2N to N+1. Now only 5N distance and angle constraints plus 2N-1 constraints on γ are needed to pair all the bases. In this symmetric case there are 12N-7 variable torsion angles plus the variable orienting angle T, so here the nucleotide system has 5(N-1) net degrees of freedom. It is sometimes useful to include additional constraints (particularly helix relations between the terminal sugars) to reduce the number of refinement cycles and to ensure that all constraints are internally consistent. More than six constraints applied to any rigidly connected section of the molecule are redundant and do not decrease the actual number of degrees of freedom in the system. A special subroutine filters the best-satisfied redundant constraints after each cycle. Table 1 lists the constraints applied in our modeling.

In both cases described above, the chromophore is treated as a rigid body, located centrally between base pairs N and N+1 (i.e. $z=Nh$) and positioned so that a normal to its planar surface makes an angle γ with the helix axis. In the symmetric case the three Eulerian angles positioning the chromophore are fixed so that the diad of the chromophore corresponds to the central diad of the model system and its variable orienting angle is constrained to be $T/2$ so the only variable associated with the chromophore is its radius. For asymmetric chromophores, the Eulerian angles are allowed to vary and no constraint is placed on the orienting angle about the helix axis, but the plane of the chromophore is still constrained to intersect the helix axis at $z=Nh$ and to maintain the characteristic value for γ. In both cases the added variables

Table 1: Applied Constraints

Constraint type	Applied to	Value	Function
Distance	GN1--CN3	2.92Å	Hydrogen bond
"	GN2--CO2	2.85Å	"
"	GO6--CN4	2.87Å	"
Angle with helix axis (γ)	all non-root bases	4.0°	Maintain stacking
Angle	two twist axis reference points & normal to base	90.0°	Maintain twist axis
Radius difference	1C1'-2NC1'	0.0	Makes ends cohelical
"	1C2'-2NC2'	0.0	"
"	1O5'-2NO5'	0.0	"
Axial difference	1C1'-2NC1'	$2Nh$	"
"	1C2'-2NC2'	$2Nh$	"
"	1O5'-2NO5'	$2Nh$	"

do not affect the nucleotide constraints and hence add no degrees of freedom to the nucleotide conformation.

In addition to requiring that the variables are compatible with the exact constraints discussed above, we also minimise the steric compression of the system, defining optimum values of the variables as those which minimise Eq. 1.

$$\Phi = \Sigma\lambda_h G_h + \Sigma\varepsilon_j \qquad (1)$$

The first summation contains the Lagrange multipliers λ_h and the constraint expressions G_h that we wish to be zero, and the second summation represents all variable non-bonded interatomic distances d_j less than a specified minimum ${}_od_j$. This approach to minimising non-bonded interactions was developed to determine packing of rigid molecules in a crystal[15] and has been adapted to the production of acceptable conformations of flexible polymer chains.[16]

In the second summation,

$$\varepsilon_j = k_j({}_od_j - d_j)^2, \quad d_j < {}_od_j$$
$$\varepsilon_j = 0, \quad d_j \geq {}_od_j \qquad (2)$$

The values of the constants k_j and ${}_od_j$ were derived for each type of interatomic interaction from Buckingham energy functions of the form

$$E = -Bd^{-6} + Ae^{xp}(-\mu d) \quad (3)$$

Values of B, A, and μ calculated by Chandrasekaran and Balasubramanian[17] were used. Each ${}_od_j$ was set to be 0.20Å greater than the value of d corresponding to minimum E to ensure that all short contacts were driven to larger values. The values of k_j were chosen so that $\partial(k_j({}_od_j-d_j)^2)/\partial d$ closely approximated $\partial E/\partial d$ in the range $d(E_{min})$ - 0.50Å$<d<d(E_{min})$. The values of k_j and ${}_od_j$ are given in Table 2.

We find that inclusion of hydrogen atoms in the structures and searching for short non-bonded contacts involving them does not significantly alter the optimum conformation for well-constrained models, but greatly slows convergence for models during refinement. The effect of hydrogen atoms can be closely reproduced by tying all torsion angles elastically to their characteristic standard values. Operationally this requires an additional term ε_e in the sum of elasticated parameters in Eq. 1:

$$\varepsilon_e = W_e\Sigma k_e({}_o\theta_e - \theta_e)^2 \quad (4)$$

where ${}_o\theta_e$ and θ_e are respectively the values of the standard and variable conformation angles, k_e is a relative weight based on the standard deviations of these angles derived from surveys of monomer and polymer structures,[12] and W_e is an overall weighting factor scaling this elasticated sum relative to the contributions from the other contact searches. Values for these parameters are included in Table 2.

Our computer program is also capable of simulating the attractive stacking forces between aromatic chromophores and the neighboring bases. In this case when the distance d_j between two atoms in adjacent aromatic systems falls between specified limits ${}_Ad_j$ and ${}_Bd_j$, with ${}_Id_j$ the "ideal" contact distance and ${}_Ik_j$ the corresponding weight, the expression for ε_j in Eq. 2 becomes

$$\begin{aligned} \varepsilon_j &= k_j({}_od_j - d_j)^2, \quad 0<d_j<{}_Ad_j \text{ or } {}_Bd_j<d_j<{}_od_j \\ \varepsilon_j &= {}_Ik_j({}_Id_j - d_j)^2, \quad {}_Ad_j<d_j<{}_Bd_j \\ \varepsilon_j &= 0 \qquad\qquad\quad , \quad d_j>{}_od_j \end{aligned} \quad (5)$$

Here values of $_{I}d_{j}$ were taken from a survey of contacts between the bases in the highly stacked polymeric system poly(dT)·poly(dA)·poly(dT),[18] and $_{A}d_{j}$ and $_{B}d_{j}$ are $_{I}d_{j}$ ± 0.20Å, respectively. When Φ (Eq. 1) has been minimised, it provides a measure of the steric acceptability of the model system.

RESULTS

We examined all possible modes of extending a polynucleotide backbone to accommodate insertion of a planar drug between two

Table 2: Elasticated Parameters

Repulsive contacts			Torsion angles*	
Atom pair	Minimum distance $(_{o}d_{j})$ Å	Weight (k_{j}) Å^{-2}	Angle (θ_{e})	Weight (k_{e}) deg^{-2}
C-C	3.60	1.10	ξ[C3-C4-C5-O4]	1.56
C-O	3.40	1.62	θ[C4-C5-O4-P]	0.59
C-N	3.45	2.17	ψ[C5-O4-P-O1]	0.51
O-O	3.20	1.65	ϕ[O4-P-O1-C3]	0.51
O-N	3.25	2.21	ω[P-O1-C3-C4]	0.39
N-N	3.30	2.97	χ [C2-C1-PuN9-PuC4] / [C2-C1-PyN1-PyC2]	1.00

Attractive contacts				
Atom pair	Optimum distance $(_{I}d_{j})$ Å	Range of application $(_{A}d_{j})$ Å	$(_{B}d_{j})$ Å	Weight (k_{j}) Å^{-2}
Drug atom-base C	3.40	3.20	3.60	10.0
Drug atom-base N	3.20	3.00	3.40	10.0
GN1-CN3	2.92	2.60	3.30	16.19
GO6-CN4	2.87	2.60	3.30	13.92
GN2-CO2	2.85	2.60	3.30	13.92

*Weights for elastic torsion angles are calculated from the standard deviations of a survey of appropriate mono- and polynucleotide structures (Ref. 12): weight = $100/(s.d.)^{2}$. Standard values $(_{o}\theta_{e})$ for these angles are the appropriate monomer or *B*-DNA values listed in Refs. 11 and 12. The overall weighting factor W_{e} is 0.002.

base pairs. The initial candidate models were selected from a computer modeling survey of dinucleoside monophosphate structures, in which all combinations of the mononucleotide average (staggered) values for ξ, ψ, and ϕ were included. This survey yielded but four candidate conformations with the property of having extended backbones with one base approximately over the other. Intermediate modeling trials, involving these candidates either with one sugar puckering changed (to C3'-*endo*) or with all sugars C3'-*exo*, eliminated several which displayed poor satisfaction of constraints or unacceptably short non-bonded contacts even after many cycles of refinement. The remaining candidates were tested in the complete tetranucleotide (N=2) plus drug model system described previously. Only two conformations emerged which satisfied all the criteria imposed: one having all sugars puckered C3'-*exo*, the other with the sugars puckered C3'-*exo* (5'→3') C3'-*endo* about the intercalation site. Otherwise the two models are conformationally similar in having both ξ (C3-C4-C5-O4) and ψ (C5-O4-P-O1) at the intercalation site adopt *trans* conformations and ϕ (O4-P-O1-C3) in the same range as in *B*-DNA. For both models the turn angle *T* is about 90° over three residues (-18° unwinding from *B*-DNA). The model incorporating a C3'-*endo* sugar is sterically less compressed in the residues adjacent to the intercalation site. Both models were further tested in a hexanucleotide (N=3) model system, where they again displayed good satisfaction of the imposed constraints (all within 0.005Å) and then the adjacent residues could adopt fairly conventional conformations, with no torsion angles exceptionally different from standard polymeric values. Again the two models proved very similar, both displaying unwinding angles of the order of -18°, with about -13° coming from the intercalation site and the remainder distributed over the adjacent residues. The marginally superior symmetric model with one altered sugar and a proflavine chromophore is shown in Figs. 2 and 3 and its conformational parameters are listed in Table 3. The shortest nonbonded interatomic contact in this model is 2.78Å.

For proflavine, there are two possible symmetric orientations for the drug chromophore: with the amino groups pointing toward either the major or the minor grooves. We found the former case to be slightly superior on the basis of attractive contacts; as

depicted in Fig. 4, this orientation allows overlap between the proflavine amino groups and the adjacent bases. We also performed a search to determine if proflavine in the intercalated mode could form specific hydrogen-bonded interactions with one of the sugar-

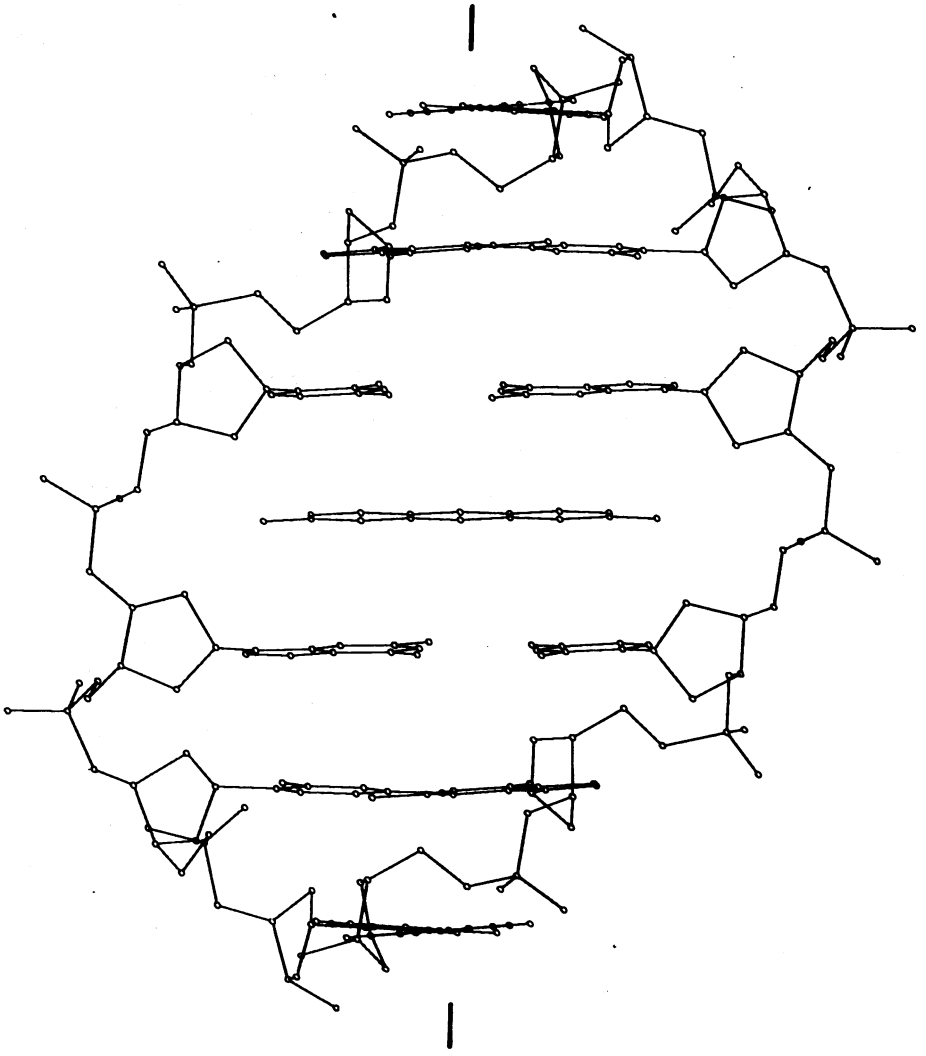

Figure 2: The optimum intercalation conformation viewed from the minor groove of the DNA along the diad axis of the proflavine chromophore. The vertical lines represent the axes of the adjacent *B*-DNA helices.

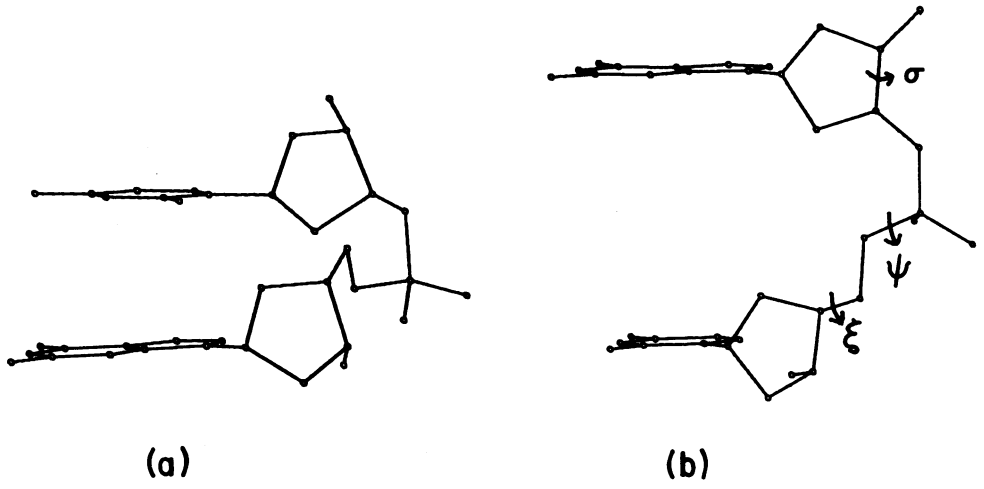

Figure 3: One residue of (a) standard *B*-DNA and (b) DNA extended at the intercalation site. The *trans* torsion angles ξ and ψ which characterize this conformation are indicated by arrows. In the least strained case, the sugar puckering is mixed, with one puckered C3'-*endo* (arrow) and all others C3'-*exo*.

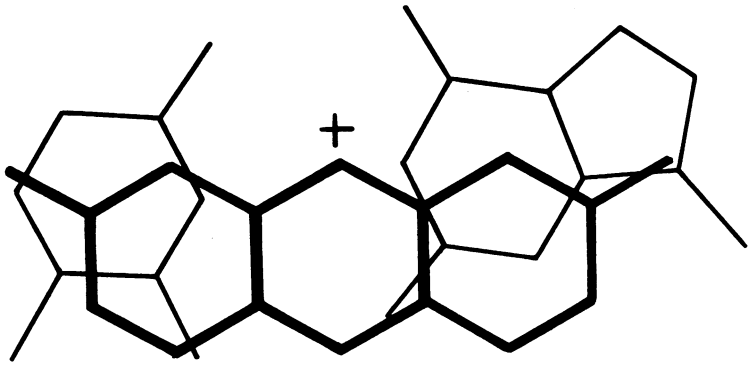

Figure 4: A symmetrically intercalated proflavine (dark lines) and an adjacent base pair viewed down the helix axis. Stacking between the proflavine and the bases is maximized with the amino groups facing the major groove. By symmetry, the chromophore has a similar stacking interaction with the other adjacent base pair. The cross represents the axis of the terminal *B*-DNA axis.

Table 3: Conformation Angles for Intercalated *B*-DNA*

	ξ	θ	ψ	φ	ω	σ	χ
Residue 1	24.9	-138.9	-61.3	-86.5	173.9	156.5	120.1
Residue 2	-173.4	-179.8	-167.9	-86.9	177.1	84.3	112.5
Residue 3	32.1	-171.0	-70.8	-104.0	178.0	156.5	-----
B-DNA[10]	36.4	-146.5	-46.1	-95.6	154.7	156.5	142.5

*Torsion angles adopting characteristic changes are underlined. Values for σ (sugar pucker) were fixed during refinement. These values are for the 17 variables of the model case N=2.

phosphate backbones, either to stabilize the binding or to mimic a nucleotide base and so to act as a mutagen. However, the phosphate-oxygen bonds at the intercalation site point away from the drug and such a direct interaction would be possible only if an amino group could form a hydrogen bond with the ester oxygen O4.

The turn angle *T* of 90° for the three residues involved in accommodating intercalation represents the least strained (energetically most favorable) conformation. We know, however, that there is some flexibility inherent in nucleotide residues and therefore tested the range of turn angles available to the optimum intercalation conformation by explicitly fixing *T* at certain values and examining the strain entailed in the refined models. The strain in a conformation is defined as $\overline{\varepsilon_e}$, the root mean square difference in torsion angle values from their appropriate standards. Fig. 5 shows the values of $\overline{\varepsilon_e}$ for a variety of intercalation models. We use the criterion that a value for $\overline{\varepsilon_e}$ exceeding 1.65 standard deviations represents an overly strained conformation; this value is determined empirically from the average $\overline{\varepsilon_e}$ for various polynucleotide structures compared to the polymer average conformation angles of ref. 11. By this criterion we note that turn angles from 84° to 95° over three residues can be accommodated without undue strain by the conformation we describe (see Fig. 5). Also we see that the intercalation model involving *trans* values of ξ and ψ but with all sugars puckered C3'-*exo* is considerably strained in the model case N=2, but in the case N=3 the necessary unwinding can be absorbed without undue strain. By contrast, we note that the proposed model for actinomycin

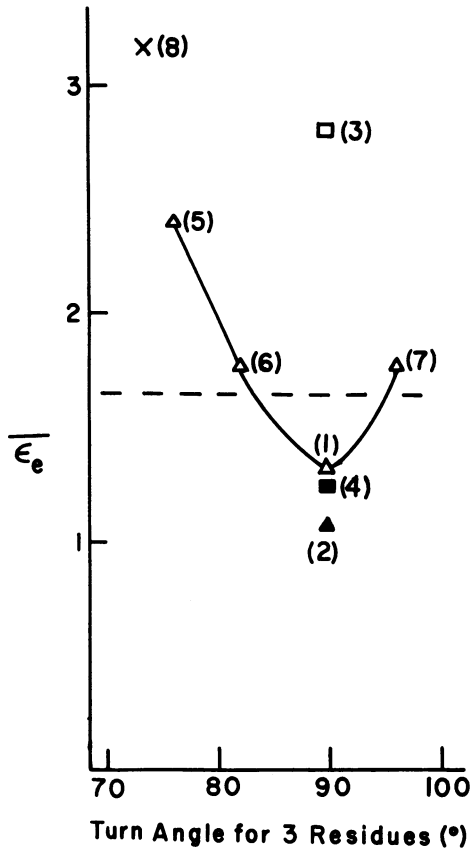

Figure 5: The steric strain $\overline{\epsilon_e}$ in the backbones of various intercalation models, measured as the root mean square difference (in standard deviations) of the backbone torsion angles from their appropriate standard values and plotted against the turn angle over 3 residues of the polynucleotide. Points 1 and 2 represent the optimum conformations for the model cases N=2 and N=3, respectively, and points 3 and 4 represent optimum conformations with all sugars puckered C3'-*exo* for the same model cases. Points 5, 6 and 7 represent refined variations of the optimum N=2 model where T has been fixed. Point 8 represents the model proposed for DNA-actinomycin D binding (Ref. 6).

intercalation[6] involves a great deal of backbone strain, with an $\overline{\epsilon_e}$ greater than 3 standard deviations for a hexanucleotide. An explicit test of this conformation (with proflavine) did not result in an improved model of this type; rather the refined model adopted the characteristic conformation we have described, even when the torsion angles were freely variable (i.e. unbound).

DISCUSSION

We have developed a computer molecular modeling system which allows explicit control of all variables and constraints and provides quantitative evaluation of the stereochemical acceptability of the model. By systematic evaluation of all conformational possibilities we have produced a sterically reasonable model which we feel is the most general and plausible mechanism for the

intercalation of planar drugs and dyes into double-helical *B*-DNA. *Our modeling conditions were chosen to simulate the effect of relatively low drug concentrations*, appropriate to biological and most solution conditions, rather than the case of drug saturation.

We note this model bears a superficial resemblance to that of Fuller and Waring.[2] The turn angle over the intercalated residue in their model is 24°, and we might expect that their characteristic conformation is as we have described. The major drawback of their model thus seems to be the assumption of one intercalation per residue, with every sugar puckered the same. The necessity we observe for having the adjacent residues unwind slightly in order to relieve strain in the molecule may provide a stereochemical rationale for the observation that approximately one proflavine molecule is bound per four bases at saturation.[19]

We note that in the proposed model for the binding of actinomycin D to DNA,[6] where the only characteristic conformational change is in the puckering of one sugar per nucleotide strand, several of the torsion angles assume nearly eclipsed values, *every* sugar ring in a hexanucleotide is distorted by an average of over 11° per ring torsion angle (i.e. nearly three standard deviations), and some of the phosphodiester bond angles are greatly distorted from their standard values. While the many interactions which the actinomycin side chains might make with DNA may be invoked to compensate for such steric difficulties, we are reluctant to admit such strain for other systems where stacking interactions are likely the main stabilizing force. Indeed, it is not unthinkable that the mechanism we present may be utilized in the binding of the asymmetric, nonplanar chromophores ethidium and actinomycin to DNA. ^{31}Pnmr studies of the binding of actinomycin D to a double-stranded hexanucleotide[20] indicate that three of the five phosphate spectra change upon binding of the drug, presumably on account of conformational changes at the intercalation site and in the two adjacent residues.

It is clear that, by itself, an alteration of sugar puckering is insufficient to provide the full 3.4Å extension required for intercalation: a C3'-*endo* sugar entails an extension between O1' and C5' only 0.5Å more than for C3'-*exo* sugars; thus the majority of the extension must come from other parts of the backbone,

either through distortion and strain or by conformation angle transitions to other staggered conformations. We prefer the latter alternative on steric and energetic principles. While the mixed sugar puckering pattern observed in the actinomycin-mononucleoside and ethidium-dinucleoside crystal structures may be attributable to the asymmetric, nonplanar natures of those chromophores, it is nonetheless interesting that such a pattern is useful in helping relieve steric strain in adjacent, nonintercalated residues.

Although dinucleosides complexed with drugs may exhibit several stable conformations in crystals, the requirement of compatability with *poly*nucleotide structures does not warrant free extrapolation from monomeric structures without careful evaluation of the steric effect of such conformations on the neighboring residues in the polymer. In particular, estimations of unwinding angles from the relative orientations of the glycosylic bonds in such structures are likely to be misleading when extrapolated to polymers where the bases are far from perpendicular to the helix axis (as in the case of *A*-RNA).

The classical definition of helical unwinding in DNA is the decrease in turn angle of a residue from that associated with a 10-fold helix. For the case of circular DNA it is apparent that not all the residues can adopt the same conformation: at least some regions must vary in order for the molecule to join with itself. Furthermore, it is known that certain alternating sequences can adopt *B*-like helical conformations with larger (8-fold) turn angles[21] and that such regions may be particularly applicable in the case of the drug ethidium, which has been shown to bind preferentially to alternating sequences.[22] Thus we consider the turn angle over the several nucleotides of the intercalation region to be a more meaningful quantity in these models than estimated unwinding angles. We also believe this study reveals a limit on the order of ±5° to the resolution attainable in physical studies of long molecules of undefined sequence and mixed conformation, due to their inherent flexibility. The careful fluorescence anisotropy decay studies of ethidium bound to defined sequences of linear DNA in the *B*-conformation[9] yield a value for the helical unwinding per intercalation nearly identical to that produced by the conformation we have described.

A characteristic feature of this model (and, in fact, of all the plausible extended candidates) is that the torsion angle ξ (C3-C4-C5-O4) falls in a range usually observed only in monomers and not in stacked helical polymers. Although it has been proposed[23-25] that variations only in the phosphodiester torsion angles φ and ψ need be considered for alternate bending conformations, it is apparent from this study that variations in ξ may also be required and even characteristic of some extended polynucleotide structures.

The high degree of stacking of the amino groups of proflavine in the intercalation site provides a rationale for the greater binding coefficients for proflavine to DNA than for the similar but unsubstituted acridines. In addition, fused four-ring DNA-binding drugs such as daunomycin,[26] ellipticine,[27] and possibly even steffimycin B[28] may also utilize a similar mechanism when binding to DNA.

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