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COMPLEXATION OF DNA WITH CATIONIC POLYMERS

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ABSTRACT. Polyethylenimine (PEI) represents the most extensively used non-viral vector for gene delivery. The complexation between nucleic acids and PEI chains is intimately related to electrostatic interactions of the positively charged amine groups with the negatively charged phosphate groups. All-atom molecular dynamics simulations of alternatively protonated PEI chains, DNA and, respectively, polyplexes thereof in solution were performed. Our results reveal an increase in gyration radius of solvated PEI chains in the presence of DNA. In order to understand the major changes in DNA properties, the impact of PEI chains on the ionic environment of DNA is described in detail. In addition, the amine-phosphate contact analysis provides valuable insight into the formation mechanism of PEI/DNA complexes.

Keywords: *polyethlenimine, cationic polymers, molecular dynamics simulations, PEI/DNA polyplexes, gene delivery systems.*

INTRODUCTION

Gene therapy is designed to introduce nucleic acids into cells for the treatment of cancer or other genetic diseases and the development of effective delivery vectors is one of the central challenges. Current gene delivery vectors are divided into two major types, viral and non-viral, with the latter ones being less immunogenic and toxic [1-3]. The use of cationic polymers as non-viral systems to condense nucleic acids into polyplexes represents a promising therapeutic strategy, and, polyethylenimine (PEI) is one of the most versatile carrier system of this class [4]. In spite of major recent advances in the development PEI-based delivery systems, there still persist numerous open questions concerning the formation of PEI/DNA polyplexes.

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Complex all-atom molecular-dynamics (MD) simulations have been employed in the last decade to elucidate the structure and properties of PEI/DNA complexes. Essentially, it was shown ([5]-[6]) that the DNA-PEI complexation is controlled by the attractive electrostatic interaction between the protonated amine groups of PEI and the phosphate groups of DNA. Ziebarth et al. [7] studied the structural differences between DNA and siRNA, and their role in the stability of PEI/DNA complexes. It should be noted, however, that the general AMBER force field [8] they have used in the simulations does not provide specific parameters for PEI.

RESULTS AND DISCUSSION

In order to study the complexation dynamics of DNA with PEI polymers, the initial structure of DNA was constructed using the Nucleic Acid Builder [9] via server http://structure.usc.edu/make-na/server.html. Specifically, a DNA strand composed of 42 bases was built in the canonical B form. In the simulations, we considered alternatively protonated linear PEI chains composed of 20 monomers. We performed three types of simulations, respectively, (a) solvated DNA strands, (b) solvated PEI chains, and (c) solvated DNA-PEI mixtures. The initial configurations for these simulations respectively comprise (a) a DNA 42-mer with the base structure described in Table 1, (b) six linear PEI chains with the mass centers forming a hexagon, and (c) a DNA strand with the center of mass placed at the PEI hexagon center and aligned parallel with the PEI chains.

Table 1. Simulated configurations

PEI			DNA		Solvation		
Size/ Prot.Frac	**No. of chains**	**No. of atoms**	**Sequence**	**Helix type**	**Water molec**	**Na^+**	**Cl^-**
20-mer 1/2	6	186 each PEI chain			31206	88	148
			CGCGAATTCGCGATATCCCGG CCGGGATATCGCGAATTCGCG	B	31489	129	89
20-mer 1/2	6	186 each PEI chain	CGCGAATTCGCGATATCCCGG CCGGGATATCGCGAATTCGCG	B	30562	86	106

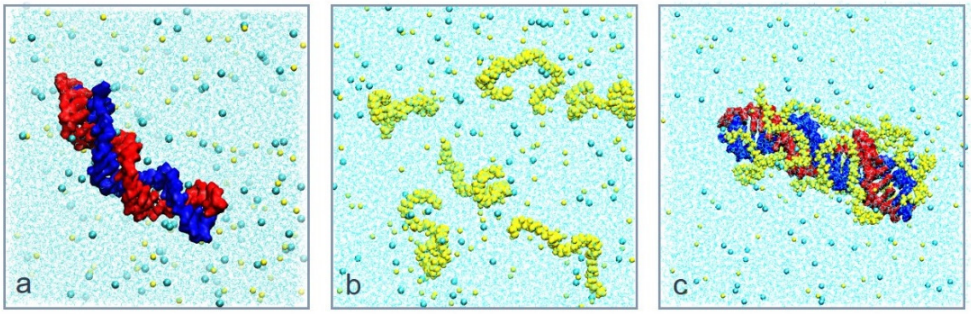

Figure 1. Intermediate configurations of a typical trajectory for: a) DNA, b) 6 alternatively protonated PEI chains, c) PEI/DNA complex.

The systems were solvated into a rectangular water box of size 100×100×100 $Å^3$, which results in a total of approximately 30000 water molecules in each system. The exact number of water molecules and neutralizing counterions are summarized in Table 1.

Isothermal-Isobaric (NPT) runs of 20 ns were performed to study the DNA behavior in solution (see Fig. 1a) and 30 ns to characterize the solvated PEI chains (Fig. 1b) and the PEI/DNA complex (Fig. 1c). All the snapshots from the simulations have been extracted using the VMD package [10]. The basic data for each simulated system are presented in Table 2.

Table 2. Details of molecular dynamics simulations

System description	No. of trajectories	Simulation length (ns)	PEI chains
PEI	4	30	6 PEI 20-mers (protonation 1/2)
DNA	3	20	
PEI/DNA complex	4	30	6 PEI 20-mers (protonation 1/2)

Figure 2 presents the time dependence of the gyration radius (R_g) for alternatively protonated PEI 20-mers in interaction with DNA. The initial linear PEI chains condense into random structures and bind to DNA. In calculating the average values of R_g, we discarded the first 5 ns of each run to allow for the complex to be formed. The average R_g of PEI chains interacting with DNA in solution is 12.65 Å, very similar with the average value of bare PEI chains (12.55 Å).

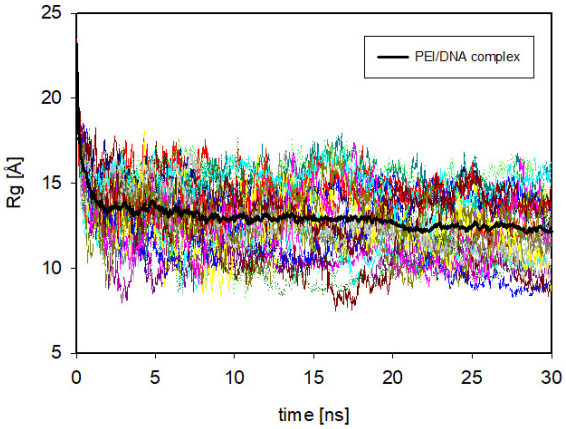

Figure 2. Time evolution of the gyration radius for 6 alternatively protonated PEI 20-mers interacting with DNA in 4 independent trajectories (black – average).

To suggest the average spatial extent of solvated PEI chains, both bare and interacting with DNA (PEI/DNA complex), probability distributions of the time-averaged gyration radius are comparatively presented in Fig. 3. Bare PEI (black curve) shows a single peak corresponding to a random folded conformation. The profile for PEI chains interacting with DNA (red curve) displays an additional peak at about 14 Å, which corresponds to PEI chains that bind to DNA (PEI/DNA complexes).

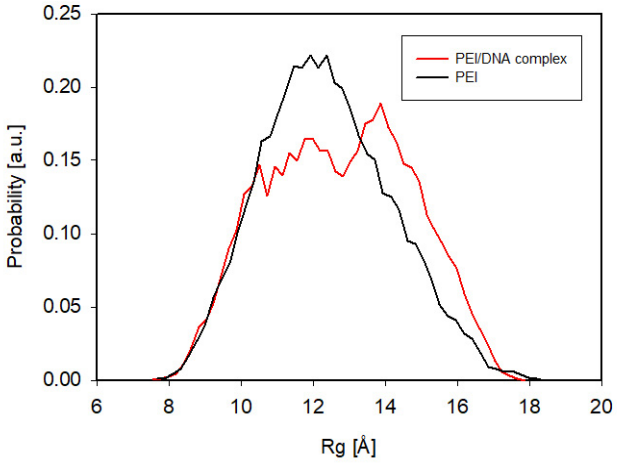

Figure 3. Probability distributions of radius of gyration for alternatively protonated PEI 20-mers (black – PEI chains in solution, red – PEI chains interacting with DNA in solution).

The binding patterns of the unprotonated vs. protonated nitrogen atoms of the PEI chains are depicted in Fig. 4. It is apparent that the number of protonated nitrogen atoms which are in direct interaction with DNA (within 4 Å), is double as compared to the number of unprotonated nitrogen atoms.

In order to characterize the electrostatics around solvated DNA, cumulative charge distributions of the solution counterions (Na^+ and Cl^-) are presented in Fig. 5, showing the amount of charge situated within a given distance from DNA. As expected, more Na^+ ions reside around bare DNA than around DNA complexed with protonated PEI chains. Conversely, the screening of the negatively charged phosphate groups by the protonated amine groups, enables complexed DNA to attract more Cl^- ions than in uncomplexed state.

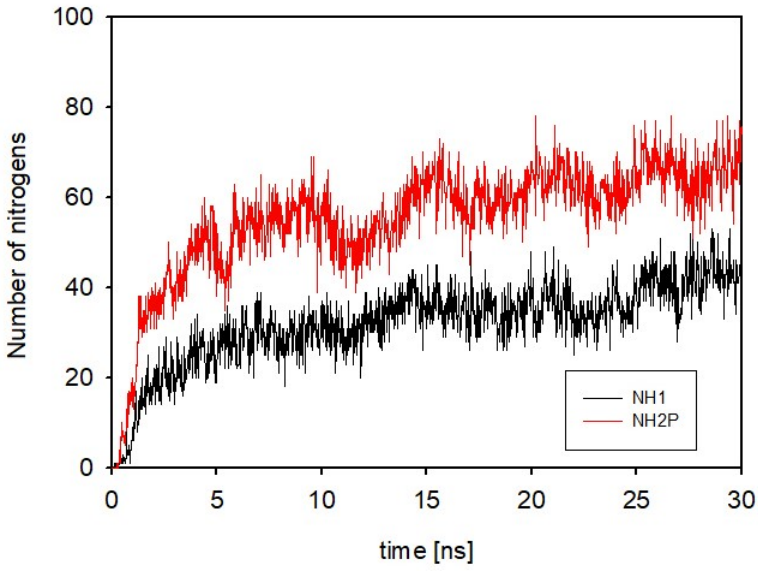

Figure 4. Time dependence of the ensemble-averaged number of unprotonated/protonated nitrogen atoms of PEI chains within 4 Å of any phosphorus atom of DNA**.**

Figure 6 presents the evolution in time of the ensemble-averaged number of Na^+ (orange) and Cl^- (cyan) ions situated within 4 Å of any atom of DNA (solvated DNA-PEI mixtures). A clear decrease in the number of Na^+ evidenced the screening effect of PEI chains on the negatively charged phosphate groups of DNA.

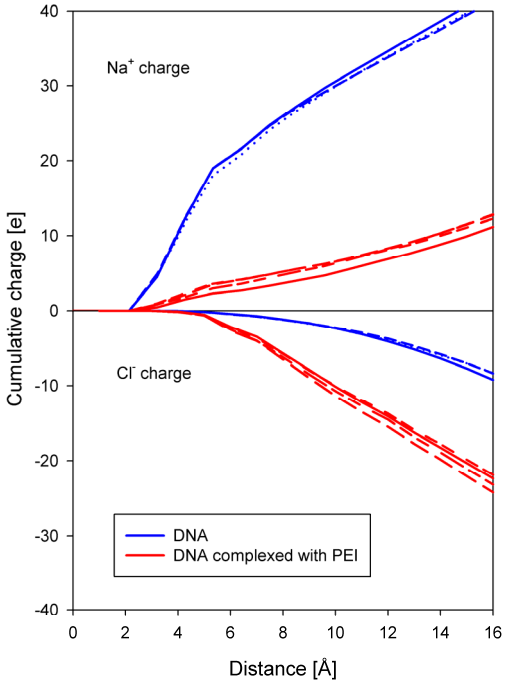

Figure 5. The cumulative charge distributions of the counterions in the vicinity of DNA (blue – bare DNA strands, red – DNA complexed with PEI chains). The curves correspond to the different trajectories.

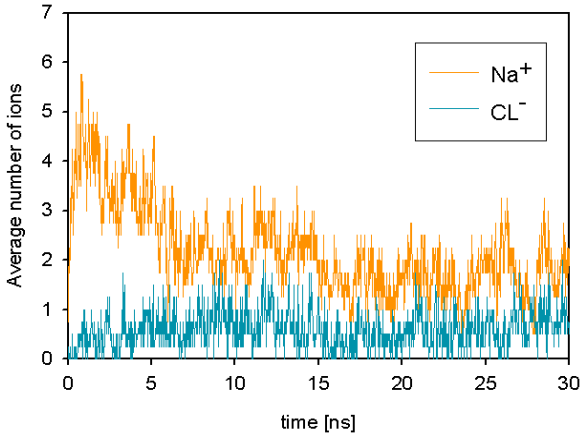

Figure 6. Time dependence of the ensemble-averaged number of counterions within 4 Å of any atom of DNA (solvated DNA-PEI mixtures).

CONCLUSIONS

In this work we performed molecular dynamics simulations of alternatively protonated PEI chains, DNA strands and DNA/PEI polyplexes in solution. The results reveal a complex behavior of solvated PEI chains in the vicinity of DNA strands. Alternatively protonated PEI chains closely package solvated DNA strands, showing a considerably higher radius of gyration than in uncomplexed state.

The protonated amine groups of PEI strongly bind to the phosphate groups of DNA, roughly outnumbering the unprotonated amine groups residing on average in the vicinity of DNA by a factor of 2.

The screening effect of the protonated PEI chains on the phosphate groups of DNA is reflected by a clear decrease in the number of attracted sodium counterions.

EXPERIMENTAL SECTION

All molecular dynamics simulations were performed with the NAMD package [11] using the CHARMM36 Nucleic Acid force field [12], [13] in conjunction with the CHARMM force field that we have recently developed for protonated PEI chains [14] [18].

In all simulations we used a time step of 2 fs combined with the SHAKE algorithm [15], [16] to constrain covalent bonds involving hydrogen atoms to fixed lengths. A cutoff distance of 12 Å was used to treat the short-ranged Lennard-Jones interactions. The particle mesh Ewald method [17] was used to treat the long-range electrostatic interactions, using a discrete mesh with a spacing of 1 Å. The temperature was kept at 310K using a Langevin thermostat with a damping coefficient of 1 ps^{-1}, while the pressure was fixed at 1 atm using a Langevin piston.

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