Coarse-grained molecular dynamics simulations of DNA condensation by block copolymer and formation of core-corona structures

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Abstract

Coarse grained molecular dynamics simulations are used to study the condensation of single polyanion chains with block copolymers composed of cationic and neutral blocks. The simulations are an effort to model complexes formed with DNA and cationic copolymers such as polyethylenimine-*g*-polyethylene glycol which have been used in gene delivery. The simulations reveal that increases in the cationic block length of the copolymer result in greater condensation of the polyanion. The ability of the complexes to form core-corona structures, with the neutral blocks of the copolymers forming a corona around a dense core formed from the charged beads is investigated. The core-corona structure is shown to be dependent on both condensation of the polyanion chain and the length of the neutral block of the copolymer. Increasing the length of the cationic and neutral blocks of the copolymer both result in improvement in the core-corona structure. The internal structure of the complex core is shown to be a function of the architecture of the copolymer. Complexes formed from linear diblock copolymers have homogeneous cores with similarly arranged cationic and anionic beads; however, complexes formed with star-shaped copolymers have a layered core structure, with anionic beads found in the center of the cores.

Keywords

gene delivery; DNA condensation; molecular dynamics simulation; block copolymers; star copolymers

I. Introduction

Oppositely charged macroions spontaneously form complexes in solution with various morphologies that depend on the flexibility, charge ratio, and types of macroions used in the system.[1] Complexes formed from flexible or semi-flexible polyelectrolytes with opposite charges have been extensively studied in order to understand the condensation behavior of charged biomacromolecules, especially DNA, in nature as well as to develop new biomedical techniques, including drug delivery and gene therapy systems. In particular, the development of non-viral based gene therapy has led to a great deal of study of polyelectrolyte complex formation. While they lack the extremely high transfection efficiencies of viral vectors, polycations, such as polyethyleneimine (PEI), have been shown to be versatile gene delivery vectors with relatively high transfection efficiencies and, in comparison to viral vectors, reduced concerns regarding safety and immunoresponses.[2,3]

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While early non-viral gene therapy polyplexes were formed from just DNA and a polycation, recent non-viral vectors have become increasingly more complex. Diblock and graft copolymers containing a cationic block, such as PEI, and a neutral hydrophilic block, such as polyethylene glycol (PEG), have been used as gene therapy vectors since the PEG block improves solubility, prevents aggregation, and reduces the toxicity of polyplexes. The PEG block of these copolymers can also be linked to ligands or peptides in order to target specific cell types and/or provide the complexes with increased membrane rupturing capabilities. Both branched and linear PEI have been used as gene therapy vectors, providing an extra layer of complexity to the structures of the DNA polyplexes. Additionally, complexes formed from synthetic linear polyelectrolytes and diblock copolymers have been studied for possible applications in drug delivery and for their ability to alter surface properties.[4]

Computer simulations have been widely used to understand the condensation of polyelectrolytes, and this introduction will discuss only a small portion of the relevant literature. Stevens[5] found that toroidal and rod-like structures were formed from condensation of a single polyelectrolyte chain in the presence of trivalent and tetravalent counterions, with the likelihood for forming toroidal structures dependent on the stiffness of the polyelectrolyte. Winkler et al. used molecular dynamics simulations of single, flexible oppositely charged chains of identical length to show that compact glasslike condensate structures formed for systems when the columbic interaction between the chains was sufficiently strong.[6] Ou et al. investigated the energetics of complex formation between two oppositely charged polyelectrolyte chains and found that the driving force of complexation is the entropy gain resulting from counterion release.[7] Dias et al.[8] showed that compact structures formed in systems of a longer polyanion chain in the presence of several shorter polycations chains and that increases in the number or length of the polycation resulted in greater collapse of the polyanion chain. In a series of Monte Carlo simulations, Hayashi et al.[9-11] studied the formation of polyplexes from a system of many polyanion and polycation chains and presented a set of simple rules that governed the number and size of the polyplexes. They suggested that during the complexation, the net charge and charge density of the polyplexes was minimized to reduce electrostatic repulsion, while the total number of polyplexes was maximized to maintain translational entropy. However, there are relatively few theoretical and simulation studies on the condensation of polyelectrolytes by copolymers composed of a neutral block and an oppositely charged block. Several studies focused on the micellization behavior of ionic diblock copolymer mixed with oppositely charged homopolymer or copolymers. Kramarenko et al.[12] theoretically studied solutions of block polymers with neutral and charged blocks mixed with shorter, oppositely charged homopolymers. They found that the micelles that formed have core-corona structures and that the resulting micelles can be either completely neutralized and partially neutralized. Later, they calculated the surface tension of complexes formed from stoichiometric mixtures of ionic block copolymers and oppositely charged homopolymers.[13] From the surface tension, they determined how the aggregation number of the micelles in the systems depended on the fraction of charges on the chains, solvent type, and salt concentration. A separate theoretical study found that solutions of ionic hydrophilic homopolymers and shorter, oppositely charged hydrophobic homopolymers form neutral complexes with a core-corona structure.[14] Finally, Feng and Ruckenstein[15] performed Monte Carlo simulations of complexation between charged/neutral diblock copolymers and oppositely charged/neutral diblock copolymers. They found that increasing the length of the charged blocks or the ratio of charged to neutral beads promoted the formation of aggregates and that self-recognition occurred between chains with matching block lengths and charges.

In this paper, molecular dynamics simulations are used to study the complexation of a single linear polyelectrolyte with block copolymers that contain oppositely charged and neutral hydrophilic blocks. The physical phenomenon we try to simulate is the condensation of long DNA with PEI-PEG diblock copolymers. We use a coarse-grained representation of DNA and

PEI-PEG diblock copolymers. Hence, the reported study bear similarity with some earlier studies, yet our focus is on the DNA condensation and the resulting complex morphology. The size and architecture of the copolymers are varied to determine how they affect complex structure. Radial distribution functions of positive, negative, and neutral beads surrounding the complex center are calculated, enabling the study of formation of core-corona structures as well as the interior structure of the core of the complex, an area that has received little attention. [4]

II. Simulation Methods

The polymer chains were modeled using a bead-spring polymer model that follows the one extensively used by Stevens and Kremer.[5,16-18] The polyanionic and diblock chains were treated with the same model. All parameters in the system are given in terms of ε, the basic unit of energy in the model, σ, the basic unit of length, and mass of bead m, the basic unit of mass This also defines the basic unit of time $\tau = (m/\varepsilon^{1/2}\sigma$. The basic unit of temperature T of the system is given by (ε/k_B), where k_B is the Boltzmann constant. Chain beads separated by a distance r interact through a purely repulsive Lennard-Jones (LJ) potential given by:

$$U_{LJ}(r) = \begin{cases} 4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6} + \frac{1}{4}\right], & r \leq r_c \\ 0, & r > r_c \end{cases}$$

(1)

with the LJ cutoff distance for all bead-bead interactions, $r_c = 2^{1/6}\sigma$, placing the chains in a good solvent. Bonded beads also interact through an attractive finite extensive, nonlinear elastic (FENE) potential given by

$$U_{bond}(r) = -1/2kR_0^2\ln\left(1 - r^2/R_0^2\right)$$

(2)

with spring constant $k = 7\varepsilon/\sigma^2$, and maximum bond distance $R_0 = 2\sigma$. The average bond length of the system, $<r>$, was 1.1σ. The chains were flexible, and, therefore, no angle bending term was included. Charged beads also interacted with a Coulomb potential given by

$$u_{ij}(r) = z_i z_j \lambda k_B T / r$$

(3)

where z_i is the valence of particle i, and λ is Bjerrum length, which is the distance at which the interaction energy between two unit charges would be equal to $k_B T$. In water at 300K, $\lambda = 7.1$Å. In this model of flexible polyelectrolytes,[16] we set $\lambda = 3.2\sigma$. This sets the Coulomb strength parameter Γ for the polyelectrolytes, $\Gamma = \lambda/<r> = 2.9$, a value representative of a strong polyelectrolyte, such as sodium poly(styrene sulfonate), but is less than the typical value associated with DNA. All charged beads carry a unit charge of the specified sign.

The simulations were performed using the LAMMPS molecular dynamics software package. [19] A Langevin thermostat with damping constant $\Gamma = 1$ was used to maintain a constant temperature $T = 1.0\varepsilon/k_B$. Long-range electrostatic interactions were included using the P^3M mesh-Ewald method. Most simulations were performed on a single polyanion chain with chain length $N_a = 64$ in the presence of multiple, shorter positively charged chains that were composed of cationic and neutral blocks. The systems are characterized by the number, type, and block length of the positively charged chains, and are designated with notation of the form QTN, where Q is the total number of positively charged beads on each cationic chain, T is the type (d = diblock linear or s = star-shaped) of the cationic chains, and N is the total number of

neutral beads on each cationic chain. In all simulations, M cationic chains were added to the system so that the total number of positively charged polymeric beads was equal to the length of the polyanion, that is $MQ = N_a$. In all but one simulation, no counterions were included. Therefore, a system labeled 16d16 would contain a polyanion chain with length 64 and 4 linear diblock chains that each had 16 positive and 16 neutral beads. The initial configurations of the systems were generated randomly. Chains were built as random walks with the requirement that the minimum next nearest neighbor distance between chain beads was 2σ, producing starting conformations that were stretched. Each simulation began with a short equilibration period using soft-core potentials to prevent high LJ energy at the beginning of the production run resulting from initial configurations with overlapping beads. This was followed by production ran for 10^7 steps, with a time step of 0.015ρ, where τ is the LJ unit of time. Hence the total simulation time during the production run was around $15 \times 10^4 \tau$. Complexation between polyanion and cationic diblock chains was typically completed within $2.0 \times 10^4 \tau$, as will be shown shortly in Figure 1 & 3. The simulation boxes were cubic with a total monomer concentration of around $1 \times 10^{-4}/\sigma^3$, and a typical box side had a length $L \approx 120\sigma$. Simulation trajectories were visualized using VMD 1.8.6.[20]

The main goal of this work is to investigate how the size and architecture of the neutral-cation block copolymer influences the condensation of DNA and the formation of core-corona structure of the complexes. For this purpose, we first monitor the complex formation during the simulation. A diblock is defined as bound to the polyanion whenever a bead on a diblock chain was within a given cutoff distance of any polyanion bead. The cutoff distance of 2.5σ, which is somewhat shorter than the Bjerrum length of the model and over twice as long as the average bond length between neighboring beads, was chosen for the bound/unbound cutoff distance. The reported simulations all form one final complex with one polyanion and varying number of diblock chains. To characterize the condensation of the polyanion, we define condensing ratio, $\alpha = \langle R_g^0 \rangle / \langle R_g \rangle$, where $\langle R_g^0 \rangle$ and $\langle R_g \rangle$ are the radius of gyration of the polyanion in the absence and presence of the condensing agents respectively. $\langle R_g \rangle$ is calculated according to the following equation.

$$\langle R_g \rangle = \sqrt{\left\langle \frac{1}{N} \sum_{k=1}^{N} (r_k - r_{cm})^2 \right\rangle}$$

(4)

The indices k run over all the beads on the chain and r_{cm} is the center of mass of the polyanion chain. The ensemble average, $\langle R_g \rangle$ is performed over the production run with simulation time from $9 \times 10^4 \tau$ to $15 \times 10^4 \tau$, well after the formation of complex.

To characterize the structure of the complex, we calculate the radial density distribution functions, $g_a(r)$, for the complex for each type of beads, $a=1$ for the polyanion beads, $a=2$ for cation beads of block copolymers, and $a=3$ for neutral beads of the block copolymers. The radial density distribution function is defined as:

$$g_a(r) = \frac{n_a(r)}{(4\pi/3)\left[(r+dr)^3 - r^3\right]\rho_a^0}$$

(5)

where $n_a(r)$ is the number of particles of type a at a distance from r to $r + dr$, from the center of mass of the polyelectrolyte complex, which included the polyanion chain and all of the bound copolymer chains, ρ_a^0 is the number density of particles of type a in the entire simulation box. These radial density distribution functions calculated for three types of beads reflect the structures of the complex in details. However, for quick assessment, we further calculated the

average distance of each type of beads $<R_a>$ (a =1, 2, 3) to the center of mass of the complex, where $<R_a>$ is calculated according to

$$<R_a(r)> = \frac{\int r\rho_a(r)\,4\pi r^2 dr}{\int \rho_a(r)\,4\pi r^2 dr} = <\sum_{i=1}^{N_a} r_i / N_a>$$

(6)

r_i is the distance of ith bead to the center of mass of the complex, N_a is the total number of beads of type a in the complex. We also calculate average size of the complex $<R_c>$, which is calculated according to the same Equation (6) except the summation is performed over all three types of beads. All ensemble averages are performed in the last half of the trajectory with simulation times from $9\times10^4\ \tau$ to $15\times10^4\ \tau$, well after the formation of complex.

III. Results

A. Complexation with linear diblock polycations

We first examine the complexation of a polyanion in the presence of short linear diblock chains. Figure 1 shows the number of diblock chains that are bound to the polyanion as a function of time at the beginning of production run for the 8d8 system, a system containing a single polyanion with length 64, 8 linear diblock chains each with a block of 8 positive beads and a block of 8 neutral beads, and no monovalent ions. A diblock chain was considered bound to the polyanion if any bead of that chain was within a given cutoff distance of any polyanion bead. The complexation of the polyanion happens quickly, with over half of the polyanion charges neutralized by bound chains within only a few thousand simulation steps. The addition of the final diblock chains to the complex occurs relatively more slowly, but a neutral complex is formed early in the simulation, well before $2\times10^4\tau$. The diblock chains that bind to the polyanion do not subsequently release from the complexes in these simulations; they remain bound throughout the simulation. The radius of gyration, R_g, of the polyanion chain as a function of simulation time is also shown in Fig. 1. The R_g of the polyanion initially decreases very rapidly, as the first diblock chains bind to it. The starting configuration of the polyanion chain resulted in a highly extended chain, and part of the initial decrease in R_g was the result of relaxation of the chain, not the result of direct interactions with the diblock chains. For comparison, $R_g{}^0$, the radius of gyration of a polyanion chain in the presence of only neutralizing monovalent counterions is also shown in Fig. 1. The average value of $<R_g{}^0>$ was calculated to be 9.67±0.03. In comparison, $<R_g>$ of the polyanion in the complexes rapidly decreases below $<R_g{}^0>$ for a chain in solution with only neutralizing counterions.

Figure 2 shows sample snapshots of the polyanion and bound diblock chains during the complex formation throughout the simulation. When the polyanion chain is bound to only a few diblock chains, the resulting configurations resemble the necklace-like structures found by Jeon and Dobrynin[21,22] in polyelectrolyte-polyampholyte complexes. The binding of the first few diblock chains does not result in the complete condensation of the polyanion; instead, the positively charged diblock beads interact with and condense only a small section of the polyanion. The condensed globules formed by a section of the polyanion and the diblock chain are connected with a stretched section of the polyanion. While the diblock chains only interact with a few of the polyanion beads at a time, they do move along the chain. For example, panels (d) and (e) of Fig. 2 show two snapshots from when the polycation chain is bound to four diblock chains. In panel (d), two of the diblocks are very close together, forming a large necklace-bead at the end of the polyanion. As the simulation progesses, one of the diblock chains translates toward the center of the polyanion chain and the polyanion has four distinct necklace beads, as shown in panel (e). When the polyelectrolyte complex approaches neutrality, the polyanion is condensed into a globule without stretched chain sections. The

proposed core-corona structure of complexes formed from polyelectrolytes and diblock polycation chains can be visually seen in Fig. 2, with the charged beads forming dense globular structure surrounded by neutral hydrophobic arms extended into the solution. The sizes of complex as well as R_g of the polyanion do not change significantly afterwards.

Figure 3 shows the number of bound diblock chains and polyanion radius of gyration for a 4d4 system and reveals that the general complexation behavior for this system is similar to the 8d8 system. Several diblock chains quickly bind to the polyanion resulting in a rapid reduction in the radius of gyration of the polyanion. Visualization of the trajectory also showed that the formation of the complex in the 4d4 system occurred much like the 8d8 system, with the complex forming a necklace-like structure when the number of bound positive beads was much lower than the charge of the polyanion. As the complex approached neutrality, a compact globular structure, such as the structure shown in Fig. 4 (a) was formed. In order to compare the effect of neutralizing counterions on complexation, Fig. 3 also includes data from a separate simulation of a 4d4(c) system, a system with the same type and number of polymer chains as the 4d4 but with additional counterions which neutralized the charges on the polyelectrolytes. The initial configuration of the 4d4(c) system included 64 positive and 64 negative singly charged beads that had the same properties as the polyelectrolyte monomers and were randomly placed in the simulation box. The presence of the counterions results in a slower increase in the number of bound diblock chains, as the counterions shield the electrostatic attraction between the polyelectrolytes. The final diblock chain does not bind to the polyanion in the 4d4 (c) system until ~ $1 \times 10^5 \tau$ into the simulation, which is beyond the maximum time shown in Fig. 3. The number of positive counterions that are bound to the polyanion, again using a cutoff distance of 2.5σ, as a function of time is also shown in Fig. 3, in order to investigate the counterion release phenomena. The initial increase in the number of bound counterions is the result of randomly placing the counterions in the simulation box, as opposed to locating them near the polyelectrolytes. The polyanion attracts a significant number of counterions to it early in the simulation that are subsequently released upon complexation with the diblock chains. However, the net charge of the complex, including the polyanion, diblock polycation, and counterion charge, remains negative until 15 of the diblock chains are included in the complex. For example, there are ~10 positive counterions bound to the polyanion when 10 diblock chains are in the complex, indicating that at least ~15 of the polyanion charges are not neutralized in the complex. In agreement with previous results,[6] the structure of the polyelectrolyte complex does not seem to be greatly affected by the presence of counterions, as the polyanion radius of gyration for both systems approaches the same value when most of the diblock chains are bound and the final structures of the simulations, as shown in Fig. 4, are similar.

Comparing typical structures shown in Figures 4 and 2 reveals that the complex formed by 4d4 does not have a well-defined core-corona structure. This difference can be seen in radial density distribution functions in the final formed complexes. In general, a prefect core-corona structures would be defined by two main characteristics: a dense core composed of only charged beads and a diffuse corona composed only of the neutral beads. Figure 5 presents the radial density distribution function for several linear diblock systems. For the 32d32 system (Fig. 5 (a)), the $g_a(r)$ of the neutral beads is wide and shallow and extends past the distance at which the $g_a(r)$ of the charged beads approaches zero, confirming that the neutral block extends into solution and forms a corona around a dense core of charged beads. As the length of the copolymer blocks gets shorter, the overlap between the $g(r)$ of the neutral and charged beads increases, and core-corona structures become less well-defined. Fig. 5 (d) shows the radial distribution functions of the 4d4 system for which the $g_a(r)$ of the neutral beads is almost entirely within the $g_a(r)$ of the charged beads. Hence, no protective layer of the corona region can be defined for 4d4 system. Figure 6 shows sample snapshots from the 32d32 and 16d16 systems. The complexes have a well-defined core-corona structure, with the longer neutral blocks sticking much further into solution than the 8d8 system as shown in Fig. 2. An additional

feature of these complexes is that the neutral blocks are typically well separated from each other; the neutral blocks of the 32d32 are often on opposite sides of the core, and the tetrahedral-like arrangement shown in Fig. 6 (b) is a common occurrence in the 16d16 system.

Another noticeable feature in the radial density distribution functions of these systems is that $g_a(r)$ for the polyanion beads follows closely with $g_a(r)$ for the polycation block of the copolymer. As will be shown shortly, this is no longer the case of star-branched copolymers. In the 32d32 complex, $g_a(r)$ is at its maximum at the center of mass of the complex for both charged beads and decays until it is near zero at a distance around 6σ. If the complex contains completely collapsed chains, then $g_a(r)$ would typically have constant density near the core and decay to zero at distance away from the center. If the polyanion somehow is not completely collapsed, then the density distribution functions may have a peak away from the center of mass as seen in 16d16 and 8d8 systems. The complex formed in the 4d4 system has a center that is less dense, as $g_a(r)$ of the charged beads has a maximum value that is only half that of the other systems and does not decay to zero until a distance of 8σ from the complex center.

Table I summarizes several parameters that quantity the ability of the diblock to condense the polyanion and to form a core-corona complex. The compaction of the polyanion is monitored through calculating the average $<R_g>$ of the polyanion in the complexes, as well a compaction factor, $\alpha=<R_g^0>/<R_g>$, where $<R_g^0>$ is the radius of gyration of the polyanion only in the presence of neutralizing monovalent counterions. In general, diblock chains with longer cationic blocks are better able to condense the polyanion, as compaction factor α increases with increasing block length. However, the values of α for the 32d32 and 16d16 systems is similar, indicating that there may be a limit to the degree to which the polyanion chain can be condensed. The rest of parameters characterize the complex size and structure. R_1, R_2, R_3 are the average radial distances of the polyanion, polycation and neutral beads in the complex and R_c is average radial distance of all beads in the complex. Table 1 shows that R_1 and R_2 are nearly the same for all the systems. The ratio $\gamma=<R_3>/<R_2>$ is a good parameter to characterize the core-corona structure. For 32d32, 16d16 and 8d8, for which the radial density distributions have revealed good core/corona structure, their γ values range from 1.8 to 2.4, much greater than one, indicates a good core-corona structure. For 4d4 system where radial density distributions indicate poor core-corona structure, its γ value is about 1.2. Based on data in Table 1, it seems $\gamma\sim1.5$ is a reasonable threshold value to differentiate core-corona structure.

The effect of varying the length of the neutral block of the diblock chain, while keeping the cationic block length constant, has also been studied through simulations of 8d32 and 8d4 systems along with 8d8. As the neutral block length decreases from 32 to 8 and 4, the size of the core slightly decreases, as reflected in small decrease in the $<R_g>$ of the polyanion and R_1 and R_2. Figure 7 shows the radial density distribution functions of these systems. As the neutral block length decreases from 32 to 8 and 4, the peak in $g(r)$ of the cationic beads shifts away from the complex center. As the neutral block length decreases, the polyanion becomes more compact, implying the presence of longer neutral blocks in the 8d32 and 8d8 systems slightly interfere with the compaction of the polyanion. The final complex in all three systems however form good core-corona structure, as their γ values varied from 1.6, 1.8 to 2.7. Increase the neutral block length from 8 to 32 significantly increases γ, as the neutral block form a good protective corona. However, when the neutral block length is kept the same, increases in the cationic block length improve the condensing capability, and thus also result in an increased γ. One can observe that γ for the 8d4 system is significantly higher than the 4d4 system, as the 8d4 forms a more condensed core.

B. Complexation with star branched copolymers

In order to further study the effects of copolymer chain architecture on complex structure, systems were prepared with star-shaped copolymers. These systems are an attempt to model

gene therapy vectors formed from branched PEI, in which several of the branches are grafted to a hydrophilic polymer, such as PEO. The star copolymers investigated here had four diblock arms. For one of the arms, the central bead of the star polymer was considered to be the first bead of that arm, while the initial bead of each of the other arms was a bead bound to the star center. Therefore, excluding the positive central bead of the start, three of the arms had length B and one arm had length B -1. Starting from the initial bead of each arm, beads 1 to $B/2$ were positively charged and bead $B/2+1$ to B were neutral. The labeling of the star chains was still dependent on the total charge of the chain, so while the system labeled 32s32 contained two star copolymers with 32 positive and 32 neutral beads, each of the four individual arms of the chain were each identical to the linear diblock chains of the 8d8 system. A 32s32 star would be composed of a positively central bead, three arms with 8 positive and 8 neutral beads, and one arm with 7 positive and 8 neutral beads. The central bead would be considered the first bead of the fourth arm.

Figure 8 shows the radial density distribution functions calculated for systems with star copolymers. In contrast with $g(r)$ for complexes formed with linear copolymers, the distributions for the anionic and cationic beads are no longer the same. The maximum in $g(r)$ for the beads of the polyanion for all of the star systems is located at the complex center of mass, and there is an additional peak located at $r \approx 2.5\sigma$ that appears only as a shoulder in the 8s8 system. The $g(r)$ for the positive beads of the copolymer has a maximum that is located at $r \approx 1.5\sigma$, between the two polyanion peaks. To help explain the layered structure indicated by the $g(r)$ of the star diblock systems, we examine Figure 9, which shows a sample snapshot of complex formed the 32s32 system. In panel (a), all of the beads in the system are shown and the complex has the familiar core-corona structure. Panel (b) of Fig. 9 shows a slightly enlarged version with same orientation of the sample snapshot shown in panel (a); however, in this image, only the positive beads of the star copolymers are shown. The positive beads of one of the star chains is shown in white, while the positive beads of the other star are shown in black. A clearly defined gap in the center of the complex between the star chains has been created by removing the beads of the polyanion from the image. The image indicates that a layered structure with the polyanion trapped between the positive beads of the stars is formed so that the beads at the very center of the complex are negative and the $g(r)$ of the polyanion chains is high. Just outside the complex center are the beads of the star copolymer that serve as the branching point of the stars. Because there are four arms on each star, the positive central bead of the star copolymer must be bound to 4 other positive beads. Therefore, the connectivity of the central bead of star chain results in a high polycation density just outside the center of the complex. This high polycation density would then attract the polyanion, resulting in the second peak of the polyanion $g(r)$ at ~ 3σ from the complex center. Complexes formed from polyanions and long-armed star copolymers have high polyanion density at the complex center, which is immediately surrounded by a region of dense positive charge resulting from the connectivity of the central beads of the star copolymers. The remainder of the complex core is composed of the positive beads of the star branches, which extend away from the middle of the core, and the polyanion chain. Finally, the neutral beads of the stars continue away from the complexes into solution, forming the corona.

Additionally, the scale of $g(r)$ for the star systems in Fig. 8 is much larger than for the linear diblock systems in Fig. 5 as the magnitude of $g(r)$ for the polyanions in the star system is very large at the center of the complexes. The neutral beads of the copolymer in the 32s32 and 16s16 systems again have a wide and shallow $g(r)$ centered several σ away from the complex center, creating a core-corona structure. In the 8s8 system, the combination of the shortness of the arms of the stars and the increased size of the complex prevents the neutral blocks from extending past the core of the complex. The complex in the 8s8 system was not condensed into a dense globular structure, but instead often had more extended conformations. For example, for brief periods in the simulation, the 8s8 complex formed a toroidal structure, as shown in

Fig. 10 (b). Toroids are common structures in complexes with semi-flexible polyelectrolytes[5,23] and in experimental condensed DNA,[2,3] but to our knowledge have not been previously reported in most simulations of flexible polyelectrolytes.[6,7]

As shown in Table 1, the R_g values of the polyanion chain in complexes with the star copolymers are dependent on the size of the stars. The size of the polyanion in the complex seems to be governed by the number of consecutive positive beads in the copolymer, not the overall charge of the chain. For example, the polyanion R_g in the 32s32 system, R_g=2.63, is close to the value, 2.74, found in the 16d16 systems, but is smaller than R_g=2.99 in 8d8 system. The cationic block lengths in each arm of the stars in the 32s32 system were 8 beads long; however, because the arms are connected, a linear stretch of 16 consecutive beads in the middle of the stars were positive, analogous to the cationic block of the copolymers in the 16d16 system. The 16s16 copolymers had the a cationic block length of 8, and the value of R_g of the 16s16 system is similar to the 8d8 system; the 8s8 has a cationic block link of 4, and R_g(8s8) $\approx R_g$(4d4) $\approx$ 4.3. The $\gamma=R_3/R_2$ ratios for the star systems again reveal that the formation of a core-corona structure is dependent on both the length of the neutral blocks of the diblock and the ability of the cationic block to compact the polyanion. While the lengths of the neutral blocks in the 32s32 and 16s16 systems were the same as the 8d8 and 4d4 systems, respectively, the corresponding γ of the star systems was greater, as the stars formed more compact cores, with lower values of R_2. Additionally, we note that, despite the differences between $g(r)$ of the polyanion and polycation beads, R_1 and R_2 remain similar for these systems. There seems to be no need to differentiate between the positive and negative beads when calculating the size of the core, even though the radial density distributions for the two types in star-branched copolymers are very different.

IV. Discussion and Conclusion

The primary goal of the simulations presented in this paper is to understand how copolymer block length and architecture affect the structure of complexes formed from DNA and PEG-PEI block copolymers. These complexes are relevant to preparation of gene therapy complexes and have been investigated by several experimental studies. Petersen et al.[24] studied the structures formed from DNA and PEI-g-PEG copolymers of different architectures and found that complexes were large and diffuse when formed from copolymers with many short PEG blocks, while copolymers with fewer and longer PEG blocks created complexes that were smaller and more compact. Additionally, they showed that complexes formed from copolymers with very short PEG blocks had high ζ-potentials, a result consistent with our simulations in which short neutral arms did not create a corona around the complex and would, therefore, not be able to shield the charged core of the complex. They also determined the ratio of amine nitrogens to DNA phosphate groups (the N/P ratio) required to neutralize the DNA charge. Neutralization occurred at N/P $\approx$ 2 for complexes formed with PEI-g-PEG, while only N/P = 1.7 was required for complexes formed with PEI homopolymers. A study of the structure and thermodynamics of the complexation of DNA by a cationic copolymer by Bronich et al.[25] had very different results. In this study, at an N/P ratio of 1, complexes of DNA and PEO-g-PEI were electrically neutral, a result that is somewhat surprising considering that PEI is not considered to be fully protonated under physiological conditions. Additionally, they did not observe condensation of the DNA, with the DNA remaining in extended threadlike particles. The differences between the two studies can to a large degree be understood by considering the size of the PEI block used to condense the DNA. Petersen et al.24 used PEI with a molecular weight of 25 kDa, the PEI used by Bronich et al.25 only had a MW of 2 kDa. The shorter PEI had fewer than 50 repeating units and was used to condense a DNA chain with ~1620 basepairs. As shown in the simulations here, chains with shorter cationic blocks resulted in less condensation of a flexible polyanion, a characteristic that would likely be even more important when considering the condensation of more rigid DNA. Differences in complexes formed from

linear PEI and branched PEI have also been widely reported,2 with highly branched chains forming smaller, but more toxic complexes. While the degree of condensation of the linear polyelectrolyte was similar for linear and branched cationic chains here, the architecture of the cation chain was found to impact the core structure in the complexes, an effect which could potentially become more important for the complexation of multiple polyanion chain.

This work may also be used to shed insight into more general study of the interactions between oppositely charged polyelectrolytes. The complexes described in this paper are part of a general class of structures that has been called complex coacervate core micelles (C3Ms), recently reviewed by Voets et al.[4] More specifically, the complexes investigated in this work are a part of a class of structures termed S-C3Ms, which refers to complexes formed from a linear polyelectrolytes and oppositely charged block copolymers. They report that investigations of the internal core structure in S-C3Ms have been extremely limited. We describe some initial investigations into the structure of the complex cores and find that, for linear diblock chains, the charged block of the diblock and the polyanion chain have similar density profiles within the complex cores. In contrast, star block chains form complexes with a layered complex core. Several experimental results are consistent with those of the simulation. For complexes formed from poly(2-methylvinylpyridinium iodide) and poly(acrylic acid)-*block*-poly(acrylamide), increasing the neutral hydrophilic block results in an increase in the hydrodynamic radius of the complex.[26] Similarly, longer neutral blocks in the simulations stick further out into solution creating larger complexes. Experimental complexes formed with very short neutral blocks have been shown to not provide an effective steric barrier to macroscopic phase separation.27 While only single polyanion chains were used here, short neutral blocks only extended slightly outside the core of the complexes, and would therefore not be a barrier to aggregation and phase separation.

In summary, molecular dynamics simulations of a single polyanion chain in the presence of several shorter copolymer chains which contained neutral hydrophilic and polycationic blocks were performed. The structure of the complexes was found to be dependent on the architecture and block length in the copolymer chains. Linear diblock chains with long blocks formed small, compact complexes with a well defined core-corona structure. As the block length decreased, the core-corona structure was disrupted, as the shorter chains lacked the ability to completely condense the polyanion and the shortness of the netural blocks prevented them from extending into solution. Very short neutral blocks were found to be located primarily within the charged core of the complex. The internal structure of the core was also investigated, and while linear copolymer chains were homogeneously dispersed with the polyanion, the core in complexes formed from star copolymers had a layered structure.

Acknowledgments

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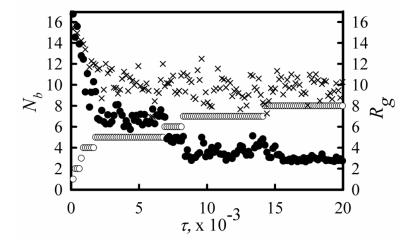27. van der Burgh S, de Keizer A, Stuart MAC. Langmuir 2004;20:1073. [PubMed: 15803680]

Figure 1.
Plot of N_b, the number of diblock copolymers bound to the polyanion (left axis, open circles), and the radius of gyration of the polyanion (right axis, closed circles) as a function of simulation time for the 8d8 system. For comparison, the radius of gyration ($\times$) of a polyanion chain in the presence of only neutralizing counterions is shown.
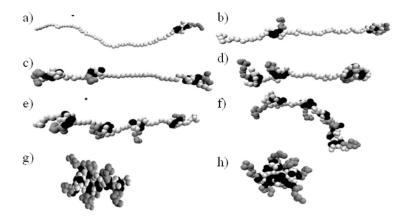
Figure 2.
Sample configurations from the 8d8 simulation with one (a), two (b), three (c), four (d) and (e), five (f), seven (g), and eight (h) diblock copolymer chains bound to the polycation. Negatively charged beads are white, positively charged beads are black, and neutral beads are gray.
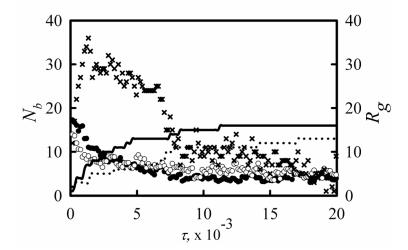
Figure 3.
The left y-axis shows the number of polycation chains, N_b, bound to the polyanion for the 4d4 (solid line) and the 4d4(c) (dotted line) systems. The number of counterions bound to the polyanion in the 4d4(c) system ($\times$) are also shown. The right y-axis shows the radius of gyration of the polyanion in the 4d4 (filled circles) and 4d4(c) (open circles) systems.
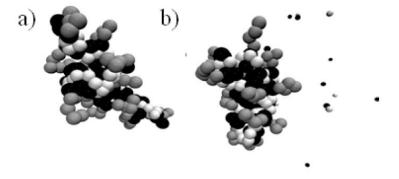
Figure 4.
Snapshots of the 4d4 system without (a) and with (b) counterions. The meaning of the bead colors is given in Figure 2. The images are not shown on the same scale; as shown in Table 1, the actual complex sizes in the two systems is similar.
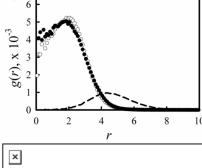
c)

Figure 5.
Radial density distribution functions around the complex center of mass for the 32d32 (a),
16d16 (b), 8d8 (c), and 4d4 (d) systems. $g(r)$ for polyanions (open circles), polycation
copolymer blocks (filled circles), and neutral copolymer blocks (dashed lines) are shown.
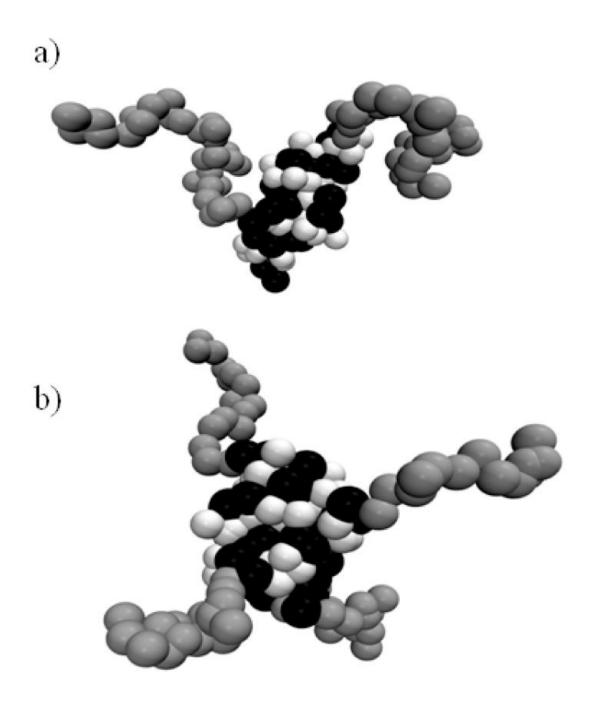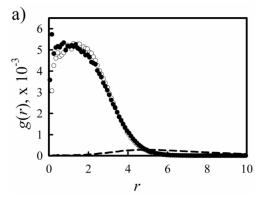
a)

b)

Figure 6.
Final configurations of complexes in the 32d32 (a) and 16d16 (b) systems.
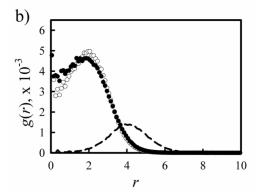
Figure 7.
Radial distribution functions around the complex center of mass for the 8d32 (a) and 8d4 (b) systems. $g(r)$ for polyanions (open circles), polycation copolymer blocks (filled circles), and neutral copolymer blocks (dashed lines) are shown.

Figure 8.
Radial distribution functions around the complex center of mass for the 32s32 (a), 16s16 (b), and 8s8 (c) systems. $g(r)$ for polyanions (open circles), polycation copolymer blocks (filled circles), and neutral copolymer blocks (dashed lines) are shown.
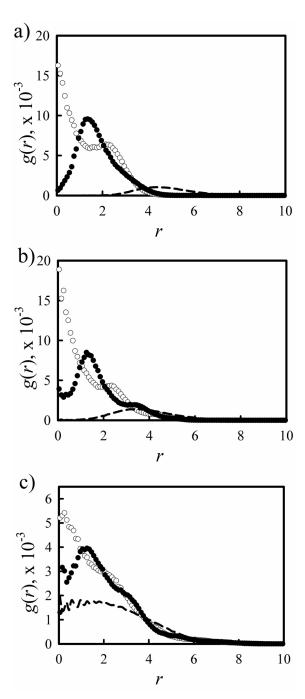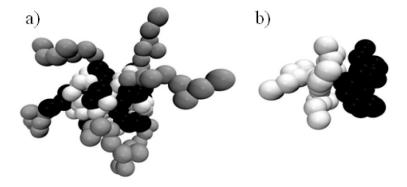
Figure 9.
Snapshot of the final configuration of the 32s32 system. In (a), all of the beads in the system are shown, with the bead coloring following the description of Figure 2. In (b), only the positive beads of the star copolymers are shown, with the positive beads of one of the star chains shown in white and the positive beads of the other star chain shown in black.
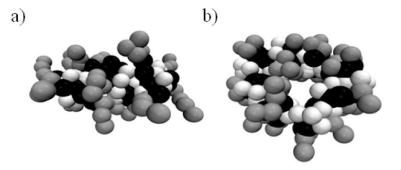
Figure 10.
Snapshots for the 16s16 (left) and 8s8 (right) simulations. The colors of the beads match the description of Figure 2.

Table 1

Characterization of degree of polyanion condensation and the formation of core-corona structures.

System	Number of copolymer chains	Architecture, length for positive (+) and neutral (N) blocks	$\langle R_g \rangle$	α	$\langle R_1 \rangle$	$\langle R_2 \rangle$	$\langle R_3 \rangle$	$\langle R_c \rangle$	γ
32d32	2	linear, 32(+), 32(N)	2.72(3)	3.5	3.03(5)	3.04(5)	7.37(10)	4.48(11)	2.4
16d16	4	linear, 16(+), 16(N)	2.74(3)	3.5	2.72(3)	2.75(4)	6.25(7)	3.91(8)	2.3
8d8	8	linear, 8(+), 8(N)	2.99(3)	3.2	2.85(4)	2.91(5)	5.35(6)	3.70(7)	1.8
8d32	8	linear, 8(+), 32(N)	3.02(3)	3.2	3.04(5)	3.10(5)	8.37(12)	6.60(15)	2.7
8d4	8	linear, 8(+), 4(N)	2.96(3)	3.3	2.81(4)	2.86(4)	4.62(5)	3.19(5)	1.6
4d4	16	linear, 4(+), 4(N)	4.32(3)	2.2	3.99(7)	4.10(8)	5.13(8)	4.41(8)	1.2
4d4(c)	16	linear, 4(+), 4(N)	4.41(3)	2.2	4.06(8)	4.15(8)	5.14(8)	4.45(8)	1.2
32s32	2	4-arm star, 8(+), 8(N)	2.63(3)	3.7	2.52(3)	2.57(4)	5.27(5)	3.45(7)	2.0
16s16	4	4-arm star, 4(+), 4(N)	3.24(4)	3.0	3.01(5)	3.02(5)	4.63(6)	3.55(6)	1.5
8s8	8	4-arm star, 2(+), 2(N)	4.31(3)	2.2	3.91(8)	3.87(8)	4.52(8)	4.10(8)	1.1

$\alpha = \langle R_g(I) \rangle / \langle R_g \rangle$ where $\langle R_g^{0} \rangle = 9.67 \pm 0.03$ is the average radius of gyration the polyanion in the absence of condensing agents; R_1, R_2, R_3 and R_C are average distances of polyanion beads, positive beads, neutral beads and all beads to complex center respectively (see text for definition). 4d4(c) system has the counter ions while all the rest of systems do not have counter ions. All stars have four diblock arms. Numbers in brackets give the errors in the last digit. $\gamma = \langle R_3 \rangle / \langle R_2 \rangle$.