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DNA–Polyelectrolyte Complexation Study: The Effect of Polyion Charge Density and Chemical Nature of the Counterions

Mojca Seru^{nik}, rtomir Podlipnik, Barbara Hribar-Lee^{*}

Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ve^{na} pot 113, SI-1000 Ljubljana, Slovenia

Abstract

Complexes of polycations and DNA, also known as polyplexes, have been extensively studied in the past decade, as potential gene delivery systems. Their stability depends strongly on the characteristics of the polycations, as well as the nature of the added salt. We present here a study of the DNA ionene complexation in which we used fluorescence, UV, and CD spectroscopy, combined with molecular dynamics computer simulations, to systematically examine the influence of the polycation charge density, as well as the influence of the nature of the counterion, on the stability of these systems. Ionenes as polycations, depending on their structural characteristics, have previously been found to possess low cytotoxicity, and are therefore particularly interesting as potential gene delivery agents. The results show that the DNA solutions in the presence of the polycation are more stable in the case of very large or very small ionene charge density, suggesting different mechanism of complexation. The computer simulations show that the ionenes with high charge density bind to the minor groove of the DNA molecules, while the ionenes with lower charge density bind to the major groove of the DNA. The nature of the counterions play only a minor role: precipitation of the DNA molecules occurs at slightly lower ionene concentration when fluoride counterion are present, compared to the bromide counterions.

INTRODUCTION

Investigating polyelectrolyte–polyelectrolyte, as well as polyelectrolyte–protein complexation has attracted much attention, mostly due to technological needs. While the main application of polyelectrolyte–protein complexes is in protein purification, cosmetics, drug release, and stabilization of enzymes, the most important driving force to study the polyelectrolyte–polyelectrolyte complexation are DNA–polycation complexes, which are under investigation in the field of gene transfection.¹ In this so-called gene therapy, the treatment is carried out by delivering therapeutic genes to diseased cells. A successful delivery system should be able to neutralize the negative charges of phosphate groups on the DNA backbone in order to avoid the electrostatic repulsion with the anionic cell surface, to compact the relatively bulky DNA structure to appropriate length scale for cellular internalization, and to protect the DNA from extracellular and intracellular nuclease degradation. There are three major problems arising in this process, preventing the effective delivery of

^{*}Corresponding Author barbara.hribar@fkkt.uni-lj.si.

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therapeutic genes to targeted tissues: low uptake of the DNA–polycation complex across the cell membrane, inadequate release of DNA molecules from the complex, and lack of nuclear targeting. Understanding these biological barriers, especially in the terms of the particularities of the delivery systems, is one of the main goals in current gene delivery research.^{2–5}

Most of the investigations considering polyion–polyion as well as polyion–protein interactions were focused on the influence of the structural characteristics of the polyelectrolyte on the complexation, such as the degree of polymerization,⁶ the charge density of the polyion,^{7,8} and their rigidity.⁹ Further, experimental parameters, such as pH, ionic strength, ion type, protein/polyelectrolyte ratio, and the conformational transition were found to influence the complexation.^{10–12} It has been established that the stability of these complexes is governed mainly by electrostatic interactions, as well as by hydrogen bonding and hydrophobic interaction.¹³ The complexes formed may undergo aggregation, or remain stable in the solution.¹ To design a good delivery system, it is however necessary to understand the physicochemical characteristics of the binding process.

Ionenes are aliphatic polyelectrolytes. The polyions are positively charged due to the quaternized nitrogen atoms in the backbone of the chain.¹⁴ By suitably guiding the synthesis, the structure and charge density (hydrophobicity) of the polyion, as well as the nature of counterions, can be systematically varied,¹⁵ which makes them an interesting substance to study the interplay of electrostatic and ion specific effects.^{16–18} Their pharmacological significance has been extensively studied in the past. The antiheparin activity of tetramethyl-6,3-ionene bromide was first reported by Barlow et al.¹⁹ in 1963. Later, the antimicrobial and antifungal effects, as well as their interaction with negatively charged biological polyelectrolytes, was systematically described by several authors.^{15,20–23} One of the more interesting potential applications of aliphatic ionenes is as potential gene delivery agents.^{24–26} Their biological activity is frequently dependent on the charge density and the structure of the ionene polyion, as well as on the nature of the counterions.^{15,21,25,26}

In the past, several studies of thermodynamic, as well as dynamic properties of aqueous ionene solutions were carried out, and the results are summarized in some more recent papers.^{18,27–29} The properties of the ionenes that proved to be crucial for their behavior are the linear charge density of the polyion, characterized by the parameter $\xi = e_0^2 / (4\pi\epsilon_0\epsilon b)$ (e_0 is the elementary charge, ϵ_0 and ϵ are the permittivities of vacuum and of solvent, respectively, and b denotes the average length in-between two charged groups of the polyion), and the nature of the counterions. Counterions are known to, depending on their characteristics, condense on the polyions to a different extent, and in this way modify the charge density of the polyelectrolyte, and consequently also the mobility of the polycations.^{16,29}

The charge density of the DNA also plays an important role in the stability studies.³⁰ In this work we systematically explored the interplay of the polyion charge density and the nature of the counterions in the process of complexation between ionenes and calf thymus DNA (ξ for a double stranded B form being 4.2,³⁰ and the distance between two successive phosphate groups being 6.8 Å³¹). We supplemented the experimental results with the molecular dynamics study of the complexation.

MATERIALS AND METHODS

Calf Thymus DNA.

Highly polymerized calf thymus DNA (~10 000 base pairs, lyophilized powder) was purchased from Sigma (USA). DNA was dissolved in water that was first deionized and then distilled two times. DNA was then extensively dialyzed against buffer (20 mM phosphate, 1 mM EDTA, pH = 7) using a dialysis tube Float-A-Lyser (Spectrum Laboratories U.S., Mw cutoff 500 Da). The starting solution of DNA was heated to 95 °C in an outer thermostat and then cooled to 5 °C at cooling rate of 0.075 °C/min to allow DNA to adopt double-stranded DNA structure. Concentration of DNA base pairs in buffer solution was determined spectrophotometrically at 25 °C, $\lambda = 260$ nm, using molar extinction coefficient $\epsilon = 13200 \text{ M}^{-1} \text{ cm}^{-1}$.

3,3-, 6,6-, 6,9-, and 12,12-ionenes.

N,N,N',N'-tetramethyl-1,3-propanediamine (99%), *N,N,N',N'*-tetramethyl-1,6-hexanediamine (99%), 1,3-dibromopropane (99%), 1,6-dibromohexane (96%), 1,9-dibromononane (97%), and 1,12-dibromododecane (98%) were purchased from Sigma-Aldrich and used as received. Dimethylamine hydrochloride (99%), tetrahydrofuran (99.9%), methanol (99.8%), and diethyl ether (99.8%) were also purchased from Sigma-Aldrich. NaF was purchased from Merck.

Aliphatic *x,y*-ionene bromides were prepared with Menshutkin reaction from *N,N,N',N'*-tetramethyl-1,*x*-alkyldiamine and 1,*y*-dibromoalkane as described in detail elsewhere.¹⁷ Polymers were purified by extensively dialysis using dialysis tubes (Sigma-Aldrich, MW cutoff 12 000 Da) against deionized water until conductivity was less than $2 \mu\text{S cm}^{-1}$. Based on the purification process, as well as the chromatographic determination, the estimated molar mass was between 20 and 100 kDa (the procedure is described in detail in ref 17). Ionene fluorides were prepared from bromide salts by ion exchange during dialysis using 0.05 M NaF as the exchanging solution (10 times) and then purified against water to remove sodium ions (until conductivity was less than $2 \mu\text{S cm}^{-1}$ and the flame test for sodium ions was negative). Ionene salts' concentrations were determined by potentiometric titrations using a standard solution of AgNO_3 for bromide and LaCl_3 for fluoride salts. For this purpose, bromide and fluoride ion selective electrodes (Merck) were used, while the saturated mercury sulfate electrode was employed as the reference electrode in all cases.

UV Measurements.

Spectrophotometric measurements were performed using a Cary 100 (Varian, Palo Alto, CA) two-beam spectrophotometer, equipped with Peltier block for temperature regulation. Absorbance was measured from 400 to 235 nm. For the turbidity observations, we used measurements at 340 nm where DNA has no absorption.

We performed titration of DNA with ionenes of different charge density (3,3-, 6,6-, 6,9-, 12,12-) and two different counterions (fluoride and bromide) at 25 °C. Measurements were made in a cuvette with optical length 0.5 cm under permanent stirring. The starting concentration of DNA was 0.2 mM, the initial volume of the sample was $V_0 = 1000 \mu\text{L}$. All

ionenes were 10 mM. After each addition of ionene, we waited for 3 min before measurement. This time of equilibration was estimated by several measurements until no further changes in the absorbance readings were observed. Circular dichroism spectra were obtained with the same sample afterward.

Fluorescence Quenching.

Fluorescence intensity was measured using PerkinElmer LS 55 Fluorescence Spectrometer. DNA solution (0.2 mM) was mixed with the ethidium bromide (purchased from Sigma-Aldrich; 20 $\mu\text{g/mL}$), and the fluorescence was set to 100%. The excitation and emission wavelengths were 535 and 599 nm, respectively.

We performed titration of DNA with ionenes of different charge densities (3,3-, 6,6-, 6,9-, 12,12-) and two different counterions (fluoride and bromide) at 25 °C directly in the fluorimetric cell under constant stirring. All ionenes were 10 mM. The time required for equilibration was found to depend on the ionene charge density and typically varies from 5 to 10 min.²⁶ The reading for each point was therefore taken 10 min after the ionene addition.

Circular Dichroism (CD).

Circular dichroism spectra were taken with AVIV 62A DS CD spectrometer (Lakewood, NJ) in wavelength range from 320 to 220 nm at 25 °C. In this range, the CD signal is sensitive to changes in secondary structure of DNA. For all measurements, the wavelength step was 1 nm, the average measuring time (for each point) was 3 s, and the bandwidth was 2 nm. Measurements were made in a cuvette with optical length 1.0 cm under permanent stirring.

Theoretical Models and Computer Simulations.

The model solution consisted of ~15 000 water molecules (TIP3P water model³²), one molecule of ionene, one molecule of DNA, and an equivalent number of sodium counterions to satisfy electroneutrality condition. The DNA molecule was modeled in Avogadro³³ chemical editor. The model double-stranded B-DNA molecule $d(CG)_{12}$ was constructed using 24 base pairs, using the AMBER14 force-field parameters³⁴ for energy minimization. The model parameters for the ionenes are taken from ref 35. The 3,3-ionene molecule was constructed out of 6 monomer units, 6,6-ionene was constructed using 5 monomer units, 6,9-ionene was constructed using 4 monomer units, and 12,12-ionene was constructed using 2 monomer units. Every monomer unit consisted of two quaternary nitrogen atoms, and the appropriate number of methylene groups (see Figure 1).

In the first step, AutoDock VINA software³⁶ was used to dock the model ionene molecules to the model DNA. The simulated annealing method was used for energy optimization. The structures with the minimum energy were then used in YASARA³⁷ software using the AMBER14 force-field to perform the molecular dynamics simulation at constant pressure 1 bar and temperature 298 K; pH was set to 7.4. The periodic boundary conditions were used, together with the particle-mesh Ewald to take into account long-range electrostatic interactions. The time step used was 2.5 fs, the length of the simulations being 100 ns.

RESULTS

Experimental Section.

The main concern of this work was to investigate how the charge density (hydrophobicity) of the polyelectrolyte and the nature of the counterion influence the complexation and the stability of the DNA molecules in aqueous solutions. The nature of the counterions was namely found to drastically affect the effective charge density of ionenes. For example, in the case of 3,3-ionenes, it has been established that the effective charge density of the polyion in the presence of fluoride counterions is approximately twice as large as in the case of bromide counterions (see, for example, Figure 7 of ref 16; the data, where available, are given in Table 1). Since the charge density of the ionenes was found to influence the degree of complexation with the DNA molecules,²⁶ as well as the stability^{24,25} of these complexes, we decided to study the complexation process as a function of the ionene structural charge density, as well as in combination with two different counterions (fluoride and bromide).

The experiments were performed for ionenes with different charge densities, and two different counterions, fluorides, and bromides, and repeated with different ionenes, as well as DNA concentrations. It has been established that the results do not depend on the concentration of the two substances separately, but on the ratio of the two concentrations, $r = c_{\text{ionene}}/c_{\text{DNA}}$. We therefore present all the results as the function of the ratio r . Here c_{ionene} is referring to the concentration of ionene monomer units, and c_{DNA} to the concentration of DNA base pairs, which makes r also the ratio between concentration of quaternized nitrogen groups of the ionene backbone and the concentration of DNA phosphate groups. Assuming all the quaternized nitrogen groups and phosphate groups are ionized, we can identify r also as the $+/-$ charge ratio.^{26,31}

The complexation between the DNA and the ionenes was checked using the fluorimetric measurements of ethidium bromide that inserts itself in-between the base pairs of the DNA double helix. Upon DNA complexation, the intercalated ethidium bromide molecules get displaced by the polycations causing the fluorescence intensity of ethidium bromide to decrease.^{38,39} As seen in Figure 2, the addition of the ionene in the DNA/ethidium bromide solution gave rapid fall in the fluorescence, regardless of the ionene, as well as of the counterion, suggesting immediate and efficient complexation (for the sake of clarity, only the results for 3,3-, 6,9-, and 12,12-ionene fluorides and 6,9-bromides are shown in the figure), as expected, considering the relatively high charge density of DNA ($\xi = 4.2$). The fluorescence decreases linearly, as observed before^{24,26} in the entire concentration range studied here.

The interaction of DNA with ionenes and the stability of the formed complexes was further monitored by measuring the absorbance of the solutions of 0.2 mM DNA (concentration of the DNA phosphate groups), as titrated with different ionene solutions (counterion concentration 10 mM). The method has been previously used to study the aggregation of protein solutions.^{40,41} When the aggregates begin to form in the solution, the turbidity increases due to the precipitates, and the absorbance of the solutions changes. Figure 3 shows the curves for DNA absorbance, as well as the absorbance of the solutions during the titration, and namely for the ionene quaternium nitrogen concentration ($c_{\text{ionene}}/c_{\text{DNA}}$).

phosphate unit concentration (c_{DNA}) ratio, r equals 0.42 and 1.25. While the DNA has an absorbance maximum around 260 nm, there is no specific absorption at larger wavelengths detected. We therefore used the absorbance at 340 nm as a measure of the turbidity of the solutions.

The results for the absorbance as a function of r are for ionene bromides and fluorides given in Figure 4.

An interesting observation is that, regardless of the counterion, the stability of the solution, as measured by its turbidity, mostly depends on the structural charge density of the ionene polyion. Surprisingly, the turbidity starts increasing very fast upon addition of the polyelectrolyte for polyelectrolytes with high, as well as with very low charge density. The exception are the polyions with intermediate charge density (6,6- and 6,9-ionenes), where the solution starts to precipitate, depending of the counterion, at r between 0.4 (6,6-ionene fluorides) and 0.75 (6,9-ionene bromides). Similar observations were observed previously for the complexation of the DNA with 3,3-ionene bromides (at $r = 0.5$),³¹ and for complexation of two-dimensional clay particles with ionene bromides (at $r \approx 0.5$).⁴² It has been shown that immediate rise of turbidity suggests 1:1 complexation, while the increasing turbidity from $r = 0.5$ is characteristic for binding stoichiometry 1/2.³¹ We therefore speculate that the differences in ionenes arise from the different binding stoichiometries, which depend on the ionene and DNA charge density, as well as the distances between charges of both types of molecules.

To analyze this further, we plotted the ratio r at which the total precipitation of the DNA occurs (the drastic increase of the absorbance at 340 nm) for all the solutions studied (Figure 5; the numerical values of r_{crit} are given in Table 1). Note that larger r effectively means larger ionene concentration at a constant DNA concentration. In the same figure we also plotted the data for the effective charge density of the ionene polyion, where available (taken from ref 16 for the ionene concentration 20 mM). Note that the effective charge density of the ionenes used here negligibly depends on the concentration in the concentration range studied.¹⁶

From the limited amount of data available for the effective charge density of the ionene polyion, one can speculate there exist a correlation between the effective charge density and the precipitating ionene concentration. In the case of 6,6-ionenes where the polyion effective charge density is approximately the same regardless of the counterion used, the precipitating concentration does not depend on the counterion. In other cases, it seems that the precipitating concentration is larger in the case of lower effective charge density. Although this observation seems counterintuitive at first, it could be explained as follows. In the pure aqueous ionene solutions, the effective charge density differs from the structural one due to the condensed counterions that electrostatically interact with the polyion. The larger effective charge density basically means less condensed counterions,¹⁶ so the polyion charges are more “exposed” to interact with the charges on the DNA, leading to lower precipitating ionene concentration.

Also seen from Figure 5 is the nonmonotonous relation between the ratio r required for total precipitation, r_{crit} , and charge density on the polyion. To explore this further, we looked at the DNA structural changes upon titration using the CD measurements. Figure 6 shows the CD spectra of the DNA–3,3-ionene bromide solutions at different concentration ratios, r .

The CD spectrum of DNA shows a minimum at 244 nm, crossover at 258 nm, and maximum at 276 nm, all characteristics for native calf thymus DNA in B form.⁴³ As the ionene is added to the solution, the maximum starts to decrease and moves to larger wavelengths, indicating the change in the DNA structure. The effect is better observed plotting the molar ellipticity at 276 nm as a function of ratio r for different ionenes. The results are shown in Figure 7.

The results are consistent with the observations from absorption spectroscopy. Ionenes with relatively low charge density (structural charge density below 1) gradually change the DNA structure until the double helix structure completely collapses at the conditions of total precipitation. The influence of ionene increases with each charge density, regardless of the counterion. The exception is, as observed before, the 3,3-ionene, which has a structural charge density above 1. Addition of 3,3-ionene to the solution influences the CD spectrum very little until the total collapse occurs, at ionene concentrations that are relatively large compared to other cases. To explain this result we decided to perform computer simulations of these systems to obtain the microscopic view of the process.

Theoretical.

Molecular modeling-based computational approaches can assist in understanding the experimentally observed behavior of the systems, and as such provide valuable information on the molecular level.⁴⁴ For example, Kondinskaia et al. showed that two distinct patterns of binding of DNA with polycation exist,⁴⁵ which has an important effect on the stability of the DNA in the aqueous environment. Extensive computer simulations of the DNA–polycation complexation exist, mostly concentrating on the influence of the polycation structure on the stability of the formed complexes.^{44–48} The purpose of our computer simulations was not to investigate the thermodynamics of the polyion–DNA complexation, but we were mostly interested in the mechanism of the polyion binding to the DNA. We therefore performed molecular dynamics simulations with model 3,3-, 6,6-, and 6,9-ionenes, and analyzed the geometry of the DNA–ionene polycation complexes, as well as their stability during the simulation. Due to the surplus of negative charges on the DNA molecule, compared to the positive charges on the ionene, the only counterions present in the simulation were the sodium cations, to preserve the electroneutrality condition. We did not include the ionene counterions in the simulation.

First we had a look at the structure of the DNA–ionene complex. Figure 8 shows typical snapshots from the simulation for structures of 3,3- (left), and 6,9- (right) ionene bromides. The van der Waals surface of the DNA molecule is shown in green, and the ionene molecule is shown with balls and sticks. One can see that two different binding patterns are observed. In the case of highly charged 3,3-ionene, being driven by the electrostatic attraction between the negatively charged phosphate groups of DNA and positively charged quaternary nitrogen in the ionene backbone, the polyion binds to the minor groove of the DNA,

maximizing the number of the negatively charged phosphate group (P) and positively charged quarternium nitrogen group (N) contacts, as previously observed for highly charged polyions.⁴⁵ On the contrary, all the other ionenes, 6,6- and 6,9-shown here, and 12,12-ionenes get embedded into the major groove of DNA, as a consequence of their hydrophobic parts of the backbone. The hydrophobic characteristic of the backbone of these ionenes has been recently established by volumetric and calorimetric measurements.²⁷

Closer examination of the formed complexes reveals that the distance between two charged groups on the 3,3-ionene is ~ 5 Å, which is approximately the same as the distance observed between two neighboring charged groups on the DNA molecule (~ 6 Å), leading to a good electrostatically driven fit of the two molecules. Further, the charges on the ionene polyion come to the close proximity of the phosphate groups on both sides of the minor group of the DNA molecule, further stabilizing the structure of the DNA molecule, as well as the complex. In the case of ionenes with lower charge density (e.g., 6,6-, 6,9-, and 12,12-), this is not the case. One can observe the stacking of the hydrophobic parts of the two molecules in the major DNA groove.

To check for the stability of the complexes during the simulation, we have analyzed the distance between the ionene and the DNA molecule. The average distance between the ionene backbone nitrogen atom and the closest DNA phosphor atom during the first 50 ns of the simulation is plotted in Figure 9. As seen from the time evolution, the 3,3-ionene binds very closely to the DNA, and the DNA complex is stable during the simulation. The distance between DNA molecule and ionenes with smaller charge density (6,6-, 6,9-, and 12,12-) is slightly larger, indicating different kinds of binding. Also, larger fluctuations in the distance are observed, indicating more loose complexes.

DISCUSSION AND CONCLUSIONS

In this work we performed a combined, experimental and theoretical study of the complexation of the DNA with cationic polyelectrolytes, ionenes, of different charge densities, as well as with different counterions. The complexation was checked, measuring the fluorescence quenching of the ethidium bromide upon ionene addition to the DNA solution. The binding of the ionene to the DNA molecule occurs almost instantly, as previously observed by Trukhanova et al. for 3,3-ionene bromides,²⁶ and namely for all the ionenes studied here, regardless of the nature of the counterion. Using the turbidity measurements of the DNA solutions titrated with the ionene solution, the conditions in the solution were determined, at which the DNA–ionene complex precipitates. The results show that these conditions can be characterized by the polyion–DNA concentration ratio, rather than individual concentrations of the substances.¹¹ However, the nature of the polyion counterions plays a minor, but important role in the process. The counterions that are condensed on the polyion to a larger extent screen the polyion charges and therefore shift the critical precipitation concentration of the ionene solution to higher values of r .

A more counterintuitive result observed was the fact that the critical r does not depend proportionately on the linear charge density of the polyion. The critical r , regardless of the nature of the counterions, for ionenes with structural linear charge density less than 1,

increases with the decreasing charge density of the polyion, as expected; polyions with lower charge density of the polyelectrolyte bind to DNA to a lesser extent due to weaker electrostatic interaction. For the 3,3-ionenes with structural linear charge density of 1.44, this is not the case. The critical r for 3,3-ionenes is approximately the same as for 12,12-ionenes with $\xi = 0.44$. The electrostatics alone cannot be used to explain this. With the help of molecular dynamics simulations, we examined the mechanism of complexation for the ionenes in question and in fact observed different mechanisms of binding of 3,3-ionene that binds to the minor groove of the DNA to maximize the number of P–N contacts, while all the other, more hydrophobic, ionenes embed into the major groove. From the CD measurements evaluating the structure of the DNA molecule upon addition of the polyelectrolyte, it can be concluded that the 3,3-ionene that binds in minor groove influences the DNA structure to a minor extent. This can explain the experimental observations.

For aliphatic ionenes being one of potential gene delivery agents, it is important to understand the mechanism of their complexation and decomplexation, as well as the stability of the DNA–ionene complexes. While it has been established before that the structure and charge density of the polyion play an important role in these processes, we have established here that the nature of the counterions has to be taken into consideration when preparing the complexes. Since more than one kind of ions are usually presented in the physiological systems, the complexation of DNA with ionenes with different counterions in the presence of different simple salts will be studied in our future work.

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Figure 1.
Ionene monomer unit. A⁻ stands for the counterion, Br⁻, or F⁻ in our case.

Figure 2. Effect of the addition of the 3,3- and 6,9-ionene fluorides and bromides on the fluorescence of the DNA/ethidium bromide solutions, shown as a function of the ionene/DNA charge ratio, r .

Figure 3. Absorbance of the DNA solution (red), as well as the solutions with the ionene quarternium nitrogen concentration vs DNA phosphate unit concentration ratio 0.42 (blue) and 1.25 (green) for the case of 3,3-ionene bromides.

Figure 4. Absorbance of the solutions as a function of r for ionene bromides (top), and ionene fluorides (bottom).

Figure 5.

Ratio r at which the total precipitation occurs, r_{crit} , as a function of the structural charge density of the ionene polyion. The red solid line shows the results for ionene bromides, and the green dashed line applies to the ionene fluorides. The symbols show the effective charge density of the ionenes as determined in the pure aqueous ionene solutions (red circles - ionene bromides; green squares - ionene fluorides). The data for effective charge density are taken from ref 16 and namely for the ionene concentration 20 mM.

Figure 6.
CD spectra of the DNA–3,3-ionene bromide solutions at different concentration ratios, r .

Figure 7. CD molar ellipticity at 276 nm as a function of ionene–DNA concentration ratio, r , for different ionene bromides (top) and fluorides (bottom).

Figure 8.

A typical snapshot from the MD simulation of the structure of the DNA–ionene polycation complexes for 3,3- (left) and 6,9- (right) ionene bromide.

Figure 9.
Average distance between the ionene backbone nitrogen atom and the closest DNA phosphor atom during the first 50 ns of the simulation.

Table 1.

Mean Charge Separation on the Ionene Chain (b),¹⁸ and Structural (ξ), and Effective (ξ_{eff}) Linear Charge Density, When Available, for Ionenes Used in This Work^a

ionene	$b/\text{\AA}$	ξ	ξ_{eff} (fluorides)	r_{crit} (fluorides)	ξ_{eff} (bromides)	r_{crit} (bromides)
3,3-	5.0	1.44	0.52	1.13	0.29	1.23
6,6-	8.8	0.82	0.36	0.80	0.34	0.80
6,9-	10.6	0.67	0.34	0.96	0.41	0.95
12,12-	16.3	0.44		1.30		1.18

^aThe data for effective charge density are take from ref 16 and namely for the ionene concentration 20 mM. r_{crit} is the critical ratio between DNA and ionene concentration (charge ratio), at which the precipitation occurs.