Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4630–4634, June 1990
Biophysics

Why do A·T base pairs inhibit Z-DNA formation?

LIEM X. DANG, DAVID A. PEARLMAN, AND PETER A. KOLLMAN†

Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143

Communicated by I. Tinoco, Jr., March 20, 1990

ABSTRACT **We have carried out free energy perturbation calculations on DNA double-stranded hexanucleotides. The sequence d(CGCGCG)$_2$ has been "mutated" into d(CGTG-CG)·d(CGCACG) with the oligonucleotide in the A, B, and Z structural forms, both *in vacuo* and in aqueous solution. In addition, model free energy calculations have been carried out in which the electrostatic charges of the H-bonding groups of the bases in the major and minor grooves of the DNA are reduced to zero as a way of assessing the relative solvation effects of these groups in the different structural forms of DNA. Finally, energy component analyses have been carried out to assess the relative roles of different intranucleotide interactions on the B → Z equilibrium as a function of base sequence. *In vacuo*, the free energy for changing a G·C to an A·T base pair is largest in the Z conformation; in the A and B conformations, the free energy cost is ≈2 kcal/mol lower (1 cal = 4.184 J). The results are similar when the simulations are run in explicit solvent: the change costs 3 kcal/mol more in the Z conformation than in the B form. These results are consistent with experimental data, where it is clear that A·T sequences are significantly more "Z-phobic" than G·C sequences. The calculations indicate that both intranucleotide and solvation interactions contribute to this Z-phobicity.**

Ever since the discovery of Z-DNA (1, 2), its physical properties and stability have been of considerable interest. The fact that alternating C·G base pairs are required for facile formation of Z-DNA is intriguing. The *syn* conformation of the guanine bases in Z-DNA suggests a clear and compelling reason why alternating pyrimidine-purine sequences are more stable in the Z conformation than nonalternating sequences: a purine base (e.g., guanine) is approximately equally stable in *syn* and *anti* conformations, while pyrimidine bases (such as thymine and cytosine), which have a C=O group at the C-2 position, are significantly less stable in the *syn* conformation because of steric interactions of the C=O with the sugar ring in the *syn* conformation (3).

Another interesting observation is that an alternating m^{5}CG double-helical DNA sequence is significantly more stable in the Z conformation than in the B conformation, relative to a nonmethylated alternating CG sequence (4). Thus 5Me substitution in cytosine potentiates Z-DNA formation. On the other hand, it is more energetically costly for a B → Z transition to take place in poly d(AC)·poly d(GT) than in poly d(GC)·d(GC), suggesting that the presence of A·T base pairs inhibits Z-DNA formation (5). This is in spite of the fact that thymine residues have the 5Me group, the addition of which to cytosine causes potentiation of Z-DNA formation. Interestingly, Ho *et al.* (6) have suggested that the somewhat different location of the methyl group in A·T base pairs in Z-DNA can rationalize the differing effects of the 5Me group in A·T and G·C pairs on stabilizing the Z-DNA conformation. They have supported this suggestion with solvent-accessible surface area calculations.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

With the use of recombinant plasmids and superhelical stress, various sequences can be forced into the Z conformation and the energetic cost of this process can be assessed (7). Thus, in principle, one can experimentally determine the free energy cost of the B → Z conformational transition for any sequence.

Theoretical calculations, using molecular mechanics and dynamics, have been used to try to rationalize the B–Z conformational free energy difference as a function of sequence. The role of the 5Me group in potentiating Z-DNA formation has been rationalized by molecular mechanics (3), and, more recently, by free energy perturbation approaches (7). Free energy calculations have also been successfully applied to study 5BrC and 8BrG contributions to Z stability in DNA and RNA double helices (8). And molecular mechanics/polyelectrolyte calculations have been applied in a useful way to understanding how salt effects potentiate Z-DNA formation, leading to a quantitative model of the salt dependence of the B–Z conformation transition (9).

Notably, some previous molecular mechanics calculations (3) have been unsuccessful in rationalizing why A·T base pairs inhibit Z-DNA formation. Encouraged by our success in simulating the consequences for Z-DNA stability of 5Me, 5Br, and 8Br substitutions by using molecular dynamics/free energy perturbation approaches, we present in this paper a free energy perturbation study of a G·C → A·T base pair mutation. Our previous studies of solvation effects on amino acid side chains and nucleic acid bases (10), as well as the relative binding free energies of 2′-AMP and 2′-GMP to ribonuclease T (11), encouraged us that even a rather large mutation such as that of C·G → T·A had a reasonable chance to be successful. Although a C·G → T·A base pair involves a large change in electronic structure–electrostatic interactions, it involves a small change in steric/van der Waals effects and conformation. Thus, it is likely that free energy perturbation methods can be usefully applied to this mutation (10).

Below, we present such free energy calculations on the double-helical hexanucleotide d(CGCGCG)$_2$ in its A, B, and Z conformations. The mutation of the third base pair from C·G to T·A was carried out both *in vacuo* and in aqueous solution. These calculations are supplemented by molecular mechanics calculations/energy component analyses to try to understand in more detail what factors play a role in the "Z-phobicity" of an A·T base pair. Finally, a set of model free energy calculations, in which the polar groups in the major and minor grooves are neutralized, allow us to qualitatively assess the role of groove solvation on the "Z-philicity" of different base pairs.

METHODOLOGY

The calculations were carried out by using an enhanced version (D.A.P., unpublished data; the calculation of intra- as well as intergroup free energies has been included in this version) of the molecular simulation program package AMBER

Abbreviation: FEP, free energy perturbation.

*To whom reprint requests should be addressed.

3.0 (12) with an all-atom parameter set (13). The free energy perturbation (FEP) technique was used (14), and the potential function that describes the interactions of DNA molecules has the following form:

$$V_{\text{total}} = \sum_{\text{bonds}} K_r(r - r_{eq})^2 + \sum_{\text{angles}} K_\theta(\theta - \theta_{eq})^2 + \sum_{\text{dihedrals}} \frac{V_n}{2}[1 + \cos(n\phi - \gamma)] + \sum_{i<j}\left\{\left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}}\right] + q_i q_j/\varepsilon R_{ij}\right\} + \sum_{\text{H bonds}}\left\{\frac{C_{ij}}{R_{ij}^{12}} - \frac{D_{ij}}{R_{ij}^{10}}\right\}. \quad [1]$$

In this equation, V_{total} is the potential energy of the system; K_r and r_{eq} are the bond stretching constant and the equilibrium bond distance; K_θ and θ_{eq} are the bond angle stretching constant and the equilibrium bond angle; V_n, n, and γ are the torsional force constant, the periodicity of the torsional term, and the phase angle; A_{ij} and B_{ij} are the non-bond (Lennard–Jones) repulsion and attraction coefficients; R_{ij} is the interatomic distance between atoms i and j; q_i and q_j are the atomic partial charges on atoms i and j; and ε is the effective dielectric constant. The simple point charge model TIP3P of Jorgensen *et al.* (15) was used to model the water–water interactions.

We have used FEP calculations to study the effects of "mutating" a C·G base pair to an A·T pair in the hexamer $d(CGCGCG)_2$ for the A, B, and Z conformations *in vacuo* and for the B and Z conformations in aqueous solution. For the calculations performed *in vacuo*, a distance-dependent dielectric constant $\varepsilon = r$ along with parameters corresponding to the hydrated counter sodium ions ($r^* = 5.0$ Å and $\varepsilon = 0.1$ kcal/mol; 1 cal = 4.184 J) were used to partially mimic solvent effects. No distance cutoff was used for the *in vacuo* calculations. The explicit solvent calculations were carried out in a periodic box run at constant temperature (300 K), and pressure (1 atm; 1 atm = 101.3 kPa). We used roughly 1500 and 1700 water molecules to solvate the Z- and B-DNA, respectively, in the aqueous solution simulations. In these calculations, counter ions ($r^* = 1.6$ Å and $\varepsilon = 0.1$ kcal/mol) were included, a nonbonded cutoff radius of 8 Å was used, and the nonbonded pair list was updated every 50 time steps. A time step of 1 fs was used and the SHAKE (16) procedure was adapted to constrain all the bond lengths to their equilibrium values. The free energy simulations were carried out on these systems by using the slow growth procedure (14) and the free energies for both the forward ($\lambda = 1 \rightarrow 0$) and reverse ($\lambda = 0 \rightarrow 1$) directions were obtained.

The details of the slow growth procedure have been presented elsewhere (14), but we briefly review the approach here. In this procedure, the free energy change associated with changing a system from state $\lambda = 0$ to state $\lambda = 1$ can be written as

$$\Delta G = G(\lambda = 1) - G(\lambda = 0) = \int_{\lambda=0}^{\lambda=1} \left\langle \frac{\partial V(q, \lambda)}{\partial \lambda} \right\rangle_\lambda d\lambda. \quad [2]$$

Here, $V(q, \lambda)$ is the potential function, q is the coordinates, and λ is the coupling parameter between state 0 and state 1. If the increments $d\lambda$ are small enough, Eq. 2 can be approximated as

$$\Delta G = \sum_M \left(\frac{\partial V(q, \lambda)}{\partial \lambda}\right)_\lambda \Delta\lambda \approx \sum_M \Delta V(q, \lambda), \quad [3]$$

where M is the number of steps and $\Delta\lambda = 1/M$. Thus, the free energy associated with changing a system from one in which $\lambda = 0$ to one in which $\lambda = 1$ can be evaluated by a molecular dynamics simulation during which the potential function slowly changes in discrete steps as the function of λ.

In most calculations, a total of 40 ps (40,000 time steps) was used in each direction. We have also carried out some 80-ps simulations to test the convergence of the calculations. We found the results of the 80-ps calculations to be the same, within the reported statistical error of the calculations (Table 1). Moreover, in the sequence $d(C_1G_2C_3G_4C_5G_6)$·$d(C_{1'}G_{2'}C_{3'}G_{4'}C_{5'}G_{6'})$, the perturbation group was taken to be the third $C_3G_{4'}$ base pair alone (Fig. 1). The sugar–phosphate backbone was not included in the perturbing group; however, both inter- and intragroup energies were included in the evaluation of ΔG (D.A.P., unpublished data).

We have also carried out 10-ps FEP calculations in which the atomic charges on the H-bonding groups in the major and minor grooves of the DNA are reduced to zero in order to assess the relative solvation effects of these groups in the different structural forms of DNA. The calculations were carried out in such a way that the total charge of the system was unchanged. Thus, the charges on O-6, O-4, and O-2 were placed on C-6, C-4, and C-2, respectively; the charges on H-6 and H-2 were placed on N-6 and N-2, respectively; and the charges on N-7 and N-3 (A,G) were distributed to C-8, C-5, C-4, C-2 as shown in Fig. 2.

Finally, the coordinates of the B- and Z-DNA helices were minimized *in vacuo* by using a conjugate gradient method and the potential function given above. All degrees of freedom

Table 1. Free energies in kcal/mol for the C·G → T·A mutation

Structure*	Condition	
	In vacuo	In solution
Isolated base pair†	18.44 ± 0.7	
Z-DNA‡	25.96 ± 1.6	38.70 ± 0.3
B-DNA‡	23.96 ± 1.0 (23.2 ± 1.2)§	35.73 ± 0.2 (36.2 ± 0.3)§
A-DNA‡	23.62 ± 0.2	
Z-DNA¶	93.87 ± 0.4	
B-DNA¶	90.15 ± 0.7	

Free energies reported are an average of forward and backward mutations, with the standard deviation of these two runs. Unless otherwise noted, simulations were run for 40 ps in each direction.
*DNA structural form.
†System is dC·dG.
‡System is $d(CGCGCG)_2 \rightarrow d(CGTCGC)$·$d(GCGACG)$.
§Results for simulations for 80 ps in each direction are in parentheses.
¶System is $d(CGCGCGCGCGCG)_2 \rightarrow d(CGCGTATAGCGCG)_2$.

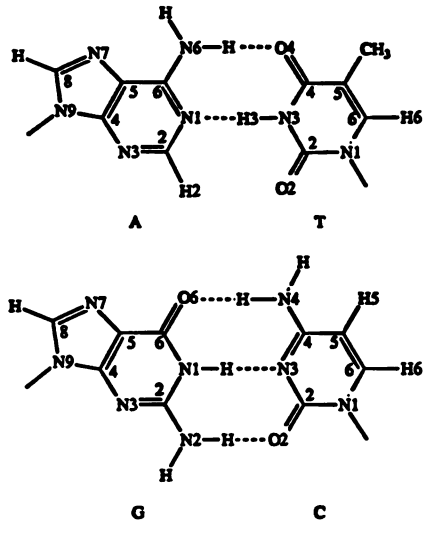

FIG. 1. The models of perturbed A·T and G·C base pairs used in the calculations.

were included. Each minimization proceeded until the rms derivative in energy was <0.1 kcal/mol. We carried out the energy component analyses on the minimized sequences $d(CGCGCG)_2$, d(CGTGCG)·d(CGCACG), d(CACACG)·d(CGTGTG), and d(CATACG)·d(CGTATG) to assess the relative roles of different intranucleotide interactions on the B → Z equilibrium as a function of sequence.

RESULTS

Table 1 contains the results of the free energy calculations both *in vacuo* and in solution. As one can see, if an isolated C·G base pair is mutated into a T·A base pair *in vacuo*, the change in free energy is 18.4 kcal/mol. This is mainly an intramolecular energy. Calculated free energy changes for the same base pair modification in various $d(CGCGCG)_2$ hexamers are all larger, suggesting that the third C·G base pair interacts more favorably with its environment (surrounding bases and sugar–phosphate backbone) than does a T·A base pair at this position. These more favorable interactions are worth 5 kcal/mol in the A and B forms and 7 kcal/mol in the Z form (see Table 1). Thus, *in vacuo*, the presence of a T·A base pair makes it 2 kcal/mol more difficult to induce a B → Z transition in a DNA oligomer than a C·G base pair at that position.

We have also carried out a mutation of the four C·G base pairs in the center of $d(CGCGCGCGCGCG)_2$ to T·A, and, as Table 1 indicates, the differential free energy cost of the $(C{\cdot}G)_4 \rightarrow (T{\cdot}A)_4$ mutation is 3.7 kcal/mol greater in Z- than in B-DNA. This is significantly less than 4 times the value of the single base mutation in $d(CGCGCG)_2$ and suggests significant nonadditivity in differential sequence stability effects on the B → Z transition.

Results for the simulations carried out in explicit solvent are in qualitative agreement with those determined *in vacuo*. The C·G → A·T mutation destabilizes the B-form helix by 35.7 kcal/mol, whereas this change destabilizes the Z-form helix by 38.7 kcal/mol. In other words, in solution a T·A base pair makes the B → Z transition 3 kcal/mol less favorable than with a C·G base pair at the same position. This can be compared to the 2 kcal/mol value determined *in vacuo*. Using the 80-ps values for B-DNA in parentheses in Table 1 would lead to free energies of 2.8 *in vacuo* and 2.5 in solution. The similarity of the vacuum and solution results implies that the Z-phobicity of A·T base pairs may be mainly intramolecular. This contrasts with the results of calculations in which a 5Me group was added to cytosine (7). In those calculations, potentiation of the Z structure is calculated to be both an intramolecular and a solvation effect.

How do the structures of these oligonucleotides change during the molecular dynamics simulations, each of which starts as canonical A-, B-, or Z-DNA? The number of dihedral angles of each type for a given simulation is given in Table 2. As one can see, sugar repuckering has occurred frequently in all structures, whereas while transitions of γ from $g^+ \rightarrow t$ occur frequently for A- or B-DNA, this dihedral angle remains in its starting value (alternating g^+ and t) in Z-DNA. Stereoviews of some of these structures are presented in Fig. 3 and illustrate that, although these structures are far from "canonical," they are still recognizably double helices of the B and Z families, both after dynamics equilibration and after the perturbation calculations.

Can one make a qualitative estimate of how much the hydrogen-bonding groups in the grooves contribute to the Z-philicity of A·T sequences? We summarize the results of perturbations in which the atomic charges on the H-bonding groups of the base pairs were reduced to zero. Fig. 2 presents the initial and final charge models for minor and major group perturbations in the C·G and T·A base pairs. These perturbation calculations were performed on the base pair at the third position of the hexanucleotides *in vacuo* and on this same system in solution. For the C·G base pair, the major and minor grooves are better solvated in the B-form than in the Z form, with a larger difference in the major groove solvation. In contrast, for the T·A base pair, major groove solvation is overall much less favorable and is more favorable in the Z form than in the B structure. For T·A, the minor groove is substantially better solvated in the B form, the same as the G·C base pair. Thus, differential H-bonding to solvent between B and Z forms appears larger for the C·G pair (favoring B) than the A·T pair. Since the results for these H-bonding groups alone do not mirror those for the free energy base pair mutations or experiment, it is likely that solvent H-bond effects are not the dominant reason for the sequence-specific effects.

To further analyze which interactions cause the differential stability of a C·G base pair compared to a T·A base pair in Z- and B-DNA, we performed energy component analyses on energy-minimized B and Z structures with sequences $d(CGCGCG)_2$. The base–base interaction differences do contribute in a significant way to the Z-phobicity of the T·A base pairs compared to G·C: both the intragroup energy of a given base pair and the interaction of this base pair (mainly with the base pair $G_2C_{5'}$) significantly favor B- over Z-DNA, when the given base pair is T·A rather than C·G. Not only do base–base interactions contribute to the Z-phobicity of the A·T base pair, but the base–phosphate interactions also make major contributions to this preference.

DISCUSSION AND CONCLUSIONS

We have carried out energy minimization and free energy calculations on DNA hexamers to attempt to assess the physical basis for the Z-phobicity of A·T base pairs. The free energy calculations, in both the absence and presence of explicit solvent, are successful in qualitatively predicting this Z-phobicity. The quantitative free energies (2–3 kcal/mol) are larger than but of the correct order of magnitude as experimental estimates (≈0.7 kcal/mol) for the differential free energy of A·T vs. G·C base pairs in B- vs. Z-DNA (19). Energy component calculations on minimized structures suggest that base–base and base–phosphate interactions are the most critical intramolecular contributors to the calculated Z-phobicity of A·T pairs.

Recently, Hartmann *et al.* (20) carried out molecular mechanics calculations on various DNA sequences in B and Z forms. They used an approach that varies the energy in

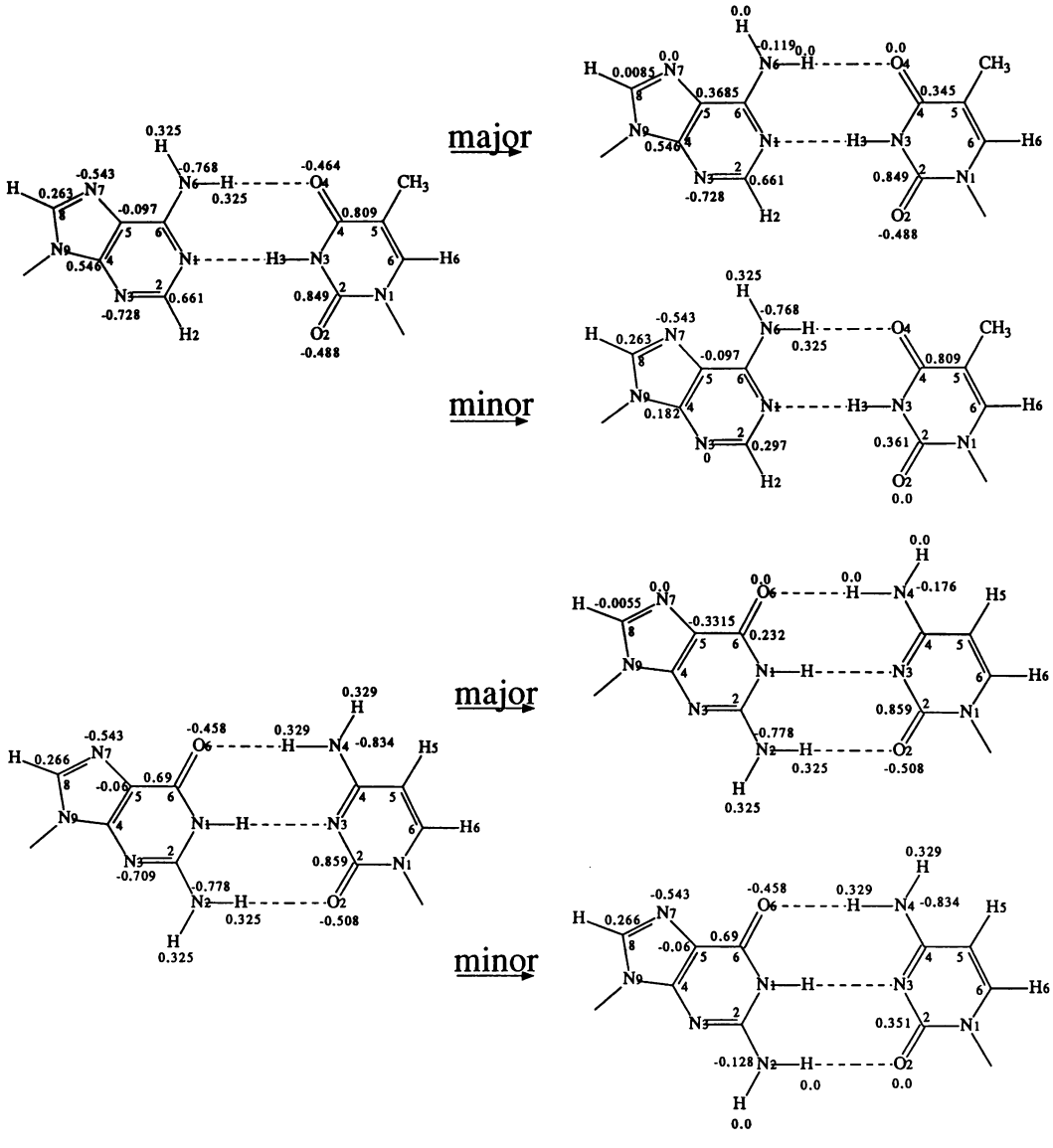

FIG. 2. The initial and final charge distributions on the perturbed base pairs used in the study of the relative solvation effects on various structural forms of DNA.

helical coordinates and imposes either a mono- or dinucleotide repeat on the system. They also used a different force field than in ref. 3. Their results lead to calculated ΔH values for the B → Z transition that are in partial agreement with the experimental data in ref. 19. In particular, mutating 1 base pair C·G → T·A reduces the relative tendency for the B → Z transition to occur, in agreement with experimental data. But mutating the second C·G → T·A is calculated to potentiate the B → Z transition, in disagreement with experiment (19). It is not clear what causes the discrepancy. It is clear that this approach (20) and ours are complementary, in that ours can more easily include explicit solvent and calculate ΔG directly, but it is also more computer intensive and less efficient at exploring conformation space widely.

Table 2. Number of conformation changes in various dihedral angles

DNA	$\alpha(10)$	$\beta(10)$	$\gamma(12)$	$\delta(12)$	$\varepsilon(10)$	$\zeta(10)$	$\chi(12)$
A*	5	3	7	6	2	1	0
B*	6	1	6	4	2	2	0
B†	4	3	5	6	4	3	0
Z*	2	0	0	3	4	2	2
Z†	0	0	1	7	3	0	0

For definition of dihedral angles, see Saenger (17). The total number of occurrences of a given dihedral angle is given in parentheses. The numbers that have changed from their standard range (g^+, t, or g^-) to one of the other three after the G·C → A·T mutation are given in this table. For example, three of the angles change from their "standard" g^- value to t and one changes to g^+ for the G·C → A·T mutation in the B-DNA hexamer in solution. In the corresponding Z hexamer, each strand has α value g^+, t, g^+, t, g^+ both before and after the mutation.

**In vacuo.*

†In solution.

We have also carried out model free energy calculations in which we reduced to zero the charges on the H-bonding groups in the major and minor grooves of B- and Z-DNA in solution. These calculations suggest that these H-bonding effects are not the dominant component in the sequence specificity of the B ⇄ Z transition. Although the simulation time used in these model calculations is short, we feel it is sufficient for the qualitative model purpose for which it was intended.

There are a number of assumptions made in these calculations. We assume that ionic strength/counterion effects, which are critical to the B → Z transition, are the same in both

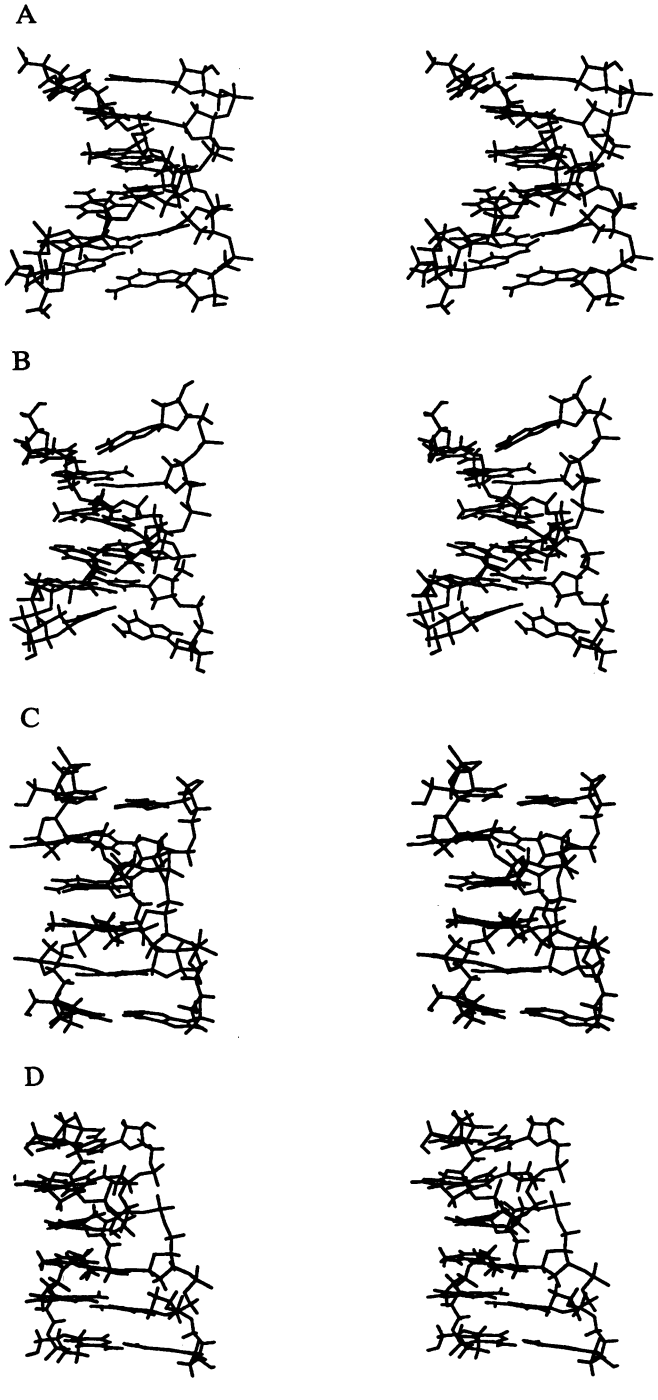

FIG. 3. (*A*) The equilibrated structure of the B-DNA in water. (*B*) The final structure after perturbation calculations of the B-DNA in water. (*C*) The equilibrated structure of the Z-DNA in water. (*D*) The final structure after perturbation calculations of the Z-DNA in water.

sequences. This has precedent in our previous simulations (7), in which quantitative agreement with experiments for the Z potentiation by 5MeC was achieved. Irikura *et al.* (21) have noted a significant difference in entropy between B- and Z-DNA, with, as expected, the latter more rigid. Although one expects DNA with A·T base pairs to have a higher entropy than that with G·C, it is unlikely that the differential entropy between B and Z structures of $d(CGCGCG)_2$ and d(CGTGCG)·d(CGCACG) is of the magnitude of the free energies calculated here. In particular, Rao *et al.* (22) have used normal mode analysis to calculate the ΔS ($Z \rightarrow B$) for $d(CGCGCG)_2$ and $d(TATATA)_2$, finding 15.9 eu and 15.4 eu, respectively. Thus, conformational entropy effects do not appear to be large enough to explain the Z-phobicity of A·T base pairs. It is important to keep in mind that the results of the calculations described here are influenced by molecular mechanical parameters and simulation protocols. The protocol used here has precedent in previous free energy calculations on DNA (7), but it certainly would be desirable to carry out the calculations with more water molecules and a larger nonbonded cutoff and to carry out the mutation over a longer simulation time. We have run the simulations in both directions and the values in Table 1 are an average, so we have some confidence in the reproducibility of our results. In addition, since the simulation described here involves mainly changes in electrostatic energies and not molecular shape, they should be more reliable than free energy calculations where major structural changes are involved.

At any rate, our calculations open a window for determining the free energy effects of sequence on DNA stability and drug binding. The calculations presented here and in refs. 7, 8, and 10 show that many useful and exciting insights can be derived from such work.

The calculations were carried out at the Pittsburgh Supercomputer Center, supported by the National Science Foundation. Research support from the National Institutes of Health (CA-25644) to P.A.K. is acknowledged, as is the use of the University of California, San Francisco Computer Graphics Lab, supported by RR-1081 (R. Langridge, principal investigator).

1. Pohl, F. M. & Jovin, T. M. (1972) *J. Mol. Biol.* **67**, 375.
2. Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., van Der Marel, G., Crawford, J. L. & Rich, A. (1979) *Nature (London)* **282**, 680–686.
3. Kollman, P. A., Wiener, P., Quigley, G. & Wang, A. (1982) *Biopolymers* **21**, 1945–1969.
4. Behe, M. & Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1619–1623.
5. Jovin, T. M., Soumpasis, D. M. & McIntosh, L. P. (1987) *Am. Rev. Phys. Chem.* **38**, 521–560.
6. Ho, P. S., Quigley, G. J., Tilton, R. F. & Rich, A. (1988) *J. Phys. Chem.* **92**, 939–945.
7. Pearlman, D. A. & Kollman, P. A. (1990) *Biopolymers*, in press.
8. Ross, W., Hardin, C., Tinoco, I., Jr., Rao, S. W., Pearlman, D. & Kollman, P. A. (1989) *Biopolymers* **28**, 1939–1958.
9. Garcia, A. & Soumpaisis, D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3160–3164.
10. Bash, P., Singh, U. C., Langridge, R. & Kollman, P. A. (1987) *Science* **236**, 564–568.
11. Hirono, S. & Kollman, P. A. (1990) *J. Mol. Biol.* **212**, 197–209.
12. Singh, U. C., Weiner, P. K., Caldwell, J. W. & Kollman, P. A. (1986) AMBER, A Molecular Mechanics and Dynamics Program (Univ. of California, San Francisco), Version 3.0.
13. Weiner, S. J., Kollman, P. A., Nguyen, D. T. & Case, D. (1986) *J. Comp. Chem.* **7**, 230–252.
14. Singh, U. C., Brown, F., Bash, P. & Kollman, P. (1987) *J. Am. Chem. Soc.* **109**, 1607–1611.
15. Jorgensen, W. L., Chandrasekhar, J. & Madura, J. D. (1983) *J. Chem. Phys.* **79**, 926–935.
16. Ryckaert, J. P., Ciccotti, G. & Berendsen, H. J. C. (1983) *J. Comput. Phys.* **23**, 326.
17. Saenger, W. (1984) *Principles of Nucleic Acid Structure* (Springer, New York).
19. Ellison, M. J., Fergon, J., Kelleher, R., Wang, A. H.-J., Habener, J. & Rich, A. (1986) *Biochemistry* **25**, 3648–3655.
20. Hartmann, B., Malfoy, B. & Lavery, R. (1989) *J. Mol. Biol.* **207**, 433–444.
21. Irikura, K., Tidor, B., Brooks, B. & Karplus, M. (1985) *Science* **229**, 571–572.
22. Rao, S., Kollman, P. & Jovin, T. (1989) in *Unusual DNA Structures*, eds. Wells, R. and Harvey, S. (Springer, New York).