Molecular dynamics of an *in vacuo* model of duplex d(CGCGAATTCGCG) in the B-form based on the amber 3.0 force field

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ABSTRACT The characteristics of 100 ps of molecular dynamics (MD) on the DNA dodecamer d(CGCGAATTCGCG) at 300 K are described and investigated. The simulation is based on an *in vacuo* model of the oligomer and the AMBER 3.0 force field configured in the manner of Singh, U. C., S. J. Weiner, and P. A. Kollman, (*1985. Proc. Natl. Acad. Sci. USA.* 82:755–759). The analysis of the results was carried out using the "curves, dials, and windows" procedure (Ravishanker, G., S. Swaminathan, D. L. Beveridge, R. Lavery, and H. Sklenar. 1989. *J. Biomol. Struct. Dyn.* 6:669–699). The results indicate this dynamical model to be a provisionally stable double helix which lies at ~3.2 Å rms deviation from the canonical B-form. There is, however, a persistent nonplanarity in the base pair orientations which resemble that observed in canonical A-DNA. The major groove width is seen to narrow during the course of the simulation and the minor groove expands, contravariant to the alterations in groove width seen in the crystal structure of the native dodecamer (Drew, H. R., R. M. Wing, T. Takano, C. Broka, S. Tanaka, I. Itakura, and R. E. Dickerson. 1981. *Proc. Natl. Acad. Sci. USA.* 78:2179–2183). The propeller twist in the bases, the sequence dependence of the base pair roll and aspects of bending in the helix axis are in some degree of agreement with the crystal structure. The patterns in DNA bending are observed to follow Zhurkin theory (Zhurkin, V. B. 1985. *J. Biomol. Struct. Dyn.* 2:785–804.). The relationship between the dynamical model and structure in solution is discussed.

INTRODUCTION

The structure and dynamics of duplex DNA is of central importance in the field of molecular biophysics. Considerable structural detail has now become available from diffraction studies on oligonucleotides in crystals (1) and from optical (2) and NMR (3) studies of the structure and certain dynamical features of oligonucleotides in solution. With the advent of supercomputers and dedicated processors, theoretical studies of the molecular dynamics (MD) of oligonucleotides have now become generally feasible and provide useful information complimentary to the above experiments. The quality and reliability of this information depends, however, on several crucial factors: the prescription assumed for the intermolecular force field, the model chosen for the representation of hydration and ion atmosphere effects in the simulation, and the nature and length of the dynamical realization.

There have already been a number of proposals for nucleic acid force fields (4-6), each of which has been used in specific studies with some degree of success. We have undertaken a series of MD simulations on the oligonucleotide dodecamer d(CGCGAATTCGCG) to characterize more comprehensively the performance of various proposed nucleic acid force fields and the sensitivity of the results to the choice of the parameters. To facilitate this, a format for the comprehensive analysis of duplex DNA structure and dynamics has been developed. The essential procedure for the analysis is based on the "curves" algorithm developed by Lavery and Sklenar (7, 8). The presentation of results is based on the computer graphics display called "dials and windows" proposed by Ravishanker et al. (9). The analysis of 30 ps of MD on the dodecamer based on the GROMOS force field was previously reported (9) as a demonstration case of DNA analysis via "curves, dials, and windows" (CDW).

We describe here an analysis of 100 ps of MD on d(CGCGAATTCGCG) from an in vacuo simulation based on the AMBER 3.0 force field. The calculation is set up in a form as similar as possible to that used in the earlier report of 100 ps of MD simulation on duplex d(CGCGA) by Kollman and co-workers (10). The present study is meant to complement the report from Kollman's laboratory, expanding the purview to a longer sequence, and a more comprehensive analysis of the conformational and helicoidal dynamics of the system. Molecular dynamics is essentially a device for probing the nature of the thermally accessible energy surface defined by the assumed force field. The intention of this study is to characterize the system in the vicinity of the (local) minimum closest to that of canonical B-form DNA as defined by the analysis of fiber diffraction by Arnott and Hukins (11), henceforth denoted as B73.

The dodecamer d(CGCGAATTCGCG) is chosen for our studies because of the extensive crystallographic structural data and analysis on this sequence reported by Dickerson and co-workers (12–16) and the fact that this sequence corresponds to a complete turn of a doublehelical DNA such that the full effect of the major and minor groove structure of the helix comes into play. The sequence GAATTC is the recognition site for the important restriction enzyme Eco Rl endonuclease (17). The structure of the dodecamer and related oligonucleotides with modified flanking sequences is the subject of parallel NMR studies by one of us (J. Srinivasan) and I. Russu.

CALCULATIONS

The studies described herein involve energy minimizations (EM) and molecular dynamics (MD) calculations on duplex d(CGCGAATTCGCG) using the program AMBER 3.0 supplied to us by Kollman (18), and using the standard prescription of nucleic acid force field parameters encoded therein. A detailed discussion of the parameterization has been given previously by Weiner et al. (4). Following the application reported by Singh et al. (10), we configure the system as a fully anionic oligonucleotide with monovalent cations placed initially at positions bifurcating the O-P-O angle at 6.0 Å from the phosphorus atom. The cations were assigned a charge of +1, a Lennard-Jones radius parameter of $\sigma = 5$ Å, a well depth of $\epsilon = 0.1$ Kcal/mol and a mass of 131 atomic mass units, which is a representation of hexahydrated Na⁺. The effect of hydration is included implicitly via the presence of a distance-dependent screening parameter R_{ii}^{-1} in the Coulombic contribution to the nonbonded interactions. There were no cutoffs applied to the potentials during the calculation.

Energy minimization was performed to locate the structure of the dodecamer corresponding to a local minimum in the vicinity of a B-form DNA. The initial structure in the minimization was taken as that of B73 (11). Restrained minimization was carried out using 10 steps of the steepest descent method followed by 40 steps of the conjugate gradient method. During this calculation, harmonic restraints with a force constant of 20 kcal/mol per $Å^2$ were applied to all nonhydrogen atoms. The resulting minimized structure exhibited an RMS deviation of 0.09 Å (excluding counterions) and 1.01 Å (including counterions) from the initial B73 structure. The minimized structure was then heated to 300 K in steps of 20 K over a time interval of 0.15 ps for 3.0 ps with the MD velocities being reassigned after each step based on the Gaussian approximation to a Maxwell-Boltzmann distribution. Then 1.2 ps of MD was performed, during which the velocities were rescaled at the end of every 0.1 ps. The MD simulation was continued for 95.8 ps. resulting in a 100-ps MD realization.

A plot of temperature and average total energies vs.

time of simulation is shown in Fig. 1. The simulation is seen to settle down fairly well after 25 ps of MD. However, it was necessary to rescale velocities at two instances during the postequilibration trajectory (at times t = 49.2 ps and 65.5 ps) due to a slight increase in the temperature of the solute over the temperature window limit of ± 10 K. These small temperature fluctuations could not, however, be definitively linked to any significant structural change. During the last 30 ps of the simulation the temperature window limit was increased to ± 20 K after which no velocity rescaling was observed. The EM was preformed on a local MicroVax computer. The MD was carried out on the CRAY XMP/48 at the Pittsburgh Supercomputing Center and required 15 h of CPU time.

RESULTS

The composite in Fig. 2 contains a sequence of structures which represents the dynamical trajectory obtained in the ~ 100 ps of MD for d(CGCGAATTCGCG) based on the AMBER force field. It is clear that although the double helix remains intact over the course of the simulation, considerable deviations of the structure from canonical



FIGURE 1 Calculated total energy, temperature, RMS deviation from canonical A72 and B73 forms (11, 20) and the crystallographic native dodecamer (12–16) (X) vs. time for 100 ps of MD on duplex d(CGC-GAATTCGCG).



FIGURE 2 A sequence of snapshots of the structure of d(CGCGAATTCGCG) during 100 ps of MD. The structure in the upper lefthand panel is the canonical B73 (11) form used as a starting configuration. The point in time the snapshot was taken is shown in the upper lefthand corner of each panel. The calculated RMS deviation of the structure from B73 is given in the lower right hand corner of each panel.

B73 are evident. We proceeded using CDW analysis to investigate the nature of these deviations, with regard to both general questions such as the extent to which the AMBER dodecamer maintains a B-form structure over the course of the simulation and details such as conformational transitions and sugar repuckering present in the trajectory.

Conformational coordinates are used in the form defined in Fig. 3. The conformational dynamics of this dodecamer are shown in Figs. 4 and 5 for the left and right strand of the duplex, respectively. The conventions adopted for this representation are fully described in the original CDW article by Ravishanker et al. (9). Briefly, each of the 206 backbone angles α , β , γ , δ , ϵ , and ζ , the exocyclic angle χ , and the sugar pucker ϕ in the molecule has a corresponding Klyne-Prelog (19) conformational wheel or, in the case of ϕ , a pseudorotation wheel. The layout of the dial system in the computer graphics is consistent with the structure per se, and the order of the dials is based on a sequence which places the phosphodiester torsion angles (α and ζ) centrally within the dial system for a given







FIGURE 4 Conformational dials for the analysis of the dynamical behavior of strand No. 1 of d(CGCGAATTCGCG). The radial coordinate is the time axis, with t = 0 ps at the center and t = 100 ps at the circumference.

nucleotide. The phosphodiester torsions for base pair steps on opposite strands are thus switched in corresponding interior positions for the left- (LHS) and righthand (RHS) set of dials. The radius of the conformational dials is taken as the 100 ps time coordinate of the dynamics, beginning from the center. Thus each dial contains a record of the dynamical trajectory of the corresponding structural parameter in the course of the simulation. The solid line in each dial specifies the initial configuration of the system before the onset of MD. The dotted lines in each of the dials are the corresponding crystallographic values for the native dodecamer. Dials for the canonical A72 (20) and B73 form of DNA are given at the bottom of the page for orientation and reference.

The analysis of the conformational dynamics of the

dodecamer over 100 ps indicate that the dihedral angle δ at the interior of the furanose sugar ring is overall relatively stable. However, conformational transitions $t \rightarrow g^+$ are observed at T7 and G12 on the LHS and C21 on the RHS, and are seen to be correlated with the sugar repuckering in ϕ (see below). The behavior at G12 is likely to be an end effect.

The parameters ϵ and ζ show some correlated dynamical behavior involving what has also been identified from earlier crystallographic studies as B_I ($\epsilon = t$ and $\zeta = g^-$) and B_{II} ($\epsilon = g^-$ and $\zeta = t$) forms (21). The initial B73 configuration is in the B_I form. Distinct $B_I \rightarrow B_{II}$ transitions are seen in the dynamics at C3, A6, and C11 on the LHS and C21, A18, and C15 on the RHS. Note that each of these transitions occur in the early stages of



FIGURE 5 Conformational dials for the analysis of the dynamical behavior of strand No. 2 of d(CGCGAATTCGCG). The radial coordinate is the time axis, with t = 0 ps at the center and t = 100 ps at the circumference.

the MD, during the heating cycle, and remain in the region of the B_{II} configuration throughout the simulation. A late $B_I \rightarrow B_{II}$ transition occurs at G16.

The phosphodiester torsion angles (ζ and α) are both $g^$ in the initial structure. The ζ parameter changes in the case of the $B_I \rightarrow B_{II}$ transition noted above. The parameter *a* shows a considerably higher degree of conformational flexibility than ζ , and is very flexible in the AATT tract. The pair (α , γ), separated by β , show a number of correlated changes such as (g^- , g^+) \rightarrow (t, t) and (g^- , g^+) \rightarrow (t, g^-) at diverse positions. This case of correlated transition is a crankshaft motion, where compensatory changes occur while the overall helix remains intact. The g^+ to t transition of the γ parameter produces a form of DNA erroneously given as a model for the fiber structure, but later found to be energetically feasible for singlestranded DNA by Broyde et al. (22) and for the double helix by Zhurkin et al. (23).

The parameter β , with few exceptions, is relatively stable, as is the exocyclic torsion χ . The sugar pucker, described by the pseudorotation angle, ϕ begins at the C3' exo for the canonical form and drifts in most cases to the conventional C2' endo state. Distinct repuckering is seen at T7 and G12 on the LHS, and C21 and G16 on the RHS. We note that sugar repuckering as well as other conformational changes appear to be more likely at the interfaces of the GC and AT tracts in the sequence.

The definition of the helicoidal parameters involved in the analysis is given in Fig. 6. Analysis of the dynamical evolution of the helicoidal parameters for the dodecamer



are shown in Figs. 7–10. A set of 20 helicoidal parameters are involved and are divided into four groups: axis-base pair (4), intra-base pair (6), inter-base pair (6), and axis junction parameters (4). The helical axis is obtained in a curves analysis by minimizing a function which describes simultaneously the change in orientation between successive nucleotides and the nonlinearity of the helicoidal axis. The optimal helicoidal solution produces the overall helical axis that best fits the conformation considered, with structural irregularity distributed between changes in the axis-base pair parameters and axis curvature. The results on helicoidal parameters pertaining to successive nucleotide base pairs are laid out in the graphics as "windows", defined on a suitable range of values for each parameter. The time axis is on the vertical, increasing from bottom to top in these figures. The dotted lines in each of the windows represent the corresponding crystal values for the native dodecamer. As with the dials, the layout of the windows represents the structure as viewed with the 5'-3' polarity of the lefthand strand running down the page and vice versa for the righthand strand. Full details on the helicoidal parameters are given (7-9).

The results for the axis base-pair parameters, X-displacement (XDP), Y-displacement (YDP), Y-displace-



FIGURE 7 Helicoidal windows for the analysis of the dynamical behavior of axis-base pair parameters for d(CGCGAATTCGCG). The vertical direction is the time axis, with t = 0 ps at the bottom and t = 100 ps at the top.

ment (YDP), inclination (INC), and tip (TIP) are shown in Fig. 7. The parameter XDP is critical in differentiating the canonical B form of DNA (-0.71 Å) from the A form (-5.43 Å). On the basis of XDP, the dodecamer definitely remains in the B range over the 100 ps of MD. There is, however, a perceptible drift in the direction of the A-DNA value for this parameter during the first 50 ps of the simulation. During the remaining 50 ps, the value of XDP remains stable. The parameter YDP is stable, although as in XDP end effects are evident at ca. G12-C13.

The parameters INC and TIP specify the orientation of the base pairs with respect to the helical axis. Here we find a significant change in structure over the course of the simulation. The dynamics commences with INC = -5.9° , and in most regions of the sequence drifts monotonically to values closely approaching that of the canonical A72 value. A rather abrupt transition to this state occurs for base pairs C1-G24 to G4-C21, and INC values are somewhat greater than those of canonical A. The *in vacuo* AMBER dodecamer thus features greater angular base pair orientation than the canonical B73 form. The complimentary parameter, TIP specifying orientation in the Y direction, slips to more positive values of ~20° at the C1-G24 end and to values of equal magnitude but opposite sign are observed for the G12-C13 end vs. 0° for canonical A72 and B73. A spike is observed in TIP and many of the other helicoidal parameters at ~77 ps. This



FIGURE 8 Helicoidal windows for the analysis of the dynamical behavior of intra-base pair parameters for d(CGCGAATTCGCG). The vertical direction is the time axis, with t = 0 ps at the bottom and t = 100 ps at the top.

indicates that some form of transient instability is present in the calculation but conventional oscillatory behavior is quickly restored. This was seen to be reproducible, but may be an end effect.

The dynamical behavior of the intra-base pair parameters is shown in Fig. 8. The linear parameters shear (SHR), stretch (STR), and stagger (STG) are all relatively stable. The angular parameters buckle (BKL) and propeller twist (PRP) show high amplitude fluctuations. The BKL parameter in the central hexamer region shows oscillatory behavior closer to the canonical value of the B-form while the outer G-C regions show considerable drift during the time evolution. Base pair opening (OPN) is relatively stable, with the mean value of OPN lying close to the canonical B-value. However, there is perceptible negative drift in OPN at base pair C1-G24 which may also be an end effect. The spike at 77 ps is evident here as well.

The inter-base pair parameters shift (SHF), slide (SLD), and rise (RIS) as shown in Fig. 9 are stable and oscillatory (except for the spike) over the course of the dynamics. The parameter RIS, also critical in distinguishing the A- and B-forms, remains very near to the canonical B-value of 3.4 Å. The parameters tilt (TLT), roll (ROL), and twist (TWS) are also oscillatory, with TWS showing a tendency to assume overwound values in the range of $40-45^{\circ}$ as compared to the canonical B-value of 36.0° . The axis parameters AXD, AYD, AIN, and



FIGURE 9 Helicoidal windows for the analysis of the dynamical behavior of inter-base pair parameters d(CGCGAATTCGCG). The vertical direction is the time axis, with t = 0 ps at the bottom and t = 100 ps at the top.

ATP exhibit oscillatory behavior in the dynamics except for possible end effects as represented in Fig. 10.

DISCUSSION

The conformational and helicoidal dynamics of the dodecamer under the conditions assumed in the present calculation indicates that the behavior of the *in vacuo* AMBER model is characterized by a high degree of fluctuation. Examining the overall trends in the dynamics, we find many parameters to be stable and oscillatory over 100 ps but some, such as INC, showing the possibility of concerted drift or oscillations of a frequency >100 ps. On the basis of the results obtained here, we cannot rule out further deviations of the structure from canonical behavior if the run length were increased by orders of magnitude.

RMS deviation for all atoms of the MD structures with respect to A and B canonical forms and the crystallographic dodecamer during the course of the simulation are shown in Fig. 1. There is a considerable drift from the canonical B73 form, 3.2 Å, during the heating and equilibration phase of the simulation. The subsequent behavior of RMS vs. time is reasonably oscillatory. On the basis of RMS deviation, the MD results agree slightly



FIGURE 10 Helicoidal windows for the analysis of the dynamical behavior of axis-junction pair parameters d(CGCGAATTCGCG). The vertical direction is the time axis, with t = 0 ps at the bottom and t = 100 ps at the top.

but significantly better with B73 than with the crystal structure of the native dodecamer (~3.6 Å) and A72 DNA (~6.8 Å). The behavior of XDP with time, however, indicates that the structures generated in the course of MD remain generally in the B-bamily. Based on the values of the critical parameters XDP and RIS, the *in vacuo* AMBER model is thus a variant of B-DNA which is slightly overwound as indicated by the values found for the parameter TWS. However, a significant deviation in

the direction of the canonical A-form is found in the helicoidal base pair parameter INC. The resulting dynamical structure is thus a hybrid, clearly B-form with respect to the position of the base pairs relative to the helix axis and A-form with respect to their orientation.

Another representation of the calculated results is shown in the cartoons of the helix axis and base pair for the sequence of the dynamical structures as shown in Figs. 11 and 12. In Fig. 11, the local helix distortions

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FIGURE 11 Calculated dynamical behavior of base pair orientations (a side view).

subsumed in the course of dynamics and indicated by AXD, AYD, AIN, and ATP are quite evident, including the apparent kink in the vicinity of C3-G22 near the primary roll point of the native dodecamer. The rosette view of the structures (Fig. 12) shows clearly that the slight contraction in a structure typical of a vacuum dynamics simulation is present here as well.

The dynamical behavior of the major and minor grooves during the course of the simulation are represented in Figs. 13 and 14, respectively. The calculated major groove width is seen to be considerably narrower (11-14 Å) than that of the canonical form (17 Å). The minor groove is



FIGURE 12 Calculated dynamical behavior of base pair orientations (a top or rosette view).

expanded in the central region of the structure. The deviations in the groove widths from canonical values observed in the calculation are contravariant to those observed in the crystal structure, where the minor groove is seen to be markedly narrowed (12-16). The end regions of the crystallographic dodecamer are, however, subject to the influence of helix packing effects, and may not be a relevant point of comparison.

Comparing further with experiment, the helicoidal analysis of the crystallographic dodecamer based on CDW was given in some detail in a previous article (9). The conformational and helicoidal parameters for the crystal structure are shown as dotted lines in Figs. 4 and 5 and 7–10. The native dodecamer shows the B_{II} form at G10 and C23, a feature not reproduced by the calculation. In the crystal structure, INC assumes values close to canonical B in the AT region, but assumes a range of values intermediate between A and B in the GC regions. The AMBER values for the A-T and G-C tracts deviate considerably from the crystal values. The behavior of TIP in the MD calculation reported here does not parallel that found for the crystal, which is near 0.0 in all cases except G10-C15 and C3-G22.

The helicoidal parameters SHR, STR, and STG agree reasonably with the crystal values. The amplitude of BKL in the calculation shows a considerably larger fluctuation than the range of values observed in the crystal structure. The values found for propeller twist, large and negative, agree quite well with the characteristic behavior in the crystal. There is significant oscillation of the PRP parameter for the central hexamer region between the crystal value and the canonical B-value. The observed sequence dependence of OPN, AT > GC is perceptible in the calculation.

Of the interbase parameters, the result of the RIS parameter is comparable with that of the crystal structure (excluding end effects), but the overwinding indicated by the parameter TWS as seen in the calculation is not systematically observed. The sequence dependence of ROL is quite well reproduced in the simulation. The axis bending in the crystal structure is seen toward the grooves (ATP) rather than the backbone (AIN), as predicted some years ago by Zhurkin (24) based on purinepyrimidine clashes. The MD shows that the dodecamer shows no particular overall preference (cf. Fig. 10). Zhurkin theory predicts bending toward the major groove at C3-G22/G4-C21 and the C9-G16/G10-C15 junctions, and toward the minor groove at the A6-T19/T7-A18 junction. The crystallographic dodecamer follows these predictions quite closely. The sequence dependence of ATP observed in the calculation also follows the pattern predicted by Zhurkin.





The dynamical behavior of the helix axis is illustrated in Fig. 15, where the inflection points at the primary and secondary roll points are quite evident. Overall, the main deviations between the MD results on this dodecamer reported here and the crystal structure of the native dodecamer are alterations in the groove widths compared with the canonical values, the calculated high-amplitude BKL, seen routinely in other MD calculations on DNAs as well, and in fine details of the base pair orientation. The sequence dependence of some of the structural irregularities observed in the native dodecamer, particularly ROL, are reproduced by this theoretical model.

The solution structure of the B-form DNA is currently a matter of considerable interest of both NMR and optical spectroscopies (2, 3). NMR results have been interpreted in several cases in terms of structures intermediate between A- and B-forms of DNA (25), and there is considerable evidence from flow linear dichroism (26) that the base pairs are tilted away from perpendicularity for B-form DNAs in solution. On this basis, the characteristics of the dodecamer d(CGCGAATTCGCG) as a theoretical model are not necessarily unrealistic. However, vibrational circular dichroism results seem to favor bases perpendicular to the axis (27). Further, information on dodecamer structure in solution is necessary before an unequivocal position can be taken on this point.

The hydrated counterions were initially placed at 6 Å from the phosphorus atom of each phosphate group. The



FIGURE 14 Calculated dynamical behavior of the minor groove width for the dodecamer. (Dotted lines) Native dodecamer.



FIGURE 15 Calculated dynamics of the helix axis for d(CGCGAAT-TCGCG). The helix axis of canonical B-DNA (*straight bold line*) and the Native dodecamer (*curved bold line*) are included for reference.

counterions were not restrained to the phosphate but completely free to move during minimization and dynamics. The distances of the counterions to the corresponding phosphorus atoms on the backbone of DNA after minimization were all found to reside at ~ 5.9 Å remaining tethered to the corresponding phosphates. By the end of the dynamics run the hydrated counterions were generally found to move significantly away from the solute. However, and exception to this is the ion associated with the phosphate between base pairs T20-C21 and C21-G22; which remained within 7.5 Å from their corresponding phosphate groups. An additional six counterions diffuse away from their initial phosphate residues but remain vicinal to other phosphate groups along the backbone. The remainder of the counterions diffuse to positions outside of the major and minor grooves, still remaining close to their corresponding as well as the surrounding intra- and interstrand phosphates. It is important to note that the counterions are not found inside the grooves due to their large van der Waal radius. There is no discernible perturbation in the intramolecular dynamics which could

be linked to the unphysical behavior of counterions. Additionally, the CDW analysis of the 100-ps simulation shows no apparent correlation between the behavior of the counterions and the internal dynamics of the solute.

A more realistic model of the environment comes from including water as well as counterions explicitly. Siebel et al. (28) described a simulation on duplex d(CGCGA) in a cluster of 830 water molecules using the AMBER force field and TIP3P water model. Explicit inclusion of water is expected to alter the intramolecular dynamics considerably as seen in parallel calculations that have been carried out using the GROMOS force field (5). A detailed comparison of the results on the *in vacuo* model reported here with those of the fully solvated model via CDW analysis is obviously the next point of interest.

SUMMARY

In this investigation, 100 ps of MD have been carried out on d(CGCGAATTCGCG), which includes a hydrated Na⁺ model for the counterions and an implicit distancedependent screening model to represent the effect of hydration. The results were analyzed in detail using CDW. The analysis reveals that the theoretical model maintains an overall B-form structure over the course of the MD trajectory, but is considerably different (~3.2 Å RMS) than the canonical form. There is a persistent nonplanarity in the base pair orientation with respect to the helical axis, significant buckling, and a slight but significant overwinding with respect to the canonical structure. The dynamical model for the dodecamer based on this in vacuo AMBER MD calculation is this a hybrid structure, B-form with respect to the position of base pairs relative to the helix axis, and more like the A-form with respect to base pair orientation. The dynamical model of DNA produced in this simulation lies distinctly closer to the structure of the canonical B-DNA than to the crystallographic form (native dodecamer). Some areas of agreement with the crystallographic dodecamer, particularly in sequence dependence of base pair ROL and in axis bending are observed, and generally correlate well with Zhurkin's theory of irregularities in the structure. The question as to whether this model contributes a reasonable proposition for the solution structure of the dodecamer or not cannot be unequivocally answered at this point in time.

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