Calculated distortions of duplex DNA by a cis, syn cyclobutane thymine dimer are unaffected by a 3′ TpA step

Michael G. Cooney¹ and John H. Miller¹,²,*

¹Molecular Biosciences Department and ²Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99352, USA

Received November 14, 1996; Revised and Accepted January 29, 1997

ABSTRACT

Molecular dynamics simulations were performed on the duplex DNA dodecamers d(CGCGAATTCGCG); d(GCGGAATTCGGC) and d(GCAGAAATTTGGC), where TT denotes a cis, syn cyclobutane thymine dimer. The constant temperature and pressure algorithm of the AMBER 4.1 molecular-modeling package was used with explicit water and counterions, periodic boundary conditions and electrostatic interactions evaluated by the particle-mesh Ewald method. Results were analyzed by the CURVES algorithm and its implementation in DIALS and WINDOWS. Calculated distortions of DNA structure by the thymine dimer were qualitatively and quantitatively similar for the two sequences. Despite the enhanced flexibility of the native TpA dinucleotide step, major deviations from the B-DNA values of helicoidal parameters were found only at the ApT and TpT dinucleotide steps in both sequences. Only the AT base pairs of the two sequences that contain the 5′ thymine of the dimers exhibited weakened Watson–Crick hydrogen bonds and anomalous stretching. Hence, we conclude that the pattern of structural perturbations responsible for recognition of cis, syn thymine dimers by repair enzymes is not sensitive to their sequence context.

INTRODUCTION

Cyclobutane pyrimidine dimers and 6–4 photoproducts are the major lesions formed in DNA exposed to ultraviolet (UV) irradiation (1). Since UV-induced mutations occur predominantly in dipyrimidine sequences, these lesions are also believed to be responsible for many of the adverse biological effects from UV radiation (2). The existence of several enzymatic repair systems to remove cyclobutane pyrimidine dimers from DNA (3) adds weight to this conclusion. The question of what features of these DNA lesions allow them to be recognized by various repair enzymes is not sensitive to their sequence context. For example, two early modeling studies based on molecular mechanics (3,6) came to dramatically different conclusions regarding the degree of bending and kinking of the helical axis caused by the cis, syn thymine dimer in the dodecamer d(CGCGAATT CGCG). Most recent structural studies, both experimental (7–12) and theoretical (13,14), have concluded that, although the local environment around thymine dimers is altered, global structural changes, such as bending of the helical axis, are relatively small. Uncertainty still exists regarding the precise nature of localized perturbations induced by the thymine dimer. Most studies agree that the 5′-AT base pair of the dimer is more distorted than the 3′-AT base pair but details of how neighboring base pairs are affected are less clear. NMR chemical shift data (10,11) suggest that distortion of neighboring base pairs is more extensive on the 3′ side of the thymine dimer than on the 5′ side. Analysis of our 500 ps molecular dynamics (MD) simulation of the CGCGAA TT CGCG sequence (14) by the CURVES algorithm (15) identified major distortions only at the ApT and TpT steps. This raised the question of whether the chemical shift changes observed on the 3′ side of the dimer (10,11) are due to more subtle structural changes or are sequence dependent.

The cis, syn pyrimidine dimer can assume two conformations depending on the pucker of the cyclobutane ring. Modeling and NMR studies of thymine dimers have most often addressed the conformation with H(6) of the 5′ thymine and the methyl group of the 3′ thymine in the axial orientation. On the other hand, crystallographic studies (16,17) have investigated the β-cyanoethyl phosphate derivative of the cis, syn dimer of the TpT dinucleotide, which has the methyl group of the 5′ thymine and H(6) of the 3′ thymine in the axial position. The existence of two puckering states for the cyclobutane ring led Taylor et al. (10) to speculate that the conformation of cis, syn pyrimidine dimers would be dependent on their local environment. We did not find any evidence for dimer flexibility in our 500 ps MD simulation of CGCGAA TT CGCG (14). Fluctuations in backbone dihedral angles and sugar pucker were significantly less at the dimer than at neighboring dinucleotide steps, which suggests to us that the conformation of the surrounding DNA duplex must adapt to the conformational demands of the thymine dimer rather than the dimer conformation adjusting to its environment.

*To whom correspondence should be addressed. Tel: +1 509 376 5803; Fax: +1 509 376 0302; Email: jh_miller@pnl.gov
If the localized perturbations in DNA structure due to a cis, syn pyrimidine dimer do reflect adaptation to a more rigid base step as a consequence of dimerization, then MD simulations of a duplex with a flexible TpA step on the 3' side of the dimer might exhibit a more symmetric distribution of distortions than we found in the CGCGAATTCCG sequence. Enhanced flexibility at TpA steps relative to other native dinucleotides is supported by hydrogen exchange experiments (18), NMR line widths (19,20), analysis of crystal structures (21), Monte Carlo calculations (22) and energy optimization of duplexes subjected to external stress (23,24). The flexibility of TpA steps in response to changes in twist (23,24) is particularly relevant to distortions induced in DNA by thymine dimers since a reduction in twist by 10–15 degrees appears to be associated with dimer formation (14).

Guided by the findings discussed above, we carried out MD simulations on the dodecamer d(GCAGAATTCAAG):d(CTTAGTTCGTC), which has a TpA step after a 6 bp run ...CGAATT... also present in the dodecamer studied in our previous work (14). We were encouraged to investigate the effects of a TpA step on distortions induced by a thymine dimer by the success of studies on the sequence dependence of native DNA structure by the same methods (25). These methods represent a significant advancement over early theoretical studies of DNA distortions by the thymine dimer (5,6) in that the bias inherent in molecular mechanics is removed by sampling a large region of conformation space around the starting structure, solvation is included realistically (26) and the electrostatic potential energy is calculated without the introduction of an arbitrary cut-off distance (27). More details on our computational methods are given in the next section.

METHODS

Cis, syn cyclobutane thymine dimers were incorporated into the standard B-DNA conformation (28) of the duplex dodecamers d(CGCGAATTCCG):d(CGCGAATTCCG) and d(GCACGAAATTCA):d(CTTAGTTCGTC) at the positions indicated by underlining. Changes in conformation, residual charges and atom types at the TT sites from the standard B-DNA values of AMBER 4.1 (29) were the same as in earlier calculations (14). Negative charges on the phosphate groups of each dodecamer were neutralized by 22 Na + ions. Each modified DNA molecule with its counterions was placed in a 68 Å × 44 Å × 44 Å box containing ~3000 TIP3P water molecules. Energy minimization and equilibration to a temperature of 300 K and a pressure of 1 atm followed the procedure described by Cheatham et al. (30). After temperature equilibration, production runs were performed at constant temperature and pressure using the AMBER 4.1 algorithm with the SHAKE constraint on bonds to hydrogen atoms, a 0.002 ps time step, and a cut-off distance of 9 Å on Lennard–Jones interactions. The particle mesh Ewald (PME) method (27) was used to calculate the electrostatic potential energy so that a cut-off distance on long-range Coulomb interactions could be avoided.

Our 500 ps MD simulation of CGCGAATTCCG (14) converged to structures with 3–4 Å root mean square deviation (r.m.s.d.) from the starting structure after ~300 ps. MD simulation of the GCACGAAATTCAAG sequence converged to a similar r.m.s.d. from its starting structure in ~350 ps. Conformations generated between 350 and 550 ns were analyzed by the CURVES algorithm (15) to obtain average values of structural parameters that deviated significantly from results expected for standard B-DNA. This analysis revealed a BII backbone configuration for the GspA6 dinucleotide step throughout the 550 ps simulation except for the time interval between 160–270 ps. To verify that the BII conformation at GspA6 was a stable feature of the GCACGAAATTCAAG duplex, we continued the simulation to 1 ns and did not observe any addition B–BII transitions at GspA6. The DIAL and WINDOWS program (31) was used to monitor the time dependence of all structural parameters of interest in this study throughout the 1 ns simulation. No systematic changes in the parameters of interest (i.e. excluding simulated thermal fluctuations) occurred in the extended MD simulation; hence, the average values calculated from the 350–550 ps time interval are characteristic of the entire simulation from 0.35 to 1.0 ns. These values were used for comparison with averages from the 300 to 500 ps interval in our previous MD simulation of the CGCGAATTCCG sequence.

RESULTS AND DISCUSSION

The analysis of irregular duplex DNA structures by the CURVES algorithm (15) involves four types of global structural parameters: 6 intra-base pair parameters, 6 inter-base or inter-base pair parameters, 4 axis-junction parameters, and 4 axis-base or axis-base pair parameters. These parameters are termed ‘global’ because they are defined relative to an overall helical axis for the fragment under study. This distinguishes them from local structural parameters that specify the position of a base or base pair relative to the preceding base or base pair in a locally defined coordinate system. To find an overall helical axis for an irregular DNA fragment, the CURVES algorithm minimizes a function of the changes in orientation of successive nucleotides relative to a global helical coordinate system as well as kinks and dislocations between helical axis segments. The resulting optimal helical axis is a three dimensional space curve that distributes axis irregularities between axis-junction and axis-base parameters, which would be undefined in a purely local description of structural irregularities.

In our previous study of perturbations to DNA structure by the cis, syn thymine dimer (14), we found the global helical axis calculated by CURVES to be well approximated by a circular arc, which allowed deviations from a straight helical axis to be characterized by a magnitude and direction of bending. When the thymine dimer is present, only a subset of the parameters defined in the CURVES analysis deviate strongly from the values expected for B-DNA. This subset includes inclination and tip from the axis-base and axis-junction groups, stretch, stagger, buckle and propeller twist from the intra-base pair group, and rise, twist, roll and tilt from the inter-base group of the damaged strand. Values of stretch for each base pair in the two modified duplexes are compared in Figure 1. In the schematic that accompanies the graph, bases are represented by rectangular solids, the z axis is tangent to the helical-axis space curve and the x axis points into the major groove. The two duplexes under investigation in this work share the sequence CGAATT that terminates with the 3’ thymine of the dimer. Base pairs that are common to the two dodecamers have been aligned in Figure 1 so that the dramatic increase in stretch associated with the base pair containing the 5’ thymine of the dimer occurs at the same point in the solid and dashed curves. This presentation facilitates comparison of the magnitude of the perturbations induced by the dimer in each duplex. Even though the dinucleotide step on the 3’ side of the dimer should be more flexible in the GCACGAAATTCAAG sequence, significant changes in stretch and stagger (not
A similar conclusion is suggested by the results for buckle shown in Figure 2. The average buckle in the base pair that contains the 5' thymine of the dimer in CGCGATTCCCGG is slightly larger than that found for GCACGATTAAAG; however, this difference is small compared to the deviation of both sequences from values near zero expected for B-DNA. For both sequences, the average propeller twist (not shown) in the base pair containing the 3' thymine of the dimer is close to the value of -13.3 degrees found in the CURVES analysis of standard B-DNA (15) while that found for the base pair containing the 5' thymine of the dimer is about -40 degrees. This asymmetry is also reflected in the Watson–Crick hydrogen bonding of the base pairs. In both simulations, hydrogen bonding in the base pair containing the 3' thymine was normal while one of the hydrogen bonds in the base pair containing the 5' thymine was stretched to 2.35 Å and reduced in angle to 130 degrees. This weakening of hydrogen bonds may be related to the recent observation (32) that the G5:A6 step in the GCACGATTAAAG sequence context (5'-CGA-3') is the same in the two dodecamers. Larger buckle in the G5:C20 base pair of the GCACGATTAAAG is correlated with increased inclination and x-displacement of the G5 base (not shown), greater twist at the GspA6 dinucleotide step in the GCACGATTAAAG sequence (Fig. 3) and more negative roll at this step (Fig. 4). These differences in our results for the two dodecamers are most likely associated with a BII to BII transition (33,34) at the GspA6 junction in the GCACGATTAAAG sequence. This transition, which involves coupled changes in the ε (C4'-C3'-O3'-P) and ζ (C3'-O3'-P-O5') backbone dihedral angles, swings the phosphate towards the minor groove relative to its symmetric position between the grooves in the more common BII state.

Swaminathan et al. (35) observed a brief BII→BII transition at the G16pA17 junction in their 140 ps MD simulation of d(CGCGAATTCCCGG)2 using the GROMOS force field with explicit water and counterions. We also found occasional BII→BII transitions in simulations of this dodecamer with and without a thymine dimer; however, the GspA6 junction in our simulation of GCACGATTAAAG remained in the BII state for 700 ps after the initial 300 ps during which the simulation was converging to a stable r.m.s.d. from the starting structure. In the absence of the dimer, the GspA6 junction was in the BII state only during the 200–230 ps time interval of a 1 ns MD simulation of the native sequence context. Structural changes associated with BII→BII transitions are small for a GpA dinucleotide step (34); nevertheless, calculated differences in the fine structures of CGCGAATTCCCGG and GCACGATTAAAG in the neighborhood of GspA5 and GspA6 respectively are generally as large as differences found in the immediate vicinity of the dimer.

The average twist shown in Figure 3 for the TpC step on the 3' side of the dimer in CGCGAATTCCCGG is closer to the value expected for B-DNA than is the case for the TpA step on the 3' side of the dimer in GCACGATTAAAG. This is the type of result...
expected from greater flexibility of the TpA step (18–24), which Finch and co-workers (21) attribute to the absence of steric hindrance imposed by the methyl group in other dinucleotide steps involving thymine. In particular, steric clashes between the methyl group and the sugar–phosphate linkage prevent significant untwisting in both ApT and TpC dinucleotide steps. Consequently, the large reduction in twist at the TpT step associated with formation of the thymine dimer is unable to propagate in either the 3′ or the 5′ direction in CGCGAA TT CGCG while the block to propagation in the 3′ direction is partially removed in GCACGAA TT AG.

Similar arguments apply to positive changes in roll (i.e. bending toward the major groove). ApT and TpC dinucleotide steps cannot deform in this way due to steric clashes between the methyl group and the sugar–phosphate backbone while TpA steps are not subject to these restraints (21). Figure 4 shows that the large positive roll at the TpT step induced by the dimer (average value >20 degrees in both cases) is reduced to 4 degrees at the TpC step in CGCGAATTCGGG and to 11 degrees in GCACGAAATTTAG. The same effect was found for the parameter axis tip (not shown), another indicator of a kink at the dimer that bends the helical axis toward the major groove. At the TpC step the 3′ side of the dimer in the CGCGAATTCGGG sequence, axis tip returns to zero (the standard B-DNA value) from an average value of 12 degrees at the dimer. The TpA step on the 3′ side of the dimer in GCACGAAATTTAG has axis tip equal to 6 degrees; down from an average value of 13 degrees at the dimer. Axis tip is slightly less than zero at the ApT step on the 5′ side of the dimer in both sequences.

CONCLUSIONS

The dependence of DNA distortions on the sequence context of a lesion is difficult to address systematically due to the very large number of cases needed for an exhaustive investigation. This paper does not attempt such a study but rather addresses a specific sequence effect; namely, the consequence of a flexible TpA step adjacent to a cis, syn cyclobutane thymine dimer on the 3′ side. The conformation of the dimer of the TpT dinucleotide dictates a large reduction in twist and large increase in roll at the dimer site. TpA is unique among dinucleotide steps involving thymine in that it can tolerate large untwisting and positive roll without steric clashes between the methyl group of thymine and the sugar–phosphate linkage (21). This mechanism explains the slower decay of twist, roll and axis tip distortions on the 3′ side of the dimer calculated for the GCACGAAATTTAG sequence relative to the CGCGAATTCGGG sequence, where TT denotes the thymine dimer. Nevertheless, it is important to emphasize that the differences in the conformations of CGCGAATTCGGG and GCACGAAATTTAG that we can with reasonable confidence attribute to the flexibility of the TpA step in the latter are small relative to the magnitude of local perturbations induced by the dimer. Results of the two MD simulations are remarkably similar with respect to both the nature and magnitude of DNA distortions induced by a cis, syn thymine dimer. This may indicate that some of the flexibility characteristic of a native TpA step is lost on association with the dimer or that there is little tendency to propagate distortions in the 3′ direction so that flexibility of the adjacent step is not very important. Our calculations raise the question of whether the presence of a dimer in the GCACGAAATTTAG sequence induces a preference for the B II backbone conformation at the GspAp4 dinucleotide step. This preference causes differences from results obtained for the CGCGAATTCGGG sequence that are at least as great as those attributed to the TpA step. Additional simulations with different starting structures are in progress to determine if this preference is an artifact of our computational method or a real effect of the cis, syn thymine dimer in the GCACGAAATTTAG sequence.
ACKNOWLEDGEMENTS

The authors gratefully acknowledge helpful discussions with Karol Miaskiewicz and Michael Kennedy. This work was supported by the Office of Health and Environmental Research (OHER) of the US Department of Energy under contract DE-AC06-76RLO 1830.

REFERENCES