A molecular mechanical study of complexes formed between 4-nitroquinoline-N-oxide and dinucleoside phosphates

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ABSTRACT.

Molecular mechanical calculations were done on complexes of 4-nitroquinoline-N-oxide (NQO) with various dinucleoside phosphates [(ApT)₂, (CpG)₂, (GpC)₂, and (TpA)₂]. Models built using proflavine (uniform C3' endo sugar puckers) and acridine orange (mixed C3' endo (3'-5') C2' endo sugar puckers) dinucleoside phosphate X-ray structures were used in the calculations. Relative binding energies, complex geometries, and various intercalator orientations in the complexes were studied. The results suggest qualitatively different geometries for pyr-(3'-5')-pur and pur-(3'-5')-pyr sequences. Specifically, we find marked distortion in some of the complexes (i.e. there is not a parallel coplanar relationship between the base pairs and intercalator), distortion of the NQO nitro group from planarity in the complexes and mobility of NQO in the intercalation site. We suggest that experimental studies of NQO-dinucleoside phosphate complexes may reveal intercalation complexes which deviate substantially more from a nearly parallel coplanar arrangement of bases and intercalator than has been previously observed.

1. INTRODUCTION

The noncovalent association of small molecules with nucleic acids has long been of interest. Lerman¹ proposed a model for the noncovalent association, or intercalation, of acridines with DNA 20 years ago. Other models for noncovalent binding of small molecules to nucleic acids also exist, such as stacking of the binding molecules along the nucleic acid backbone and external binding.² Since Lerman's proposal, extensive research has been done in the area of nucleic acid intercalation and much more is now known about the general characteristics of intercalation.²

The compound 4-nitroquinoline-N-oxide (NQO) is of biological interest due to its well documented carcinogenic and mutagenic effects.^{3,4} We are especially interested in NQO because, as an intercalator, it has some unique features. For example, NQO is one of the few two-ring intercalating agents known. Most intercalators are three- or four-ring compounds (e.g. proflavine, daunomycin). Additionally, NQO is unusual in that it is an uncharged intercalator. Most

intercalating agents are positively charged at physiologic pH. These factors, as well as the lack of an X-ray structure for any $(N_1pN_2)_2$:NQO complex, have inspired us to study this molecule.

Previous theoretical studies on NQO intercalation have been performed by Ornstein and Rein.⁵ They have examined the intercalation of NQO and a number of NQO metabolites in dinucleoside triphosphate minihelices. Their work differs significantly from ours in several ways. They used an ethidium intercalation complex X-ray structure to construct their starting models. They constrained the dinucleoside triphosphate minihelix to the starting geometry, allowing only the NQO molecule to move during refinement. They also constrained the nitro group of NQO in a planar orientation relative to the aromatic ring system. Finally, they did not examine various orientations of NQO in the intercalation site. In our calculations, we have allowed the minihelix to relax during energy refinement and we have not constrained the NQO nitro group to a planar geometry. A complete description of our methods appears in the procedure section.

In addition to the theoretical work of Ornstein and Rein, some NMR work has been done by Winkle and Tinoco⁶ on $(N_1pN_2)_2$:NQO complexes. They used ring current shift theory⁷ to predict the orientation of NQO in the intercalation site. We have used these proposed NQO orientations as starting geometries for some of our calculations.

Our objectives in this work are to investigate 1) the geometry of the drug-dinucleoside phosphate complexes and 2) the binding affinities and total energies of the complexes. Within the realm of our geometry studies we wish to examine the effects of different intercalation models, major vs. minor groove orientations of the NQO nitro group, and geometry in relation to sequence specificities. In studying the energetics of the complexes, we wish to look at absolute binding affinities and binding affinity in relation to sequence specificity.

2. MOLECULAR MECHANICS

All calculations were performed using AMBER⁸, a set of computer programs designed to construct models of molecular systems, alter the models to various desired conformations, and refine the models using an empirical potential energy function. The empirical energy function includes terms for bond stretching, bond bending, and non-bonded interactions:

$$E = \sum_{\text{bonds}} k_b (r - r_b)^2 + \sum_{\text{angles}} k_a (\theta - \theta_a)^2 + \sum_{\text{dihedrals}} \frac{k_d}{2} [1 + \cos(n\phi - \gamma)] \\ + \sum_{\text{non-bonded}} \left[B_{ij} r_{ij}^{-12} - A_{ij} r_{ij}^{-6} + \frac{q_i q_j}{\epsilon_{ij} r_{ij}} \right]$$

where non-bonded terms are summed over all atom-atom pairs, *i* and *j*, excepting those atoms involved in 1-2 and 1-3 interactions. Hydrogen bonding was modeled using the electrostatic term of the empirical energy function. Some test calculations were also done using an explicit 10-12 hydrogen bonding potential.

Values for the parameters k_b , θ_a , k_a , r_b , k_d , n , ϕ , r , B_{ij} , and A_{ij} depend on atom type. The parameter values for all atoms within the nucleic acids have been published previously.⁹ Additional parameters required for atoms in NQO are listed in Table 1 (atomic charges are from CNDO/2 calculations). Two values of ϵ_{ij} were used, giving two force fields for the calculations: (1) $\epsilon_{ij} = 1$ and (2) $\epsilon_{ij} = r_{ij}$. These two models have been used previously to study DNA structures for the dinucleoside phosphates. The $\epsilon_{ij} = r_{ij}$ model has also been used to study proflavine and acridine orange intercalation and was found to give reasonable results. The torsional parameter X-CA-N-X was derived by carrying out calculations on nitrobenzene. For this molecule the experimental rotational barrier is 3.0 ± 1.5 kcal/mole.¹⁰ Our calculations gave a rotational barrier of 3.0 kcal/mole.

Initial structures were generated with the aid of CHEM¹¹, an interactive computer graphics program designed to allow visualization and ready manipulation of molecular structure and conformation.

TABLE 1*

ATOM	TYPE	CHARGE	ATOM	TYPE	CHARGE	ATOM	TYPE	CHARGE
C1	CD	0.028	N6	N*	0.251	NC9	N	0.467
C2	CD	0.037	O8	O	-0.410	O91	O2	-0.334
C3	CD	0.020	C7	CD	0.051	O92	O2	-0.34
C4	CD	0.048	C8	CD	0.074	C10	CB	0.044
C5	CC	0.056	C9	CA	-0.005			

Table 1: Atomic charges and atom types for NQO.

* Additional parameters not given in reference 9 include: (bonds) CA-CD, CB-CD, CC-CD, CD-CD, CB-CC $k_b = 450$, $r_b = 1.39\text{\AA}$, CA-N $k_b = 450$, $r_b = 1.49\text{\AA}$, CC-N* $k_b = 450$, $r_b = 1.374\text{\AA}$, N-O2 $k_b = 450$, $r_b = 1.20\text{\AA}$, N*-O $k_b = 450$, $r_b = 1.30\text{\AA}$, N*-CD $k_b = 450$, $r_b = 1.35\text{\AA}$; (angles) X-CA-X, X-CB-X, X-CC-X, X-CD-X, X-N-X, X-N*-X $k_a = 70$, $\theta_a = 120^\circ$; (dihedrals) X-CA-CD-X, X-CB-CD-X, X-CC-CD-X, X-CD-N*-X $k_d = 25$, $\gamma = 180^\circ$, $n = 2$, X-CB-CC-X, X-CC-N*-X $k_d = 25$, $\gamma = 180^\circ$, $n = 4$, X-CD-CD-X $k_d = 25$, $\gamma = 180^\circ$, $n = 1$, X-CA-N-X $k_d = 3.6$, $\gamma = 180^\circ$, $n = 4$; and (van der Waals) values A_{ij} and B_{ij} for atom type O2 calculated from Slater-Kirkwood parameters $r_{vdw} = 1.60$, $n_{eff} = 7$, and $\alpha = 2.14$.

Figure 1: NQO numbering system used.

3. PROCEDURE

Two model structures were used, based on a) Sobell's structure¹² for $i^5\text{CpG}:\text{proflavine.H}^+$ and b) Sobell's structure¹² for $i^5\text{CpG}:\text{acridine orange.H}^+$. These two models are examples of "uniform pucker" and "mixed pucker" intercalation sites. Elsewhere we have demonstrated that starting with the Sobell $i^5\text{CpG}:\text{proflavine.H}^+$ structure or the Neidle¹³ $d(\text{CpG}):\text{proflavine}$ structure gave essentially identical results for proflavine and acridine orange intercalation.¹⁴ The cytosine iodine atoms and the ribose 2'-hydroxy groups of Sobell's structures were deleted to produce deoxydinucleoside phosphates. NQO was substituted for proflavine or acridine orange in the intercalation site. NQO was positioned in the intercalation complexes using geometries proposed by Winkle and Tinoco⁹ or by adjusting the intercalator so as to give apparently reasonable structures.

The force fields used in these calculations consider only hydrogen atoms attached to heteroatoms. Since the X-ray structures on which our models were based do not include hydrogen positions, the necessary hydrogen atoms were added using standard bond lengths and angles.⁹ Finally, the structures were adjusted to provide good hydrogen bonding contact between bases of the minihelix. Models for $(\text{ApT})_2:\text{NQO}$, $(\text{GpC})_2:\text{NQO}$, and $(\text{TpA})_2:\text{NQO}$ complexes were constructed in an analogous manner with the torsional angles of the dinucleoside phosphate backbones set to the values found in Sobell's CpG crystal structures.

For each dinucleoside phosphate sequence, models were constructed with major and minor groove projections of the NQO nitro group. Thus, a total of 16 model structures were prepared. These structures were used as starting geometries for energy refinement. The refinement was necessary to adjust all model structures to a common basis (see, for example, the procedure section of reference 14). Each structure was refined using force fields (1) and (2). Convergence criteria included that the rms derivative of the energy function relative to atomic coordinate changes should be less than 0.1 kcal/A as well as minimal change in the energy during the final stages of refinement.

In addition to the above calculations, several extra models of $(CpG)_2:NQO$ complexes were refined. In these models NQO was placed in various positions and orientations within the intercalation site to test the effect of starting geometries on final results and to gain some indication of NQO "mobility" in the intercalation site.

4. RESULTS

Table 2 shows the total energy after refinement for each model studied. In previous studies¹⁴ satisfactory results have been obtained with a dielectric constant $\epsilon_y = r_y$ and, for the sake of brevity, we will focus most of our attention on the results obtained with this force field.

For analysis purposes, the nucleotides were broken down into groups of atoms (see figure 2 for group labeling pattern). Table 3 contains some components of the total energy obtained from the group analysis of the complexes using $\epsilon_y = r_y$. The drug-helix interaction energy is the sum of interaction

TABLE 2

Model	Proflavine Model		Acridine Orange Model	
	$\epsilon = 1$	$\epsilon = r$	$\epsilon = 1$	$\epsilon = r$
CpG major groove*	-345.9	-140.2	-337.3	-132.1
CpG minor groove	-341.7	-134.2	-336.8	-129.5
CpC major groove	-344.5	-142.3	-335.0	-132.5
CpC minor groove	-343.0	-140.6	-336.6	-131.7
ApT major groove	-242.0	-91.6	-236.3	-90.4
ApT minor groove	-239.6	-90.3	-234.3	-87.5
TpA major groove	-239.2	-94.0	-236.9	-96.6
TpA minor groove	-234.7	-93.4	-239.4	-96.4

Table 2: Total energies of various complex models (energies in kilocalories).

* Major groove refers to a major groove projection of the NQO nitro group. Likewise, minor groove refers to a minor groove projection.

Figure 2: Group labeling pattern.

TABLE 3

Model*	Total*	Intra.*	Inter.*	Drug-helix*	Nuc.*	Corrected*	Angle*
CpG pro.major	-140.2	-28.8	-111.4	-69.3	-62.1	-133.2	43°
CpG acr.major	-132.1	-23.4	-108.7	-66.7	-62.0	-132.1	19°
CpG pro.minor	-134.2	-28.7	-105.5	-64.3	-61.2	-127.5	305°
CpG acr.minor	-129.5	-24.3	-105.2	-64.9	-60.3	-129.5	39°
GpC pro.major	-142.3	-23.6	-118.7	-66.1	-62.6	-131.0	16°
GpC acr.major	-132.5	-24.5	-108.0	-65.5	-62.5	-132.5	340°
GpC pro.minor	-140.5	-24.4	-116.1	-63.9	-62.2	-129.4	44°
GpC acr.minor	-131.7	-25.4	-106.3	-63.9	-62.4	-131.7	326°
ApT pro.major	-91.6	9.2	-100.8	-51.5	-49.3	-79.9	317°
ApT acr.major	-90.4	8.1	-98.5	-53.5	-45.0	-90.4	336°
ApT pro.minor	-90.3	9.1	-99.4	-50.1	-49.3	-78.0	39°
ApT acr.minor	-87.5	10.4	-97.9	-52.6	-45.3	-87.5	34°
TpA pro.major	-94.0	10.1	-104.1	-55.5	-48.6	-81.8	25°
TpA acr.major	-96.6	13.7	-110.3	-55.0	-55.3	-83.3	34°
TpA pro.minor	-93.4	5.7	-99.1	-54.4	-44.7	-87.5	312°
TpA acr.minor	-96.4	17.0	-113.4	-55.7	-57.7	-83.0	17°

Table 3: Components of total energy (in kilocalories) for various complex models calculated with force field (2).

* Key to headings.

Model - pro.major indicates a proflavine geometry with a major groove orientation of NQO group. Likewise, acr.major indicates an acridine orange structure with a major groove orientation of NQO nitro group, etc.

Total - The total energy of the complex.

Intra. - The intra-group interaction energy.

Inter. - The inter-group interaction energy.

Drug-helix - The drug-minihelix interaction energy.

Nuc. - The nucleotide interaction energy.

Corrected - The total energy corrected for intrachain hydrogen bonding.

Angle - The out of plane distortion angle of the NQO nitro group in the complex model (angle C10-C9-NC9-O92).

energies of NQO with each of the 10 groups in the dinucleoside phosphate minihelix. The nucleotide interaction energy is the total inter-group interaction energy for the 10 groups in the dinucleoside phosphates. The drug-helix and nucleotide interaction energies sum to give the total intermolecular energy. The intramolecular energy is the sum of all intra-group energies. The intra- and intermolecular energies sum to give the total energy. Table 3 lists these values as well as a corrected total energy term (see below) and out of plane distortion angles for the nitro group of NQO.

Examination of the structures and component energy terms showed that intramolecular hydrogen bonds formed between terminal hydroxy groups and phosphate oxygens of the dinucleoside phosphate backbone. This intramolecular hydrogen bond formation occurred only in the proflavine geometry models with the exception of the $(TpA)_2$:NQO complexes where all models displayed intramolecular hydrogen bonds. We have made corrections for the intramolecular hydrogen bonding because we wish to compare the relative stabilities of the complexes as they would exist in a biological system (i.e. in an aqueous environment). Although some of the complexes might form intramolecular hydrogen bonds in solution, those which did not would exhibit hydrogen bonding between terminal hydroxy groups and water molecules. Thus, the aqueous environment would have a leveling effect on hydrogen bonding differences between various complexes.

To correct for this intramolecular hydrogen bonding, a portion of the deoxyribose-phosphate interaction energy terms was subtracted in the models where intramolecular hydrogen bonds formed. The corrections needed to account for the intramolecular hydrogen bonding were determined by comparing deoxyribose-phosphate interaction terms for groups exhibiting hydrogen bonding vs. groups with no hydrogen bonding. The deoxyribose-phosphate interactions of the NQO complexes were also compared to deoxyribose-phosphate interaction terms for dinucleoside phosphate minihelices with no intercalator. These dinucleoside phosphate minihelices displayed no intramolecular hydrogen bond formation.⁹ Using these comparisons, corrections were made for the intramolecular hydrogen bonding and the corrected total energies are listed in Table 3. We also subtracted all deoxyribose-phosphate interaction energy terms in the dinucleoside phosphate:NQO complexes. Both of these "correction techniques" predict the same preferred model in all cases. Thus, we feel reasonably confident that our hydrogen bonding corrections are appropriate.

After the correction for hydrogen bonding is made, we find that $(CpG)_2:NQO$ and $(TpA)_2:NQO$ complexes prefer the proflavine model geometry while $(ApT)_2:NQO$ and $(GpC)_2:NQO$ complexes prefer the acridine orange model geometry. Note also that the refined CpG and TpA structures are qualitatively similar as are the refined ApT and GpC structures (see figure 3). In the CpG and TpA complexes, NQO is centrally located in the intercalation site and stacks between the purine bases. The pyrimidine bases distort noticeably, encapsulating NQO in the intercalation site. In the ApT and GpC complexes, NQO slips to one side of the intercalation site and stacks between the purine and pyrimidine bases of one dinucleoside phosphate strand. The other dinucleoside phosphate strand collapses slightly, reducing the size of the intercalation site. The distortion of the ApT and GpC complexes is much less than the distortion of the CpG and TpA complexes. Table 4 lists the backbone torsional angles for the structures shown in figure 3.

Table 5 gives relative ΔE values for the NQO-minihelix interaction. These ΔE values are obtained by subtracting the total energy of a dinucleoside phosphate minihelix from the corresponding dinucleoside phosphate minihelix:NQO com-

Figure 3 : Refined structures for the energetically most favorable model in each sequence. Structures are: (A) $(CpG)_2:NQO$, (B) $(GpC)_2:NQO$, (C) $(ApT)_2:NQO$, and (D) $(TpA)_2:NQO$.

TABLE 4

Model ^a	Angle	Strand 1		Strand 2	
		Initial Values	Refined Values	Initial Values	Refined Values
(CpG) ₂ pro.major	χ	27°	36°	28°	53°
	ε	195°	199°	197°	197°
	θ	296°	300°	295°	290°
	ψ	265°	275°	295°	304°
	φ	247°	257°	216°	182°
	ω	55°	44°	60°	57°
	χ'	86°	71°	69°	27°
(GpC) ₂ acr.major	χ	19°	16°	15°	26°
	ε	216°	197°	226°	196°
	θ	295°	290°	284°	291°
	ψ	291°	298°	296°	312°
	φ	237°	236°	229°	186°
	ω	58°	76°	61°	74°
	χ'	96°	105°	86°	54°
(ApT) ₂ acr.major	χ	19°	18°	15°	40°
	ε	216°	196°	226°	191°
	θ	295°	297°	284°	290°
	ψ	290°	297°	298°	312°
	φ	236°	237°	229°	178°
	ω	59°	72°	61°	70°
	χ'	95°	108°	86°	38°
(TpA) ₂ pro.minor	χ	27°	26°	28°	65°
	ε	194°	200°	196°	191°
	θ	296°	299°	296°	287°
	ψ	284°	281°	295°	305°
	φ	247°	255°	216°	176°
	ω	55°	46°	59°	53°
	χ'	85°	58°	66°	12°

Table 4 : Backbone torsional angles of best minihelix complex in each sequence (results of force field (2) calculations).

^a - See Table 3 legend for description of model notation.

TABLE 5

Uncorrected Values			Corrected Values ^a			Comparison Values	
Model ^a	E	ΔE	Model	E	ΔE	OR ^a	WT ^a
CpG pro.major	-140.2	-17.2	CpG pro.major	-133.2	-10.0	-16.2	-5.92
GpC pro.major	-142.3	-15.3	GpC acr.major	-132.5	-5.6	—	-5.39
ApT pro.major	-81.6	-14.5	ApT acr.major	-80.4	-13.3	-10.6	-5.65
TpA acr.major	-86.6	-22.7	TpA pro.minor	-87.5	-13.6	-19.4	-5.61

Table 5 : Total energies and binding energies for various complexes (in kilocalories).

^a Corrected values refer to energy terms corrected for hydrogen bonding. Note that the hydrogen bond correction changes the model preference in some cases.

Model - See Table 3 legend for description of model notation.

OR - Calculated ΔH values for NQO intercalation in minihelices. A GpC minihelix was not tested. Taken from Ornstein and Rein⁶.WT - Experimental ΔG values for NQO intercalation in minihelices. Taken from Winkle and Tinoco⁶.

plex. Again, the corrected values correspond to structures for which intramolecular hydrogen bonding interactions have been subtracted. Note that intercalation in $(CpG)_2$ is preferred over $(GpC)_2$ while ΔE values for intercalation in $(ApT)_2$ and $(TpA)_2$ are similar ($(TpA)_2$ being slightly preferred over $(ApT)_2$). These calculated ΔE values are in reasonable agreement with experimental ΔG values of Winkle and Tinoco.⁸

Inspection of the corrected total energy terms in Table 2 shows that for the CpG complexes the proflavine model structure with a major groove orientation of the NQO nitro group is the preferred model. For both GpC and ApT the preferred model is an acridine orange geometry with a major groove orientation of the nitro group. TpA prefers a proflavine geometry with a minor groove nitro group projection. Winkle and Tinoco⁸ predict major groove orientations for the NQO nitro group in the $(CpG)_2:NQO$ and $(GpC)_2:NQO$ complexes and minor groove orientations of the nitro group in the $(ApT)_2:NQO$ and $(TpA)_2:NQO$ complexes.

In analyzing the various CpG complexes, we have looked at all the intra- and inter-group interaction energies. We find that the proflavine major groove model is preferred over the proflavine minor groove and acridine orange models because in the former model, NQO interacts well with all four bases of the minihelix. All other CpG complex models have good NQO interactions with only three of the four bases (i.e. the two guanines and one cytosine). We examined the quality of fit between intercalator visually by calculating¹⁵ and displaying the molecular surfaces for the complexes. This showed that for CpG:NQO only the proflavine major groove model refined to give a structure free of gaps between component molecules. In the other models, the molecular surfaces clearly show gaps between the van der Waal's surfaces of NQO and one of the pyrimidine bases. In the proflavine major groove model, the nitro group is essentially sandwiched between the amino groups of the cytosine residues. The nitro group appears to exert a favorable attractive force on the amino hydrogens (the amino hydrogens are indeed distorted approximately 5° out of the plane of the cytosine bases in the direction of the nitro group). This distortion of the cytosine amino group hydrogens may weaken the hydrogen bonding of the base pairs, thus allowing the cytosines to fold around the NQO molecule more efficiently. The base pairing interactions are weaker by about 0.3–1.5 kilocalories/mole base pair in models with strong interactions between the NQO nitro group and the cytosine amino groups. In the models with a minor groove NQO nitro group projection, the nitro group does not interact with the cytosine amino groups. The nitro group interaction with the cytosine amino groups in the acridine orange major groove model is negligible, perhaps due to the larger

size of the intercalation site in the acridine orange geometries. In the models with little or no nitro group interaction with the cytosine amino groups, the cytosine bases distort much less (they do not fold around the NQO molecule) and this, we believe, explains why only three of the four bases can interact efficiently with NQO in these models.

As discussed in the molecular mechanics section, hydrogen bonding was modeled using an electrostatic term in the empirical energy function.⁹ To test the reliability of this approach, we also did a number of calculations using an explicit 10–12 hydrogen bonding potential.¹⁶ The potential was calibrated to yield a value of 3 kcal. per hydrogen bond in addition to electrostatic effects. As a final test, we modeled the hydrogen bonds between the base pairs as weak covalent bonds (the following additional parameters were needed for these calculations : $k_b = 5.0$, $k_a = 0$, $k_d = 0$, r_b for all hydrogen bonds = 1.8A). All three models gave qualitatively similar results. Both the relative energies and geometries for the complexes were essentially independent of modeling technique. We feel these results suggest that hydrogen bonding structure in the complexes can be described adequately using an electrostatic description.

The reasons for the observed model preferences in the other complexes are much less clear than in the case of the CpG series. The GpC structures favor a major groove projection of the NQO nitro group, possibly due to interactions between the nitro group and the cytosine amino groups. The acridine orange model seems to be preferred over the proflavine model due to its somewhat better intra-group energy terms. In the ApT complexes, the acridine orange models display better NQO–base interactions but the reasons for this are unclear. The acridine orange major groove structure also has the best intra-group energy terms. Interaction of the adenine 6–amino hydrogens with the NQO nitro group appears to be less important than the nitro–cytosine amino interactions in the CpG series, but the nitro group interaction with the amino hydrogens of adenine may play some role in distorting the bases from coplanarity. The TpA complexes are most difficult to analyze. It appears that the proflavine minor groove model is preferred in the TpA sequence because the intra-group energy terms are much better for this model compared to the other TpA complex models. Note from the structure in figure 3 that the TpA model is extremely distorted (see also the list of backbone torsional angles in table 4). Careful observation of the molecular surfaces calculated for the TpA structures indicates that this distortion reduces the size of the major groove to such an extent that the minor groove actually becomes the larger of the two

grooves. The molecular surfaces suggest that the NQO nitro group prefers a minor groove orientation in the TpA models because the nitro group can be more easily accommodated in the minor groove (less steric crowding).

In our group interaction analysis, we have observed that purine-NQO interactions are generally more important than pyrimidine-NQO interactions. Values for NQO-base interactions are as follows: $(CpG)_2$:NQO, NQO-guanine interactions -16.58 and -15.58 kcal, NQO-cytosine interactions -9.38 and -9.04 kcal; $(CpC)_2$:NQO, NQO-guanine interactions -14.37 and -9.56 kcal, NQO-cytosine interactions -11.73 and -10.77 kcal; $(ApT)_2$:NQO, NQO-adenine interactions -15.43 and -10.43 kcal, NQO-thymine interactions -10.58 and -8.30 kcal; $(TpA)_2$:NQO, NQO-adenine interactions -16.99 and -16.27 kcal, NQO-thymine interactions -5.95 and -4.41 kcal. Winkle and Tinoco¹⁷ and Okano et. al.¹⁸ have observed this preference of NQO for purine mononucleotides and mononucleosides, especially guanine, in earlier experimental work.

The ΔE values in Table 5 suggest that NQO should bind much more tightly to $(CpG)_2$ than to $(CpC)_2$. Analysis of the inter-group interaction energy terms suggests that NQO has generally better interactions with the bases in the $(CpG)_2$ minihelix. Again, we feel that the geometry of the two complexes explains the preference for $(CpG)_2$. Recall that NQO is centrally located in the intercalation site of the $(CpG)_2$ minihelix; thus, it can interact with all 4 bases. In the $(CpC)_2$ minihelix, NQO slides to one side of the intercalation site and the interactions with the bases of the opposite strand are not especially good.

To investigate the "mobility" of NQO in the intercalation site, additional models of the $(CpG)_2$:NQO complex were constructed with a variety of starting positions and orientations for NQO. Figure 4 shows a diagram of final NQO orientations and the corresponding refined energy for each structure. As can be seen from the diagram, the barrier to complete rotation of NQO from a major groove to a minor groove orientation projection is only about 6 kilocalories. The barrier for "sliding" (i.e. movement of NQO from one side of the intercalation site to the other) is only about 2-3 kilocalories. These small energy barriers to movement indicate that there may be considerable mobility in the intercalation complexes. Considering the small size of the intercalating agent, this does not surprise us and we suggest that experimental studies be undertaken to investigate this.

In all our refined structures we found that the NQO nitro group was distorted from a planar orientation relative to the NQO ring system. The distortion angles for the various complex models are listed in table 2. Comparison of the

Figure 4 : Mobility of NQO in the intercalation site of the (CpG)₂ minihelix. The arrows indicate the orientation of the NQO nitro group. The numbers are the relative energies (uncorrected) for the various models. (P) denotes a proflavine model structure and (A) denotes an acridine orange model structure. The absolute lowest energy for the proflavine model is -140 kcal.; for the acridine orange model, -134 kcal.

NQO intra-group interaction energy to the size of the distortion suggests the lowest energy structure for NQO is one with the nitro group approximately 60° out of plane. We have done *ab initio* quantum mechanical calculations on NQO and related molecules which support this finding.¹⁰

5. DISCUSSION

Careful study of our refined complex structures indicates that the models based on a proflavine X-ray geometry form intramolecular hydrogen bonds (in the TpA complexes the acridine orange models also form intramolecular hydrogen bonds). These hydrogen bonds increase the stability of the proflavine models relative to the acridine orange models. Dearing et. al.¹⁴ found similar hydrogen bonding patterns in their study of proflavine and acridine orange intercalation. Correction for this hydrogen bonding using two different methods suggests that CpG and TpA complexes prefer a proflavine intercalation geometry (uniform C3' endo sugar pucker) while GpC and ApT complexes prefer an acridine orange intercalation geometry (mixed C3' endo(3'-5')C2' endo sugar pucker). Earlier studies¹⁴ have suggested that three-ring intercalators may prefer a mixed sugar pucker (i.e. acridine orange geometry) because of

better stacking interactions. Our present results imply that the mixed sugar pucker is less crucial for NQO intercalation. Due to the smaller size of the intercalator, the minihelix can distort to give good NQO–base interactions even with the uniform sugar pucker model.

Our calculated relative binding affinities (ΔE values in Table 5) are in reasonable qualitative agreement with the relative free energies found by Winkle and Tinoco⁶ (see Table 5) as regards $(CpG)_2$ vs. $(CpC)_2$ and $(TpA)_2$ vs. $(ApT)_2$ even though the range of our calculated ΔE values is much larger than the range of their experimental ΔG values. However, our calculations are not consistent with their findings that NQO binding to $(ApT)_2$ and $(TpA)_2$ is intermediate in free energy between NQO binding to $(CpG)_2$ and $(CpC)_2$. One explanation for this discrepancy lies in the fact that the AT isomers have notably smaller equilibrium constants for minihelix formation.²⁰ The experimental ΔG values reflect the energetics of both minihelix formation by the dinucleoside phosphates and NQO intercalation in the minihelix. Thus, intercalation in $(ApT)_2$ and $(TpA)_2$ will be less favorable relative to $(CpG)_2$ because of the smaller minihelix formation constants. Our calculated ΔE values only consider the energies of the reaction $(N_1pN_2)_2 + NQO \rightarrow \text{complex}$. Thus, the intercalation into $(ApT)_2$ and $(TpA)_2$ is calculated to be more favorable than has been observed. This hypothesis could be tested by temperature and solvent dependence experiments of NQO association with the four dinucleoside phosphates.

Within the CG isomer sequence, we attribute the preference of NQO for $(CpG)_2$ to better overall interaction of NQO with the bases. The figures of the refined structures (figure 3) seem to support this hypothesis. In the AT isomer sequence, the best $(ApT)_2$:NQO complex model has slightly better NQO–base interactions but the best $(TpA)_2$:NQO complex model has slightly better intra–group energy terms. These two factors cancel each other and, as a result, we see no significant preference of NQO for either $(ApT)_2$ or $(TpA)_2$. Ornstein and Rein⁵ found in their calculations that NQO binding was much preferred in TpA minihelices over ApT minihelices (see Table 5). However, they used a constrained ethidium complex minihelix model in their calculations whereas we allowed our minihelices to relax. Ethidium is known to prefer pyr–(3'–5')–pur sequences which may explain why their model predicts NQO intercalation to be more favorable in the TpA sequence vs. the ApT sequence.

We agree with Winkle and Tinoco⁶ on NQO nitro group orientation (major vs. minor groove projection) in all but the $(ApT)_2$:NQO complex although the position of NQO in our calculated structures differs somewhat from the position

of NQO in their proposed structures. Group analysis reveals that our (ApT)₂:NQO major groove complex is preferred because the intra-group energy terms are most favorable for this model. Winkle and Tinoco have predicted the structures of the complexes using NMR ring current shift calculations. There are at least two possible reasons for discrepancies between our predicted NQO orientations and those of Winkle and Tinoco. First, and probably most important, our structures represent local energy minimum structures for each complex. The NMR shifts represent a time-average of all possible NQO orientations in the complexes. We have already noted that our calculated barriers to various types of NQO motion in the intercalation site are small. Thus, it is reasonable to assume that NQO may move about rather freely in the intercalation complex. If so, the NMR shifts measured by Winkle and Tinoco are a time-average of all possible NQO positions in the intercalation complex. A second reason may be due to the geometry of our refined structures. In their assignment of the NQO position based on NMR shifts, Winkle and Tinoco assumed a parallel coplanar arrangement of the intercalator and base pairs. Our structures clearly do not exhibit this property.

In a number of our refined structures, interactions between the oxygen atoms of the NQO nitro group and the amino hydrogens of cytosine, guanine, and, possibly, adenine bases appear to be rather important in the intercalation complexes. We have proposed that these interactions may be responsible for distorting the base pairs, to produce better interactions between the bases and NQO. The N-oxide oxygen atom of NQO may also participate in interactions with various amino hydrogens in the bases but these interactions are apparently less important than the nitro group interactions. Previously, Paul and Montgomery²¹ have suggested the possibility of hydrogen bond formation between the NQO N-oxide oxygen and amino hydrogens of the bases. We feel that the interactions of the nitro and N-oxide oxygen atoms with amino hydrogens of nucleic acid bases may be an important component of NQO intercalation.

Our observation that NQO interacts more strongly with purines seems to be well supported by previous experimental work. Overall, our calculations have successfully reproduced a number of observed experimental results regarding NQO interactions with nucleic acids. We feel this indicates that our approach to modeling NQO-dinucleoside phosphate minihelix interactions has been reasonable.

Finally, we wish to emphasize that our calculations are essentially gas

phase calculations. The dielectric constant can be modified to simulate an aqueous environment. However, until solvation effects can be included in calculations in a feasible and physically realistic manner, the best we can hope for with molecular mechanical calculations is to make qualitative predictions within a series of interest. We feel that the results of our calculations can give some insight into the interaction of NQO with nucleic acids, particularly as regards differences between NQO and other more "traditional" intercalators such as proflavine, acridine orange, and ethidium. For example, our calculations have suggested that the NQO group can move about freely in the intercalation complexes. This is in contrast to larger intercalators which are more limited in their motion in intercalation complexes. We also find noticeable distortion of the dinucleoside phosphate minihelix in our structures. This has not been observed in X-ray structures of dinucleoside phosphate intercalation complexes with larger intercalating agents. Hogan et. al.²², employing electric dichroism, have suggested a significant tilting of ethidium, 9 amino-acridine, proflavine and the actinomycin chromophore during DNA intercalation. This tilt, which averages approximately 20° for the various drugs, is significantly greater than that observed in crystal structures of dinucleoside-drug complexes^{12,13}. This apparent discrepancy may be due to crystal packing effects, differences in lengths of the DNA, or ambiguities in the interpretation of the dichroism data. Our calculations suggest that the tilting for NQO should be greater than that observed in the above three-ring intercalators, given that the comparison is made under similar experimental conditions (solution or X-ray, similar length of DNA helix, etc).

We are presently performing molecular mechanical calculations on interactions of NQO and other intercalators with double-stranded hexanucleotides. Although the hexanucleotide complexes may be more biologically realistic models of DNA intercalation complexes in some respects, we feel that dinucleoside phosphate complexes are also valid and informative when considering intercalation activity near replicating DNA.¹⁴

6. SUMMARY

In conclusion, we find that NQO intercalates preferentially in $(CpG)_2$ minihelices relative to $(CpC)_2$ minihelices while there is essentially no preference for $(TpA)_2$ over $(ApT)_2$. Our calculations suggest that the nitro group of NQO distorts out of plane in the intercalation complexes. We also find rather strong interactions between the NQO nitro group oxygens and amino hydrogens

of the nucleic acid bases. Our results imply that there may be considerable mobility in the intercalation complexes, which could be demonstrated experimentally using NMR relaxation techniques. Finally, we suggest that experimental studies of NQO-dinucleoside phosphate intercalation complexes may reveal an intercalation geometry that deviates substantially more from a nearly parallel coplanar relationship between the intercalating agent and all the bases of the minihelix than has been previously observed.

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