Structural effects in overcharging in complexes of hyperbranched polymers with linear polyelectrolytes

Sergey Lyulin,*[a] Kostas Karatasos,[b] Anatolij Darinskii,[a] Sergey Larin[a] and Alexey Lyulin[c]

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New insight is provided by a combined theoretical and simulational approach regarding the effects of structural characteristics of the constituents, on the overcharging phenomena in complexes formed by hyperbranched polymers with linear polyelectrolytes.

Complex formation between a polyelectrolyte (PE) and a macroion (*e.g.* between DNA and protein) is a usual phenomenon in living matter,[1] whilst playing a key role in novel nanoscale applications such as protein separation and colloidal stabilization.[2,3] As a consequence of such complexation *overcharging* may take place, in which the countercharge carried by the polyelectrolyte exceeds that on the surface of the macroion resulting in charge inversion. Charge inversion is of particular importance in biological systems through which close proximity between initially like-charged entities (*e.g.* the negatively charged DNA and the cell surface) becomes possible.[4] Moreover, the presence of a linear polyelectrolyte provides stabilization of the formed complexes as well as protection of candidate guest molecules,[5] which are among the most expedient attributes in gene transfer or in targeted drug-delivery applications.[6] Hyperbranched polymers (HBP) appear as particularly promising candidates for the formation of interpolyelectrolyte complexes (IPEC), due to their high functionality and the versatility on the control of their chemical and geometrical characteristics.[7] On the above accounts, exploration of the properties of HBP-PE complexes appears as a challenging task not only in terms of a scientific, but also in terms of a practical point of view. As we demonstrate in this work, by using computationally efficient coarse grained models, one can predict the behavior of HBPs of distinctly diverse topologies, and illustrate in which cases HBPs can act either as cost-effective alternatives of regular branched dendrimers, or as components with sensitively tunable structural and overcharging properties in applications involving IPECs. To this direction, the goal of the present paper is to communicate results from a combined simulational and theoretical effort on the charge inversion phenomenon in non-covalent HBP-PE complexes, focusing on the effects of the topology of the HBP constituent and the size of the linear PE on the structural characteristics of these systems, as well as on the occurrence of charge inversion. Our findings offer a deeper understanding of the mechanisms involved, demonstrating possible routes for controlling the above attributes.

Previous experimental work on IPECs involving HBPs has focused on molecules bearing a perfect dendritic topology, referred to as dendrimers,[8,9] while overcharging phenomena in complexes formed by a penetrable dendrimer with charged terminal groups (macroion) and oppositely charged linear polyelectrolyte (polyion), have recently been discussed by some of the authors.[10] Although these efforts shed light on the properties of individual neutral and charged HBPs[11,12] as well as on the behavior of IPECs involving perfect dendrimers, similar studies for complexes between non-regular shaped HBPs with oppositely charged linear PEs are not as yet available. Our recent study of electroneutral complexes HBP - linear PE[13] serves as a base for the present work.

To this end, we have performed Brownian dynamics simulations of freely-jointed generic bead-rod model[11] of HBP with tri-functional core. Both HBP and linear PE are represented by beads with friction coefficient ζ connected by rigid rods of length l. The total amount N of monomers in the simulated HBPs was equal to $N = 94$ which corresponds to the 4th generation ($g = 4$) of a perfect dendrimer. The branching ratio of the inner monomers is defined by their degree of branching,[14] DB, related to the probability of branching of each monomer[12]

$$DB = \frac{2D}{2D + L} \tag{1}$$

Here D is the number of branched monomers and L is the number of linear (non-branched) monomers, excluding terminal groups. We have simulated HBPs with degree of branching $DB = 0.5$, which is typical in synthesis of HBPs.[15] N and DB define the number of the HBP terminal groups, N_T, ($N_T = 25$ in the present simulations) at fixed functionality of the branching points and core,

$$N_T = 2 + DB(N/2 - 1) \tag{2}$$

With fixed N the same values of the parameter DB may represent branched polymers with distinctly different topology. In order to uniquely define the branched structure we have utilized an additional parameter, the topological distance-based Wiener index WI,[12]

$$WI = \frac{1}{2}\sum_{i,j=1}^{N} d_{ij} \tag{3}$$

where d_{ij} is the number of monomer segments separating beads i and j. With functionality fixed by DB and N, WI defines the specific topology of the HBP.

Two topological extremes of HBPs have been examined as illustrated in Fig. 1. HBP with a relatively low WI corresponds to a rather

[a]*Institute of Macromolecular Compounds, Russian Academy of Sciences, Bolshoj pr. 31, St. Petersburg, 199004, Russia. E-mail: serge@macro.ru; Fax: +7 812 3286869; Tel: +7 812 3285601*
[b]*Physical Chemistry Laboratory, Chemical Engineering Department, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece. E-mail: kkaratas@auth.gr; Fax: +30 2310996222; Tel: +30 2310995850*
[c]*Group Polymer Physics, Eindhoven Polymer Laboratories and Dutch Polymer Institute, Technische Universiteit Eindhoven, P.O. Box 513, MB Eindhoven, 5600, The Netherlands. E-mail: a.v.lyulin@tue.nl; Fax: +31 40 2445253; Tel: +31 40 2474253*

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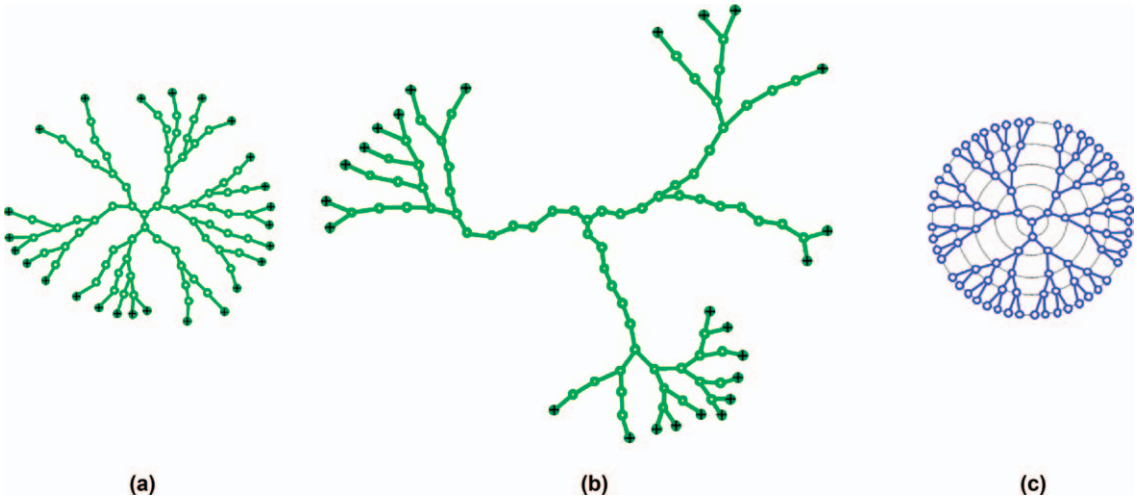

Fig. 1 Simulated HBPs. (a) HBP_{min} with minimal Wiener index ($WI = 37\,670$), (b) HBP_{max} with maximal Wiener index ($WI = 60\,351$) and (c) regular $g = 4$-generation dendrimer ($DB = 1$) with the same molecular weight $N = 94$ and $WI = 31\,293$ (note that the linear polymer chain with $N_{ch} = 94$ has $WI = 156\,849$).

compact dendrimer-like structure (HBP_{min}, with preferable branching close to the core) while HBP with a relatively high WI represents a less compact, star-like structure, (HBP_{max}, with preferable branching at the periphery). Each terminal group of the simulated HBP is charged with a positive unit charge, while each monomer of the linear PE with length N_{ch} was assigned a negative unit charge. Such a type of HBP ionization is close to the neutral pH values.[10] For electrical neutrality of the complex the length N_{ch} of the PE should be $N_{ch} = N_T$. In present simulations the PE length N_{ch} is varied between 25 and 80, *i.e.* larger or equal to the value necessary for electroneutrality. The results are compared to those for simulated complexes[10] formed by perfect dendrimers of $g = 3$ generation bearing $N_T = 24$ charged terminal groups.

More details on the implemented Brownian dynamics algorithm and used potentials can be found elsewhere.[10] We employ dimensionless quantities where length l, energy k_BT, time $\zeta l^2/k_BT$, and translational friction of a single bead $\zeta = 6\pi\eta_s r_h$ are set to unity. Here k_B is the Boltzmann constant, T is the absolute temperature, r_h is the hydrodynamic radius of a single bead, and η_s is the solvent viscosity. Excluded-volume interactions are modelled by the Lennard-Jones potential with parameters $\sigma = 0.8$ and $\epsilon = 0.3$.[10] In the present study only the dilute solutions have been considered, so counter ions are not taken into account explicitly; the electrostatic interactions between all charged beads are treated in the Debye–Hückel approximation with Bjerrum length $\lambda_B = l$ and Debye screening length $r_D = 8.96l$ which is comparable to the size of macroions. Such a high value of r_D was chosen to represent a state of almost non-screened electrostatic interactions. Since the main attention in this study is paid to the difference in the overcharging between HBP and regular dendritic polymers, the same parameters as before[10] have been adopted for electrostatic and excluded-volume interactions.

Similar to what was found in complexes formed by regular dendrimers, charge inversion is observed for the simulated complexes formed by both HBP_{max} and HBP_{min} as well, when $N_{ch} > N_T$. Applying the same adsorption criterion as in the case of dendrimer models[10] the amount of adsorbed polyion monomers, N_{ads}, is found to be always larger than $N_T = 25$ which is required for electroneutralisation. At $N_T \leq N_{ch} \leq 50$ the value of N_{ads} increases

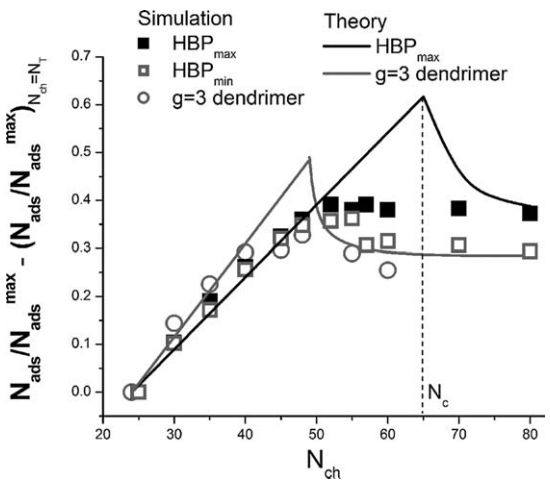

Fig. 2 Polyion-length dependence of the amount of the adsorbed monomers for simulated HBP complexes and a $g = 3$ dendritic complex. The results are normalized with respect to the maximum value of adsorbed monomers, N_{ads}, and shifted by the corresponding value at $N_{ch} = N_T$. Vertical dashed line shows the critical length at which polyion tail releases. The symbol sizes are larger than error bars.

monotonically with polyion length as shown in Fig. 2. This behaviour corresponds to the full adsorption of the linear PE and appears to be common for complexes formed by hyperbranched polymers of extremely different topology ranging from the perfect compact dendrimers to the rather open structure HBP_{max} models.

The topology-insensitive initial part of the N_{ads} (N_{ch}) dependence is mainly dictated by the macroion net charge. The maximum adsorption is reached at some critical value of the polyion length, $N_{ch} = N_c$. Compared to their dendritic analogues, the critical length for HBP complexes is moderately larger ($N_c \cong 45$ for a $g = 3$ dendrimer[10] and $N_c \cong 55$ in the present study). In the complexes formed by perfect dendrimers a further increase of the PE length leads to the polyion tail release and a consequent decrease of the number of adsorbed monomers.[10] A similar behavior is observed here, with the difference that the maximum of adsorption is rather smeared out as

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portrayed in Fig. 2. For HBP_{min} the onset of the decrease of the N_{ads} starts at larger values of N_{ch}, compared to the value observed in complexes formed by perfect dendrimers. For complexes formed by HBP_{max} however, such a decrease is absent, Fig. 2. Instead, a saturation of the adsorption curve is observed in the range of the simulated polyion lengths $\sim 2N_T = 55 \leq N_{ch} \leq 80$.

We suggest that for the complexes formed by star-like HBP_{max}, the N_{ads} (N_{ch}) dependence is related to the change of the HBP size which is linked with the increase of the polyion length. The mean-squared gyration radius R_g^2 of the HBP in a complex as a function of the linear PE length is shown in Fig. 3. The behavior of the HBP_{min} model is similar to that of the perfect dendritic molecules of the same size: complexation merely incurs a slight reduction in dimensions compared to the neutral analogues, Fig. 3. The size of the HBP_{min} in a complex remains unaltered upon further increase of the length of the polyion. The response of HBP_{max} however is distinctly different. For short polyions, $N_T < N_{ch} < N_c$, the size of the HBP_{max} decreases significantly to values lower than the size of the individual neutral HBP_{max}. This implies that complex formation not only triggers the decrease of dimensions of the linear PE, but leads to a severe reduction of the HBP_{max} size as well. In addition, the size of the HBP_{max} appears to be influenced by the chain-tail length. When the chain length N_{ch} becomes larger than N_c the size of the HBP_{max} reaches the dimensions of the individual neutral HBP_{max}.

To further understand these findings, we have resorted to the correlation theory developed by Nguyen and Shklovskii[16] who considered complexes formed by a structureless rigid sphere (macroion) and a linear persistent polyelectrolyte model with some radius a. In order to incorporate the effects on the HBP size introduced by the length of the polyion, we have extended their theoretical description to the case of spherical macroions with variable radius. To the first approximation we have not taken into account the anisotropy imparted to the HBP shape, which is discussed later. Instead, an isotropic spherical macroion with variable radius $R_0 + dR$ is considered. In contrast to the case of rigid sphere we examined the two-dimensional surface of the free-energy $F(L_1,dR)$ for a macroion complexed by a linear polyelectrolyte chain of length L. L_1 is the length of the adsorbed part and Λ is the polyion length required for the electrical neutrality. Energy in this equation is in units of $(e/l)^2/\epsilon_s$, where ϵ_s is the dielectric constant of the solution. In eqn (4) the first term is the self-energy of the PE with length L_1, the second term is the correlation energy between adsorbed PE monomers, the third term is the Coulomb energy due to the macroion net charge, the fourth term is the self-energy of the chain tail, and, finally, the fifth term is the energy of interaction between the polyion tail and the spherical complex. The Nguyen and Shklovskii[16] expression for the free energy is modified in eqn (4) by adding the term $F_{add} = \kappa \cdot dR^2$ which describes the change of energy arising from an elastic deformation of the macroion. κ is the effective elastic constant, and $dR = R - R_0$ is the difference between an instantaneous value of R and the non-perturbed macroion radius R_0.

$$F = L_1 \ln\left(\frac{R_0 + dR}{a}\right) - L_1 \ln\left(\frac{L_1}{R_0 + dR}\right) + \frac{(L_1 - \Lambda)^2}{2(R_0 + dR)} + (L - L_1) \ln\left(\frac{L - L_1}{a}\right) + (L_1 - \Lambda) \ln\left(\frac{L - L_1 + R_0 + dR}{R_0 + dR}\right) + \kappa \cdot dR^2 \quad (4)$$

When the length of the adsorbed part L_1 is much larger than the macroion radius R, the solution of the minimization problem of eqn (4) with respect to parameters L_1 and dR leads to the following system of equations,

$$\begin{cases} (L_1 - \Lambda)\left[(R_0 + dR)^{-1} - (L - L_1 + R_0 + dR)^{-1}\right] + \ln\left(\frac{R_0 + dR}{L_1}\right) = 0 \\ \frac{2 \cdot L_1}{R_0 + dR} - \frac{(L_1 - \Lambda)^2}{2 \cdot (R_0 + dR)^2} - \frac{L_1 - \Lambda}{R_0 + dR} + 2\kappa \cdot dR = 0 \end{cases} \quad (5)$$

which is solved numerically. For short polyions as shown in Fig. 4a, there exists only one minimum of the free energy $F(L_1,dR)$ which corresponds to the fully adsorbed state, i.e at $L_1 = L = 40$. Upon increasing of the polyion length L, a second minimum in the free-energy $F(L_1,dR)$ appears as illustrated in Fig. 4b. The non-global minimum at $L_1 = L \sim 75$ corresponds again to the fully adsorbed state. The position of the global minimum shifts to values of $L_1 \sim 55$ smaller than the chain length L, *i.e.* the polyion tail releases. Note that both minima appear at non-zero values of dR meaning that the size of the macroion is influenced by the polyion length, in qualitative agreement with the present computer simulations.

In order to make quantitative comparisons the values for the following parameters have been taken from the present Brownian dynamics simulations: $a = \sigma/2 = 0.4$, $L = N_{ch}$, $\Lambda = N_T$ and $R_0 = \sqrt{\frac{5}{3} R^2_{g,HBP_{max},neutral}}$. The elastic coefficient $\kappa = 8.07$ has been chosen to fit the simulated value of the gyration radius of the HBP_{max} in a complex with the shortest PE, $N_{ch} = 25$. This value of κ is used for all other simulated polyion lengths. The predictions of the theory, both for rigid non-deformed macroion and elastic macroion, have been compared with the Brownian dynamics results as shown in Fig. 2. For complexes comprised by perfect (practically non-deformed) dendrimers the position of the maximum in the dependence $N_{ads}(N_{ch})$ is close to the nominal maximum observed in simulations. For complexes formed by the (stretched) HBP_{max}, the location of the maximum corresponds to the plateau in the dependence $N_{ads}(N_{ch})$, after the polyion tail release. Theory also predicts the existence of a sharp maximum which is not observed

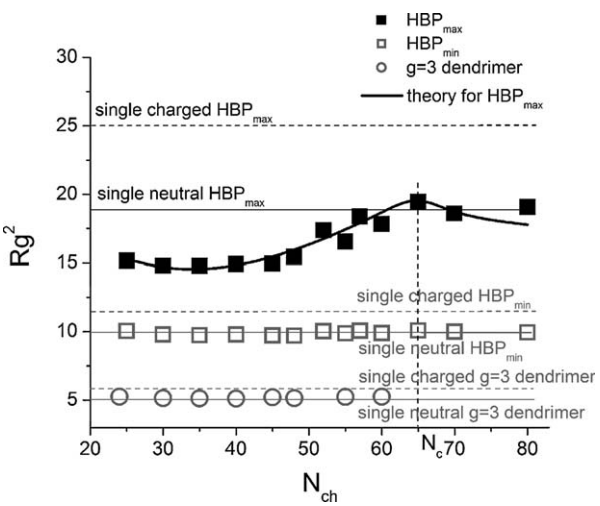

Fig. 3 The polyion-length dependence of the HBP gyration radius R_g^2 in complexes with PE of different lengths. The symbol sizes are larger than error bars. Vertical dashed line shows the critical length at which polyion tail releases.

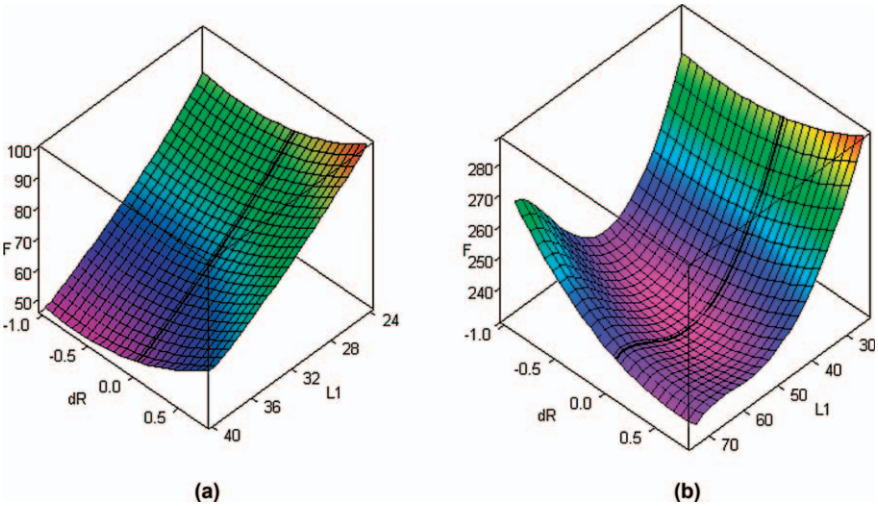

Fig. 4 Dependence of the complex free energy on the adsorbed chain length L_1 and dR. (a) $L = 40$ (before tail release), absolute minimum is colored in purple; (b) $L = 75 > \Lambda$ (after tail release) where two minima (non-global at $L_1 \sim 75$ and global at $L_1 \sim 55$) are seen.

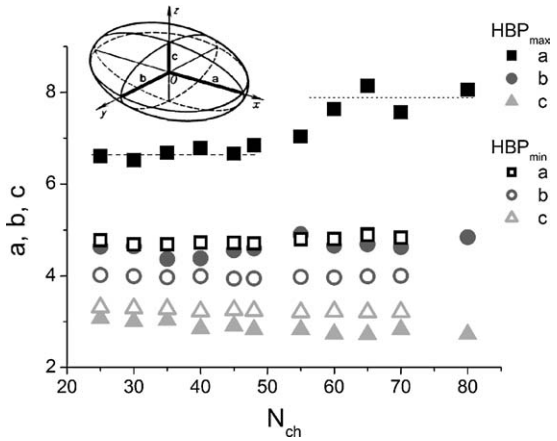

Fig. 5 Polyion-length dependence of the length of the semi-axes (a, b, c) of the ellipsoid of inertia for HBP_{max} and HBP_{min}. Lines denote average values of the longest semi-axis, a, for HBP_{max} before (dashed) and after (dotted) tail release.

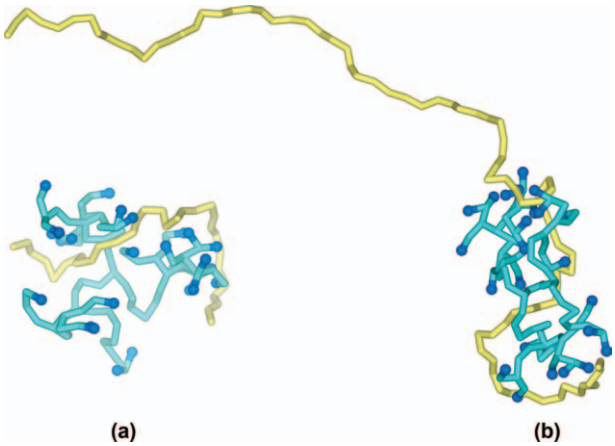

Fig. 6 Snapshots of complexes formed by HBP_{max} and PE of different lengths: a) $N_{ch} = 30$ (HBP_{max} has a sphere-like shape) and b) $N_{ch} = 80$ (HBP_{max} has a cylinder-like shape).

in the simulations. The presence of this maximum should be related to the disregarding of the shape anisotropy in the correlation theory. Analysis of the effective ellipsoid of inertia (Fig. 5) shows that HBP_{max} in a complex assumes a rather anisotropic shape.

For HBP_{max} the degree of the shape anisotropy is much larger compared to that of HBP_{min}. It increases after the maximum of adsorption is reached and the corresponding polyion tail is released. Significant changes in the anisotropy results to the change of R_g^2 discussed above, Fig. 3. Summarizing our findings we would like to point out that before the start of the change of HBP_{max} anisotropy ($N_{ch} < 55$), the adopted theory of sphere with flexible radius correctly describes the overcharging of HBP_{max} (both as the R_g^2 and N_{ads} dependences on N_{ch}). This theory does not take into account the anisotropy change of the macroion and therefore overestimates overcharging more than 100% (after $N_{ads} > 55$). Contrary to that result our BD simulation predicts overcharging only about 50% in a good agreement with the theoretical predictions for an infinite cylinder.[17] Sphere-like shape of HBP_{max} in complex with short PE ($N_{ch} = 30$, Fig. 6a) and cylinder-like as in complex with long PE ($N_{ch} = 80$, Fig. 6b) are confirmed by snapshots shown in Fig. 6. Thus, during the increase of the PE chain we can observe 3 regimes of HBP_{max} overcharging.

1) $N_n = 25 \leq N_{ch} \leq 55$. For this regime full adsorption of the chain is preferable, HBP_{max} may be described by correlation theory for the sphere with variable radius.

2) $55 \leq N_{ch} \leq N_c = 65$. Here again full adsorption of the chain remains preferable, however, a further chain adsorption does not take place; instead, increasing of the chain length leads to the increase of HBP_{max} anisotropy, most probably due to the PE stiffness which is essentially controlled by the mutual repulsion between its charges. HBP_{max} may thus be considered as a cylinder. At $N_c = 65$ the increase of the anisotropy ceases and the tail release occurs.

3) $N_c = 65 \leq N_{ch} \leq 80$. Further increase of the chain length leads to the increase of the chain tail. HBP_{max} becomes significantly anisotropic (close to cylinder in shape) compared to the initial state.

Thus, HBP_{max} is sensitive to the maximum of PE adsorption that corresponds to the increase of HBP_{max} anisotropy. This occurrence may in principle be experimentally verifiable.

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In spite of the rather narrow interval of simulated parameters and model potentials which may be far from the realistic biological situation we believe that the results based on the adopted models provide new insight in HBP-PE complexation, indicating that the properties of charged HBP_{min} with preferable branching close to the core and minimal Wiener index in complexes with oppositely charged linear PE are close to those for regular dendrimers of the same charge. Some slight differences are probably related to size-mismatching rather than to structural details. In contrast, complexes formed by the non-regular HBP_{max} with maximal Wiener index and preferable branching at the periphery exhibit markedly different behavior mainly due to the less compact interior. After the complex formation the size of the HBP_{max} decreases significantly compared to that of a single neutral HBP_{max}, indicating that complex formation induces a reduction of the dimensions of both linear PE and HBP. A similar effect is observed in the size of HBP_{min} only to a minor degree. The computer simulations and the analytical calculations show that charged macroions adsorb an amount of the linear PE which exceeds that required for the electrical neutrality, *i.e.* charge inversion takes place. Upon further increase of the PE length, tail releases at some critical value of the PE length N_c. Equilibrium properties of the complexes formed by regular dendrimers or the similar in topology HBP_{min} are practically not influenced by the increase of the PE length and by the tail-release mechanism. On the contrary, the behavior of the charged HBP_{max} appears sensitive to the tail release: this critical PE length corresponds to the state of the complex with maximum anisotropy of HBP_{max}. Such different properties of HBP_{max} and HBP_{min} are strongly related to their distinctly different branching pattern: the "dendritic" structure of HBP_{min} is clearly more compact near the core, and thus cannot be compressed easily. HBP_{max} possesses much more "free" space inside; charged terminal beads are distributed over the entire HBP structure. Hence, a linear polyelectrolyte has to adapt its conformation in order to "neutralize" these charges, which finally leads to structural reorganization of both components of the complex.

The parameters utilized in the present work correspond to a dilute solution; counterions are accounted implicitly. In more concentrated solutions with explicit counterions present, the effects discussed in this study are expected to be more pronounced: explicit counterions will decrease the strength of electrostatic repulsions between PE charges in complex, so the overcharging of HBP would be even stronger.

The observed sensitive response of the HBP_{max} to the size of the adsorbed linear PE can be potentially exploited in applications where the control of size and effective charge are crucial, while HBP_{min} systems can be regarded as cost-effective alternatives for applications in which regular dendrimers are so far being used.

Acknowledgements

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