Conformational flexibility of dinucleoside dimers during unwinding from the B-form to an intercalation structure

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ABSTRACT

Intra-dinucleoside dimer conformational energy calculations were performed on a series of conformations corresponding to the unwinding of a dinucleoside dimer from the B-form type structure. Conformational states were characterized in terms of the distance, d , between parallel bases. All 16 possible sequences for (G) and (C) and/or (A) and (T) bases were considered. Both free space, and free space plus aqueous solvation energies were computed. It was found that the B-form like conformer is the most stable structure in free space and in an aqueous medium. However, the energy as function of d is base pair sequence dependent. This sequence dependence suggests a possible inherent specificity for intercalation of the dinucleoside dimer with a drug molecule.

INTRODUCTION

Alkylation and intercalation processes involving DNA are experimentally observed to be preferentially specific to base-pairs of guanine (G) and cytosine (C). N7 alkylation of (G) is far and away the most commonly observed base alkylation site with N3 of both (G) and adenine (A), and O6 of (G) reported as minor sites of alkylation (1). Krugh was the first to suggest that intercalation of drugs between adjacent base-pairs might be sequence-specific, and preferentially disposed toward base-pairs of (G) and (C) (2). Additional support for such specificity has come from solution studies (3), as well as the general observation that (G) and (C) containing dinucleoside monophosphate-drug complexes are most easily crystallized (4).

Alkylation specificity has been attributed to the relative chemical reactivities of individual base sites. We (5,6), like others (7,8), have sought to test this hypothesis by performing chemical reactivity calculations using semi-empirical molecular orbital methods. Base-site reactivity does appear to be an important factor in alkylation specificity of the aziridinium and methyldiazonium ions (5,6).

DNA intercalation processes involve unwinding and extending the double

helix (9). The specificity for intercalation between certain base pairs has been investigated by ourselves (10) and others (11-16). This intermolecular process is not yet understood with respect to both mode and specificity of interaction.

Recently, there has been considerable progress in identifying new possible DNA conformations. These studies have shown that crystal structures, which differ from the Watson-Crick base pair A,B, and C structures, do exist (17,18). There also have been several theoretical calculations on DNA conformation (15,19-24) which support the concept that DNA can be, at least locally, a "floppy" macromolecule.

The apparent conformational flexibility of DNA, and the observed base-sequence specificity for intermolecular interactions, leads one to wonder if there is a cause-effect relationship between flexibility and intermolecular interactions. That is, is there a base pair sequence specificity with respect to conformational flexibility? This is the question we address in this paper.

METHOD

1. Dinucleoside Monophosphate Dimer Structures

The backbone and base pair valence geometries of each dinucleoside were frozen at the crystallographic bond lengths and angles reported for B-form type DNA from x-ray data (25,26). The base pair geometries were held rigid in all conformational states considered. Hydrogens were added using "standard" bond lengths and angles (27).

The consequence of keeping the base pairs rigid forces us to ignore "breathing" and related interchain motions in a DNA molecule. The elimination of this artificial constraint, however, would make the calculations impractical. Hence, our work is tainted with this limitation.

Three additional constraints were used to generate a family of dinucleoside monophosphate dimer conformations;

1. The base pairs were held parallel to one another for all inter-base pair separation distances, d . This restriction destroys the exact conformational symmetry of chemically symmetric B-form dimers.
2. The distance between each dinucleoside backbone was held fixed so that different base pairs could be directly deleted and/or inserted without altering the backbone conformation of each oligomer.
3. The type of sugar phosphate ring puckering was held fixed in the generation of dinucleoside dimer conformations. Dinucleoside conformational sets were generated for both the C2' *endo* sugar phosphate puckering, as

found in B-form type DNA (25), and the alternate C3' *endo* (3'-5') C2' *endo* puckering (for $d > 5.7 \text{ \AA}$) as first found by Sobell and coworkers (28,29) in dinucleoside-drug intercalator crystal complexes.

The geometry of a representative dinucleoside monophosphate dimer, using the three structural constraints, is shown in Figure 1. An implicit assumption made in generating the dinucleoside dimer structures is that each conformation represents an "unwinding" of the B-form type DNA conformation. A set of torsion angles can be determined that yield a dinucleoside dimer possessing a pre-stated distance, d , between base pairs. Consequently, it is convenient to characterize the conformation of these structures in terms of d . Further, conformational flexibility can be measured in terms of the intra-dimer energy as a function of d . The ring closure equations are given in ref. 31. Figure 2 illustrates some possible results of unwinding a $d(T_pC) \cdot d(G_pA)$ dinucleoside dimer under these constraints.

The ring closure equations do not require that all backbone torsion angles need be uniquely specified for the imposed structural constraints. This is fortunate from the point of view of generating stereochemically acceptable conformations. Once a set of torsion angles were found to satisfy the structural constraints, we were able to perform a perturbation analysis of the torsional angles to simultaneously identify a local conformational state having a "low" intra-dinucleoside dimer energy. The "low" energy conformations do not necessarily correspond to stable energy minima in the thermodynamic sense.

We took advantage of the observation that the torsion angles ϕ and χ are the principal degrees of freedom in observed intercalation crystal complexes (30). That is, these angles were allowed to vary over a wider range of values than other torsion angles in the perturbation analysis. Generally, the perturbation analysis employed to obtain a "low" conformational energy

Figure 1. The dinucleoside monophosphate dimer geometry for $d(T_pC) \cdot d(G_pA)$ with the B-form type DNA sugar ring puckering (looking into the minor groove). Nomenclature for the torsional angles are the same as Berman et. al. (30).

Figure 2. A sequential set of views of $d(TpC) \cdot d(GpA)$ unwinding. (Looking into the major groove and looking from the top) These are the lowest energy structures realized at the respective d values for a particular puckering.

results in a minor change in the value of d from that originally selected.

2. Molecular Energetics

Molecular mechanics potential functions, as reported in refs. 32 and 33,

were used to compute both isolated and aqueous solvation intra-dinucleoside dimer conformational energies. A specialized component of the MASS (molecular assembly software system) option in our VAX version of CAMSEQ-II (27) was used to make the energy calculations.

The potential functions used to compute the isolated (vacuum) energies are well-documented, and generally yield reasonable and accurate results (27, 32,33). However, the model to compute aqueous solvation energetics is not quantitatively reliable. The energies derived from these calculations must be viewed as meaningful only in so far as trends can be discerned.

Hence, while the aqueous solvation model has been described and documented elsewhere (32-34), it is worthwhile to both describe and critically evaluate the model here. The essential features of the model are:

A hydration sphere is centered about each of the set of solute groups composing the molecule. Each hydration sphere is initially assumed to contain its corresponding maximum number of water molecules. Consequently, the model actually computes the aqueous desolvation energy as a function of chemical structure and conformation of the molecule.

Four parameters characterize the solvation thermodynamics and geometry of each hydration sphere. First, the size (radius) of the hydration sphere is determined from aqueous solvation computer simulation calculations (34). Next the number of water molecules in a hydration sphere is taken from available experimental hydration studies (33) when possible, or, again from computer simulation hydration experiments. The free energy of hydration of a water molecule in a hydration sphere is taken from experimental data carried out on congeneric sets of molecules (32,33). Lastly, the mobility of a water molecule in a hydration sphere is estimated from computer simulation experiments (33,34).

Change in the aqueous solvation free energy is assumed to be a linear function of the overlap volume of a pair of interacting hydration spheres. The total aqueous solvation energy of a molecule corresponds to the sum of all pairwise hydration sphere overlaps. Since the pairwise hydration sphere overlap volumes depend upon conformation, the total aqueous solvation energy is a function of conformation. Scheraga and coworkers have developed (35) and extended (36) a similar hydration sphere model.

There are three critical features of the aqueous solvation model which limit its quantitative usage:

1. One or more of the values of the four hydration sphere parameters may be in error.

2. The linear relationship between pairwise hydration sphere overlap volume and change in solvation energy may not be reasonable over a wide range of overlap volumes.
3. The model neglects triplet, and higher order, hydration sphere overlap volumes. Aqueous solvation energetics is not simply proportional to pair-overlap volumes of hydration spheres. Correspondingly, the total molecular solvation energy is not simply the sum of all pairwise interactions in a molecule.

The effect of the pairwise-overlap-volume approximation is to over-estimate changes in aqueous solvation energy. This is a point to keep in mind when considering the numerical ranges in the results presented in the next section.

RESULTS AND DISCUSSION

Figure 3 contains plots of the vacuo (free-space) energy, V , aqueous solvation plus free space energy, T , and the difference, $(T-V)$ as a function of distance, d , between base pairs. $(T-V)$ is nearly equal to the aqueous solvation energy. However, solvent dictated conformational effects can change the value of the free-space energy in the T calculation from that in the V computation. All sixteen possible dimer dinucleoside sequences, involving guanine (G) and cytosine (C) base pair(s) and/or adenine (A) and thymine (T) base pair(s) are reported. The intradinucleoside dimer energies have been computed for conformations having d approximately equal to 3.38 (the B-form type structure), 4.0, 4.6, 5.3, 5.8, 6.0, 6.6, and 6.8 Å (the intercalation structure, which varies with sequence and is not necessarily the Sobell structure). Slight deviations from these d values results from the perturbation analyses to "minimize" the energy. Each specified point on each plot corresponds to the lowest energy structure found from a set of at least four different dinucleoside dimer conformations in the B-form type sugar ring puckering, plus at least four conformations in the C3' *endo* (3'-5') C2' *endo* puckering mode as found by Sobell and coworkers (28,29). Mixed sugar ring puckering was considered only for conformations having $d > 5.7$ Å because of stereochemical considerations.

Three general observations can be made from these figures:

1. Both the free space, V , and total, T , energies increase with increasing unwinding (larger d values) of the dinucleoside dimer. This suggests that the B-form type conformation is preferred for all sequences, at least when longer range intramolecular interactions, as in a DNA polymer are

neglected. Loss in attractive dispersion potential and complementary electrostatic interactions between the base-pairs are mainly responsible for the loss in stabilization energy when d increases. These findings are consistent with other studies (24) in which the stability of the B-DNA is attributed the steric potential which confines the double helix to the region of a 10-fold helix for 3.38 Å base-pair separation.

2. The unwinding energy curves indicate that all dinucleoside dimer sequences have to overcome an "activation" barrier to unwinding located at about $d = 4.6$ Å. The height of this barrier is sequence dependent. This barrier is due to steric repulsions (bad contacts) between base atoms and the hydrogen atoms on the adjacent deoxyribose sugar ring. This barrier might be lowered, but not eliminated, by allowing non-parallel base-pair geometries while unwinding.
3. Inclusion of aqueous solvation energetics enhances the propensity of the B-form-type structure to unwind. This enhancement is sequence specific. However the aqueous solvation energy curve flattens out for $d > 5.5$ Å for all dimer sequences. This indicates that the solvation shells are filled. In essence, for $d > 5.5$ Å there is virtually no overlap between solvation shells of the adjacent base pairs. This, in turn, suggests that once d is greater than 5.5 Å, the aqueous solvent adds virtually no extra stabilization energy to the system.

We have defined five molecular descriptors from an analysis of fig. 3 which are;

ΔE_B - the aqueous solvation energy for the B-form type structure ($d = 3.38$ Å).

$\Delta E_{6.7}$ - the aqueous solvation energy for the most opened ($d \sim 6.76$ Å) dimer structure.

$T_{6.7}$ - the total energy of the totally unwound dimer.

T_{MIN} = the total energy of the unwound conformer which is an energy minimum for the particular path realized in our calculations.

T_{BAR} = the barrier energy to unwinding from the B-state.

Figure 3. The free space (V), free space plus aqueous (T), and their difference (T-V) energies as a function of the distance between base pairs (d) for all 16 dimer sequences. Note, the lines connecting the various points have been smoothed out for some of the graphs at $d \approx 5.8$ and ≈ 6.6 Å. The high energies are due to steric interactions between the bases and the sugar rings. However, a simultaneous unwinding and change in sugar ring puckering at these distances would alleviate such steric overlaps. Our current unwinding algorithm cannot, however, handle changes in ring puckering during unwinding.

Differences between selected pairs of these descriptors also provides useful information concerning relationships between structure and flexibility. The particular differences we found useful are;

- $(\Delta E_{6.7} - \Delta E_B)$ - the relative aqueous solvation of the completely opened and B-form type conformation.
- $(T_{BAR} - T_B)$ - the "activation" energy to go from the B-form type conformation to the more opened forms.
- $(T_{BAR} - T_{MIN})$ - the "activation" energy to go from the opened forms to the B-form type structure.
- $(T_B - T_{MIN})$ - the relative stability of the B-form type conformation to the preferred unwound conformer.
- $(T_B - T_{6.7})$ - the relative stability of the most opened form to the B-form type structure; in essence this is a measure of the relative intramolecular stability of a dinucleoside dimer when intercalated by another molecule.

The values of ΔE_B , $\Delta E_{6.7}$, $(\Delta E_{6.7} - \Delta E_B)$, $(T_{BAR} - T_B)$, $(T_{BAR} - T_{MIN})$, and $(T_B - T_{6.7})$ as function of sequence are given in Table I. An analysis of the data in Table I suggests some general trends;

1. Dinucleoside dimers containing only (C) and (G) base pairs are most soluble in water in both the B-form type structure (ΔE_B) and in the most opened conformation ($\Delta E_{6.7}$). These dinucleosides also gain the most amount of solvation energy when completely opened (see $(\Delta E_{6.7} - \Delta E_B)$). Correspondingly, this suggests that (A) and (T) containing sequences will be less soluble than dimers containing (C) and (G) base pairs. This is qualitatively consistent with experimental solubility measures of isolated bases (37). The solubility parameters, used as additive features, would predict the solubility of (A) and (T) base pairs to be 4.7×10^{-2} molar and the (G) and (C) base pairs to be 6.76×10^{-2} molar in an aqueous solution.
2. The "activation" barrier at about $d \approx 4.6 \text{ \AA}$ is strongly sequence specific. However, all (G) and (C) containing dinucleosides have the highest relative unwinding barrier energies (see $(T_{BAR} - T_B)$).

The location, with respect to d , and well-depth of the opened form minimum energy conformer is also sequence dependent. These relative energy minima are deepest for all (C) and (G) containing dinucleosides (see $(T_{MIN} - T_{BAR})$ and d).

3. The difference in energy between the B form type conformation and the completely opened structure $(T_B - T_{6.7})$ is strongly sequence specific.

Table I

Characteristic molecular energetics associated with unwinding dinucleoside dimer sequences. All energies have dimensions of kcal per mole of dinucleoside dimer.

Sequence	ΔE_B	$\Delta E_{6.7}$	$(\Delta E_{6.7} - E_B)$	$(T_{BAR} - T_B)$	$(T_{BAR} - T_{MIN})$	d_{MIN}	$(T_B - T_{MIN})$	$(T_B - T_{6.7})$
$\begin{smallmatrix} 3' \\ (CG) \\ 5' \end{smallmatrix} \begin{smallmatrix} 5' \\ (CG) \\ 3' \end{smallmatrix}$	-2.8	-9.0	-6.2	47.5	16.5	5.33	-30.0	-43.0
$\begin{smallmatrix} (CG) \\ (GC) \end{smallmatrix}$	-3.0	-9.8	-6.8	61.9	50.2	6.65	-10.0	-12.0
$\begin{smallmatrix} (GC) \\ (CG) \end{smallmatrix}$	-4.4	-8.3	-3.9	32.1	16.5	5.97	-16.9	-31.0
$\begin{smallmatrix} (GC) \\ (GC) \end{smallmatrix}$	-4.2	-8.8	-4.6	51.9	26.2	6.59	-19.0	-40.0
Avg.	-3.6	-8.89	-5.38	48.35	27.35	6.14	-18.75	-31.50
Std. Dev.	.71	.54	1.17	10.74	13.77	.54	7.26	12.09
$\begin{smallmatrix} (AT) \\ (AT) \end{smallmatrix}$	-1.5	-6.3	-4.8	31.8	10.8	6.76	-24.0	-24.0
$\begin{smallmatrix} (AT) \\ (TA) \end{smallmatrix}$	-2.9	-6.6	-3.7	41.3	18.7	6.76	-21.0	-21.0
$\begin{smallmatrix} (TA) \\ (AT) \end{smallmatrix}$	-1.7	-5.7	-4.0	27.8	15.8	6.59	-13.0	-36.0
$\begin{smallmatrix} (TA) \\ (TA) \end{smallmatrix}$	-2.9	-6.3	-3.4	31.7	18.2	6.59	-13.0	-31.0
Avg.	-2.25	-6.23	-3.98	33.15	15.88	6.68	-17.75	-28.00
Std. Dev.	.65	.33	.52	4.97	3.13	.09	4.87	5.87
$\begin{smallmatrix} (AT) \\ (CG) \end{smallmatrix}$	-2.7	-8.0	-5.3	44.8	13.1	6.02	-36.0	-24.0
$\begin{smallmatrix} (AT) \\ (GC) \end{smallmatrix}$	-2.3	-7.6	-5.6	54.7	34.5	6.76	-19.0	-19.0
$\begin{smallmatrix} (TA) \\ (CG) \end{smallmatrix}$	-3.4	-7.2	-3.8	30.7	15.3	5.97	-16.0	-33.0
$\begin{smallmatrix} (TA) \\ (GC) \end{smallmatrix}$	-3.0	-7.6	-4.6	50.5	49.4	6.65	0	-36.0
Avg.	2.85	-7.6	-4.83	45.18	28.08	6.4	-17.75	-28.00
Std. Dev.	.4	.28	.69	9.07	14.87	.33	12.77	6.82
$\begin{smallmatrix} (CG) \\ (AT) \end{smallmatrix}$	-1.6	-7.9	-6.3	39.9	17.5	5.33	-22.0	-22.0
$\begin{smallmatrix} (CG) \\ (TA) \end{smallmatrix}$	-2.4	-7.9	-5.5	43.2	26.5	6.59	-16.0	-16.0
$\begin{smallmatrix} (GC) \\ (AT) \end{smallmatrix}$	-3.5	-7.3	-3.8	29.7	13.4	6.76	-16.0	-45.0
$\begin{smallmatrix} (GC) \\ (TA) \end{smallmatrix}$	-4.2	-6.9	-2.8	32.6	11.0	5.32	-21.0	-43.0
Avg.	-2.93	-7.5	-4.6	36.35	17.1	6.01	-18.75	-31.5
Std. Dev.	1.0	.42	1.38	5.43	5.9	.69	2.77	12.7

ΔE_B = T-V for B-form structure, D = 3.38 Å.

$\Delta E_{6.7}$ = T-V for conformer having D = 6.76 Å

T_{BAR} = Total energy of maximum barrier height

$T_{6.7}$ = Total energy of D - 6.76 Å conformer state

T_{MIN} = Total energy of the unwound minimum energy conformer

d_{MIN} = Separation distance between base-pairs in Å for which the unwound conformer having local minimum energy T_{MIN} is found.

The sequence $\begin{pmatrix} CG \\ GC \end{pmatrix}$ has the lowest absolute value of the energy difference, $|(T_B - T_{6.7})| = 12.0$ kcal/mole. The second and third lowest absolute value of the energy difference, 16.0 and 19.0 kcal/mole are for the dimers $\begin{pmatrix} CG \\ TA \end{pmatrix}$ and $\begin{pmatrix} AT \\ GC \end{pmatrix}$, respectively. The $\begin{pmatrix} CG \\ GC \end{pmatrix}$ dinucleoside dimer has most often been used to form dinucleoside dimer-intercalator crystal complexes. The large variation in the energy difference $(T_B - T_{6.7})$, as a function of sequence, suggests intercalation may be sequence specific.

The unwinding energy is sequence dependent primarily because of, a) inter-base pair interactions, and b) the bases interacting with the sugar mono-phosphate backbone. Base-backbone interactions are responsible for the observation that the "minimum" energy unwound backbone conformation for one sequence is not necessarily the "minimum" energy unwound conformation for another sequence.

Critical assumptions have been made, with regard to both molecular geometry and energetics, which must limit the conclusions drawn from this study. These assumptions are:

1. The dinucleoside dimer conformations used are representative of those realized in unwinding. A specific corollary to this general requirement is that dimers not having parallel base pairs are similar in energetics to the parallel base-pair structures used in this study. Clearly we have not generated all possible structures at each d value.
2. The aqueous solvation energetics are reliable in discerning trends between dimer sequence and conformational energy.
3. Ionization and counterion effects were not considered in the energy calculations.

Further, if we wish to make inferences from this work about DNA structure, as we do, it is necessary that (1-3) and higher order nucleic acid residue interactions can be neglected. This is probably not the case. Nevertheless, the trends obtained in these studies, and the magnitudes of energy differences in the trends, suggest reasonable evidence that the results are qualitatively meaningful.

The energy differences computed as a function of d are probably too large for V,T, and (T-V). We have already identified the source of overestimating aqueous solvation energy differences in the METHOD section. The electrostatic term used in calculating the free space energy may be the source of overestimation of energy differences involving V. The charge distributions and/or dielectric and screening terms may need to be modified.

It is to be noted that Nuss et al. (15) calculate the difference in the

free space energy of a B-form type structure and an open conformation to be about 15 to 30 kcal/mole. Kinetic measurements suggest that unstacking free energy of activation is about 15 kcal/mole for proflavin-DNA intercalation (38). We predict a range of sequence-dependent unwinding barrier energies of 33 to 68 kcal/mole, in free-space, and 28 to 62 kcal/mole when aqueous solvation energy is added to the free space values. We also find the difference in free-space energies of the B-form type conformation and an open minimum energy conformation to be sequence specific ranging from 12 to 45 kcal/mole. These findings are consistent with the theoretical results of Nuss et al. (15). Our predicted barrier energies, however, are too large when compared with the kinetic measurements. The overestimation of the barrier potential may be due, in combination, to inadequate representation of steric repulsion at short contact distances in our potentials, and incomplete searching for the lowest energy path to unwinding. We do believe, however, that the trends supporting the barrier height to be sequence-dependent are meaningful.

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