Communications to the Editor

A Nanosecond Molecular Dynamics Trajectory for a B DNA Double Helix: Evidence for Substates

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We report here the results of the three molecular dynamics (MD) simulations on the d(CGCGAATTCGCG) duplex, two for 500 ps and one extended to 1 ns, as a response to serious questions about the sensitivity of the results to trajectory length and choice of starting structure.

In 1992 we reported a 140-ps MD on the d(CGCGAATTCGCG) duplex including water and counterions.[1] This simulation utilized the GROMOS force field,[2] and irreversible base pair opening events were observed, as with other force fields.[3,4] A second simulation employed a harmonic restraint function with GROMOS to assure that Watson–Crick base pairing was maintained intact. The results in the latter case were found to be consistent with available experimental data in comparison both with crystal structure data[1] and with 2D-NOESY buildup curves obtained from NMR spectroscopy.[5,6] Subsequent studies of hydrogen bond interaction energies in Watson–Crick base pairs revealed that GROMOS underestimates these interactions. When a hydrogen bond potential[7] was added, the energies compared closely with corresponding experimental data and *ab initio* quantum mechanical calculations.[8] The simulations described herein are based on GROMOS with this modification.

The current studies also utilize a longer range switching function, which feathers the truncation of potentials over the length scale from 7.5 to 11.5 Å and eliminates the tendency of charged groups to cluster at the cutoff limit when potentials are truncated too abruptly.[9] We treat the effect of counterions implicity, using a reduced charge of –0.24 eu on the phosphate groups.[10] All current simulations are carried out using free MD on the dodecamer duplex surrounded by ~3500 water molecules in a hexagonal prism elementary cell of constant volume, treated under periodic boundary conditions to model dilute aqueous solution. Velocities are rescaled when necessary to produce an average kinetic energy corresponding to 300 K.

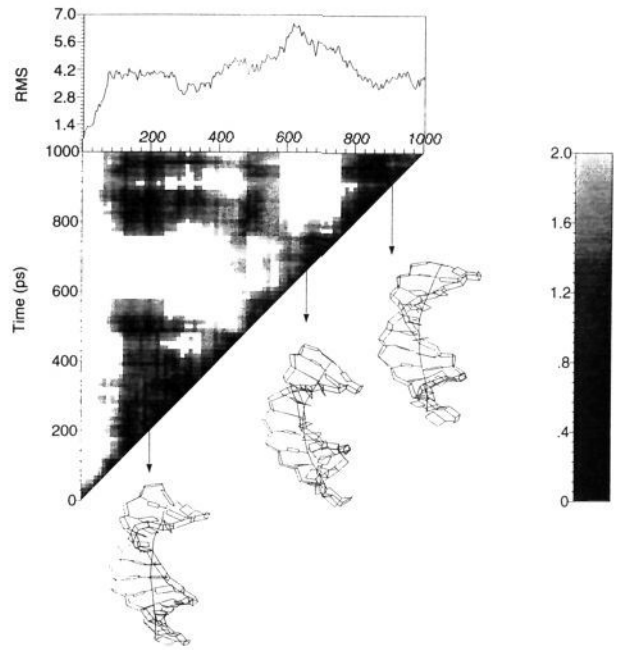

Figure 1. 1D rms map (top) and 2D rms map (center) for base pairs in the 1-ns MD simulation of the d(CGCGAATTCGCG) duplex. Shaded areas in the 2D rms map indicate regions of low rms (<2 Å) and hence the similarity of structures. Average MD structures for each of three putative substates are shown at bottom.

The three new simulations on the d(CGCGAATTCGCG) duplex were performed under protocols identical except for startingstructure and length. The starting configurations were (a) the canonical B80[11] fiber form of DNA (500-ps trajectory), (b) the Drew–Dickerson[12] crystal form (500-ps trajectory), and (c) the protein-bound form observed in the complex of d(CGCGAATTCGCG) with the restriction enzyme *Eco*RI endonuclease[13] (1-ns trajectory). The simulations were performed using the program WESDYN[14] and analyzed by means of various utilities available in MD Toolchest.[15] All three simulations were found to converge to essentially similar MD behavior, indicating that the results, at least for this system, are not sensitive to the choice of starting structure. The extreme kink in the *Eco*RI dodecamer was found to relax within the first 20 ps of MD, indicating the protein-bound form to be a strained conformation rather than a metastable intermediate.[16]

The MD based on the *Eco*RI form was extended to 1 ns. The stability and convergence behavior of the simulation was monitored by one-dimensional (1D) and two-dimensional (2D) root mean square deviation (rms) maps (Figure 1). After an initial

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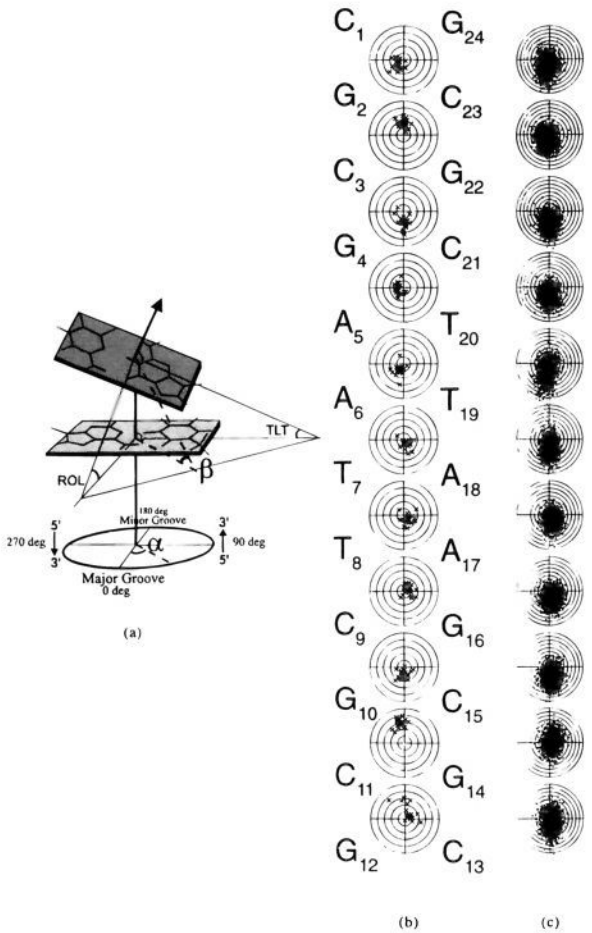

Figure 2. Bending dials analysis[17] of the crystal structures of the d(CGCGAATTCGCG),[19] compared with corresponding results from the 1-ns MD simulation: (a) definition of bending dial (seen in a perspective view at bottom) in terms of base pair tilt and roll; (b) bending dials analysis for 20 crystal structures;[19] and (c) bending dials analysis for the MD structures. Each ring represents a 5° increment in axis deflection. To make trends in the analysis more discernible, crystal structure dials extend to 25°, while MD dials extend to 45°.

equilibration period, the MD structure resides for ~300 ps in a form ~4.5 Å rms from canonical B form DNA. The system then makes a distinct transition to a new form, still in the B family but somewhat more distant (~7.5 Å rms) from the canonical form, where it remains for 180 ps. A rapid (~1.5 ps) reversible base pair opening event occurs in this structue at the T7 step, concomitant with displacements in helicoidal roll and twist. Then the dynamical structure transits to a third form, where it resides at the termination of the run. The third form, as evidenced by a cross peak in the 2D rms map, bears a strong resemblance to the first, indicating that the MD results appear to describe an incipient dynamical equilibrium among putative dynamical substates of the B family.

Validation of the MD results was pursued by a comparison of calculated and observed helix bending characteristics. To analyze axis bending in a given structure, the magnitude β and angular direction of bending α are computed from deviations in the helicoidal parameters roll and tilt, Figure 2a. The values of β and α for a given step can be projected onto a polar plot or "bending dial" (Figure 2a).[17]

The bending dials for 20 reports of the d(CGCGAATTCGCG) crystal structures are shown in comparison with the MD results in Figure 2b. The results show that bending toward the minor groove occurs at the G2–C3 step, followed in the succeeding step C3–G4 by a bend toward the major groove. A similar effect is seen in the other flanking sequence. The MD results from the 1-ns trajectory axis deformations are shown in Figure 2c and indicate that bending toward the major groove occurs at the G2–C3 and C9–G10 steps. However, the adjoining step does not distinctly show the corresponding roll into the minor groove as observed crystallographically. The effect of crystal packing, which can be significant in DNA oligonucleotides,[18] is not considered in the calculations.

The occurrence of substates on the nanosecond time scale for the d(CGCGAATTCGCG) duplex indicates that MD on DNA must be carried out for at least an order of magnitude longer than previously expected, and perhaps even longer, to fully sample the thermally bound conformational surface of the B-form DNA double helix.

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