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Supramolecular complexes of DNA with cationic polymers: The effect of polymer concentration

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ABSTRACT

Supramolecular complexes of DNA with cationic polymers are of tremendous importance as these polymers can successfully be used in gene therapy as delivery vectors of genetic material. In this work we employ atomistic molecular dynamics simulations to get an insight into the impact of polymer concentration on the structural and electrostatic properties of the complexes of DNA and cationic polymers. Four linear cationic polymers of different chemical structure and protonation state were studied: polyethylenimine (PEI), poly-L-lysine (PLL), polyvinylamine (PVA), and polyallylamine (PAA). For all considered polymers our computational findings clearly demonstrate that increasing the polymer concentration leads to the overcharging of the DNA molecule. This is relevant as the overall positive charge of the DNA-polycation complex is considered to be a prerequisite for efficient binding of the polyplexes to negatively charged cell membranes. However, the concentration effects themselves are found to be sensitive to the chemical structure of a polymer. In particular, flexible PEI chains, being characterized by the high affinity to DNA's phosphate groups, are able to bind to DNA in an independent manner. As a result, DNA and PEIs form compact complexes with the largest cumulative positive charge among all four types of cationic polymers under study. In contrast, PVA chains show the affinity to the major groove of DNA due to the hydrophobic nature of their backbones. This leads to stronger interactions with the DNA molecules and to the competition between PVA chains for DNA binding sites. This competition is responsible for the observed saturation in the PVA-induced neutralizing of the DNA charges when the PVA concentration increases. As for PLL and PAA, both these polycations are found to be less effective in neutralizing the charge of the polyanionic DNA molecules as compared to PEI and PVA. PLL has relatively long side chains, so that some of them cannot access DNA phosphates and reside in aqueous solution. In turn, PAA has a low protonation level, leading to a weak electrostatic binding to DNA. Overall, our findings can relate the properties of supramolecular DNA-polycation complexes at elevated polymer concentrations with the chemical structure of a polycation and therefore can be useful for the development of novel, highly efficient polycation-based delivery vectors of DNA.

1. Introduction

Gene therapy is largely based on introducing nucleic acids into the target cells. By their nature, nucleic acids (especially gene-encoding plasmid DNA) represent large polyanionic macromolecules and normally cannot be delivered into cells without delivery vectors (or vehicles). By far the majority of the delivery vectors have been of viral nature [1] and had therefore virus-specific safety issues such as high cytotoxicity and immunogenicity [2]. To overcome this, there has been a continuous search for non-viral alternatives [3]. Among others, water-soluble cationic polymers represent a very promising class of non-viral delivery vectors for gene therapy [4,5]. Cationic polymers are able to efficiently condense polyanionic nucleic acids through electrostatically-driven formation of supramolecular DNA/RNA-polymer complexes and can easily be designed to have a specific chemical structure and a charge distribution [4,6].

Numerous representatives of cationic polymers studied as non-viral delivery vectors include poly-L-lysine [7], poly-L-ornithine [8],

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poly-L-arginine [9], linear and branched polyethylenimine [10], poly (amidoamine) dendrimers [11], and poly (methacrylates) [12]. Polypeptide-based polymers such poly-L-lysine and poly-L-arginine, being biodegradable, are characterized by a relatively low transfection activity. In turn, polyethylenimine is often considered as the "gold standard" among cationic polymers as it combines both low toxicity and high transfection efficiency. The latter is normally attributed to the ability of polyethylenimine to change its protonation state within endosomes (in contrast to e.g. poly-L-lysine) [10]. To improve the transfection efficiency of pure polymers, copolymers of polycations have also been considered. In particular, block-copolymers of poly-L-lysine with polyethyleneglycol [13] and chitosan [14] as well as copolymers of polyethylenimine with polyethyleneglycol [15] and uranoic acid [16] were recently explored.

As in many other areas of research, computer modeling has successfully complemented existing experimental studies of cationic polymers as delivery vectors of nucleic acids [17]. To this end, atomic-scale molecular dynamics simulations are of much importance as they provide a detailed, molecular-level insight into the interactions of nucleic acids with polymers, which is not easily accessible through experimental techniques. Most relevant computation studies have considered complexes of DNA/siRNA with polyethylenimine (PEI) [18–21], poly-L-lysine (PLL) [18,21–25], and poly-L-arginine [26]. Although the primary focus of these studies was on atomistic details of binding patterns between nucleic acid and polycations, some work has been done on exploring the effects of polymer concentration [18,20,23,27] and even on polycation-mediated DNA aggregation [28,29]. In addition to formation of "nucleic acid - cationic polymer" complexes, atomic-scale computer simulations were also applied to study the opposite process, namely the DNA-polycation decomplexation via addition of divalent salt to aqueous solution [21,25].

Recently we employed atomic-scale MD simulations to systematically study formation of complexes of DNA with four linear polycations of different chemical structure and protonation: polyethylenimine (PEI), poly-L-lysine (PLL), polyvinylamine (PVA), and polyallylamine (PAA) [30]. The last two polycations, PVA and PAA, being polymers with hydrocarbon backbones and short side chains bearing amine groups, were examined experimentally in terms of their use for transfection delivery vectors [31,32]. Our simulations demonstrated that for most polymers (PEI, PLL, and PAA) their complexes with DNA were mainly stabilized by the electrostatic attraction between polymer's protonated amine groups and negatively charged phosphate groups of DNA in line with a number of previous computational studies [18,19]. However, for PVA we found an alternative binding pattern when a polycation gets embedded into the DNA major groove [30]. Note that the situation of a dilute polymer solution was considered in Ref. [30]: a DNA fragment interacted with a single polymer chain. In this paper we make the next step, namely, we vary systematically the polymer concentration in aqueous solution with DNA, so that binding of DNA with multiple polycation chains will be explored. This allows us to assess the effect of polymer concentration on the binding pattern of a polycation with DNA. Furthermore, elevating polymer concentration will make it possible to study overcharging of DNA-polycation complexes: the overall positive charge of the complex is believed to facilitate its binding to the negatively charged cell membrane and correspondingly enhance transfection activity [33].

2. Materials and methods

Atomic-scale molecular dynamics simulations were employed to study complexes of DNA with cationic polymers (CPs). Each simulated system consisted of a single DNA fragment and several chains of cationic polymers. We considered linear cationic polymers of four different types: polyethylenimine (PEI), poly-L-lysine (PLL), polyvinylamine (PVA), and polyallylamine (PAA), while a Dickerson's dodecamer (d (CGCGAATTCGCG)$_2$ [34,35] was used as a DNA fragment, see Fig. 1 for chemical structures of DNA and cationic polymers. An initial configuration of DNA was taken from Refs. [36,37]; a DNA fragment contains 12 base pairs and has the total charge of $-22e$ under physiological conditions. Based on experimental data available, the protonation level of polymers under the same conditions (pH 7) was set as follows: 100% for PLL, 50% for PEI [38,39] and PVA [40], and 20% for PAA [38]. As each linear cationic polymer consisted of 20 monomer units, this protonation level led to the total charge of $+20e$ for PLL, $+10e$ for both PEI and PVA, and $+4e$ for PAA. The distribution of protonated and deprotonated monomers for all four polymers can be found elsewhere [30].

An initial configuration of each simulated system was built up according the following protocol. A single DNA fragment was centered in the simulation box, and several copies of cationic polymers were placed around the DNA with a DNA-polymer distance no less than 1 nm (the distance was measured between closest DNA and polycation atoms). The DNA-CP system was solvated then with ~35000 water molecules, and DNA and polycation counterions (Na^+ and Cl^- ions) were added to the system for electroneutrality. The number of polymer chains in the system was varied in the range from 2 to 10, depending on the type of a polycation (or on the charge of a polymer chain) with the aim to achieve the polymer concentration at which the overall charge of cationic polymers exceeds the DNA charge, see Table 1 for the complete list of simulated systems. The total number of atoms in the DNA-polycation systems amounted to ~99500. Furthermore, for the sake of comparison, we considered a reference polymer-free DNA system that consisted of a Dickerson's dodecamer and 6815 water molecules (the system DNA in Table 1).

Similar to our previous work [30], the DNA-polycation systems were described in the framework of the AMBER family of force-fields. We used AMBER parmbsc0 force-field [41] for DNA and AMBER99 force-field [42] for cationic polymers. Partial charges for PLL were taken from the standard AMBER99 force-field, while for PEI, PVA, and PAA we used partial charges that were previously computed with the use of *ab initio* calculations [30,43]. Water was represented by the TIP3P model [44], and the ion parameters developed by Joung and Cheatham [45] were used for monovalent Na^+ and Cl^- ions.

The DNA-CP systems were simulated in the NPT ensemble at $P = 1$ bar and $T = 300$ K. We used the isotropic Parrinello-Rahman barostat [46] and the velocity-rescaling thermostat [47] to control pressure and temperature, respectively. The Lennard–Jones interactions were cut off at 1 nm. The particle-mesh Ewald method (PME) was employed to handle the electrostatic interactions [48]. Most DNA-CP systems were simulated for 500 ns with the time step of 2 fs, see Table 1 for details. Production runs were preceded by energy minimization and short runs with position restraints applied to DNA and cationic polymers. Structural characteristics were calculated over last 100 ns of the production runs (except for the DNA-PAA-10 system where last 50 ns were used for averaging). The Gromacs 4.6.5 suite was used in all simulations [49].

3. Results and discussion

To follow the kinetics of formation of a complex between DNA and cationic polymers, we calculated the distance between centers of masses (COM) of a DNA molecule and a polymer chain for each chain in the system. In our previous work we demonstrated that single chains of all four types of linear polycations (PLL, PEI, PAA, and PVA) were able to form stable complexes with DNA on a time

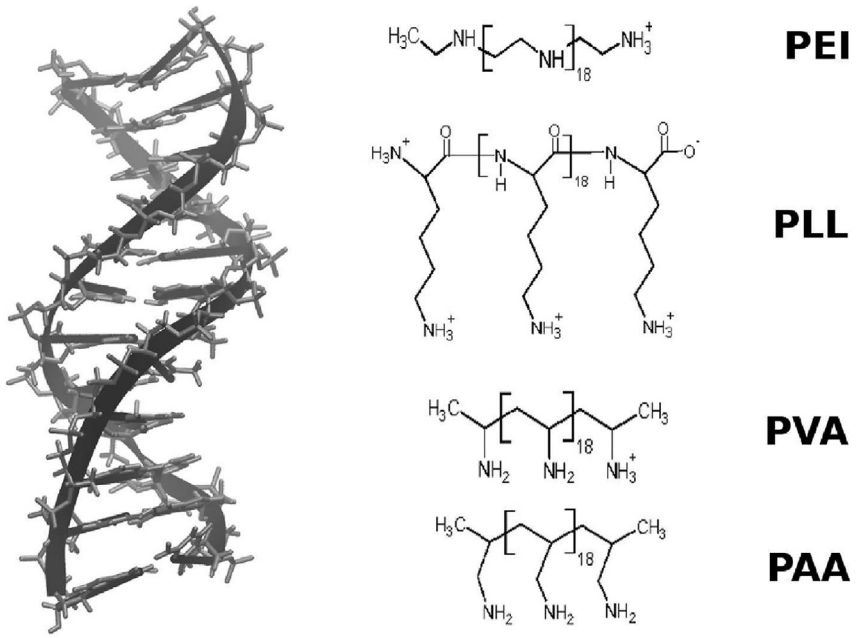

Fig. 1. Chemical structures of DNA (left) and cationic polymers (right) studied in MD simulations of DNA-polycation complexes: polyethylenimine (PEI), poly-L-lysine (PLL), polyvinylamine (PVA), and polyallylamine (PAA).

Table 1
Simulated systems: DNA with cationic polymers (CPs).

System	no. of CP chains	charge ratio DNA/CP	Simulation time [ns]
DNA	–	–	200
DNA-PEI-2	2	22/20	500
DNA-PEI-3	3	22/30	500
DNA-PEI-4	4	22/40	500
DNA-PLL-2	2	22/40	300
DNA-PVA-2	2	22/20	500
DNA-PVA-3	3	22/30	500
DNA-PVA-4	4	22/40	500
DNA-PAA-2	2	22/8	500
DNA-PAA-4	4	22/16	500
DNA-PAA-6	6	22/24	400
DNA-PAA-8	8	22/32	500
DNA-PAA-10	10	22/40	100

scale of 10–20 ns. Once the complex has been formed, it remained stable over the course of simulations (up to 1 μs) [30]. As we proceed to show, the stability of the DNA-polycation complex depends strongly on the polymer concentration and on the chemical structure/protonation state of a cationic polymer. In Fig. 2 we plot the distances between COMs of DNA and polycation chains for several representative systems. In the case of two poly-L-lysine chains (the DNA-PLL-2 system) both chains demonstrate fast and stable complexation with a DNA molecule: the initial binding takes ~10 ns and the complex is stable for at least 300 ns. Note that the formation of a stable complex is observed despite the fact that the overall charge of PLL chains exceeds the DNA charge almost by a factor of 2, see Table 1. Similar conclusions could also be drawn from the time evolution of the DNA-polycation COM distance for polyethylenimine. Stable binding to DNA is witnessed for all three considered systems with PEIs, including the DNA-PEI-4 system, see Fig. 2. Again, despite a considerable excess of the charge of PEI chains with respect to the DNA charge (40 vs 22), all four PEI chains are bound to the DNA duplex. The results for both PLL and PEI systems are in line with previous MD simulations [18,20].

The situation changes drastically for polyvinylamine and polyallylamine. Both these polymers have the hydrocarbon backbones, which differ them from PLL and PEI whose backbones contain amine groups and are therefore hydrophilic even if the amines are not in the protonated state, see Fig. 1. The difference in the chemical structures of these two classes of cationic polymers is most clearly seen for PEI and PVA, two polymers with the same protonation level (50%). Indeed, while 2 and 3 PV A chains are able to bind simultaneously to a DNA fragment, for the DNA-PVA-4 system one of the chains can temporarily get detached from the complex, in contrast to the DNA-PEI-4 system, see Fig. 2. This could be due to a relatively strong binding of PVA chains to the DNA duplex, leading to a competition between different PVA chains. In turn, somewhat weaker interactions of PEI chains with DNA make it possible for PEI chains to bind independently to DNA. Interestingly, a similar difference in concentration effects of PEI and PVA was also observed previously in MD simulations of adsorption of cationic polymers on model bacterial (anionic) membranes [43]. As for polyallylamine, its low protonation level (20%) plays a crucial role as the small charge of PAA chains limits strongly their ability to bind to a DNA molecule. As a result, when the number of PAA chains in solution exceeds 4 (or, in other words, when the overall charge of PAA chains becomes large enough to neutralize the charge of DNA), one can witness the formation of rather unstable DNA-PAA complexes in which polymer chains can detach readily from the complex (Fig. 2) and reside in the water phase. As an illustration, in Fig. 3 we showed snapshots of complexes of DNA with polycation chains of different chemical structure and protonation state.

The formation of stable DNA-polycation complexes could change the conformation of the canonical B-form of the DNA dodecamer used in simulations, especially at elevated polycation concentration. To this end, for each DNA-polycation system we calculated the root mean square deviation (RMSD) of DNA from the initial (reference) structure. In polycation-free solution the RMSD was found to equal 0.22 ± 0.04 nm in line with previous computational studies [30,41]. In the presence of polycations the root mean square deviation of DNA from the reference structure remained stable for all the DNA-polycation systems considered and varied in

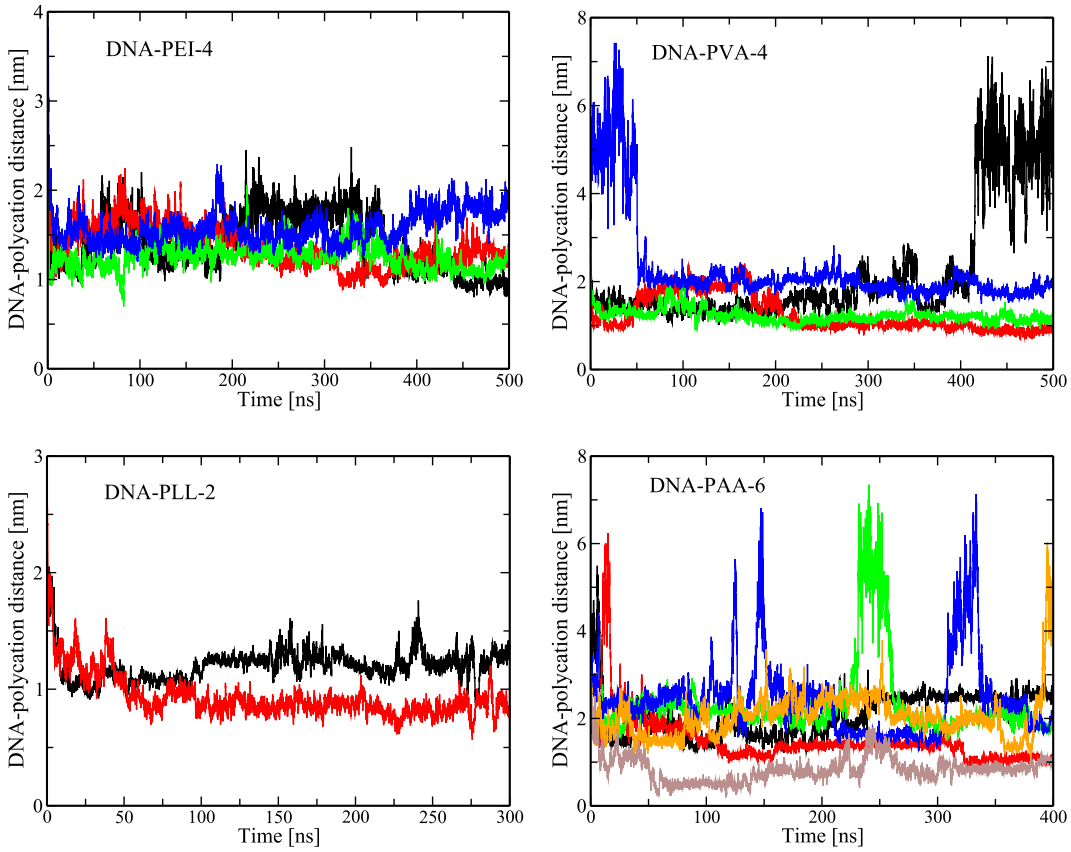

Fig. 2. Time evolution of the distances between centers of masses of the DNA molecule and polymer chains. Shown are results for DNA-PEI-4, DNA-PVA-4, DNA-PLL-2, and DNA-PAA-6 systems. Results for different chains are shown in different color. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

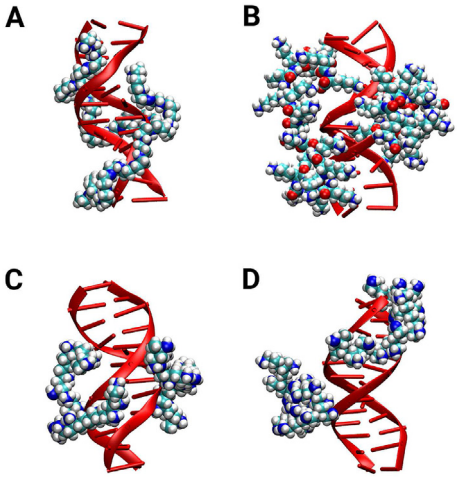

Fig. 3. Representative snapshots of complexes of DNA with two polycation chains for DNA-PEI-2 (A), DNA-PLL-2 (B), DNA-PVA-2 (C), and DNA-PAA-2 (D) systems. DNA is shown in red; polycations are represented by spheres of different color. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the range from 0.19 ± 0.02 nm (the DNA-PVA-4 system) to 0.31 ± 0.05 nm (the DNA-PAA-4 system). Therefore, one can conclude that the canonical B-form of DNA is preserved in all DNA-polycation systems. Interestingly, in some systems the strong binding of polycations slightly reduces the RMSD value as compared to the polycation-free situation.

To further characterize the structure of DNA-polycation complexes, we calculated several radial distribution functions (RDF) of protonated amine groups of polymers with phosphate atoms and selected electronegative atoms of grooves of a DNA molecule. This allows us to get insight into the details of the DNA-polymer interactions. In our previous simulation study of DNA in dilute polymer solution we identified two binding patterns in DNA-polycation systems: (i) electrostatic attractive interactions between polycation's protonated amine groups and phosphate groups of DNA and (ii) embedding of a polycation into the DNA major groove [30]. The former was observed for PEI, PLL, and PAA (and was also reported in a number of earlier computational studies [17–20,24]), while the latter was strictly PVA-specific [30].

Now, when the polymer concentration is considerably elevated, the situation becomes more complicated as both binding patterns can be witnessed simultaneously in the systems under study. In particular, although most PEI chains in the complex still follow the binding pattern (i), one can find the substantial number of contacts of PEI's amine groups with electronegative atoms of the DNA grooves, see Fig. 4 (top). In turn, one can witness PVA chains that are bound to the DNA molecules mainly via contacts of protonated amine groups with phosphate groups of DNA (Fig. 4 (bottom)). Such behavior can be explained by saturation of binding sites when the polymer concentration increases. In the case of DNA-PEI systems the polymer crowding nearby DNA promotes contacts of PEI chains also with the DNA grooves. As far as the DNA-PVA systems are concerned, embedding of some PVA chains into the major groove of DNA prevents the rest of chains from contacts with groove atoms,

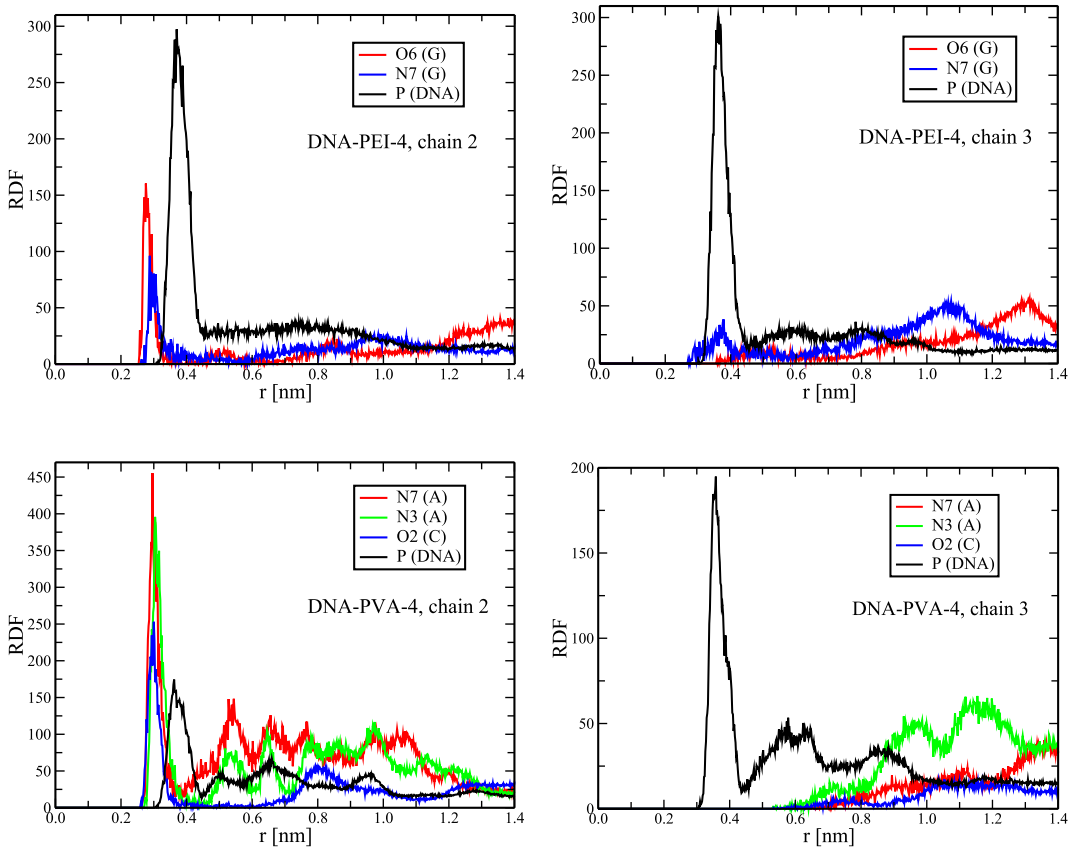

Fig. 4. Radial distribution functions (RDF) of nitrogen atoms of polymer's protonated amine groups with phosphate atoms and selected electronegative atoms of the grooves of the DNA molecule. Shown are results for two individual chains of DNA-PEI-4 (top) and DNA-PVA-4 (bottom) systems.

so that these chains are forced to interact with DNA's phosphates.

As DNA is a polyanionic molecule, one of the crucial characteristics of polycation-based vectors is the ability to establish contacts of polycation's protonated amine groups with negatively charged phosphate groups of DNA. In Fig. 5 we present the average number of such DNA-polycation contacts for all the systems considered. Overall, out of four polycations at hands, PEI demonstrates the strongest ability to form contacts with negative charges of DNA: increasing the number of PEI chains in solution leads to an almost linear increase in the number of PEI-DNA contacts, see Fig. 5. Interestingly, we do not observe a saturation in the number of contacts when the number of PEI chains is increased from 2 to 4. The ability of PVA chains to establish contacts with DNA phosphate groups is somewhat lower as compared to PEI for the systems with 2 and 3 PV A chains. This could be explained by the above mentioned affinity of PVA chains to the major groove of DNA. However, further increase in the PVA concentration does not increase the number of contacts most likely due to the increased competition between different PVA chains for binding to the DNA duplex, which leads to the appearance of PVA chains residing mainly in the water phase. Therefore, there is a pronounced difference in establishing contacts with DNA charged groups for these two cationic polymers that have the same protonation level. As for the DNA-PAA systems, PAA chains form noticeably smaller numbers of contacts with DNA's phosphate groups due to their smaller charge as compared to PEI and PVA chains ($+4\,e$ vs $+10\,e$), see Fig. 5. Furthermore, one can see the saturation of the number of DNA-PAA contacts when the number of PAA chains in the system amounts to 8. It is also instructive to compare the numbers of contacts in different polymer systems with the same overall charges of polycations. A thorough comparison of the systems with the maximal ratio of polycation/DNA charges (DNA-PLL-2, DNA-PEI-4, DNA-PVA-4, and DNA-PAA-10, see Table 1) clearly demonstrates that PEI chains are able to establish the maximal number of contacts with DNA charges. In turn, although PVA chains form considerably less contacts with DNA as compared to PEI, they turn out to be more effective in doing that as compared to PLL chains (we recall that the charge of a PLL chain is twice larger than that for PVA). The origin of this difference is most likely due to the fact that PLLs have relatively long side chains, so that some of them are not bound to DNA and prefer to stay in the water phase [30]. Finally, the lowest number of

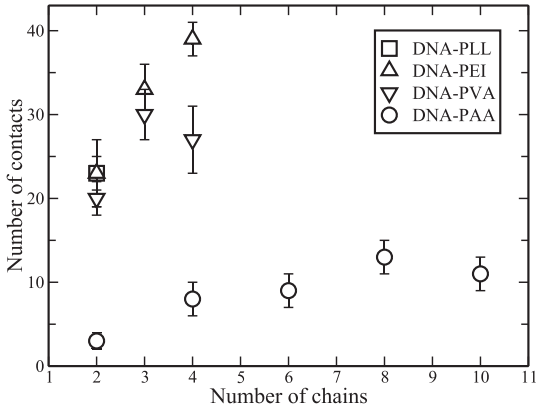

Fig. 5. Average number of contacts of protonated amine groups of polycations with phosphate groups of DNA as a function of the number of polycation chains in the DNA-polycation complex.

contacts is observed for the DNA-PAA-10 system in line with the above mentioned weak electrostatic attraction between DNA and PAAs.

In addition to the number of DNA-polycation contacts, it is very instructive to explore the interaction energies [50] between DNA and various components of the system such as polycation chains, water molecules and Na counterions, see Table 2. First of all, as the interactions of DNA with polycations, water and ions are mainly driven by electrostatics, the contributions of the electrostatic interactions prevail over the contributions due to Lennard-Jones interactions. Furthermore, in all cases one can observe a substantial contribution to the interaction energies of DNA due to water molecules. This energy is the largest for the polymer-free solution of DNA and drops upon binding of polycations to the DNA molecules because the polycations push water molecules from DNA to bulk water. Obviously, this DNA dehydration is maximal for the DNA-PEI-4 system, i.e. for the system with the tightest DNA-polymer complexation, see Table 2. In turn, contributions due to DNA counterions are relatively small, which is a sign of a weak binding of monovalent ions to DNA. The most interesting energy component is related to the interactions of DNA with polycations and can directly be linked with the number of DNA-polycation contacts, see Fig. 5. The corresponding energies for DNA-PEI systems demonstrate gradual growth with polycation concentration, while the same quantity for DNA-PVA systems is characterized by saturation when the number of PVA chains exceeds three polycations per DNA dodecamer, see Table 2. In turn, a much smaller charge of PAA chains leads to noticeably smaller binding energies between DNA and PAA polycation as compared the rest of cationic polymers considered.

Most of the results for the number of DNA-polymer contacts and the interaction energies are directly translated to the ability of polycations to neutralize the charge of DNA. To characterize this ability, we calculated cumulative charges of the polymer solution around DNA [18,20]. This quantity includes all the charges in the system except DNA charges (cationic polymers chains and Na and Cl ions). As an illustration, in Fig. 6 we show the typical behavior of various contributions to the cumulative charge as a function of the distance from the DNA helical axis. Tight binding of three PEI chains to DNA is responsible for a steep increase of the cumulative charge at small distances from the DNA molecule; at larger distances this charge gradually decreases due to contributions of Na and Cl ions up to the value ($+22\ e$) needed to neutralize the DNA charge, see Fig. 6.

When it comes to the efficiency of polycation-induced neutralization of the polyanionic DNA molecule, two factors could play role. First one is the distance at which polycations neutralize the DNA charge: the smaller the distance, the more compact the DNA-polycation complex. And the second factor is related to the

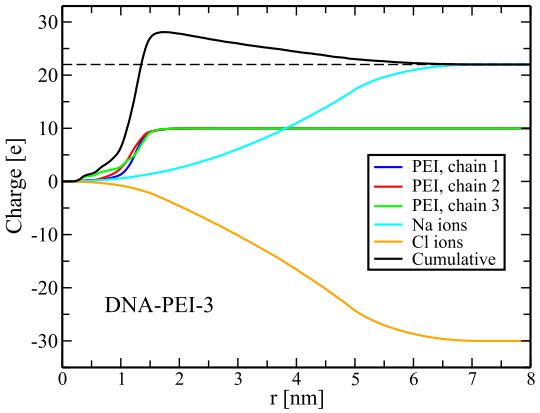

Fig. 6. Component-wise charges in the polymer solution surrounding DNA as a function of the distance from the DNA helical axis for the DNA-PEI-3 system. Shown are results for PEI chains, Na and Cl ions, and also the cumulative charge (DNA charges are not included). The dashed line corresponds to the charge needed to neutralize the DNA charge ($-22\ e$).

overcharging of the complex as a whole since the overall positive charge of the complex is believed to promote binding of the complex to the negatively charged cell membrane [33]. In Fig. 7 (top) we show the cumulative charges (polycations and ions) as a function of the distance from the DNA helical axis for all considered systems. First of all, it is obvious that PEI is the most efficient cationic polymer from the point of view of the above criteria: one can easily see that the DNA-PEI-4 system gives the shortest distance at which the DNA charge is neutralized and also the largest positive charge of the DNA-polycation complex. Although PVA is rather close to PEI at moderate polymer concentrations (systems with 2 and 3 chains), its cumulative charge saturates already in the system with 4 chains due to the above mentioned competition between PVA chains: the interactions between DNA and PVA chains are strong, so that one of the chains is not able to bind tightly to the complex and to contribute to the cumulative charge.

Interestingly, the maximal cumulative charge in the system with two PLL chains is rather close to the charges of the DNA-PEI-3 and DNA-PVA-3 systems although the overall polymer charge of the DNA-PLL-2 system is larger ($+40\ e$ vs $+30\ e$). Furthermore, the distance at which PLL chains balance the DNA charge is much larger as compared to DNA-PEI-3 and DNA-PVA-3 systems, see Fig. 7 (top). This implies that the ability of PLL chains to effectively neutralize the charge of DNA is rather limited most likely due to the fact that PLL is a bulky cationic polymer with relatively long side chains, so that not all of the chains can access DNA's charged groups.

As far as DNA-PAA systems are concerned, one can see some overcharging of the complex only starting from the system with

Table 2
Electrostatic and Lennard-Jones (shown in brackets) components of the interaction energies [kJ/mol] of DNA with cationic polymers, water molecules, and DNA's counterions.

	DNA - Polycations	DNA - Water	DNA - Na ions
DNA	–	-8976 ± 229 (-591 ± 75)	-316 ± 158 (14 ± 15)
DNA-PEI-2	-1223 ± 116 (-409 ± 46)	-7533 ± 203 (-488 ± 70)	-32 ± 56 (2 ± 5)
DNA-PEI-3	-1936 ± 204 (-464 ± 55)	-6795 ± 270 (-497 ± 67)	-17 ± 42 (1 ± 4)
DNA-PEI-4	-2333 ± 170 (-571 ± 53)	-6275 ± 235 (-507 ± 72)	-3 ± 16 (0)
DNA-PLL-2	-1570 ± 190 (-432 ± 44)	-7106 ± 271 (-487 ± 72)	-4 ± 19 (0)
DNA-PVA-2	-953 ± 116 (-235 ± 36)	-7963 ± 208 (-539 ± 74)	-38 ± 68 (2 ± 5)
DNA-PVA-3	-1559 ± 124 (-409 ± 43)	-7511 ± 207 (-484 ± 71)	-10 ± 30 (0)
DNA-PVA-4	-1496 ± 172 (-435 ± 48)	-7322 ± 249 (-471 ± 72)	-16 ± 39 (1 ± 3)
DNA-PAA-2	-325 ± 90 (-338 ± 43)	-8587 ± 228 (-461 ± 77)	-163 ± 117 (8 ± 11)
DNA-PAA-4	-727 ± 117 (-522 ± 46)	-8125 ± 220 (-374 ± 74)	-99 ± 91 (5 ± 9)
DNA-PAA-6	-813 ± 157 (-755 ± 60)	-7911 ± 261 (-308 ± 78)	-52 ± 74 (3 ± 7)
DNA-PAA-8	-907 ± 199 (-625 ± 97)	-7923 ± 312 (-347 ± 79)	-35 ± 57 (1 ± 5)
DNA-PAA-10	-902 ± 131 (-625 ± 57)	-7903 ± 225 (-343 ± 72)	-46 ± 69 (2 ± 6)

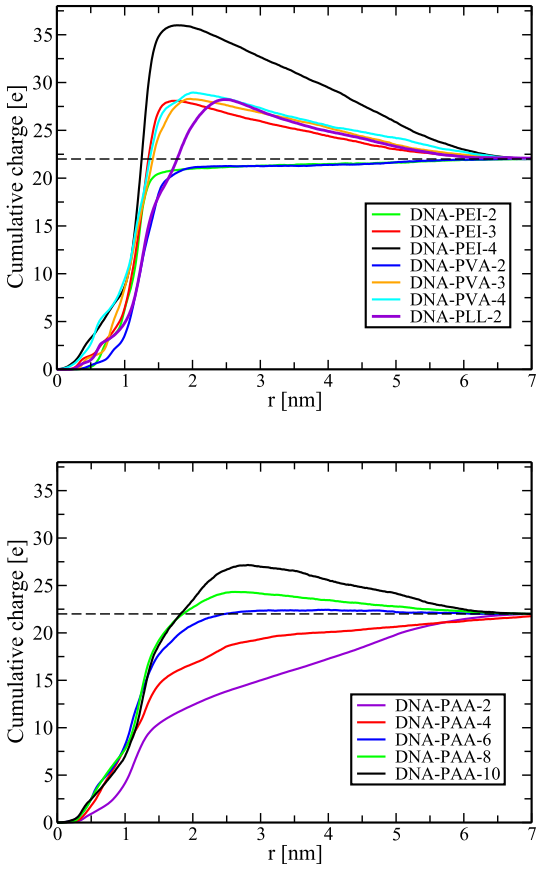

Fig. 7. Cumulative charges (polycations and Na and Cl ions) as a function of the distance from the DNA helical axis. Shown are results for PEI, PVA, and PLL (top) and PAA (bottom) systems. The dashed line corresponds to the charge needed to neutralize the DNA charge.

6 PAA chains (we recall that the charge of a PAA chain equals +4 *e*), see Fig. 7 (bottom). Although the maximal cumulative charge of the DNA-PAA-10 system is comparable with PVA systems, PAA chains neutralize DNA at noticeably larger distances. In fact, this distance is rather close to what was observed for PLL (Fig. 7). This implies that complexes of DNA with PAA are also characterized by a relatively large size but the underlying reason for this differs from PLL: in contrast to PLL, the protonation level of PAA chains is small, so that the attractive interactions of PAA with DNA are rather weak.

4. Conclusions

In this work we employed atomistic molecular dynamics simulations to get an insight into the impact of polymer concentration on the structural properties of supramolecular complexes of DNA and linear cationic polymers. Four linear polycations of different chemical structure were considered: polyethylenimine (PEI), poly-L-lysine (PLL), polyvinylamine (PVA), and polyallylamine (PAA), see Fig. 1. To the best of our knowledge, the polymer concentration effects for complexes of DNA with PVA and PAA chains have never been studied with the use of computer modeling.

Our findings clearly demonstrate that the polymer concentration effects depend strongly on the chemical structure of linear polycations. Out of four polymers considered, PEI is found to neutralize the polyanionic DNA molecules in the most efficient way. The flexibility of PEI chains along with their affinity to charged phosphate groups of DNA allow PEI chains to bind to DNA in an almost independent manner. As a result, DNA-PEI complexes, being the most compact, are characterized by the largest positive overall charge of the complex, which is considered to be a prerequisite for the efficient binding of polyplexes to cell membranes [33]. The differences related to the polycation's chemical structure is most clearly seen when comparing concentration effects of PEI and PVA polymers. These two linear polycations have the same protonation level (50%) but differ in the hydrophobicity of their backbones, see Fig. 1. In contrast to PEI, PVA chains show affinity to the major groove of DNA [30] and are characterized by stronger interactions with DNA. When polymer concentration increases, these strong interactions give rise to the competition between different PVA chains for binding sites on DNA. Therefore, the PVA-induced neutralizing of the DNA charges saturates rather quickly: already the forth PVA chain cannot bind tightly to the DNA dodecamer in contrast to PEI. Consequently, the PVA-DNA complexes are less compact and of smaller overall positive charge, see Fig. 7 (top). The practical implication of these features of the concentrated solutions of PVA polycations would be a much lower transfection activity of PVA, which is indeed in line with experimental data available [32].

Our computational results also can provide a molecular-level explanation for the relatively low transfection efficiency of pure PLL, which was observed experimentally [13,14]. Despite its highly charged nature (PLL's protonation level is 100%), PLL chains are not able to neutralize DNA charges effectively: the total number of DNA-PLL contacts is significantly smaller as compared to both PEI and PVA at the same DNA/polymer charge ratio, see Fig. 5. This is most likely due to a larger size of the PLL polymer: a noticeable portion of PLL's relatively long side chains cannot access DNA phosphates and reside in aqueous solution. As a result, the overall size of the DNA-PLL complex exceeds considerably those for PEI and PVA, see Fig. 7 (top), making PLL polycations less effective transfection agents.

Finally, as far as PAA polycations are concerned, they demonstrate rather weak binding to the DNA dodecamer, which could be explained by their low protonation level (20%). The corresponding DNA-PAA complexes are large and unstable and characterized by a rather weak overcharging of the polyanionic DNA, see Fig. 7 (bottom). However, it should be noted that the computational findings regarding the properties of the concentrated solution of PAA chains cannot be linked in a straightforward manner with their transfection activity as PAA is known to be a relatively efficient delivery vector [32]. Therefore, in addition to the size of the DNA-polycation complex and the ability of a polycation to overcharge the DNA molecule, there must be other important factors that affect the transfection activity of polycation-based DNA delivery agents.

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