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Dielectric Response of Triplex DNA in Ionic Solution from Simulations

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ABSTRACT We have analyzed a 1.2-ns molecular dynamics simulation of 51 mM d(CG·G)$_7$ with 21 Na$^+$ counter-ions and 1 M NaCl in water. Via the dipole fluctuations, the dielectric constant for the DNA is found to be around 16, whereas that for the bases and sugars combined is only 3. The dielectric constant for water in this system is 41, which is much smaller than 71 for pure SPC/E water, because of the strong restriction imposed on the motion of water molecules by the DNA and the ions. Also addressed in the present work are several technical issues related to the calculation of the dipole moment of an ionic solution from molecular dynamics simulations using periodic boundary conditions.

INTRODUCTION

Triple helical DNA has attracted considerable attention in biophysical chemistry in the past few years (Cheng and Pettitt, 1992). This is largely due to the potential a triplex-forming oligonucleotide has as a therapeutic agent in gene regulation (Bischofberger and Shea, 1992). Triplex-forming oligonucleotides as drugs can bind selectively to a target segment of a long double helical DNA in cells to form a short triple helical DNA sequence. This can aid in gene regulation by blocking the transcription process. A better understanding of the physical aspects of triplex DNA would be beneficial to overcoming some of the current problems associated with binding and specificity in random sequences.

One of the important properties associated with the stability, conformation, and function of any nucleic acid oligomer is the spatially varying dielectric response, both within and immediately outside the DNA. The dielectric response significantly influences the strength of the electrostatic interactions among various parts in complex molecular systems. All DNA contains modestly polar bases, less polar sugars, and negatively charged phosphates. In the case of triple helix formation, the charge density of the backbone is increased by roughly 50% over duplex DNA. How the system responds to this increased repulsion is not completely understood. Cations migrate to the vicinity of the triplex DNA in order to partially balance the negative charges of the phosphates. Additional salt is present to favor the formation of triplex DNA (Cheng and Dyke, 1993). Clearly, the interactions of charges and the binding free energies in this complex system depend on the spatial and time-dependent dielectric response.

Experimental measurement of the dielectric constant typically limits itself to the overall response of a sample to the applied external electric field (Grant et al., 1978). It is difficult to measure or even uniquely define the dielectric constant of specific parts of a biological macromolecule. On the other hand, modeling studies may be done without explicit solvent molecules by assuming different and empirical dielectric functions that have spatial dependencies. The importance of dielectric constants in determining the structure and the thermodynamics of electrostatically interacting systems is quite evident in the spread in results obtained from different electrostatic conditions (Brooks et al., 1988; Smith and Pettitt, 1994).

The presence of ions in a system complicates the electrostatic interactions; this is true even for pure ionic solutions without the biological macromolecules. In a continuum sense, ions affect the Poisson equation by involving the Boltzmann factor in the source term to reflect the screening around the test charges. At low ion concentration, the resultant effective force law becomes the normal Coulomb force reduced by the Debye-Hückel factor, which decays exponentially with distance (Jackson, 1975). At high ion concentration, the new interactions can only be described numerically by calculations including correlations. Furthermore, ions affect the dielectric constant, which in turn affects the strength of the Coulomb forces and the Debye screening length. One must distinguish the static dielectric constant of the solution from the static dielectric constant of the solvent. In concentrated saltwater solutions, the former is infinite solely because the ions are capable of conducting electricity, or equivalently, forming infinitely polarizable cation-anion pairs in an external static electric field (Jackson, 1975; Stillinger and Lovett, 1968). The solvent dielectric constant is not only finite but also smaller than the value of pure water because the motion of water is restricted by the ions. The dielectric decrement of water in the presence of ions has been well characterized, in the limit of infinite dilution, by the Debye-Hückel theory as extended by Hückel in 1925 (Harned and Owen, 1958) and measured experimentally for various salts at many concentrations (Franks, 1973). (Unfortunately, Hückel's formula predicts a negative water dielectric constant at high enough ion con-

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centrations (Harned and Owen, 1958).) The experimental measurements of the dielectric constant of water in ionic solutions involve the extrapolation to zero frequency of the external electric field and the separation of the diverging (flux) component, which is proportional to the conductivity divided by the frequency.

Pure electrolytes discussed above provide a simple example of separation of the dielectric constant in terms of components. For a more complex system containing a biological macromolecule in ionic solution, a similar separation is meaningful for the following reasons. 1) The component dielectric constant of the macromolecule can be approximately extracted from experimentally measurable quantities. For example, a value for myoglobin was obtained based upon the effective volume of a myoglobin molecule and the dielectric decrement of water as a function of myoglobin concentration (South and Grant, 1972). 2) The separation of the overall dielectric constant into self-terms for individual components (component dielectric constants) and cross-terms among components is well defined mathematically. We shall give a derivation in the following section and show by calculation that the cross-terms are negligible as compared to the self-terms when the components are grouped appropriately. It should be emphasized that one can justify the last point only after performing the actual simulations, calculations, and comparisons. If the cross-terms were to dominate the overall dielectric constant, component dielectric constants or the self-terms would be of little relevance to either experiment or theory. 3) There is a use for the resultant information in popular continuum model calculations. Researchers performing Poisson-Boltzmann calculations and implicit-solvent molecular modeling need reliable output from explicit-solvent simulations regarding the spatially varying dielectric constant from deep inside a macromolecule to the bulk of the solvent. An analysis in the same spirit has previously appeared on the subject of protein dielectric contributions (Smith et al., 1993). The derivation and computation of cross-terms were not considered in that case, however.

Although the dielectric constant in an inhomogeneous system is a property of the state of the entire system, we show that it is possible heuristically to separate components and, thereby, simultaneously calculate the contributions of the macromolecule and the explicit solvent to the total dielectric constant, and to evaluate any cross-terms from molecular dynamics (MD) simulations. This can be done via the calculation of fluctuations of the dipole moments (Neumann et al., 1984; Neumann, 1986b). One obstacle to such a direct approach is that the simulation must be on the order of nanoseconds to attain convergence, even for pure water (Neumann, 1986a).

In this study, we calculate and partition the dielectric response of a system containing a triplex DNA, water molecules, and ions from MD simulations. Discussions will be presented on several theoretical issues involved in the calculation. These relate mainly to the effect of the periodic boundary condition (PBC), the ionic contribution, convergence, and the choice of origin of the coordinate system in the calculation of dipole moments. Our results on the response of bases, sugars, phosphates, water molecules, and ions in the system provide information on the dielectric characteristics for different regions in the DNA-ion-water complex.

THEORY

The dielectric constant ϵ of a system surrounded by an infinite dielectric medium, which provides a reaction field to the interior system and has a dielectric constant ϵ_{RF}, is related to the dipole fluctuations (in SI units) by (Neumann et al., 1984; De Leeuw et al., 1980; Smith et al., 1993)

$$\frac{(\epsilon - 1)(2\epsilon_{RF} + 1)}{(2\epsilon_{RF} + \epsilon)} = \frac{\langle \vec{M}^2 \rangle - \langle \vec{M} \rangle^2}{3\epsilon_0 V k_B T} \equiv \alpha. \tag{1}$$

Here $\vec{M}$, V, T are the total dipole moment, the volume, and the temperature of the system, respectively, and ϵ_0 and k_B are the electric permittivity of vacuum and the Boltzmann constant. We define a positive definite quantity α in the equation above for convenience in subsequent discussions. It is independent of the system size in the thermodynamic or macroscopic limit, i.e., it is *intensive.*

Two special formulas for ϵ, denoted as ϵ_1 and ϵ_2 below, emerge from Eq. 1. They are

$$\epsilon_1 = 1 + \alpha, \tag{2}$$

as $\epsilon_{RF} \rightarrow \infty$, and

$$\frac{(\epsilon_2 - 1)(2\epsilon_2 + 1)}{3\epsilon_2} = \alpha, \tag{3}$$

as $\epsilon_{RF} = \epsilon$. One solution of ϵ_2 in Eq. 3 is given by

$$\epsilon_2 = (3\alpha + 1 + \sqrt{9\alpha^2 + 6\alpha + 9})/4. \tag{4}$$

The other solution for the quadratic equation of ϵ_2 (Eq. 3), which gives $\epsilon_2 < 0$, is dropped because such a solution is not physically meaningful.

It is clear that $\epsilon_1 = \epsilon_2 = 1$ as $\alpha \rightarrow 0$. On the other hand, $\epsilon_2 = 3\epsilon_1/2 = 3\alpha/2$ as $\alpha \rightarrow \infty$. If the same α is used in Eqs. 2 and 3, then one has $\epsilon_1 \leq \epsilon_2 \leq 3\epsilon_1/2$ in general and $\epsilon_2 \approx 3\epsilon_1/2$ for $\epsilon_1 > 10$. For example, ϵ_2 is approximately 14 when $\epsilon_1 = 10$. We should note, however, that α and $\vec{M}$ can be influenced self-consistently by ϵ_{RF} in actual simulations (Neumann et al., 1984).

Now let us turn to the calculation of the total dipole moment for a system of N particles (atoms and ions) defined as

$$\vec{M} = \sum_{i=1}^{N} q_i \vec{r}_i, \tag{5}$$

where q_i and $\vec{r}_i$ are the partial charge defined in the force field and position vector of the ith particle. For a system composed of particles belonging to m different groups, it is often convenient to decompose $\vec{M}$ into m group components as follows

$$\vec{M} = \sum_{k=1}^{m} \vec{M}_k, \tag{6}$$

$$\vec{M}_k = \sum_{i=N_{k-1}+1}^{N_{k-1}+n_k} q_i \vec{r}_i. \tag{7}$$

Here n_k is the number of particles in the kth group and $N_k = \sum_{l=1}^{k} n_l$. Similarly, the mean square deviation of the dipole moment, denoted as $\Delta(\vec{M}) \equiv \langle \vec{M}^2 \rangle - \langle \vec{M} \rangle^2$, can also be expanded in terms of the m components,

$$\Delta(\vec{M}) = \sum_{k=1}^{m} (\langle \vec{M}_k^2 \rangle - \langle \vec{M}_k \rangle^2) + 2 \sum_{l=k+1}^{m} \sum_{k=1}^{m} (\langle \vec{M}_k \cdot \vec{M}_l \rangle - \langle \vec{M}_k \rangle \cdot \langle \vec{M}_l \rangle). \tag{8}$$

For the triplex simulation, the particles may be partitioned into five ($m = 5$) chemical groups: k = 1, 2, 3, 4, and 5 for the base, sugar, phosphate, water, and ion groups, respectively. As is well known, the dipole moments of the neutral species ($\vec{M}_1$, $\vec{M}_2$, $\vec{M}_4$, and $\vec{M}$) are independent of the location of the origin of the coordinate system, whereas those of the charged phosphate ($\vec{M}_3$) and ion ($\vec{M}_5$) groups are not (Jackson, 1975).

To obtain a meaningful value for the dielectric constant of the DNA that is charged in the phosphate group, special attention must be paid to the choice of the origin. For a similar case of protein molecules, it has been argued that the proper choice for the origin should be the center of drag of the molecule, which coincides with the center of mass if nonlinear hydrodynamic terms are neglected (Kirkwood and Shumaker, 1952).

Following the convention of Kirkwood and Shumaker, we choose the instantaneous center of mass of the DNA as the origin in order to eliminate the conductance component of M_3, the dipole moment of the phosphate group, due to the translational motion of the DNA. Having made this choice, the same origin may also be used for the calculation of $\vec{M}_5$, the dipole moment of the ion group, to obtain the same total dipole moment $\vec{M}$ from either Eq. 5 or from Eqs. 6 and 7. Physically, this choice amounts to fixing or immobilizing the DNA, as for instance in a chromosome, and considering only the fluctuations. If the DNA is mobile, it, like all charged species, will yield a finite conductance in the long time limit.

As shown in the Appendix (Moving Ions Under PBC), species moved by PBCs must be neutral in order not to cause artificial discontinuities in the dipole moment as time progresses. To consider the relevant fluctuations for all species, even those whose contributions must diverge such as the ions, the effects of the PBCs on the trajectory must be taken into account. One method of obtaining a continuous total dipole moment $\vec{M}$ is to unfold the trajectories of the ions and atoms out of the central simulation box as these particles move across the boundaries (De Leeuw and Perram, 1981). This method was originally proposed by De Leeuw and Perram and will correct the discontinuities in the dipole moment. This strategy is the same as the one commonly used in the calculations of diffusion constants (via mean square displacements) from MD simulations. Although dipole moment and mean square displacement are different quantities, both of them involve the positions of particles and encounter the same problem of discontinuity in position caused by periodic boundary conditions. The diffusion constant and mean square displacement are mentioned here only for comparison because these appear more frequently in the literature on molecular dynamics simulations and thus may be more familiar to the reader. Note that the neutrality of water molecules does not cause any problems if each molecule is moved as a unit when applying PBC. Systematic unfolding of all particles will not change the water contribution to the total dipole moment. We note that an essentially equivalent method of generating the unfolded positions of ions and atoms is to integrate the velocities over time, with initial positions within the central simulation box (Caillol et al., 1989a).

A question arises regarding the correct value of the volume, V, in Eq. 1, where $\vec{M}$ is the total dipole moment, when the trajectories of particles are unfolded into the neighboring boxes around the central box. V should be the volume of only one such identical box because the effective volume of all particles, including ones unfolded, should add up to the volume of the central box. It is correct and convenient to add the effective volumes of the images inside the central box even for particles that are treated as outside the box in the calculations of dipole moments.

One may try to obtain a convergent dielectric constant ϵ by calculating α in Eq. 1 for more and more neighboring boxes around the central simulation box. And it may appear that the statistics acquired this way should stay the same, if not become better. However, as pointed out in the Appendix (Multiple Boxes Under PBC), the consequence of such a practice is much worse because the ensemble average of the fluctuations then does not correspond to the Hamiltonian intended to describe the single box system: α becomes extensive and the dielectric constant becomes strongly dependent on the number of boxes used. Therefore, one must consider only one of the identical images for each particle in the calculations.

As also shown in the Appendix (Minimum-Image Approach), it is problematic to apply the minimum-image convention to the double sum (over the number of particles) converted from the single sum in Eq. 5. Although a similar practice is widely used for the calculation of pressure in MD simulations (Allen and Tildesley, 1987), it does not work for dipole moments because of the different physical functions with parity involved.

MODEL

We have recently completed a 1.155-ns MD simulation of a triple helical DNA, d(CG·G)$_7$, with 37 Na$^+$, 16 Cl$^-$, and 837 water molecules in a 32Å × 32Å × 32Å box using PBCs at 300 K. This produces a system that is 51 mM and approximately 1 M in DNA and salt concentrations, respectively. The intramolecular bonds were constrained via the SHAKE algorithm (Ryckaert et al., 1977), and all atoms were allowed to move in a force field with parameters for the DNA, water, and ions taken from CHARMM 22 (Brooks et al., 1983), SPC/E (Berendsen et al., 1987), and Chandrasekhar et al. (1984), respectively, using the velocity Verlet algorithm (Allen and Tildesley, 1987) with a time step of 2 fs. All electrostatic interactions were calculated via the Ewald technique with $\kappa = 10/L$, where L is the box length, and the number of lattice vectors is set to 515 in the reciprocal-space sum (De Leeuw et al., 1980; Allen & Tildesley, 1987; Ewald, 1921). This simulation, which is an extension of the rigid-DNA study presented by Mohan et al., (1993), provided an opportunity to obtain the dielectric response in and around the triplex DNA. More details of the simulation can be found in the work of Mohan et al. (1993) and Weerasinghe et al. (1995).

We sort the 3′ CH and 5′ CH$_2$ atoms into the extended "phosphate" group to have a net charge of $-e$ per group when using the partial charge assignments in the force field employed. This leads to 10 atoms in a nonterminal "phosphate" group. For the phosphate-terminated 5′ end, we include the 5′ CH$_2$ and the terminal H atoms, resulting in 9 atoms for the terminal "phosphate" group. Thus the total number of "phosphate" atoms in d(CG·G)$_7$ is $3 \times (6 \times 10 + 9) = 207$. The total number of base atoms in d(CG·G)$_7$ is $7 \times (12 + 2 \times 15) = 294$. The remaining 180 atoms in the triplex DNA belong to the reduced "sugar" group.

RESULTS

The calculation of dipole fluctuation requires a much longer simulation time as compared to calculations of other properties such as the radial distribution functions, the vibrational frequencies, or the diffusion constants. Two reasons are responsible for the special requirement: 1) There is only one value for the dipole moment at each MD time step; thus statistics can be accumulated only over time, not over the number of atoms. 2) The dipole moment changes with the orientation of the molecules; and the rotation of molecules is usually the slowest physical process in conformationally stable molecular systems. Although our system is well characterized as conformationally stable on this time scale (Weerasinghe et al., 1995), the total simulation time may only be adequate for obtaining a modest precision estimate.

Table 1 presents the components of the mean square deviation of the dipole moment in Eq. 8. These are the values averaged over the entire simulation of 1.155 ns. For the water group, the square of the average dipole moment (87.5) is much smaller than the corresponding mean square deviation (432.6); however, the former is much larger than the latter for the base, "sugar," "phosphate," and ion groups. The rotational and translational motions of water molecules have been sampled sufficiently within 1 ns; thus, the average dipole moment of the water molecules becomes comparatively small and affects the mean square deviation only slightly. On the other hand, the DNA macromolecule does not rotate by more than a few degrees within 1 ns; thus, the mean square deviation of the dipole moment is very small as compared to the square of the average dipole moment, because the latter barely changes during the entire simulation.

TABLE 1 Components of the mean square deviation of dipole moment ($\vec{M}$) in units of (eÅ)^2 obtained from molecular dynamics simulations of d(CG·G)$_7$, a triple helical DNA, with 21 Na$^+$ ions and 1.0 M NaCl in water

Group A	Group B	$\langle \vec{M}_A \cdot \vec{M}_B \rangle - \langle \vec{M}_A \rangle \cdot \langle \vec{M}_B \rangle$	$\langle \vec{M}_A \cdot \vec{M}_B \rangle$	$\langle \vec{M}_A \rangle \cdot \langle \vec{M}_B \rangle$
Base	Base	2.7	65.1	62.4
"Sugar"	"Sugar"	0.7	7.1	6.4
"Phosphate"	"Phosphate"	25.8	570.9	545.1
Water	Water	432.6	520.1	87.5
Ion	Ion	2422.6	12227.4	9804.8
Base	"Sugar"	0.2	9.7	9.5
Base	"Phosphate"	2.9	95.2	92.3
Base	Water	3.5	76.4	73.0
Base	Ion	3.4	62.9	59.5
"Sugar"	"Phosphate"	1.5	52.9	51.4
"Sugar"	Water	−0.4	13.7	14.1
"Sugar"	Ion	0.2	219.8	219.7
"Phosphate"	Water	4.2	127.8	123.6
"Phosphate"	Ion	−31.6	1964.7	1996.3
Water	Ion	−17.6	162.3	180.0

To illustrate the quality of convergence for the quantities in Table 1, we plot the accumulative averages for the base (Fig. 1 *A*), "sugar" (Fig. 1 *B*), "phosphate" (Fig. 1 *C*), water (Fig. 1 *D*), and ion (Fig. 1 *E*) groups as functions of time. Note the changes in scale. The dipole fluctuation for water converges best among the five groups, as shown in Fig. 1 *D*. The dipole fluctuation of ions (Fig. 1 *E*) is several orders of magnitude larger when compared to Fig. 1 *A* (base) or Fig. 1 *B* ("sugar"). Such a huge fluctuation does not settle by the end of the simulation, where the increase of 50 (eÅ)^2 after 1000 ps is hundreds of times larger than the changes in base (Fig. 1 *A*) and "sugar" (Fig. 1 *B*). The fluctuation for ions shows the expected overall divergence (Fig. 1 *E*), which will be discussed later. Fig. 1, *A* (base) and *B* ("sugar"), displays very sharp initial rise in the first 50 ps for the two neutral groups in the DNA. By contrast, the charged third group (Fig. 1 *C* for "phosphate") is slow in its initial rise and is clearly correlated most strongly to the ions (Fig. 1 *E*). We should point out that the truly small variations near the end in Fig. 1, *A* and *B*, 0.5 (eÅ)^2 for base and 0.1 (eÅ)^2 for "sugar," are indicative that the DNA is very stable during the entire simulation without rapid changes in conformation. This is in accord with a more complete structural

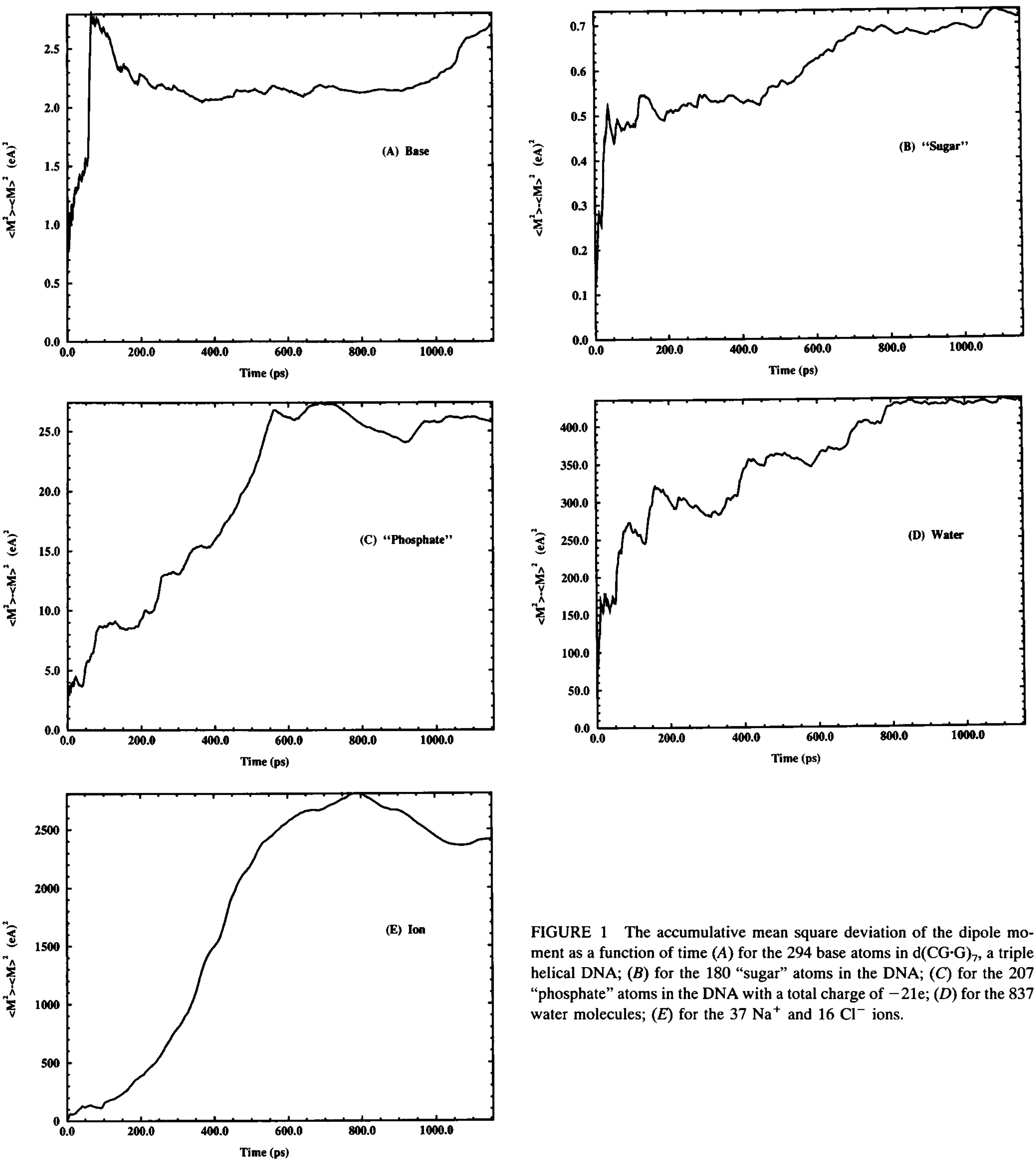

FIGURE 1 The accumulative mean square deviation of the dipole moment as a function of time (*A*) for the 294 base atoms in d(CG·G)$_7$, a triple helical DNA; (*B*) for the 180 "sugar" atoms in the DNA; (*C*) for the 207 "phosphate" atoms in the DNA with a total charge of −21e; (*D*) for the 837 water molecules; (*E*) for the 37 Na$^+$ and 16 Cl$^-$ ions.

analysis of the DNA, including root mean square (rms) deviation, radius of gyration, hydrogen bond distances, dihedral angles, helical rise, and twist between bases, etc. (Weerasinghe et al., 1995). Such analyses point unambiguously to the stability of the model DNA in this simulation. For example, the rms deviation of positions from the initial model built structure for all 681 atoms (including hydrogens) in the DNA is only 1.89 ± 0.33 Å during the entire simulation and 1.71 ± 0.19 Å for the last 400 ps, with most of the deviation already acquired during the first 70 ps of equilibration from a model-built structure (Weerasinghe et al., 1995).

We also calculated the temporal autocorrelation function of the total dipole moment of water. By performing an exponential fit to the decaying correlation function for correlation times within 1–6 ps (Neumann, 1986a), we found the dipole relaxation time of water in the triplex system to be 9.7 ps. As compared to the reported values for pure water at 293 K, this relaxation time is longer than that from another calculation, 6.5 ps for the TIP4P model (Neumann, 1986a) and closer to the experimental values of 9.3–9.4 ps (Hasted, 1973; Franks, 1973). Comparison with the other model calculation indicates that the presence of DNA and ions slows this relaxation process. Because of lack of good statistics, we did not calculate the dipole relaxation times for groups other than water.

It can be seen from Table 1 that the cross-term between two groups is always much smaller than the larger of the two self-terms of the two groups. For example, the base-"sugar" cross-term for the mean square deviation (0.2) is much smaller than the self-term of the base group (2.7). Therefore, it is usually a reasonable approximation to neglect the cross-terms when estimating the combined dipole fluctuations of two groups. This observation is crucial in justifying the separation of the total dipole fluctuation according to groups or components. Note that the cross-terms of "sugar"-water, "phosphate"-ion, and water-ion for the mean square deviations are negative, whereas all other cross-terms are positive.

Two qualitatively different sum rules related to Table 1 have been proposed recently (Chandra and Patey, 1994, Caillol et al., 1986, 1989a,b). One states that for $\langle \vec{M}_A \cdot \vec{M}_B \rangle / (3\epsilon_0 V k_B T)$ the sum of the water-cation and water-anion cross-terms should be zero (Chandra and Patey, 1994); the other states that the sum should be negative and have the same magnitude as the water-water self-term (Caillol et al., 1989a). It should be noted that both sum rules were derived assuming that the average dipole moment of water is zero. Therefore, it is more appropriate to test the data for mean square deviation in Table 1 against these sum rules. The value of $3\epsilon_0 V k_B T$ converted to the same units used in Table 1 is 14.1 $(e\text{Å})^2$. From Table 1, the mean square deviations of the dipole moment divided by $3\epsilon_0 V k_B T$ are 30.7, −1.3, and 0.3 for water-water, water-ion, and water-"phosphate," respectively. Clearly, the sum of the water-ion and water-"phosphate" cross-terms (−1) is much closer to zero rather than the negative of the water-water self-term (−30.7). Thus, data in Table 1 are in better agreement with the former (Chandra and Patey, 1994) of the two sum rules instead of the latter (Caillol et al., 1989a). We attribute the remaining difference between the simulated result and the prediction by Chandra and Patey (1994) to the statistical errors of the simulations. However, an accurate error estimation (e.g., from time block averages) for the data in Table 1 cannot be made because of the limited simulation time. Therefore, we cannot rule out either one of the sum rules conclusively.

TABLE 2 **The dielectric constant, $\epsilon(V, \epsilon_{RF})$, obtained from molecular dynamics simulations of d(CG·G)$_7$, a triple helical DNA, with 21 Na$^+$ ions and 1.0 M NaCl in water**

Group	$\epsilon(V_{Box}, \infty)$	$\epsilon(V_{Box}, \epsilon)$	$\epsilon(V_{Group}, \infty)$	$\epsilon(V_{Group}, \epsilon)$
Base	1.2	1.2	3.4	4.2
"Sugar"	1.1	1.1	2.0	2.3
"Phosphate"	2.8	3.4	33.0	48.5
Water	31.8	46.7	41.3	61.0
Ion	173.5*	259.2*	3568.9*	5352.3*
Base + "sugar"	1.3	1.3	3.1	3.7
"Sugar" + "phosphate"	3.1	3.8	20.6	29.9
DNA	3.7	4.7	15.5	22.3
Base + water	32.5	47.7	38.3	56.4
Base + "sugar" + water	32.5	47.8	36.2	53.3
DNA + water	35.6	52.4	37.3	55.0
"Phosphate" + ion	170.8*	255.2*	1609.1*	2412.7*
All particles	201.5*	301.3*	201.5*	301.3*

The final results are in the column labeled by $\epsilon(V_{Group}, \infty)$.

*Very large values. Formally, these values should be infinite because of the diverging displacements of the ions at progressively increasing times; however, they are finite in the table because of the finite simulation time of 1.155 ns.

Table 2 presents the dielectric constants of various parts of the DNA-ion-water system according to either Eq. 2 ($\epsilon_{RF} \rightarrow \infty$) or Eq. 4 ($\epsilon_{RF} = \epsilon$). The volume in Eq. 1 was taken as either that of the entire central simulation box (V_{Box}) or the effective volume of the particles in a particular group (V_{Group}). As we shall see, the only correct column in Table 2 is column 3, labeled as $\epsilon(V_{Group}, \infty)$, containing values that are both consistent with the model Hamiltonian used in the simulation and intrinsic to the individual groups listed in the table.

The correct choice for ϵ_{RF} is ∞ because this is the value chosen in the simulations when performing the Ewald sum of the electrostatic interactions. In addition, $\epsilon_{RF} = \infty$ is reasonable based on the physics of any system with mobile ions or charges. Therefore, the results reported in columns 1 and 3 of Table 2, labeled respectively as $\epsilon(V_{Box}, \infty)$ and $\epsilon(V_{Group}, \infty)$, are consistent with the model Hamiltonian used for the triplex simulation. Columns 2 and 4, which report the results of dielectric constant calculations with $\epsilon_{RF} = \epsilon$, do not correspond to the model Hamiltonian and are included only for comparisons. In Table 2, the dielectric constants in the two columns using Eq. 4 ($\epsilon_{RF} = \epsilon$) are consistently larger than those in columns using Eq. 2 ($\epsilon_{RF} \rightarrow \infty$) with the same V. The enhancement factor changes between 1 (as $\epsilon \rightarrow 1$) and 3/2 (as $\epsilon \rightarrow \infty$), depending on the actual magnitude of the dielectric constants, as discussed earlier in the theory section. Such comparisons validate the analysis in the theory section.

For the system dielectric constant of the entire central simulation box, the choice of V in Eq. 1 should be V_{Box}, as discussed in the theory section. However, the computation of the dielectric constant of the whole system is of no value in the sense that the correct result is known to be infinite because of the ions, as discussed earlier in the introduction for the simpler case of pure ionic solutions. For this reason, one has to decompose the dielectric constant to reveal the intrinsic group dielectric constants of water and DNA that are overshadowed by the infinite ionic contributions. To judge from Eq. 1, such a decomposition should involve two steps: 1) decomposition of the total dipole fluctuation according to Eq. 8 with results presented in Table 1, and 2) decomposition of the total volume V_{Box} as explained below. Using V_{Box} in Eq. 1 for each group, one would conclude incorrectly that α, or $\epsilon - 1$ according to Eq. 2, is simply additive among groups. Consider a sealed conducting tank filled with water having $\epsilon = 78$ or $\alpha = 77$. Each half of the water does contribute equally to the dipole fluctuation; but this should not lead to $\epsilon - 1 = \alpha = 77/2$ or $\epsilon = 39.5$ for each. The correct normalization volume, i.e., half that of the tank, must be used to reach the correct answer of $\epsilon = 78$ for each half. Thus, a meaningful group dielectric constant can be obtained from Eq. 1 only by using the actual volume that the group occupies in space, i.e., V_{Group}. The group volumes as defined add up to give the system volume: $\Sigma_{\text{Group}} V_{\text{Group}} = V_{\text{System}} = V_{\text{Box}}$. The volume-weighted average of the group dielectric constants (ϵ_{Group}) gives approximately the system dielectric constant: $\Sigma_{\text{Group}}[(\epsilon_{\text{Group}} - 1)V_{\text{Group}}]/\Sigma_{\text{Group}} V_{\text{Group}} = \epsilon_{\text{System}} - 1$. Small nonzero cross-terms in Eq. 8 will modify this relation slightly. Large cross-terms would invalidate such a separation conceptually and mathematically.

In Table 2, the correct choice for the volume should be that of the particles in the group (V_{Group}) because it is the dielectric property of a certain group that is of interest here. The other choice, using the volume of the box (V_{Box}), gives the group contribution to the dielectric constant of the entire system, which depends on the group concentration. For example, as the concentration of the DNA approaches zero or as $V_{\text{Box}} \rightarrow \infty$, the dielectric constant for the DNA obtained using V_{Box} approaches unity. Thus, the dielectric constant obtained using V_{Box} is not representative of the intrinsic dielectric property of a group such as the DNA. The final results we present in Table 2 are, therefore, in the column labeled $\epsilon(V_{\text{Group}},\infty)$. We list the other three columns to demonstrate how much the dielectric constant varies with the values of ϵ_{RF} and V in Eq. 1.

The partial volumes for various groups in the system are estimated according to the procedure outlined below. The entire box can accommodate 1096.5 water molecules at a density of 1 g/cm^3. It was previously calculated that the volume excluding the DNA in the box can accommodate 890 water molecules (Mohan et al., 1993); thus, the DNA occupies an effective volume equivalent to that of 206.5 water molecules. The value V_{Group} for water is the volume of 837 water molecules at a density of 1 g/cm^3, i.e., around 76% of V_{Box}. Such a choice of volume is consistent with the findings of Neumann et al. (1984) in the special case of a homogeneous neutral liquid, say water, where the group volume, the volume of water, and the volume of the box are identical. Because there are only 837 water molecules in the box, we assign the remaining volume of 53 water molecules to be the effective volume of the ions in the box. Within the DNA, we simply assume that each of the base, "sugar," and "phosphate" groups occupies a volume proportional to the number of atoms in the group discussed earlier. Although there exists a little ambiguity in the assignment of effective volumes for the particle groups (because of real partial molar volumes), the columns using $V = V_{\text{Box}}$ in Table 2 do define the lower limits for the dielectric constants, displaying the consequences for the worst choice of volume in most cases.

From the final result column in Table 2 labeled $\epsilon(V_{\text{Group}}, \infty)$, one can see that the dielectric constants of the base and "sugar" groups are at least one order of magnitude smaller than those of the "phosphate" and water groups. The base group has a larger dielectric constant (3.4) than that of the "sugar" group (2.0) because the former is more polar, as evidenced by values in the last column of Table 1, 62.4 for the former versus 6.4 for the latter. The dielectric constant of 15.5 for the DNA is clearly dominated by the contribution from the "phosphate" group, because the value for the base and "sugar" groups combined is only 3.1. This is in accord with the results from protein dielectric calculations (Smith et al., 1993) where inclusion of surface, ionized residues greatly increases the overall macromolecule dielectric constant.

The dielectric constant of water molecules in the DNA-ion-water system, 41.3, is much smaller than 71, the value for pure water (Reddy and Berkowitz, 1989), using the same SPC/E potential. Experimental measurements show that the dielectric constant of water decreases from 78 at zero salt concentration to 64 and 40 in 1 and 4 M NaCl, respectively, and that it can be decreased to as low as 18 in 13 M LiCl (Behret et al., 1975; Franks, 1973). The trends of these measurements have been reproduced qualitatively by calculations based upon integral equation theories (Levesque et al., 1980; Kusalik, 1987; Perkyns and Pettitt, 1992). In the presence of the macromolecule and the ions, water molecules cannot rotate as freely as they do in the homogeneous system, resulting in a weaker dielectric response. We assume that the fractional change of the dielectric constant of water from 0 to 1 M NaCl is the same for both the simulation and the experiment. Then it should be reduced from 71 to 58 solely because of 1 M NaCl in the simulation. Further reduction of the dielectric constant to 41.3 can then be explained by the presence of the triplex DNA macromolecule. Thus, it appears that the effect on the dielectric constant of water due to the DNA plus counter-ions at this high effective concentration is slightly larger than that due to the ions.

Note the diverging dielectric constant of the ion group, $\epsilon(V_{\text{Group}}, \infty) \approx 3600$ at 1.1 ns, in Table 2. This and all other values denoted by an asterisk in Table 2 should actually be infinite because of the diverging displacements of the ions at progressively increasing times; however, these values are finite in the table because of the finite simulation time of 1.1 ns. That the static dielectric constant is infinite is a quite general conclusion for any system in which the lowest resonance frequency for the charges is zero (Jackson, 1975; Stillinger and Lovett, 1968). This can equivalently be reformulated in terms of conductance or flux correlations (Caillol et al., 1986; Jackson, 1975). The limited time scale sampling prevents attaining the predicted value for the ions. In addition, the restricted mobility of the DNA in the artificial high-concentration, highly ordered environment characterized by the use of PBCs and our choice of coordinate system (centered on the DNA) restricts the possibility of observing an infinite dielectric constant for the phosphates, as would be characteristic of a true oligonucleotide as opposed to a spatially restricted polyelectrolyte.

CONCLUSIONS

We have discussed the treatments of ions and macromolecules in the calculation of dielectric constants from molecular dynamics simulations using periodic boundary conditions. To avoid discontinuities in the dipole moment as time progresses, one can "unfold" the trajectories of ions. This is similar to the technique in calculation of diffusion constants. The ionic contribution to the dielectric constant in the triplex simulation is formally infinite because ions diffuse in this system.

We obtained a molecule-motivated decomposition of the dielectric constants for a complex DNA-ion-water system based on the small size of the cross-terms. The group dielectric constant of water is an experimentally observable quantity in the case of pure electrolytes (without the DNA); such results are yet to be reported from simulations of pure electrolytes. Currently, the group dielectric constants of base and sugar are not observable in experiments; the same is true for group dielectric constants within protein systems. However, their consequences in Poisson-Boltzmann and other continuum calculations of biomolecular systems are profound. Thus as a simplifying or unifying concept in the area of electrostatic phenomena, these partitions are found to be widely useful.

The water molecules in this system exhibit a dielectric constant that is only 58% of that for pure water, because of the presence of the DNA and ions, which restrict the motion of water. The overall dielectric constant for the DNA is around 15, still less than one-half the value for the spatially restricted water molecules. Within the DNA, the base and sugar atoms have a dielectric contribution of only 2–3, which is usually characteristic of a nonpolar or hydrophobic environment on the interior of proteins (Smith et al., 1993). The dipole moments and solubilities of the nucleotide bases are, however, very different from that of the core amino acid residues of an aqueously soluble protein. The restriction or hindrance of the dipole fluctuations by base pairing and base stacking are responsible for the low dielectric interior of DNA. This is likely to hold for both duplex and tetraplex interiors, as similar conditions for hydrogen bonding and base stacking are present in these systems as well.

APPENDIX

Moving ions under PBC

Consider the change in total dipole moment when the positions of n particles in an N-particle system are moved across the box by $\vec{L}$ because of the PBC ($|\vec{L}|$ = box length). The new dipole moment corresponding to $\vec{M}$ in Eq. 5 becomes

$$\vec{M}_{\mathrm{L}} = \sum_{i=1}^{n} q_{\mathrm{i}}(\vec{r}_{\mathrm{i}} + \vec{L}) + \sum_{i=n+1}^{N} q_{\mathrm{i}}\vec{r}_{\mathrm{i}} = \vec{M} + \left(\sum_{i=1}^{n} q_{\mathrm{i}}\right)\vec{L}. \tag{A1}$$

Apparently, $\vec{M}_{\mathrm{L}}$ and $\vec{M}$ are identical if and only if $\sum_{i=1}^{n} q_{\mathrm{i}} = 0$. This means that species to be moved by PBC must be neutral not to cause artificial discontinuities in the dipole moment as time progresses. Individual ions will certainly cause problems under the PBC since they are charged.

Multiple boxes under PBC

Let $\vec{M}_{\mathrm{p}}$ the dipole moment of the pth box among a total of C boxes around the central simulation box. In parallel to Eq. 8, one can decompose the mean square deviation (Δ) of $\vec{M}_{\mathrm{C}} = \sum_{p=1}^{C} \vec{M}_{\mathrm{p}}$ as follows

$$\begin{aligned}\Delta(\vec{M}_{\mathrm{C}}) &= \sum_{p=1}^{C} (\langle \vec{M}_{\mathrm{p}}^2 \rangle - \langle \vec{M}_{\mathrm{p}} \rangle^2) \\ &\quad + 2 \sum_{q=p+1}^{C} \sum_{p=1}^{C} (\langle \vec{M}_{\mathrm{p}} \cdot \vec{M}_{\mathrm{q}} \rangle - \langle \vec{M}_{\mathrm{p}} \rangle \cdot \langle \vec{M}_{\mathrm{q}} \rangle).\end{aligned} \tag{A2}$$

Under the PBC, all of the values of $\vec{M}_{\mathrm{p}}$ are equal to $\vec{M}$, the total dipole moment of the central box, at any given time; thus, one has $\langle \vec{M}_{\mathrm{p}} \cdot \vec{M}_{\mathrm{q}} \rangle - \langle \vec{M}_{\mathrm{p}} \rangle \cdot \langle \vec{M}_{\mathrm{q}} \rangle = \Delta(\vec{M})$ for all values of p and q, resulting in $\Delta(\vec{M}_{\mathrm{C}}) = C^2\Delta(\vec{M})$. The quantity corresponding to α in Eq. 1 but for C boxes around the central box becomes

$$\alpha_{\mathrm{C}} = \frac{\Delta(\vec{M}_{\mathrm{C}})}{3\epsilon_0(CV)k_{\mathrm{B}}T} = C\frac{\Delta(\vec{M})}{3\epsilon_0 V k_{\mathrm{B}}T} = C\alpha. \tag{A3}$$

The supposedly intensive quantity α is now extensive, i.e., directly proportional to the system size, in Eq. A3. This is clearly an artifact of the replication, as the Hamiltonian is for one box, but the ensemble average corresponds to the replicated system; in a real system C times in size as the central box, one should have $\langle \vec{M}_{\mathrm{p}} \cdot \vec{M}_{\mathrm{q}} \rangle - \langle \vec{M}_{\mathrm{p}} \rangle \cdot \langle \vec{M}_{\mathrm{q}} \rangle = \delta_{\mathrm{pq}}\Delta(\vec{M})$ where $\delta_{\mathrm{pq}} = 1$ if $p = q$ and $\delta_{\mathrm{pq}} = 0$ if $p \neq q$, correctly yielding $\Delta(\vec{M}_{\mathrm{c}}) = C\Delta(\vec{M})$ and $\alpha_{\mathrm{c}} = \alpha$.

Minimum-image approach

One seemingly plausible method of correcting the discontinuities in the dipole moment due to the PBC is to convert the single sum in Eq. 5 into a double sum over the number of particles and then make use of the minimum-image convention. For example, there exists a mathematical identity,

$$\sum_{i=1}^{N} \sum_{j=1}^{N} (q_{\mathrm{i}} - q_{\mathrm{j}})(\vec{r}_{\mathrm{i}} - \vec{r}_{\mathrm{j}}) = 2N \sum_{i=1}^{N} q_{\mathrm{i}}\vec{r}_{\mathrm{i}} - 2\left(\sum_{i=1}^{N} q_{\mathrm{i}}\right) \sum_{i=1}^{N} \vec{r}_{\mathrm{i}}. \tag{A4}$$

And the entire DNA-ion-water complex satisfies the charge neutrality condition $\sum_{i=1}^{N} q_{\mathrm{i}} = 0$. Thus, Eq. A4 provides an alternative expression for the dipole moment in Eq. 5 that reads

$$\vec{M} = \frac{1}{2N} \sum_{i=1}^{N} \sum_{j=1}^{N} (q_{\mathrm{i}} - q_{\mathrm{j}})(\vec{r}_{\mathrm{i}} - \vec{r}_{\mathrm{j}}). \tag{A5}$$

Consider the two groups of particles involved in the minimum-image convention, with particle positions $\{\vec{r}_{\mathrm{i_0+1}}, \ldots, \vec{r}_{\mathrm{i_0+n_1}}\}$ for one group and

$\{\vec{r}_{j_0+1},\ldots,\vec{r}_{j_0+n_b}\}$ for the other. As the separation between the two groups passes through one-half the box length, attention is switched to new images of the former group at $\{\vec{r}_{i_0+1}+\vec{L},\ldots,\vec{r}_{i_0+n_a}+\vec{L}\}$ ($|\vec{L}|$ = box length), with the latter group held fixed in space. Thus, $(\vec{r}_i - \vec{r}_j)$ becomes $[(\vec{r}_i + \vec{L}) - \vec{r}_j]$ for $i_0 + 1 \leq i \leq i_0 + n_a$ and $j_0 + 1 \leq j \leq j_0 + n_b$ on the right hand side of Eq. A5, yielding

$$\begin{aligned}\vec{M}_I &= \vec{M} + \frac{1}{2N}\sum_{i=i_0+1}^{i_0+n_a}\sum_{j=j_0+1}^{j_0+n_b}(q_i - q_j)\vec{L} \\ &= \vec{M} + \frac{n_a n_b}{2N}\left(\frac{1}{n_a}\sum_{i=i_0+1}^{i_0+n_a} q_i - \frac{1}{n_b}\sum_{j=j_0+1}^{j_0+n_b} q_j\right)\vec{L}.\end{aligned} \tag{A6}$$

It is now apparent that $\vec{M}_I$ and $\vec{M}$ are identical if and only if the two groups of particles involved in the application of minimum-image convention have the same average charge. Therefore, for a system containing cations and anions, minimum-image convention will cause artificial discontinuities in the dipole moment as time progresses. Note that the minimum-image approach does make the discontinuities associated with crossing over boundaries disappear; however, it also creates new discontinuities as the separation between two groups of particles passes through one-half the box length. Thus, such an approach is problematic.

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