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Interpolyelectrolyte Complexes between Starlike and Linear Macromolecules: A Structural Model for Nonviral Gene Vectors

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Molecular dynamics simulations are used to probe the structural organization of nonstoichiometric interpolyelectrolyte complexes (IPECs) formed by oppositely charged starlike and linear polyelectrolytes (PEs) in dilute aqueous solution. We demonstrate that undercompensated star-IPEC consists of a denser coacervate core and a charged starlike corona. Two distinctive populations of star branches—completely embedded in a coacervate core and stretched in a lyophilizing corona—are found. The scaling arguments support the stability of IPEC with partitioned star branches.

Interpolyelectrolyte complexes (IPECs) are formed by the electrostatically driven association between oppositely charged polyelectrolytes (PEs).^{1,2} Water-soluble IPECs found in a limited range of nonstoichiometric compositions are valuable candidates for the targeted delivery of biologically active molecules^{3,4} because of their enhanced safety compared to that of the viral vectors.

The advances in controlled radical polymerization have allowed for the synthesis of macroions with various topologies and well-controlled dimensions.^{5–8} Recently, a novel type of water-soluble IPECs formed upon association between linear PEs and oppositely charged, branched PEs (stars and molecular brushes),⁹ and starlike micelles assembled from asymmetric ionic/hydrophobic diblock copolymers^{10,11} have been discovered. Such IPECs with excess branched PEs are soluble in a wide range of solution composition and have almost constant size close to that of the original branched macroion.

The aim of this letter is to unravel the organization of IPECs formed upon association of branched and linear PEs. We focus here on a complex comprising one star-branched PE and a variable number of oppositely charged linear polyions. By using MD

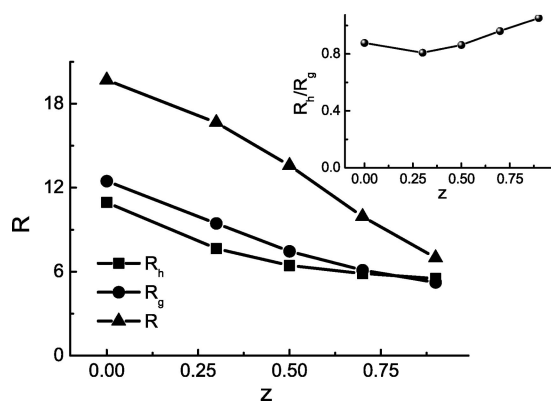


Figure 1. Radius of gyration R_g , hydrodynamic radius R_h , average distance between star end monomers and star center R , and R_h/R_g (inset) as a function of degree of neutralization z .

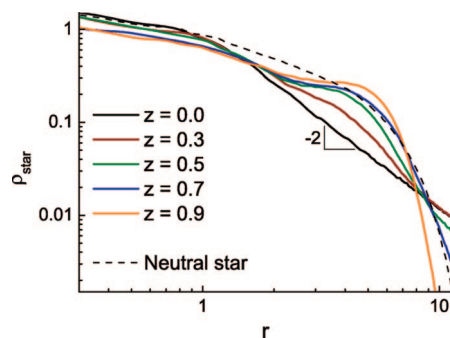


Figure 2. Number density of monomer units of the star-branched polyion in the IPEC as a function of radial distance r from the star center at different values of z (indicated). The density profile in a neutral (noncharged) star is indicated by a dashed line.

simulations and scaling arguments, we demonstrate the stability of an inhomogeneous core–shell–corona structure of such an IPEC.

Model and Simulation Method

The coarse-grained bead–rod model was used to represent both the star-branched and the oppositely charged linear PEs. The star comprised $p = 10$ arms with $N = 30$ beads (monomer

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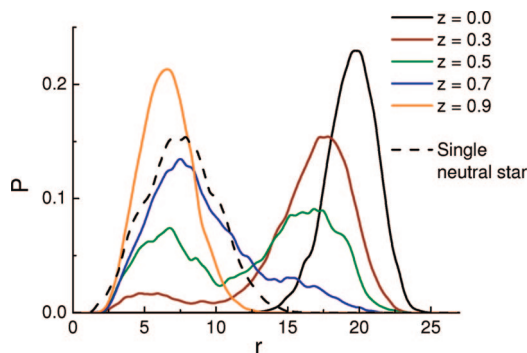


Figure 3. Fraction of the end-monomer units located a distance r from the center of a star at different values of z .

units) in each arm. The number of monomers in a linear (guest) chain, m , was equal to that in a star arm, N . All of the beads carried the absolute value of electric charge e (the degrees of ionization for negatively charged star branch, α , and for positively charged guest chains, α' , were equal and set to unity). The number f of guest chains in the system was varied so that the degree of complex neutralization $z = f/p$ changed from zero (for a bare PE star) to unity for the stoichiometric, $f = p$, IPEC. To preserve the simulation cell electroneutrality, the corresponding number of counterions was added. The simulations were performed using the stochastic dynamics method.¹² The solvent was modeled as an effective viscous medium, and the system was coupled to a heat bath. All of the particles had the same friction coefficient.

The excluded volume interactions between any nonbonded particles were described using the repulsive Lennard-Jones potential¹³

$$U_{\text{LD}}(r) = \begin{cases} 4\epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 + \frac{1}{4} \right], & r \leq r_0 \\ 0, & r > r_0 \end{cases} \quad (1)$$

where $\sigma = 1$ for polymer beads, $\sigma = 0.2$ for counterions, and $\sigma = 0.6$ for a pair bead-counterion; $r_0 = 2^{1/6}\sigma$ is a cutoff distance. In addition, the nonbonded beads of the guest chains and of the star arms experienced an attractive Yukawa-type potential¹²

$$U_{\text{att}}(r) = \begin{cases} -\epsilon \left(\frac{\sigma}{r} \right) \left[1 - \left(\frac{\sigma}{r_c} \right)^2 \right], & r_0 < r \leq r_c \\ 0, & r > r_c \end{cases} \quad (2)$$

with a cutoff distance of $r_c = 2.5\sigma$. The value of $\epsilon = 0.5$ (that corresponds to close to theta-solvent conditions), was used in all of the simulations.

Electrostatic interactions between charged particles were described by the Coulomb potential

$$U_C = l_B \sum_m \sum_{i \neq j}^N \frac{q_i q_j}{|\vec{r}_{ij} + \vec{m} L_{\text{box}}|}$$

where $\vec{r}_{ij} = \vec{r}_i - \vec{r}_j$, \vec{m} is the identification of a particular image cell, L_{box} is the side length of the periodic cell, q_i is the charge of the i th particle in e units, and $l_B = e^2/4\pi\epsilon_0\epsilon_r k_B T$ is a Bjerrum length ($l_B = \sigma = 1$ in all simulations). The particle mesh Ewald method^{14,15} was used to evaluate the electrostatic interactions.

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The equations of motion were solved using the Newtonian iteration procedure with the time step $t = 0.01\sigma(m/\epsilon)^{1/2}$. (For technical details, see ref 16.) The unit of time in the simulation is defined by $\tau = \sigma(m/\epsilon)^{1/2}$, and the lengths are expressed in units of σ . The reference temperature $T = \epsilon/k_B$ was equal to unity. The systems were equilibrated from the initial configuration during 2×10^5 time steps. Production runs of 2×10^5 time steps were carried out after equilibration. This time is much larger than the maximum relaxation times of star arms and linear chains ($\sim 10^4$ and $\sim 5 \times 10^3$ time steps, respectively).

Results

Figure 1 demonstrates the contraction of IPEC upon an increase in the degree of neutralization z . The radius of gyration R_g , the hydrodynamic radius R_h , and the average distance R between the branch end monomer and the star center all decrease monotonously as a function of z . R_h was calculated using the expression for the Zimm model in the Kirkwood–Riseman approximation.¹⁷ The ratio R_h/R_g is presented in the inset. Recall that $R_h/R_g \approx 0.66$ for the Gaussian chain, 0.93 for a regular many-arm star in a theta solvent,¹⁸ and 1.29 for a uniform hard sphere. As expected, R_h/R_g is close to 0.9 for a bare PE star ($z = 0$). At large values of z , the R_h/R_g ratio monotonously increases, approaching the value for a uniform hard sphere (dense stoichiometric IPEC, $z = 1$). The minimum in the R_h/R_g ratio is found at intermediate z values corresponding to experimentally observed complexes.

Figure 2 shows polymer density profiles for a star-branched polyion. The polymer density decay in a bare PE star follows the power law r^{-2} , where r is the distance from the star center (Figure 2). Such a dependence is expected in a PE star at low solution salinity.¹⁹ For comparison, the polymer density profile for a noncharged (neutral) star is also presented. The corresponding exponent is close to -1 in accordance with the theoretical prediction for a neutral star in a theta solvent.²⁰ In the vicinity of the star center, polymer density profiles for neutral and bare charged stars match each other. In this region, the monomer density is so large that the steric interactions dominate the electrostatic repulsions between monomers and determine the local star structure.²¹

An increase in the number f of guest PE chains (an increase in z) leads to the progressive modification of the radial distribution of the star monomers (Figure 2). An inflection point and subsequently a plateau appear on the polymer density profile at intermediate values of r . The level of the plateau region is virtually constant whereas its width increases as a function of z . Closer to the external IPEC boundary, the concentration of star monomers decreases, retaining the same power law ($\sim r^{-2}$) as in a bare PE star. The polymer density profiles of guest chains (not shown) exhibit a pronounced maximum whose width and position approximately coincide with those for the plateau on the density profiles of the star monomers. A weak but non-negligible penetration of the guest chains in the central region of the star is also observed.

The presented simulation results demonstrate that nonstoichiometric star IPECs comprise three distinctive regions. The middle plateau region corresponds to the coacervate domain where a majority of the guest monomers are found. Here the charge due

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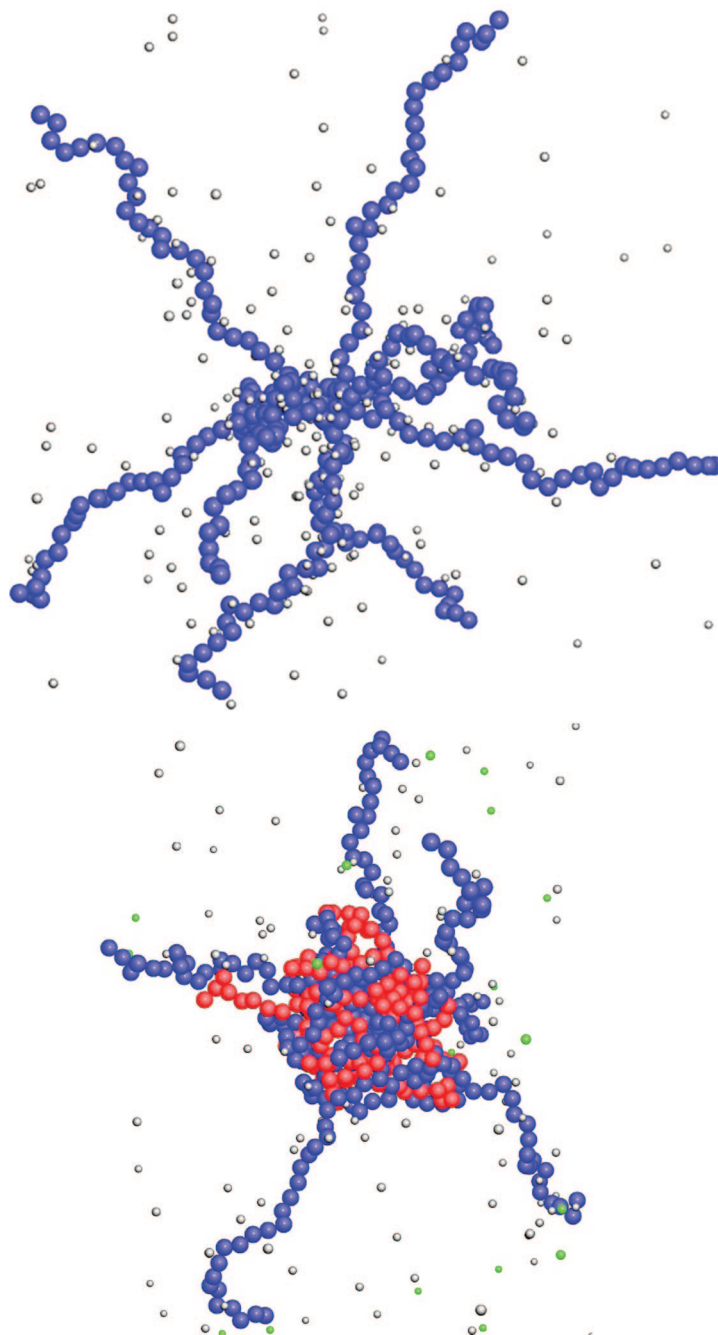


Figure 4. Snapshots of typical conformations of a bare PE star and star IPEC at $z = 0.5$.

to star branches is locally compensated charge by guest PE chains. The density in this region is controlled by the balance of the fluctuation-induced electrostatic attraction between oppositely charged PEs and ternary steric repulsions between monomers.²²

The guest PE chains also penetrate the dense central region, where they partially compensate the charge due to star branches. However, in this region local IPEC structure is determined by the nonelectrostatic repulsions between monomers. Under close to theta-solvent conditions (chosen in the simulations), ternary contacts dominate, and the central IPEC region is identified as a theta region.²¹

Finally, the periphery of the IPEC is decorated by a corona formed by the star branches. In the corona region, the concentration of monomers due to guest chains is low, and the local charge

density due to star branches is significantly undercompensated. Therefore, similarly to a bare PE star, the corona extension is controlled by a combination of the osmotic pressure of counterions and the Coulomb interactions balanced by the entropic elasticity of the branches. As a result, the star branches are stretched almost uniformly, and the polymer density decays as r^{-2} .

An important insight into IPEC structure is provided by radial distributions of the end monomers depicted in Figure 3. For a bare PE star, $z = 0$, a single sharp peak indicates fairly equal and uniform stretching of the star branches.²³ For comparison, a similar distribution is presented in a noncharged star (dotted line in Figure 3). Here, the peak is broader and is shifted to

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smaller values of r , indicating weaker radial extension of the corona in a neutral star. For $z \geq 0.3$, a single maximum splits into two: the proximal maximum corresponds to the star branches completely embedded in the coacervate domain whereas the distal one corresponds to the branches constituting the depleted corona of IPEC. A decrease in the distal maximum and a corresponding increase in the proximal one are indicative of progressive repartitioning of the branches from the corona to the coacervate region upon an increase in z . The segregation of star branches into two populations forming the condensed (coacervate) and the extended (corona) domains is clearly seen in the snapshots presented in Figure 4. A similar effect of chain segregation was also predicted for PE chains tethered to an oppositely charged interface,²⁴ core-shell spherical polyampholyte brushes,²⁵ and solutions of block polyampholytes.²⁶

To rationalize the origin of segregation in a star IPEC, we consider the complex free energy that comprises the contributions due to the coacervate domain (core) and extended corona, $F_{\text{IPEC}} = F_{\text{core}} + F_{\text{corona}}$. Assuming that $\alpha = \alpha' \leq 1$, we introduce here the number of guest monomers $n = fm/p$ per branch of the PE star. The degree of neutralization $z = n/N < 1$ coincides with the charge ratio in the solution of monodisperse IPECs. The core comprises equal numbers fm of cationic and anionic monomers and is characterized by the volume fraction τ and surface tension γ . Both are related to the correlation length ξ , which is a function of α and salt concentration in the solution c_s . Consistent with the MD simulations, we focus below on a low-salt case.

The coacervate core, similar to a salt-free polyampholyte globule, constitutes a melt of closely packed electrostatic blobs of size $\xi \approx b/\alpha^{2/3}l_B^{1/3}$, where b is the monomer size.²⁶ Each blob contains g monomers of either a cationic or anionic nature and exhibits Gaussian statistics, $\xi \approx bg^{1/2}$. The energy of the electrostatic repulsion within a blob and of the electrostatic attraction between oppositely charged blobs is on the order of thermal energy kT . The volume fraction inside the core is $\tau \approx gb^3/\xi^3 \approx \alpha^{2/3}l_B^{1/3}$, and the surface tension is $\gamma \approx b^2/\xi^2$. The core contribution to the IPEC free energy comprises the energy of fluctuation-induced attraction between oppositely charged PEs and the free-energy losses due to the core/corona boundary, $F_{\text{core}}/k_B T \approx -pn/g + (pn/\tau)^{2/3}\gamma$, where the numerical coefficients on the order of unity are omitted.

To specify the corona contribution, we examine two possible scenarios: (1) each of the branches in IPEC contributes n monomers to a coacervate core, leaving $(N - n)$ monomers in the corona and (2) the core comprises entirely $p_c = pn/N = pz$ star branches whereas the rest $p - p_c = p(1 - z)$ of the branches form a depleted corona. In both cases, $N \gg 1$, $z < 1$, and $R_{\text{core}} \ll R_{\text{corona}}$. If $p, p - p_c \gg \alpha^{-1/2}l_B^{-1} \geq 1$, then the corona is found in the osmotic regime, and a majority of the $\alpha p(N - n)$ mobile counterions are localized inside the corona to compensate for the IPEC charge.¹⁹ The osmotic pressure of the counterions induces stretching of the coronal chains. In a boxlike model, the coronal chains are stretched equally and uniformly, and the free energy of the starlike corona comprising p branches of length N each equals

$$\frac{F_{\text{corona}}}{k_B T} = \alpha p N \left[\frac{3}{2} + \ln \frac{3}{4\pi e \alpha^{1/2} N^2} \right] \quad (3)$$

Therefore, in the first scenario, $F_{\text{corona}}^{(1)}/k_B T = \alpha p(N - n)[^{3/2} + \ln(3p/4\pi e \alpha^{1/2}(N - n)^2)]$, whereas in the second scenario, $F_{\text{corona}}^{(2)}/$

$k_B T = \alpha p(N - n)[^{3/2} + \ln(3p(N - n)/4\pi e \alpha^{1/2} N^2)]$. Clearly, one finds a lower free energy when the branches partition between the two domains, $F_{\text{corona}}^{(1)} - F_{\text{corona}}^{(2)} \approx (z - 1) \ln(1 - z) > 0$. Because the condition of strong localization of counterions in the IPEC corona is violated when $p_c \rightarrow p$, a similar analysis should be performed for IPECs with delocalized counterions. For a charged corona comprising p branches of length N each,

$$\frac{F_{\text{corona}}}{k_B T} \approx N p^{5/3} (\alpha^2 l_B)^{2/3} \quad (4)$$

By comparing the two scenarios ($F_{\text{corona}}^{(1)}/k_B T \approx (N - n)p^{5/3}(\alpha^2 l_B)^{2/3}$ and $F_{\text{corona}}^{(2)}/k_B T \approx N(p - p_c)^{5/3}(\alpha^2 l_B)^{2/3}$), one finds $F_{\text{corona}}^{(1)}/F_{\text{corona}}^{(2)} = (1 - z)^{-2/3} > 1$. Therefore, segregation of the star branches is also thermodynamically favorable in the case when the Coulomb repulsions dominate in the IPEC corona.

Conclusions

The MD simulations and the scaling arguments demonstrate that nonstoichiometric star IPECs formed at a charge ratio of $z < 1$ consist of three distinctive domains: (i) a central region dominated by steric short-range repulsions between monomers, (ii) a coacervate domain containing a majority of the guest monomers, and (iii) a coronal region formed by a fraction of PE star branches that carry the uncompensated charge of the whole aggregate. The central region is similar to that in a bare PE star. It extends upon an increase in number p of the star branches. The coacervate domain is characterized by the local compensation of charge and almost uniform density distribution. A finite fraction of PE star branches are completely embedded in this domain to compensate the charge of guest PE chains. The coronal region formed by the remaining star branches determines the hydrodynamic radius of an IPEC. The interactions between coronas provide aggregative stability of the complexes in the range of $z < 1$. The predicted nonmonotonous behavior of the R_h/R_g ratio as a function of $z \sim f$ reflects repartitioning of the star branches between the coacervate domain and the corona: the formation of a dense IPEC region in the middle part of the star and depletion of the coronal domain. Note that a salt-free solution of star IPECs phase separates when z exceeds a certain critical value (≈ 0.5) and therefore IPECs with $z > 0.5$ can hardly be examined experimentally.⁹ The MD simulation of a single star IPEC enables us to analyze the IPEC evolution over the whole range of z values.

The trends discussed in this letter are also expected in IPECs formed between linear chains and other types of branched macroions (molecular and colloidal PE brushes, starlike and wormlike micelles with PE coronas, etc). The results may stimulate the design of nonviral gene delivery systems employing branched and micellar macroions. Clearly, the solution salinity is a crucial factor in the efficiency of a vector system.²⁷ We aim to explore the effect of ionic strength on the structure of star IPECs in forthcoming publications.

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