Molecular Dynamics Simulations of DNA with Protein's Consistent GROMOS Force Field and the Role of Counterions' Symmetry

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Abstract: Model solvent effects, related to DNA stability in water, are explored with molecular dynamics (MD) simulations: (i) hydrophobicity and (ii) salt modulated electrostatic effects. The 2.6 Å resolution X-ray coordinates of the DNA oligomer from Zif268 are used to seed the MD simulations. The molecular model contains fully charged and geometrically unrestricted 10 base-pairs DNA in a 2640 water molecules bath with 18 Na-ions at 298 K. (i) The hydrophobicity correction affects the water−oxygen repulsive ($\sqrt{C12(Ow,Ow)}$) parameter that transforms the "hydrophilic" united carbon atom in the old GROMOS-87 force field into an hydrophobic one. The root mean square (rms) deviations stay below 2.8 Å, and the 600 ps-averaged and regularized structure elicits the positive effect of this correction on the structure. (ii) The electrostatic effects are probed by constructing a distribution of counterions placed in between phosphate groups at the end of a collective equilibration; salt (co-ion) effects are modeled by imposing a weak harmonic constraint (0.58 kcal mol^{-1} Å^{-2}) to the equilibrated set of counterions. A 1 ns trajectory shows rms deviations from X-ray below 1.7 Å for all atoms at 600 ps and below 2.3 Å in the time span up to 1 ns; counterions fluctuations are large enough to allow for DNA bending and conformational changes. The quality of this simulation can be appreciated from diferent averaged and regularized structures. The structural results are comparable to those obtained with state-of-the-art force fields using Ewald summation technique for an oligonucleotide of similar size.

Introduction

Understanding molecular and dynamic determinants of recognition and stability of nucleic acids and complexes with proteins and other biomolecules may help solve important problems in biology, chemistry, biochemistry, and biophysics. In this context, the potential role of molecular dynamics simulations (MD) in condensed phases is well recognized.[1−8] Protein MD simulations with GROMOS force field[9] have been fairly successful,[10] but applied to DNA they have failed[11,12] or have had more limited success when *ad hoc* hydrogen bonded potential energy function and phosphate charge screening were introduced.[3,13] In contrast, there has been a formidable progress in accurately modeling DNA with programs such as AMBER[12] and CHARMM.[14] In this paper, we examine solvent dependent corrections within the GROMOS framework that lead to trajectories of a DNA oligomer with reasonable behavior in the nanosecond time scale. The results suggest that the intramolecular part of the GROMOS force field may turn out to be a suitable starting point to represent DNA and proteins on an equal footing.

Hydrophobic forces, hydrogen bonding, and stacking interactions play fundamental roles in DNA secondary structure stability.[15] Recently,[16,17] a number of authors have remarked that carbon−water interaction parameters in GROMOS-87 gave the united carbons (CHn) too much of a hydrophilic character, which was reflected in unrealistic protein behavior in water.[17] Modification of one parameter, the [C12(Ow,Ow)]$^{1/2}$ controlling hydration of a biomolecule via the repulsive part of the Lennard-Jones potential, was shown to be necessary to correct the main deficiency of the original GROMOS potential as applied to proteins.[17,18] Based on these results, we conjecture that previous simulations of DNA[11,13] ought to be affected by the "hydrophilic" default of the force field. To test this hypothesis, MD simulations are carried out on a B-like DNA, (5′-GCGTGG-GCGT-3′) duplexed with its complementary sequence (5′-ACGCCCACGC-3′), whose structure is known in a complex with a zinc-finger protein, Zif268.[19]

It is well-known that DNA conformation is modulated by the surrounding environment, in particular, it is strongly

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dependent upon salt concentration.[15,20] This factor has been modeled by several authors via the introduction of crystal symmetries using Ewald's sum techniques.[5,6,8,21] This procedure creates an electric field onto the DNA-counterion system "buffering", as it were, the otherwise large fluctuations of the electric forces in the central motif. The sources of such a field are external to the simulated motif. In this paper, a different buffering approach is used. The ion atmosphere[22] beyond the simulated motif is assumed to favor a more symmetric positioning of the counterions thereby respecting in average the backbone helicity. Here, the resultant electrostatic forces are low, so that very weak constraints acting upon them may simulate the attraction put up by salts (co-ions) outside the electrically neutral DNA-counterions motif. Now, if the hydrophobicity correction is a main factor to improve the DNA fluctuation pattern with the GROMOS force field, the inclusion of salt-effect model should produce more reasonable structural and atomic fluctuation patterns. To test this hypothesis, a trajectory of 1 ns was calculated in water.

Counterion Model

In general, DNA being a polyelectrolyte, the MD simulations are beset with the electrostatics problem.[21] This is independent from the C6−C12 parameter limitations. This problem is handled here with a model constructed in two stages: (1) selecting an initially unstable symmetrized counterion distribution that respects the helical nature of the phosphate backbone;[23] (2) followed by a cooperative ion restrained equilibration process. The initial position of a counterion was chosen to be 7 Å away the bisectrix of the triangle O1−P−O2 of each phosphate. Several runs of equilibration with the DNA position restrained ended with a couple of counterions located in-between two consecutive phosphate groups at about 5−6 Å distance from each other. Analyses of the standard 600 ps trajectory also show several counterions fluctuating in such a neighborhood. This position is very special in the sense that the attractive forces acting on the counterion are almost cancelled by the opposite effects of the phosphates. The final construction of the ion-distribution was made by constraining the ions to move collectively toward DNA at places where they are at those minimum potential energy sites located in-between consecutive phosphate groups. The target distances were imposed after extensive graphic analyses of free equilibration runs.

The existence of salt concentrations beyond the cybotactic region that surrounds the DNA-counterions is one of the factors usually neglected in MD simulations. The electrostatic effect created by co-ions[22] found in an external homogeneous salt distribution can be thought of as setting up a (weak) force acting against counterion implosion toward the DNA. Such implosion can be seen in the standard simulation. Note that for the symmetrized ion distribution herein constructed, there is an electrostatic force attracting the counterion to the DNA. We simulate then the external forces with a very weak position constraint acting on the counterions. The selected harmonic constant, 0.58 Kcal mol^{-1} $Å^{-2}$, contributes to a caging-like effect put up by the nearest-neighbor phosphate groups without "freezing" the DNA structure to an extent that would invalidate the present results.

MD Computing Details

The DNA crystallographic coordinates at 2.1 Å resolution, taken from Zif268 DNA−protein complex,[19] were used to seed the MD calculations. The system contains 454 DNA atoms, 18 Na^+, and 2640 SPC water molecules in a 55.6 Å box-length with octahedric periodic boundary conditions. After the counterions were equilibrated, the solute coordinates were optimized during 1000 steps of steepest descent energy minimization, plus 500 additional steps of conjugate gradient. Initial velocities, required by the leap-frog algorithm, were obtained from a Maxwell−Boltzman distribution calculated for the target temperature 298 K. The simulations at constant volume were run with SHAKE for the stretching degrees of freedom, time step 2 fs, a Berendsen-bath with temperature coupling ($\tau = 0.01$ ps) and 12 Å cut-off was set for computing the long range coulombic interactions between charged groups; no switching function was used. The nonbonded pair list was updated every 10 steps.

The two trajectories used a restraintless DNA model and a modified water−oxygen repulsive coefficient[17] $[C12(Ow,Ow)]^{1/2} = 793$ kcal mol^{-1} $Å^{-12}$. The standard simulation procedure employs PROION instead of the symmetrized arrangement. Proion is a subroutine replacing the first atom of solvent molecules by atomic ions, at places with lowest (positive ions) respectively highest (negative ions) Coulomb potential.

The time series for the different energy types (not shown) correspond to well equilibrated trajectories.

Results

Standard Simulation. Hydrophobicity effects are sensed in the first trajectory (600 ps long) with the help of the RMS deviation from the X-ray structure and shown in Figure 1. Here, there is an average rms deviation of about 1.5 Å up to 250 ps. An apparent conformational change takes place and the rms fluctuation keeps below 2 Å until 440 ps. Thereafter, the system fluctuates in the range below 2.8 Å. The base-pair distances that are characteristic of hydrogen bonding are roughly conserved to within 70%. The averaged, regularized (for definitions see ref 12), structures at 200 ps and cumulated 600 ps are shown in Figure 1. One can see that, while the helical shape of the backbone is conserved, there is a decrease of the minor groove size, which tends to collapse, and a general bending to the major groove side. Some disorder appears at the C5′ end of the second chain. A stationary structural fluctuation pattern at about 250 ps was found. Thus, correcting for hydrophobicity tends to yield reasonable structural results with the otherwise standard GROMOS intramolecular force field (A test trajectory of 700 ps using GROMOS 87 parameters presents a rms deviation from the X-ray structure above 3 Å, after roughly 590 ps, showing a separation of strands already described by Beveridge et al.[4]).

Symmetrized Counterion Simulation. External Electrostatic Field Model. A one nanosecond trajectory was calculated with the symmetrized counterions model. Figure 2 shows the rms deviations for both sugar-phosphate backbone and all atoms. They stay below 1.7 Å during the 0−600 ps trajectory. The donor−acceptor distance between base-pair atoms shows a potential retention of ca. 15−17 over 28 bonds during the first 500 ps. At about 600 ps the system starts drifting away, very slowly, so that the rms deviation reaches a maximum value of 2.4 Å at about 890 ps. This is followed by a slight decrease of the rms that keeps fluctuating about 2 Å. All these changes reflect different subtle conformational changes.

The averaged, regularized structures over 200 ps and cumulated at 1 ns are shown in Figure 2. Looking at the averaged over 200 ps structures in Figures 1 and 2, the effects of

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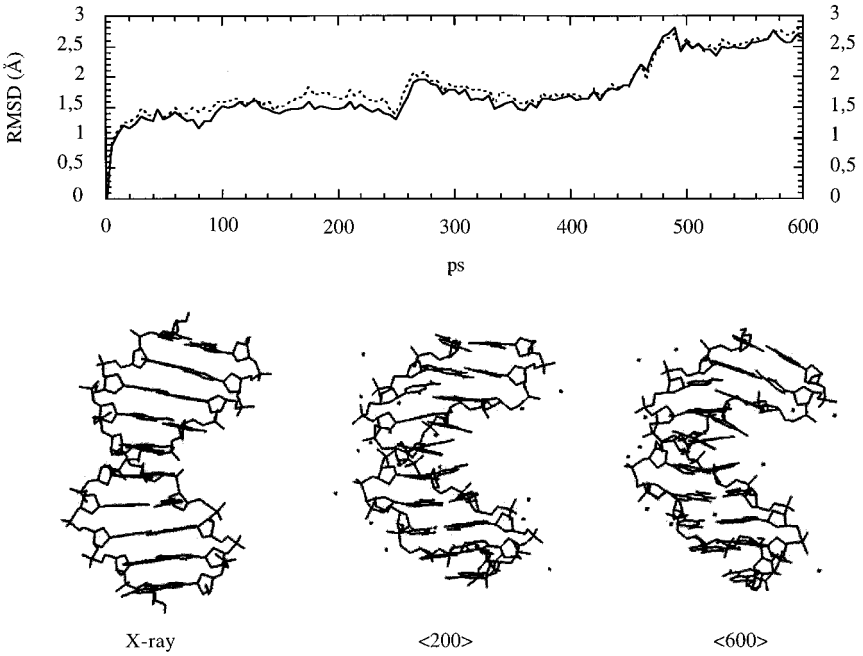

Figure 1. RMS deviation (RMSD) for sugar-phosphate backbone atoms and all atoms (dashed and solid lines, respectively), obtained from the 600 ps long standard trajectory and structures of X-ray and the averaged accumulated structures at 200 and 600 ps.

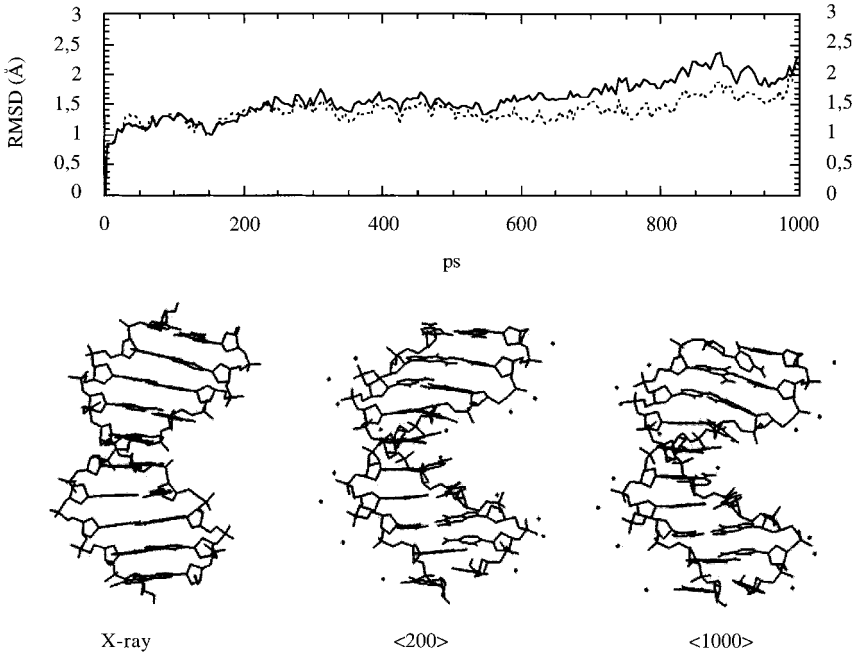

Figure 2. RMS deviation (RMSD) for sugar-phosphate backbone atoms and all atoms (dashed and solid lines respectively), obtained from the 1000 ps long trajectory using an initially symmetrized counterion distribution trajectory and the structures for X-ray and the averaged accumulated at 200 and 1000 ps.

symmetrization can be tenuously appreciated, in keeping a better organized sugar-phosphate backbone, this observation is also supported by the rms deviation graphics from the same figures, where the rms deviation backbone atoms values for the standard and symmetrized simulations are 1.7 and 1.4 Å, respectively. The 1 ns averaged structure has some disorder at the 5′-end of the first chain, and a characteristic overall bending toward the major groove can also be observed, but, otherwise, it is of excellent qualitative appearance.

The counterion fluctuation pattern is of special importance. In Figure 3 is shown the Na−Pi and Na−P_i+1 distances fluctuation averaged over the whole set of counterions. The image obtained from these results is that of relatively large fluctuations of the counterions in-between the phosphate groups. The rms fluctuation for each distance is 5.4 and 5.5 Å, respectively. Another analysis showing the coordinates fluctuations for two selected counterions is also shown in Figure 3. It is quite apparent there that the ions are not rigidly bound but fluctuate with relatively large amplitudes.

Discussion

The trajectories reported here, together with the testing work for proteins by Daura et al.,[17] strongly suggest that a proper treatment of solvation may heal some of the gross limitations shown by GROMOS-87. For DNA, appropriate models to treat the electrostatics are always necessary as they originate in external sources. The approach suggested here leads to a fairly equilibrated counterion distribution that can be taken as a

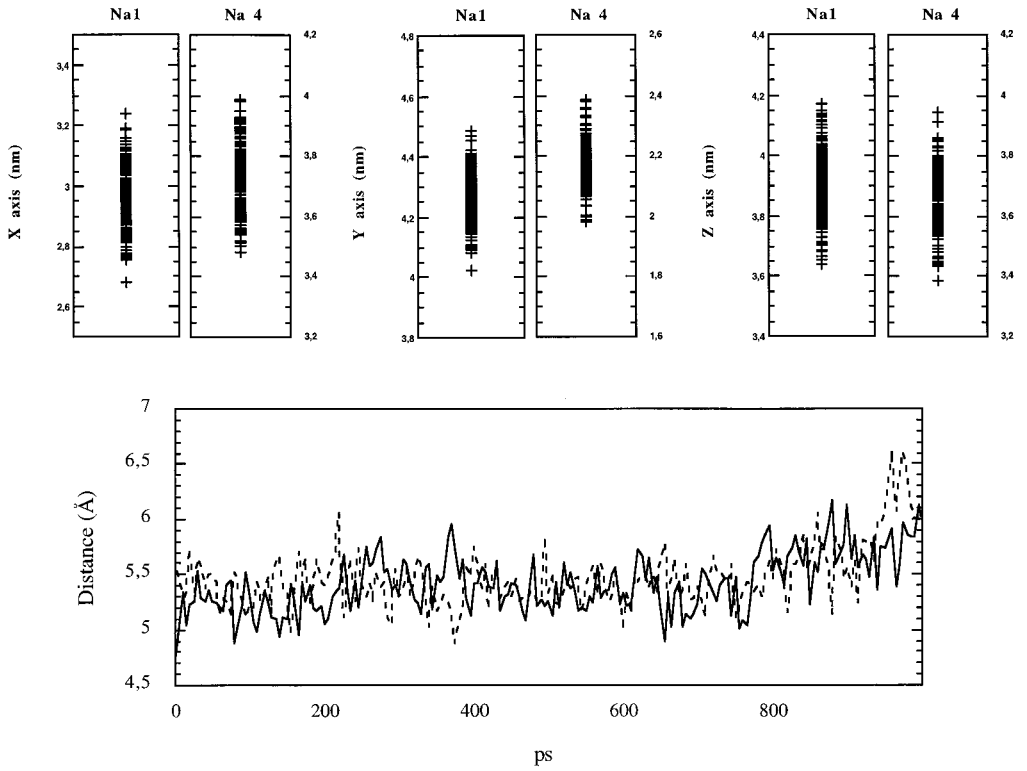

Figure 3. (a) Coordinate fluctuation pattern along the three axes *X*, *Y*, and *Z*, observed in the symmetrized simulation, for the selected ions Na 1 (interacting with the 5′-end) and Na 4 (interacting with the central part of the chain). (b) Computed Na−P_i (solid line) and Na−P_i+1 (dashed line) distances averaged over all counterions as a function of time for the symmetrized simulation.

structural feature to be used in constructing DNA−protein complexes where counterions overlapping with the protein are discarded. The present potential would handle all atoms in a DNA−protein complex on equal footing.

Circular dichroism (CD) studies on Zif268 DNA−protein complex have been reported by Elrod-Erickson et al.[24] A comparison of the CD spectrum of the free DNA and the complex shows that protein binding enhances the height of the maximum found at 275 nm. These authors infer the existence of a conformational change from what they called the $B_{(enlarged\ groove)}$-DNA (our starting point) to a possibly more canonical B-DNA. From our symmetrized simulation we have calculated averaged conformations over time slices of 200 ps, leading to structures in which the high frequency fluctuations were damped down, so that large structural changes can be sensed; its animation with the mdFRODO graphic program[25] shows a distinct global conformational change. This is also apparent from the pictures in Figure 2. At the same time, the MD trajectory shows a fairly conserved global helical topology, besides some apparent disorder at the 5′-end of the first chain.

Interestingly, the simulation results are compatible with CD experiments in the following sense. Shi and Berg have documented a DNA unwinding induced by zinc finger protein binding.[26] This is indirectly confirmed by the CD measurements. Therefore, a simulation of the DNA motif starting from the X-ray coordinates of Zif268[19,24] should elicit some kind of conformational rearrangement. This is what is sensed in the 1 ns trajectory. Whether the experimental change and the simulated one go in the same direction is difficult to say at this stage. There is not sufficient structural experimental data yet.

It is well acknowledged that a correct description of the electrostatics for this type of polyelectrolyte is a necessary condition to get realistic atom fluctuations and average structures. Ewald's technique has been successfully used with other force fields.[5,6,12] Possible artifacts have been discussed recently.[8,12] Here, we tested an alternative model counterion-distribution (not to be mistaken with the procedure used to get it) yielding atom fluctuation patterns of a similar quality that those reported by Cheatham and Kollman.[12] These latter were obtained with a state-of-the-art force field.[7,12] If the results presented here are confirmed by further structural analyses of helical parameters (twist, roll and tilt, pucker) and new sequences studied, then GROMOS might well be joining the family of successful force field presently available. This would open good prospects to simulate protein−DNA complexes using a uniform force field. In fact, results obtained from a 1 ns trajectory of the Zif268 complex by Roxtröm et al. in our laboratory (unpublished results), DNA and Zif268 both have about 1.3 Å rms deviation, would confirm the quality of the present approach.

Auffinger and Beveridge[27] discerned artifacts in the ion−ion distribution function at about 16 Å corresponding to the external limit in the twin-range cutoff of a MD simulation on a 1 M aqueous NaCl solution. For our standard simulation (without switching function) and selected snapshots, the maxima of the Na−Na distance distribution functions (not shown) are found to coincide with the cutoff of 12 Å. This artifact is not found in the symmetrized simulation. The corresponding distribution peaks about 9 Å during the full trajectory. The width at half height fluctuates over relatively large ranges, and only a small fraction of ions populate the region beyond the cutoff.

In conclusion, the stability of the simulated DNA oligomer studied here appears to depend to a great extent on a correct representation of solvation effects. This does not mean that

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further refinements of the GROMOS parameters are not needed for a proper representation of nonbonded interactions.[17,18] Among conspicuously absent effects, there are the stacking forces, which would add a marginal stabilization contribution to the DNA simulated structure as well as improved treatments of puckering fluctuations.

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