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ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · OCTOBER 1999

Impact Factor: 3.3 · DOI: 10.1021/jp9927496

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The Journal of Physical Chemistry B

 VOLUME 103, NUMBER 46, NOVEMBER 18, 1999

LETTERS

Molecular Dynamics Study of a Lipid−DNA Complex

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Received: August 3, 1999; In Final Form: September 20, 1999

Lipid−DNA complexes are of topical interest because of their potential for use as vectors in gene therapy. Herein, molecular dynamics simulations have been carried out to probe the nature of lipid−DNA interactions and thereby provide a complement to recent experimental and theoretical studies. Specifically, we have investigated the DNA duplex $d(CCAACGTTGG)_2$, in its canonical B-form, intercalated into a lipid bilayer consisting of a neutralizing binary mixture of cationic (dimyristoyltrimethylammonium propane−DMTAP) and zwitterionic (dimyristoylphosphatidylcholine−DMPC) lipids. Surprisingly, both lipids are involved in neutralizing the anionic DNA phosphate groups. The electrostatic interactions between the cationic trimethylammonium (TAP) and zwitterionic phosphocholine (PC) headgroups of the two lipids allow the PC headgroups to orient out of the bilayer plane and thereby also become available to screen the negative charges on the DNA.

1. Introduction

Human gene transfer is potentially an important biomedical application in which an expression of extracellular DNA is transferred to the nucleus of cells to replace or add genes.[1−3] At present, the most common method of gene delivery uses viral-based carriers of DNA.[1,4,5] It has been known for some time that binary mixtures of suitable cationic and neutral lipids can form stable complexes with DNA and hence can likely be used as synthetic carriers of DNA.[6] Recently, the study of lipid−DNA complexes has received considerable attention.[2−5,7−19] X-ray studies by Safinya et al.[7,8] discovered that mixtures containing the unsaturated neutral DOPC (dioleoylphosphatidylcholine) and the cationic DOTAP (dioleoyltrimethylammonium propane) lipids form a novel multilayer structure with alternating lipid bilayers and DNA monolayers, in which the DNA chains form a two-dimensional smectic phase, intercalated between lipid bilayers. Rädler and co-workers[9,10] used binary mixtures of the saturated neutral and cationic lipid DMPC and DMTAP to complex with DNA. They showed that these intercalation complexes exist in two lamellar phases, the gel phase (L_β^c) at low temperatures and the fluidlike lamellar phase (L_α^c) at higher temperatures.

Although the study of lipid−DNA complexes has received increasing attention, the development of nonviral vectors still exists only at a rudimentary level. This is due, in part, to lack of detailed understanding of the complex structure and the nature of interactions between the DNA and the lipids. A number of theoretical calculations have been carried out that help rationalize the observed structures of the lipid−DNA complexes.[14−18] Atomistic-based molecular dynamics (MD) simulations can also play an important role in elucidating the structural and other properties of such systems and are a natural complement to less-detailed theoretical models.[14−18] Although there have been several reports of MD simulations of DNA in aqueous solution,[20−25] we are unaware of any attempt to study lipid−DNA complexes. Accordingly, we report herein the salient results of a MD simulation of a binary mixture of DMPC and

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10.1021/jp9927496 CCC: $18.00
Published on Web 10/30/1999

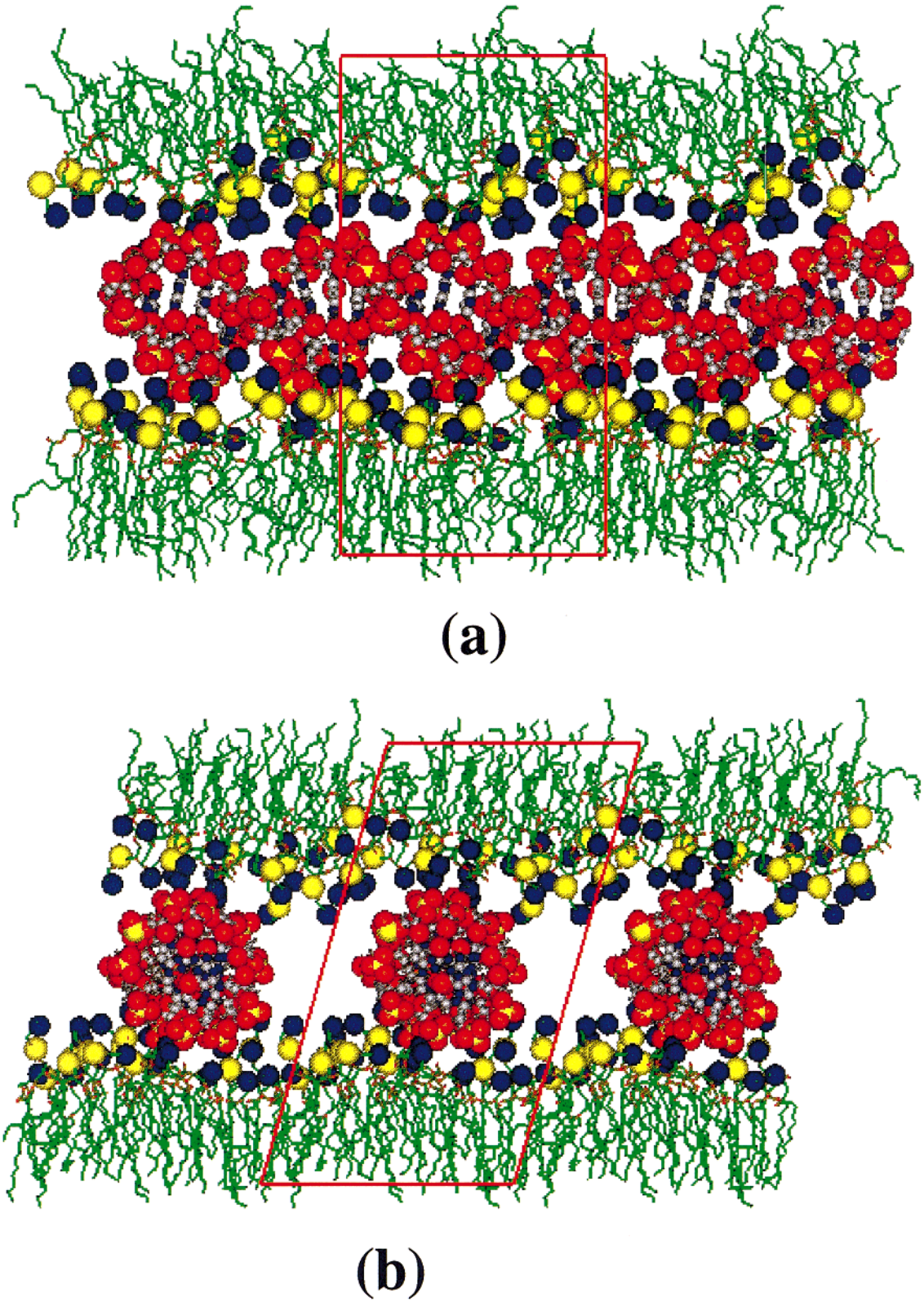

Figure 1. Configuration of the DMPC/DMTAP−DNA complex after about 5.5 ns of MD simulation. Two different views are shown: (a) perpendicular to, and (b) along, the DNA axis. The DNA and the lipid headgroup P and N atoms are drawn as spheres, while the lipid chain atoms are drawn as sticks. The atom coloring scheme is N, blue; O, red; P, yellow; C (DNA), gray; C (lipid), green, and H, dark gray. The water molecules and the lipid H atoms are not shown, and the system is replicated once on both sides of the central simulation cell for visual clarity.

DMTAP in its liquid crystalline L_{α}^{c} phase intercalated with a canonical B-form of the DNA duplex $d(CCAACGTTGG)_2$.[22] Anticipating our results, we will see that the neutral and cationic lipids are both involved in screening the negative charges on the DNA.

2. System Setup and Simulation Details

To carry out the MD simulation described herein we employed a recently developed molecular dynamics package (PINY-MD)[26] with the force field and the potential parameters

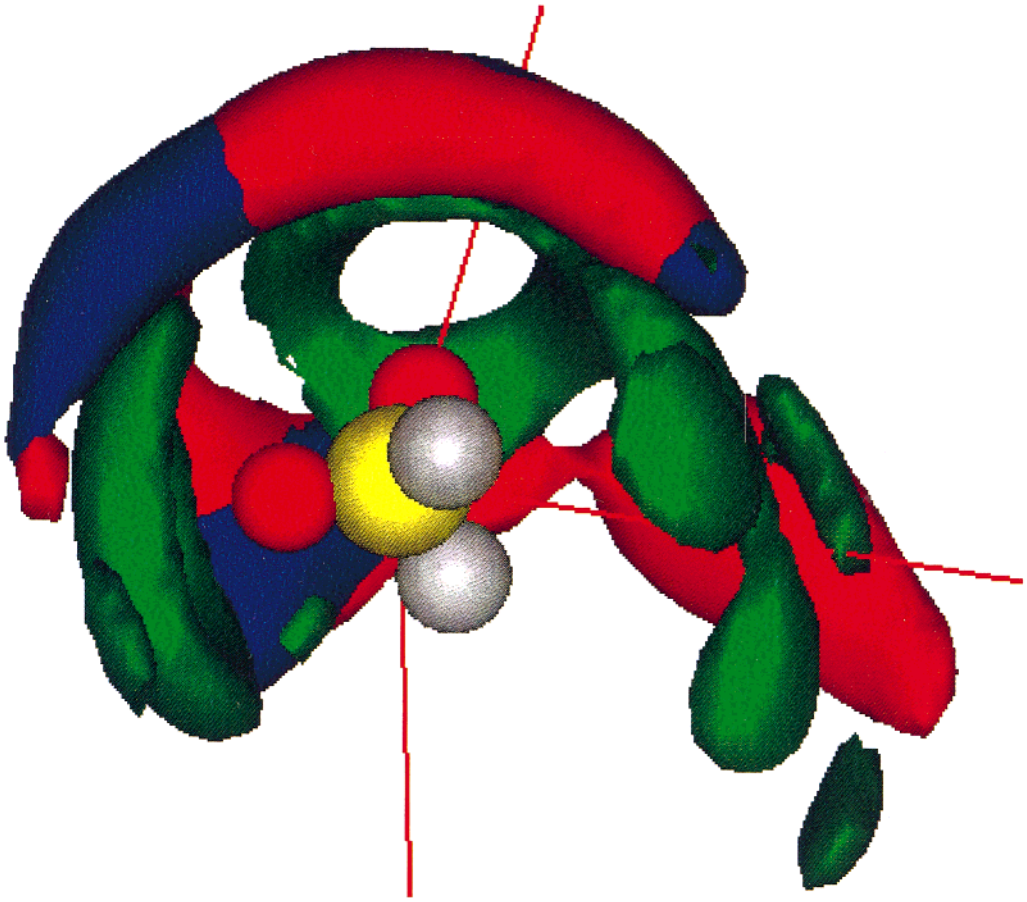

Figure 2. Ensemble average density isosurfaces of the lipid headgroup nitrogen atoms and the water oxygen atoms around a representative DNA phosphate group. The probability densities of the cationic lipid (DMTAP) and neutral lipid (DMPC) nitrogen atoms are drawn in red and blue, respectively, while the water density surface is drawn in green. The DNA phosphate group is at the center of the figure with the nonbonded and bonded oxygen atoms drawn as red and gray spheres, respectively.

from the latest version of CHARMM (all-hydrogen) for nucleic acids[27,28] and lipids.[29] The TIP3P model[30] was employed for water, which is consistent with the chosen lipid and nucleic acid force fields.

The initial configuration of the system was constructed in several stages. First, a configuration was taken from a well-equilibrated fully hydrated liquid crystalline bilayer phase of DPPC (dipalmitoylphosphatidylcholine).[31] Two end carbon atoms were removed from each hydrocarbon chain of the lipids and the resulting DMPC bilayer system was then equilibrated for 500 ps at constant temperature ($T = 50$ ° C) and pressure ($P = 0$) in a fully flexible simulation cell. This DMPC bilayer structure was used to build the initial DMPC-DMTAP mixed lipid configuration. Next, the dimensions of the simulation cell were adjusted to accord with the experimental values of the DNA inter-helical distance (d_{DNA}), the lamellar repeat distance (d_L), and the repeat distance along the DNA axis or the pitch of the helix (d_p). The x and z dimensions of the cell are $d_{DNA} =$ 36.8 Å and $d_L = 64$ Å, respectively, corresponding to the L_α^c phase at 50 °C,[10] while that for the y dimension was adjusted to $d_p = 34$ Å, corresponding to canonical B-DNA. The simulation cell was monoclinic in shape, with angle $\beta = 74°$. At this stage the simulation cell contained 44 DMPC lipids (22 per layer) and 1218 water molecules. Then, to neutralize the net charge of the anionic phosphate groups of the DNA, 20 DMPC lipids (10 per layer) were randomly changed to DMTAP by replacing the PC headgroups with TAP groups. Finally, the DNA duplex $d(CCAACGTTGG)_2$ was inserted between the two lipid layers with its axis parallel to the y direction by carefully removing any overlapping water molecules. The resulting simulation cell contains 24 DMPC, 20 DMTAP, 1003 water molecules, and the DNA duplex. The simulation system refers to the isoelectric point, as the number of cationic lipids was taken to be equal to the number of anionic phosphate groups of the DNA. Note that, there was no external counterion present in the system.

A short MD run of 10 ps was carried out keeping the DNA rigid. Next, the DNA was made flexible and the whole system was slowly heated to 50 °C over a period of about 60 ps. The final configuration from this run was then equilibrated for 1.4 ns at constant volume and a temperature of 50 °C. This equilibration period was followed by a NVT production run of 4 ns duration. The latter utilized the Nosé−Hoover chain thermostat extended system method. A recently developed reversible multiple time step algorithm[32] allowed us to employ a MD time step of 6 fs. This was achieved using a three-stage force decomposition into intramolecular (torsion/bend-bond), short-range intermolecular, and long-range intermolecular forces. During this decomposition the intramolecular forces were updated every 0.5 fs, whereas the short-range intermolecular forces were updated every 2 fs. Electrostatic interactions were calculated by using the particle-mesh Ewald (PME) method.[33] The minimum image convention[34] was employed to calculate the Lennard−Jones interactions and the real-space part of the Ewald sum, using a spherical truncation of 7 and 10 Å, respectively, for the short- and the long-range part of the force

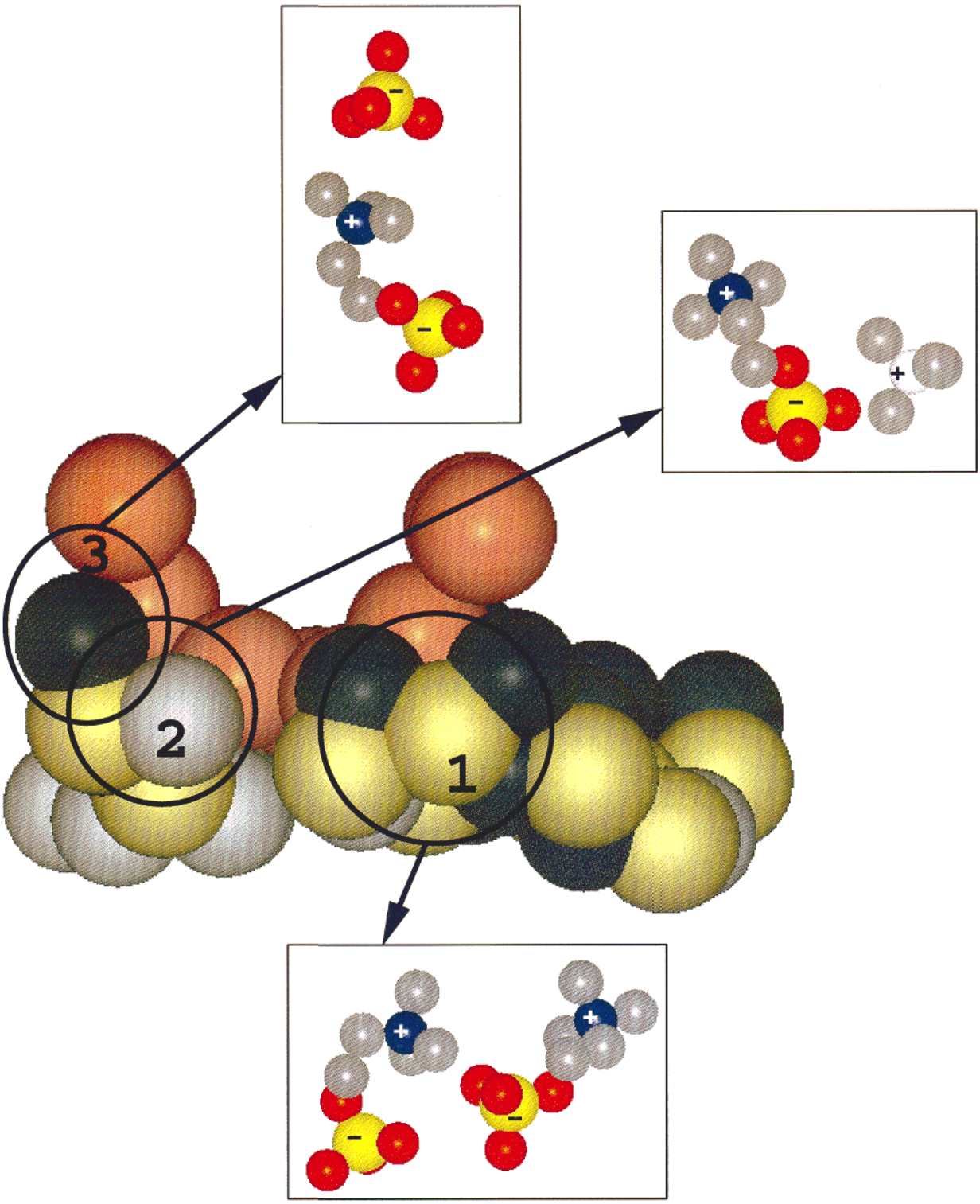

Figure 3. A snapshot from the simulation illustrating how $N(CH_3)_3^+$ (blue) and PO_4^- (yellow) of the zwitterionic PC headgroups and $N(CH_3)_3^+$ (gray) of the TAP groups interact with the phosphate groups (red) of the DNA. Region 1 shows $P^- - N^+ - P^- - N^+$ contacts between the PC groups. Region 2 shows substitution of N^+ of a PC group by the N^+ of a TAP group, while Region 3 demonstrates how such substitution leads to change in orientation of the PC group, bringing it into close proximity to the DNA phosphate. For clarity, the spheres are drawn with artificially enhanced radii of 3.6 Å (blue and gray) and 3.9 Å (red and yellow). The insets show the explicit arrangement of the atoms in the PC and TAP groups as well as the DNA phosphate group within the three regions. These inset atoms are colored as P, yellow; O, red; PC group N, blue; TAP group N, light gray; C, dark gray. Hydrogen atoms and water molecules are omitted for visual clarity.

decomposition. SHAKE/ROLL and RATTLE/ROLL methods[32] were implemented to constrain all bonds involving H atoms to their equilibrium values. To maintain one-dimensional periodicity of the DNA strand, a special feature of the code was used, so that the end residues were bonded to the appropriate image residues across the boundary of the simulation cell.

3. Results and Discussion

The configuration of the system at the end of the run spanning about 5.5 ns is shown in Figure 1. It is evident from Figure 1 that the structure of the DNA in the complex remained stable over the entire simulation. The average root-mean-square

positional deviation of all the non-hydrogen atoms of the DNA structure was measured against the reference DNA structure in its canonical B-form, and found to be about 1.6 Å. Calculation of the mean-square displacement of the center of mass of the DNA showed that, it undergoes a slow translational diffusion in the bilayer plane (*xy*). The calculated value of the two-dimensional diffusion coefficient (D_{xy}) was found to be of the order of 10^{-7} cm^2 s^{-1}. This value of D_{xy} is of the same order as that reported from a recent experimental study on self-diffusion of DNA on the surface of a fluid lipid bilayer supported on a glass substrate.[35] The most interesting and important feature to note from Figure 1 is the structure of the lipid−DNA interface. It is clear that significant undulations developed near the lipid headgroup region of the interface as compared to the nearly flat interface of the lipid bilayer.

Knowledge of the lipid−DNA interface, particularly the spatial arrangement of the lipid headgroups around the negatively charged phosphate groups of the DNA, is likely crucial to understand the stability of such complexes. To gain insight into such three-dimensional distributions, we have calculated the local density isosurfaces of the lipid headgroup nitrogen atoms as well as the water molecules around the DNA phosphate groups. These distributions are illustrated in Figure 2. To generate the isosurfaces, first, the instantaneous positions of the atoms in question were replaced by normalized Gaussian distributions with a width of 0.4 Å, to obtain a smooth effective local density. A surface of the nuclear distribution was then obtained by linear interpolation of the contours generated over last 4 ns of the trajectory. The contours shown enclose approximately 50% of the total density.[36,37] The density isosurfaces reveal that a large fraction of the cationic lipids, are bound to the DNA phosphate groups either directly or bridged by water molecules. However, the most interesting feature is the significant population of the zwitterionic PC headgroup nitrogens of DMPC in proximity to a DNA phosphate group.

To gain more insight, we estimated the number of PC and TAP groups that are nearest neighbors to the nonbonded oxygen atoms of the DNA phosphate groups, by integrating up to the first minimum (∼5.5 Å) of the appropriate radial distribution function. We observed that on average there are approximately 0.3 TAP and 0.3 PC groups per nonbonded DNA phosphate oxygen atom within this distance. Thus, our results predict the existence of TAP and PC groups with about equal probability around the DNA phosphates. From a similar estimation, we found that there are roughly 2.5 water molecules per nonbonded DNA phosphate oxygen atom within a typical nearest neighbor distance of ∼3.3 Å, which confirms the existence of water molecules bridged between the lipid headgroups and the DNA phosphates.

The driving force behind the surprising distribution shown in Figure 2 is the attractive electrostatic interaction between the cationic TAP headgroup and the anionic phosphate of the zwitterionic PC headgroup of DMPC. In Figure 3 we show a snapshot from the simulation illustrating the lipid−lipid and lipid−DNA contacts (salt bridges). For clarity, we have shown only the N^+ and P^- atoms of the PC and N^+ atoms of the TAP groups corresponding to one monolayer and the phosphate groups of the DNA, which are close to that layer. Figure 3 clearly shows the existence of three distinct types of contact bridges as marked by circular regions. Region 1 shows a $P^- -N^+ -P^- -N^+$ type of bridge between the PC groups, typical of a pure lipid bilayer structure.[38] Similar bridged configurations are identified in Region 2, where the N^+ of the PC group is replaced by the cationic N^+ of the TAP group. The existence of such configurations unambiguously demonstrates the presence of strong electrostatic interactions between the PC and TAP headgroups. This leads to the formation of contact pairs of DMPC−DMTAP lipids with the P^- end of the $P^- -N^+$ dipole approximately at the same distance (∼4.5 Å) from the PC headgroup N^+ as from N^+ of the TAP group. Such interactions also induce a change in orientation of the $P^- -N^+$ headgroup dipoles of DMPC with the N^+ part pointing away from the bilayer plane and hence coming into more effective contact with the anionic DNA phosphate, as illustrated in Region 3 of the snapshot. The explicit arrangements of the atoms in these three regions are shown in the insets of Figure 3.

From our simulation, the average angle between the $P^- -N^+$ dipole vector and the bilayer normal was found to be ∼50° (± 8°). This finding is in good agreement with experimental results on similar lipid systems.[11,39] The NMR studies of Scherer and Seelig[39] showed that while the $P^- -N^+$ dipole of pure phospholipid membranes remains approximately parallel to the membrane plane, addition of cationic amphiphiles cause the N^+ end to change the orientation of the dipole by more than 30° toward the water phase. Recently, Zantl et al.[11] studied the thermotropic phase behavior of the DMPC/DMTAP−DNA complex and compared this with the neat binary lipid mixtures. Their results also indicate a need to invoke a strong interaction between the PC and TAP headgroups.

4. Summary

We have carried out a simulation of the isoelectric DMPC/DMTAP−DNA complex, using a current generation all-atom force field and a state-of-the art MD algorithm. On the time scale of the simulation the DNA structure remained stable in the lipid environment. The most significant observation was the existence of strong interactions between the cationic TAP and zwitterionic PC headgroups of the two lipids. This in turn allows the PC headgroups to come in close proximity to the DNA and effectively compete with the cationic lipids in neutralizing the anionic phosphate groups. This observation gives direct support to interpretations based on a number of recent experiments.[7−13] Additional MD simulations are under way in our laboratory to investigate larger systems. This will enable us to focus on several crucial issues, such as, the equilibrium DNA spacing and correlations between the DNA rods in adjacent lattices.

Acknowledgment. The authors gratefully acknowledge Alex MacKerell for providing us the latest version of the all-atom CHARMM force fields and Doug Tobias for providing a DPPC bilayer configuration. We also thank J. Rädler for many stimulating discussions and for providing us his pre-prints. We are also grateful to Tom Lubensky and C. R. Safinya for their suggestions and critical comments about the manuscript. This research was supported by generous grants from the NIH and NSF.

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