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Attractive Hydration Forces in DNA-Dendrimer Interactions on the Nanometer Scale

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Abstract

The energetic contribution of attractive hydration forces arising from water ordering is an interesting but often neglected aspect of macromolecular interactions. Ordering effects of water can bring about cooperativity in many intermolecular transactions, in both the short and long range. Given its high charge density, this is of particular importance for DNA. For instance, in nanotechnology, highly charged dendrimers are used for DNA compaction and transfection. Hypothesizing that water ordering and hydration forces should be maximal for DNA complexes that show charge complementarity (positive-negative), we present here analysis of water ordering from molecular dynamics simulations and free energy calculations of the interaction between DNA and a nanoparticle with a high positive charge density. Our results indicate not only that complexation of the dendrimer with DNA affects the local water structure, but also that ordered water molecules facilitate long range interactions between the molecules. This contributes significantly to the free energy of binding of dendrimers to DNA and extends the interaction well beyond the electrostatic range of the DNA. Such water effects are of potentially substantial importance in cases when molecules appear to recognize each other across sizable distances, or for which kinetic rates are too fast to be due to pure diffusion. Our results are in good agreement with experiments on the role of solvent in DNA condensation by multivalent cations and exemplify a microscopic realization of mean-field phenomenological theories for hydration forces between mesoscopic surfaces.

Keywords

Hydration forces; DNA dendrimer interactions; free energy calculations; molecular dynamics simulations

Introduction

Water is essential to nearly all reactions involving biological molecules, and yet the role of solvent is often overshadowed by that of the macromolecules themselves. This situation deserves reexamination, for instance, in the case of the genetic transactions of the cell. As a

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Supporting Information Available

A movie showing the dynamics of water ordering during simulation, as well as additional figures depicting dipole moments, ionic rearrangements and profiles for distinct dendrimer orientations are available as Supplemental Material. This material is available free of charge via the Internet at <http://pubs.acs.org>.

consequence of the highly negatively charged backbones of DNA duplexes, it has been suggested that reorganization of water molecules around DNA in the presence of cations may facilitate interactions between DNA segments even more than bridging by ions or bending of the DNA.¹ Ordered waters have also been implicated in long range interactions between lipid bilayers,^{2,3} in facilitating binding of charged proteins to DNA,⁴ and in modulating a host of other crucial association events in adhesion of bio-materials and tissue response.⁵ In the colloidal and surface-science literature such water-ordering mediated interactions have long been coined with an overall phenomenological term: hydration forces (see Ref.⁶ for a review).

There is evidence accumulating from biophysical experiments that hydration forces between charged macromolecules may be dominant in the nanometer range. The standard theoretical treatment of colloidal particles in aqueous electrolyte solutions that goes beyond the Debye-Huckel model is the so-called Derjaguin-Landau-Verwey-Overbeek (DLVO) theory.^{7,8} According to DLVO theory, it has been thought that interaction between nanometer-scale particles can be well described by electrostatic and electrodynamic (i.e., van der Waals) forces, that is, with a Poisson-Boltzmann (PB) theory. In PB approaches, electrostatic forces dominate at long inter-particle distances, while van der Waals interactions operate at the relatively shorter range. In these calculations, water is treated as a smooth medium with a continuous dielectric function. However, experiments on both biological molecules and colloidal particles show behavior that does not fit with PB equations in the 10–20 Å range. At separations less than a few nanometers the forces between colloidal particles become more complex and even oscillate.^{9,10} Repulsive forces measured between lipid bilayers and attractive forces measured between DNA molecules are significantly stronger than expected from electrostatic interactions in this same range.⁶ In order to reconcile the experimental data with traditional electrostatic theory, it is necessary to consider the effects of the local solvent structure (i.e., structural water ordering) at these distances. Early phenomenological modeling to explain these findings by implicating water ordering was proposed by Marcelja and Radi^{11,12} and involved a mean-field free energy expansion in terms of a water order parameter. It has also been proposed that the nanometer range forces, both attractive and repulsive, are due to hydrogen bonding networks in the solvent induced by the surfaces of the interacting molecules.^{6,10} Determining the nature of these hydration networks is potentially essential for a complete understanding of biomolecular interactions.

Numerous studies in the past three decades have focused on the mechanism of DNA condensation by multivalent cations, ranging from small di-, tri-, and tetra-valent ions^{13–16} to DNA-binding proteins^{17–21} and large nanoparticles with multiple charges such as PAMAM dendrimers.^{22–24} Such condensation is necessary for packing of DNA into viruses²⁵ and, in nanotechnology, it mediates the transfection of therapeutic genes into target cells.^{26,27} Possible contributions to DNA condensation include ion bridges between adjacent DNA strands,^{24,28} collapse of the DNA backbone due to neutralization of the phosphate ions,^{29–31} bending or wrapping of DNA around large proteins or nanoparticles,²² and rearrangement of solvent molecules around the DNA.¹ This last mechanism, the effect of water structure on condensation, has been the least well-characterized. DNA is known to order approximately three layers of solvent, causing the water molecules to orient so that their dipoles lie along the direction of the DNA's electric field.^{32,33} These ordered waters lead to strong repulsive hydration forces between DNA strands.³³ Two decades ago, Rau and Parsegian had proposed that rearrangement of these solvent molecules was responsible for an observed decrease in the decay length of repulsive hydration forces between DNA strands in the presence of polyvalent cations, and that this rearrangement could even lead to attractive hydration forces between DNA strands.¹ Further studies by Todd et al.³⁴ found that the attractive hydration force between cation condensed DNA strands was

approximately twice the repulsive force. Attractive hydration forces have also been detected between lipid bilayers,^{2,3} and between collagen helices.³⁵

We describe in this paper evidence from molecular dynamics simulations that water plays an integral role in the condensation of DNA by PAMAM dendrimers, both in the long range interactions between the DNA and dendrimers, and in the effect of the interaction on the local water structure around the DNA-dendrimer complex. Polyamidoamine (PAMAM) dendrimers are large branched polymers whose size and charge can be easily controlled by adding successive branches and conjugating chemical groups to the terminal amines.^{36,37} Like DNA, large generation dendrimers (i.e., highly charged dendrimers) have been shown to have an affect on the orientation of surrounding waters.³⁸ Given the high positive charge density on amine-terminated dendrimers at physiological pH, it is possible that ordered waters could facilitate interactions between dendrimers and other charged molecules, such as DNA. Here, potentials of mean force (i.e. free energy profiles) were calculated for the interaction between generation-3 PAMAM dendrimers and a 24 base-pair double strand of DNA using the umbrella sampling method.³⁹ Simulations were run using one dendrimer with positively charged amines on all 32 terminations and one with half of its amine terminations randomly replaced with neutrally charged acetamide groups. Analysis of the behavior of the waters in our systems indicates that not only do both dendrimers affect the solvent shell of DNA when bound, but that positive hydration forces contribute to the free energy of the interaction between the all-amine dendrimer and DNA at large distances. These hydration forces extend the interaction between the molecules by approximately 10 Å and account for nearly a third of the total interaction energy, suggesting that long-range hydration forces could be a key component of molecular recognition in interactions between highly charged molecules. Comparison to experiments on DNA condensed by multivalent cations indicates that the free energy contributions of ordered waters obtained from our simulations are in the range expected based on the measured values. We also make connections between the results of our simulations and a mean-field phenomenological model describing the interaction contributions of water ordering.

Simulation Methods

Dendrimers were generated using parameters obtained as described in C. V. Kelly et al.⁴⁰ The amine terminations for both dendrimers were assigned a charge of +1. For the mixed-termination dendrimer, 16 of the 32 terminations were chosen at random and the amine was replaced with a neutral acetamide group. The DNA sequence used was a two-times repeat of the Drew-Dickerson sequence.⁴¹ The DNA-dendrimer systems were fully solvated using TIP3P water molecules⁴² in a rectangular box with a minimum of 14 Å padding around the molecules; 46 sodium ions and either 32 or 16 chloride ions were added to balance the charge of the DNA and dendrimer. Simulations were run in NAMD with the CHARMM 27 parameter set⁴³ with a 2-fs time step, using constant number of particle, volume and temperature conditions and periodic boundaries. Electrostatics were calculated using the particle-mesh Ewald method.⁴⁴ Non-bonded interactions were truncated with a cutoff of 14 Å and a switching function at 8 Å.⁴⁵ The systems were minimized for 1000 steps of steepest-descent minimization with the DNA and dendrimer held fixed and then for 4000 steps of the adopted basis set Newton-Raphson method with decreasing harmonic restraints on the dendrimer and DNA. The system was then equilibrated for 50 ps with a harmonic restraint of 0.5 kcal/mol/Å² applied to the heavy atoms of the dendrimer and DNA, so that only the solvent and ions were free to move. The systems were equilibrated for another 50 ps with a temperature coupled to a heat bath of 300 K under constant volume conditions with no restraints. In all simulations the base pairs at the ends of the DNA segment were restrained with a harmonic potential to prevent fraying.

For umbrella sampling simulations, the reaction coordinate used was the distance between the center of mass of the dendrimer and the center of the DNA, defined as the center of mass of the middle two base pairs. A harmonic potential with a force constant of $2.5 \text{ kcal/mol/\AA}^2$ was applied to this reaction coordinate. The series of windows for umbrella sampling were centered at reaction coordinate values from 12–60 $\text{\AA}$, starting from the initial structures and progressing in 1 $\text{\AA}$ increments sequentially (the last configuration in each window was used as the starting configuration of the next window). To increase sampling accuracy, two parallel set of simulations were run with 2×200 ps per window and a total number of 98 windows for each of the two dendrimer types. Potential of mean forces (PMFs) (i.e., free energy profiles along the interaction coordinate) were calculated using the weighted histogram analysis method.^{46,47}

To test whether water effects seen in the umbrella sampling simulations were not artifacts of the umbrella sampling constraints, 20 unconstrained simulations were also run on the all-amine dendrimer-DNA system. The simulations were run with initial center-of-mass (COM)-to-COM distances between 49 and 51 $\text{\AA}$. Simulations were run for 1 ns and if contact had not been made between the molecules within that time, for an additional 1 ns.

Results

Potentials of Mean Force

Figures Figure 1A and B show the starting structures for the two systems at 50 $\text{\AA}$. The amine terminations are highlighted in red and acetamide terminations are highlighted in blue. The potentials of mean force as a function of the COM to COM distance are shown in panel C. If the interaction between the dendrimer and DNA were due only to the electrostatic interactions between the positively charged dendrimer amine terminations and the negatively charged DNA phosphates, then one would expect that replacing half of the dendrimer terminations with neutral acetamide groups would reduce the magnitude of the interaction free energy by a factor of two, roughly. However, this was not the case in our simulations. The total free energy change for the all-amine terminated dendrimer is -13.5 kcal/mol , whereas the free energy change for the amine-acetamide dendrimer is -4.6 kcal/mol . This is a factor of almost 3. The all-amine terminated dendrimer also interacts with DNA at much longer distances than the mixed termination dendrimer. These differences are even more apparent when the free energies are plotted as a function of the shortest distance between the dendrimer and DNA (Fig Figure 1D). The PMF for the all amine-dendrimer has three distinct regimes, while the amine-acetamide dendrimer has only two. In the first of these regimes, at hydrogen bonding distance of 1.9–5.0 $\text{\AA}$, the all-amine dendrimer has a free energy change of -8.4 kcal/mol and the amine-acetamide dendrimer has a free energy change of -3.8 kcal/mol . In the second regime, between 14.0 and 5.9 $\text{\AA}$ the free energy changes are -3.0 kcal/mol and -1.7 kcal/mol , respectively. At these short distances, the free energy of the all-amine dendrimer is close to twice that of the amine-acetamide dendrimer, as expected. The bulk of the difference lies at large distances, in the region between 17.2 and 22.3 $\text{\AA}$. In this third regime, the free energy change for the all-amine dendrimer is -4.3 kcal/mol , while the amine-acetamide dendrimer shows no change.

We have used a particle-mesh Ewald summation method that in principle accounts for an infinite range of interactions with no cut-off. Nilsson and coworkers have studied extensively the range beyond which electrostatics make little contribution to stand-alone DNA molecules in solution. It was found that a cutoff of at least 12 $\text{\AA}$ is required to maintain the structure of B-DNA, but increasing the cutoff through 18 $\text{\AA}$ does not improve the results.⁴⁸ Consequently, we expect little effect from electrostatic interactions beyond a minimum distance of 14 to 16 $\text{\AA}$. Figure Figure 2A shows the electrostatic potential between the two molecules as a function of center-to-center distance. The energy was calculated

using snapshots of just the DNA and dendrimer from the simulations and does not take into account screening effects from water and ions. In reality the energy would be much lower due to these effects. The electrostatic attraction between the molecules decreases approximately as a function of distance squared, as illustrated by the black lines. The electric energy change for the mixed termination dendrimer is approximately half that for the all-amine dendrimer, exactly as expected. Interestingly, while interactions at large distances (from 40 – 50 Å) account for –4.3 kcal/mol of the free energy for the all-amine dendrimer-DNA system, nearly a third of the entire free energy change, the electrostatic energy change in this region is only about one tenth to one fifth of the total change. The correlation between the free energy change and electrostatic potential energy is plotted in Figure Figure 2B. For the mixed termination dendrimer, this correlation is linear, with a slope of 0.0012. For the all-amine dendrimer (red points in Fig. Figure 2B, the first part of the dependence can also be fit to a line with slope 0.0012. However, the last part of 4.2 kcal/mol of free energy, which corresponds to long distance interactions, shows an interesting upward trend that does not correlate similarly with the electrostatic energy as the first part: a large vertical jump in free energy (3.4 kcal/mol) is apparent at long distances, and the initial correlation with the electrostatic energy is lost. We attribute this final free energy contribution to the hydration forces, i.e., long range interactions facilitated by ordered waters.

Evidence of Ordered Waters between Dendrimers and DNA

As described above, there is little effect that is expected from electrostatic interactions beyond 14 Å from individual charges (for this system COM-to-COM distances of about 40 Å) and yet nearly a third of the total free energy change for the all-amine dendrimer occurs at distances beyond the expected electrostatic range of these simulations. These interactions exist instead in the range at which previous studies predict that hydration forces become important.^{6,10} Contour plots of the solvent-site dipole moment as a function of COM-to-COM distance between the dendrimer and DNA are shown in Figure Figure 3. The solvent-site dipole moment is calculated by dividing the system into a grid with spacing 3.0 Å × 3.0 Å × 3.0 Å and taking the average dipole moment of all the waters to occupy a cube on the grid during the 200 ps simulation for each umbrella sampling window. In bulk water, these dipole moments should average to 0.0. A nonzero dipole moment indicates that the waters in a given position are orienting in a certain direction due to an electric field.

The DNA has a dramatic effect on the water molecules, as evidenced by a bright ring of strongly ordered waters within approximately 9.2 Å, or three water layers, wide. Furthermore, the ordering effect appears to be propagated, to a lesser degree, for another 6.2 Å, or approximately two layers of water. The all-amine dendrimer also has an ordering effect on 1–2 water layers, though it is not as strong as the DNA's. The mixed termination dendrimer, on the other hand, shows almost no evidence of ordering water. The contour plots show a bridge of strongly ordered waters beginning to form between the all-amine dendrimer and DNA at distances as great as 60 Å; see Supplementary Information for a video of the vectors of ordered water dipoles during the interaction. This effect is particularly strong between 50 and 40 Å. There is no such bridge in the amine-acetamide dendrimer system. Interestingly, however, the ordered water shell around the DNA is disrupted by both dendrimers in the complexed structure, even on the side facing away from the interaction site.

Figure Figure 4 shows a side view of the dipole vectors for solvent sites around the DNA and dendrimer systems over several center-to-center distances. Only those sites with a dipole moment greater than 2.0 Debyes are shown. The presence of ordered waters between the branches of the all-amine dendrimer and DNA is clearly visible. We also quantified the extent of the polarization of waters between the dendrimer and DNA via the average dipole

moment per water molecule calculated for a box between the molecules of volume $30 \text{ \AA} \times 30 \text{ \AA} \times$ as a function of both COM-to-COM distance and minimum distance (Supplementary Figure 2). The dipole moment is considerably higher for the all-amine dendrimer, although both systems show an interesting pattern in which the dipole moment peaks at a center-to-center distances of 47 and 36 and minimum distance of 21 and 8, with a decrease in between these distances.

Connections to Theoretical Calculations of Hydration Forces

According to the order parameter formalism of hydration forces developed by Marcelja and Radi^{11,12} –a phenomenological theory that involves a Landau expansion of the free energy density functional in powers of the order parameter profile and its gradient– the repulsive and, respectively, attractive hydration pressure between two homogeneous surfaces are

$$P_{\text{rep}} = \frac{R}{4\sinh^2(L/\lambda)} \quad \text{and} \quad P_{\text{attr}} = -\frac{A}{4\cosh^2(L/\lambda)} \quad (1)$$

where L is the distance between the surfaces, λ is the characteristic decay length for water ordering, and R and A are force coefficients which incorporate the extent of water ordering at the surfaces. For separation distances L much greater than λ , these interactions become exponentials $P_{\text{rep}} = Re^{-L/\lambda}$ and $P_{\text{attr}} = -Ae^{-L/\lambda}$. While these are adequate for ideally complementary surfaces, for systems of more complex geometry, in the long length limit a geometry dependent prefactor may be required, as done in Refs.^{1-3,34,35} By definition, the PMF of a system is equal to the integral of the mean force. Therefore, per unit area, the relationship between hydration force and their free energy contribution can be described^{2,49} by

$$P_{\text{rep}} + P_{\text{attr}} = \frac{d\Delta G_{\text{hyd}}}{dL}. \quad (2)$$

Since the surfaces in our system are complementary, i.e. the ordering of water between the molecules is in the same direction at both surfaces, we can assume that the attractive force is significantly larger than the repulsive force.¹ Additionally, the region in which we assume attractive water forces dominate is at an intermolecular distance of 17–22 $\text{\AA}$, which we expect to be much greater than λ . Therefore, we can simplify the formula for the free energy contribution for our system to

$$\Delta G_{\text{hyd}}(L) = -\int_{\infty}^L Ae^{-(L'/\lambda)} f(L') dL' \quad (3)$$

where $f(L')$ is a geometry-dependent function that describes the structures of the two surfaces.

The mean force for the all-amine dendrimer, calculated by taking the numerical derivative of the PMF is shown in Figure 5. At the distances observed in our simulation, the effects of geometry appear to be negligible, thus the region which we attribute to attractive hydration forces, 40–51 is exponential in shape, as predicted by the order parameter model (Figure 5 inset). The exponential fit gives a value of $345 \pm 5.78 \text{ pN}$ for A and $9.0 \pm 0.5 \text{ \AA}$ for λ . This λ value is approximately twice that calculated from experiments on the interaction between DNA strands condensed by small multi-valent cations, which find λ values ranging from 4.0–5.0 $\text{\AA}$.^{1,2,34} This may be due to the fact that we have two surfaces with complementary charges, which reinforce each other's effect on water structure, as

opposed to the experiments cited above, which involve two strands of DNA with some of their charges neutralized by multivalent cations. It is also possible that our simulations overestimate λ due to errors in the water model, which are discussed in the next section.

Measurements of DNA condensation by cations predict a free energy contribution of attractive hydration forces falling between the limits of 1 kcal/mol/basepair between DNA strands,¹ and 2 kcal/mol/basepair in the case where the charges are completely complimentary. Our calculation of a contribution from attractive hydration forces of 3.4 – 4.3 kcal/mol per the 3–4 basepairs with which the dendrimer interacts directly falls nicely in between the above two limits and is in good agreement with theoretical predictions. Furthermore, ITC experiments on DNA interactions with poly(amidoamine) polymers indicate that both electrostatic and non-electrostatic interactions contribute to binding.^{50,51} In these studies it was presumed that the non-electrostatic contributions were direct hydrogen bond pairs between the molecules. Our results demonstrate that hydrogen bond networks in the solvent between the molecules are another potential candidate for this energetic contribution. Experiments such as the ones described in references^{1,2,34} could potentially be used to verify our free energy contribution estimations.

Discussion

While different water models reproduce various experimental parameters well, no water model perfectly replicates the behavior of water in experiments entirely. For example, while the TIP3P model used herein has a dielectric constant close to the experimentally determined one,⁵² it has an artificially high correlation between adjacent dipoles, as estimated via the Kirkwood correlation factor.⁵³ This tendency may lead to an exaggeration of the effect of a local electric field on the orientation of waters. While it is therefore possible that our simulations overestimate the contribution of water to the system, we believe that the difference between the all-amine dendrimer and the amine-acetamide dendrimer is too substantial to be a mere artifact of the model. Furthermore, the good agreement with experimental measurements indicates that the behavior of waters in our simulations are likely a good approximation of reality.

We have also considered the possibility that the way our simulations were run could contribute to an artificial ordering of waters. The distance between the dendrimer and DNA is harmonically constrained in each umbrella sampling window, however, the waters and ions are free to move. To test whether artificially holding the molecules apart could lead to the formation of ordered water structures via hypothetical kinetic trapping intermediates that would not form under normal conditions, we ran a series of 20 constraint-free simulations on the all-amine dendrimer and DNA starting from a center-to-center distance of 50 Å. If the average dipole moment of the waters between the molecules for all these simulations is compared to our umbrella sampling data, it is considerably lower. However, slightly over half of the DNA-dendrimer systems in these simulations complex very quickly, within 1 ns, while the rest take 1.5 ns or longer. If we separate the “fast” simulations from the “slow” ones, we find that the faster simulations have a inter-molecule dipole moment that closely matches that of the umbrella sampling simulations, while the slower ones have a lower, roughly constant dipole moment (see Supplementary Information Figure 3). Compared to the unbiased simulations, the umbrella sampling simulations appear to represent an ideal case in which the molecules remain oriented with respect to each other in a way that maximizes the ordering of waters between the systems. The results of the unbiased simulations indicate that the “percolation” of water orientation takes place on the sub-ns time scale, fast enough to be observable even without constraints on the DNA and dendrimer. The difference in water ordering for the fast and slow DNA-dendrimer

interactions also implies that these water-bridges have an effect on the rate of interaction between the dendrimer and DNA.

Full convergence of dendrimer internal rotations is difficult to achieve in our umbrella sampling simulations. Using simple Brownian rotation diffusion considerations for a spherical model of the dendrimer in solution, one can estimate the characteristic time for orientational de-correlation, i.e., the rotational correlation time, τ , as the average time it takes the dendrimer to rotate one radian as a function of the dendrimer size. By the Stokes-Einstein-Debye equation, we get $\tau = 4\pi\eta r^3/3k_B T$, where η is the solvent viscosity, r the (hydrodynamic) radius of the dendrimer, k_B Boltzmann's constant and T the temperature. This yields a time of approximately 4–5 ns/rad, or about 150–200 ns for a full rotation that exposes all the spherical surface of the dendrimer. The estimate is 3 orders of magnitude longer than the time for the PMF simulation to converge in each window. And this estimate for the rotational time is just a lower bound, at distances far away from DNA. When the dendrimer interacts with DNA, one expects energy barriers for rigid body rotations because of the asymmetry of the dendrimer. As the referee points out, the end point the dendrimer is bound in different orientations (see below). Thus one expects that these barriers can easily increase the overall tumbling time by another order of magnitude. Therefore full rotational sampling is indeed hard to achieve in the simulation because the time average in the simulation does not cover all relative orientations a bulk experiment would sample (the ensemble average). However, the major orientations of the dendrimer with respect to DNA are mainly of two types. The dendrimer, while roughly symmetrical in one dimension (say, along the longitude), is overall more ellipsoidal than spherical in the latitude. The interaction will be different for a “head-on” vs. a “side-on” orientation of the dendrimer with respect to the DNA helix (see Fig. 6 in Supplementary Material). This difference between the two different orientations has been quantified in our previous work.⁵⁴ Close to the DNA (see the 20–30 Å range), the two orientations lead to different PMF profiles because the binding geometry is different. However, at distances farther from the DNA (beyond 30 Å), the two orientations lead to essentially the same curves. This is the region of interest for the present study of hydration forces: the region of similarity includes the range 40–50 Å (see the jump in Fig. 1D, and see also Fig. 3A, specifically the 40 Å and 50 Å panels) which contains the signature of the hydration forces. It is true that the different relative orientations lead to different bound states. There are certainly several favorable binding states, since the dendrimer-DNA interactions are non-specific, and we expect all to contribute to the short-range ensemble average. While near-range details may vary from system to system (in the binding free energy, the various binding poses etc.) our study does show that in the longer-range hydration forces can be important whenever charged macromolecules are on-route to bind DNA nonspecifically. The importance of hydration forces, remain hence pertinent as the essence is to point to the general mechanism of water-mediated interactions.

It is interesting to note the implications of our findings in the case of protein-DNA interactions. Unlike dendrimers, which have a uniform charge distribution, many proteins which interact with DNA have solvent-exposed charged groups only in the DNA-binding region. If water-bridging is present in such systems, as simulations by Hamasaki et al.⁴ seem to indicate, the appearance of such bridges only when the protein is correctly oriented with respect to the DNA could facilitate binding of the molecules in the proper orientation. Furthermore, such water effects may also contribute to sequence recognition, since the hydration pattern of DNA has been shown to depend upon its sequence.^{55–59} Bridging by ordered waters could also help to further explain the high reaction rates for many biomolecular complexes in which the rate is diffusion limited. Electrostatic steering has been proposed as one possible explanation for observed rates,^{60–67} but electrostatic effects are not long range enough to adequately explain such behavior. Our results suggest that

ordered waters may extend interactions significantly beyond the electrostatic range and may have a similar steering effect, but at greater distances.

Conclusions

We have presented an analysis of the effects of ordered waters on the interaction between highly charged nanoparticles and DNA from umbrella sampling simulations. Water ordering was found to be a driving force for the interaction between the DNA molecule and the dendrimer with the higher charge-density. Ordered waters were found to form a bridge between the all-amine dendrimer and DNA at distances beyond which the molecules would be expected to interact directly through electrostatics. The mean force of interaction at these distances has an exponential behavior that is in good agreement with the phenomenological order-parameter formalism for hydration forces developed by Marcelja and Radi¹¹ and with experimental data on molecular interactions in the range at which the hydration effects are observed.^{6,10} Our simulations also confirm that large, multivalent cations can affect the arrangement of water molecules around DNA when in complex with it. This has implications for understanding the interaction mechanisms of many systems which involve molecules with high-charge density, such as DNA condensation by cations, nanoparticle interactions, and DNA-protein interactions. This is especially interesting in the case of DNA binding by proteins, as the results of our simulations suggest that, at least in some cases, water may not only facilitate long-range interactions, but also help to orient the protein appropriately with respect to the DNA. Future work should address whether this type of water ordering behavior is present in DNA-protein systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Starting structures for the dendrimer-DNA systems at 50 $\text{\AA}$. **(A)** All-amine terminated dendrimer with charge +32; **(B)** Dendrimer with half amine and half acetamide terminations (charge +16). Amine terminations are highlighted in red, acetamide in blue. Close-up of the chemical structure of the core of the PAMAM dendrimer and one branching iteration shown in-between. **(C)** Potentials of mean force for the systems as a function of the biased reaction coordinate, the center-to-center distance between the dendrimer and DNA. **(D)** Potentials of mean force as a function of the minimum distance (edge-to-edge) between the dendrimer and DNA.

Figure 2.

(A) Electrostatic interaction energy between the dendrimer and DNA as a function of distance. The energy decreases roughly as a function of distance squared (black lines). The total electrostatic energy change for the all-amine dendrimer from 52 to 17 Å is -12,323 kcal/mol, roughly twice the change for the mixed termination dendrimer, -6,216 kcal/mol. (B) Correlation of the free energy of the interaction with the electrostatic interaction energy between DNA and each of the two dendrimers (color coding same as in (A)). For all-amine dendrimer, correlation is roughly linear initially (for the first 9 kcal/mol of free energy). Ensuing, there is a large vertical jump in free energy at long distances (3.4 kcal/mol) that does not correlate with the electrostatic energy. The correlation for the mixed termination dendrimer is linear as well, with approximately the same slope as the first part of the all-amine dendrimer correlation, and a decorrelation/smaller jump in free energy at large distances.

Figure 3.

Top view down the DNA helical axis with contour plots of solvent-site dipole moments for (A) all-amine terminated (+32 charge) dendrimer and (B) amine-acetamide terminated (+16 charge) dendrimer; position of dendrimer and DNA marked by white arrows. Significant polarization (bright red-orange) of waters between the dendrimer and DNA are seen at distances of 40 to 60 Å for the all-amine terminated dendrimer only.

Figure 4. Sites of ordered waters. Red arrows represent electrical dipole moment vectors greater than 2 Debye for (A) the amine terminated dendrimer, and (B) the mixed-termination dendrimer.

Figure 5.

Mean force profile for the all-amine dendrimer. The region from 40–51, which corresponds to the attractive hydration forces (due to water ordering), is shown in the close-up in the inset. The mean force in this region fits an exponential of the form $Ae^{-L/\lambda}$, where $L = r - r_{\text{DNA}} - r_{\text{dend}}$ is the edge to edge distance (COM-COM reaction coordinate distance r minus the radii of the DNA and dendrimer), $A = 345 \pm 5.78$ pN and $\lambda = 0.9 \pm 0.5$ Å.