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MULTISCALE MOLECULAR MODELING AND RATIONAL DESIGN OF POLYMER BASED GENE DELIVERY VECTORS

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Summary

We used multiscale modeling technique and investigated several issues in non-viral based gene delivery using polyethylenimine (PEI). Atomistic molecular dynamic simulations captured the spontaneous complex formation between a short PEI chain and a poly-(L) lysine (PLL) chain with DNA helices. Atomic structures obtained for DNA/PEI and DNA/PLL complex compared very well with X-ray diffraction data. A computational Monte Carlo titration model was developed and was used to investigate the protonation state of PEI. The PEI was found to have about 55% of amine groups protonated at physiological condition, thereby supporting the potential hypothesis of “proton sponge effect” of PEI. Coarse-grained modelings were used to find optimum design of block copolymer, polyethyleneglycol (PEG)-b-PEI that would condense DNA and form core-corona structures to provide better biocompatibility.

Keywords

polyethylenimine; molecular dynamics simulations; protonation; copolymers; multiscale modeling

Introduction

Positively charged polyethyleneimine (PEI) possess great transfection efficiencies as gene delivery vectors.[1] The success of PEI as non-viral based gene delivery vector is attributed to its “proton sponge effect”.[2–3] However, there are still many obstacles that need to be overcome before PEI or its derivatives may be used in clinical trials of gene therapy. What currently impedes the development of polymer based gene delivery systems is a lack of quantitative structure-function relationship, which is primarily due to a lack of structural information of polyplex formed in gene delivery. Measurement of particle sizes and charges can be done fairly easily. Microscopy can also help visualize the nanoscopic particles. However, structural information at higher resolutions are difficult to obtain. Also difficult to assess is the stability and dynamic responses of polyplex particles. In order to rationally design polymer based gene delivery vectors, computational modeling can be of great help by providing critical information inaccessible to experiments. We used multiscale modeling technique and investigated different aspects of problems encountered in gene delivery.[4–6]

Experimental Methods

Molecular Dynamic Simulations

Atomistic molecular dynamic simulations were performed using AMBER molecular simulation package with AMBER Pamr99 force field. The force field for the repeating units in PEI, protonated and unprotonated forms, were developed based on AMBER gaff module. The canonical B-form DNA structure was created with the nucgen module of AMBER. The DNA sequence used in all simulations was the self-complementary Drew-Dickerson

dodecamer, d(CGCGAATTCGCG)₂, with the first 12 bases belonging to strand 1 and the final 12 bases belonging to strand 2. We investigated two types of polycations, poly(L) lysine (PLL) and PEI with PEI chain modeled in two different protonation states, namely all protonated or 50% protonated. Monovalent counterions were added to the system using LEaP to neutralize the charges on polycations. All systems were solvated in ~25,000 TIP3P water molecules in a rectangular box. Coarse-grained molecular dynamic simulations were performed with LAMMPS. The chains were modeled by simple bead-spring models in implicit water solvent with explicitly counter ions.

Computational Monte Carlo Titration

A coarse-grained model of PEI chain was first developed based on atomistic model of PEI chain. The coarse grained model of PEI was represented by a bead-spring chain with each bead representing a monomer of PEI centered at the nitrogen atom of the amine group. The beads were connected via harmonic bond and harmonic angle bending potentials. The parameters needed in these potentials were obtained by fitting the distributions obtained from the atomistic dynamic simulations. Purely repulsive Lennard-Jones potential was included to prevent overlap between non-bonded beads. The Monte Carlo titration includes the random displacement of the two beads plus random switch of the charge of the two beads. The move is accepted or rejected according to the Metropolis rule by considering the total energy change during the proposed move. If the charge of the beads is switched, the associated energy of changing the charge is governed by

$$\Delta E_{\text{tit}} = \sum_{j=1}^M W_{ij} q_i q_j \pm k_B T (\ln 10) (\text{pH} - \text{pK}_0)$$

, where W_{ij} is the electrostatic interaction energy between sites i and j , M was the total number of beads in the chain, and pK_0 was the intrinsic pK_a of site i as an isolated amine group. For W_{ij} , we used screened Coulomb interaction with a distance dependent dielectric constant.

Results and Discussion

Figure 1 presents a typical snapshot of the complexes formed at the end of molecular dynamic simulations. The runs have been repeated for several times. Although the exact atomic contacts vary from run to run, but some consistent trends have been observed. We found most of the interactions in the complexes were between the negatively charged DNA phosphate groups and positively charged amine groups of the polycation; however, there were significant interactions between electronegative sites in the major and minor grooves of DNA and the polycations. The fully protonated PEI(20) chain lines up with phosphate groups on both strands of the DNA duplex with a section of the polycation forming a bridge across the minor groove. In contrast, a section of the 50% protonated 50%-PEI(20) chain lies in the minor groove of the DNA. The PLL chain, in which the charged amine groups are on side chains several bonds away from the polymer backbone instead of along the backbone as in PEI, has several amine groups sticking away from the DNA chain, reducing the number of positive charges that are close to the DNA phosphate groups.

Figure 2 presents the results of Monte Carlo titration of a PEI chain with that obtained from experiments by Smits et al. [7]. The X-axis is the averaged fraction of protonated amine sites, α , at the given pH. The computational titration curve at salt concentration $C_s=0.01\text{M}$ matched well with the experimental curve obtained at $C_s=0.1\text{M}$. The salt dependence however was not captured correctly. Monte Carlo titration also provided detailed information as to which amines are protonated. We found that end beads are more likely protonated than the inner beads. At $\alpha = 0.5$, the chain has nearly an ordered structure, with every other beads being protonated.

Coarse-grained molecular dynamic simulations were used to investigate DNA condensation by PEI-PEG block copolymers using generic bead-spring chain models. Coarse-grained molecular dynamic simulations can cover much longer time and length scales. In the coarse-grained simulations, one can readily observe the complex formation and collapse of DNA chain. Details of results can be found in Ref. 5. In the future, we plan to develop consistent coarse-grained models of three highly investigated polycations, PEI, PAMAM and chitosan. Molecular dynamic simulations of DNA condensations with derived coarse-grained models for these three polycations will be performed. The relative structures and stabilities of formed polyplexes will be compared.

Conclusion

Multiscale molecular modeling can provide critical information inaccessible to experimental studies on the structure and dynamics of polyplex particles. With structural information provided through multiscale modeling, better design of polymer based gene delivery systems can be achieved.

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Figure 1.

Typical snapshots of complex formed late in the trajectory for (a) PLL, (b) PEI(20), and (c) 50%-PEI(20) simulations. The polycation is shown in a space-filling model, while the DNA is shown as sticks-and-balls

Figure 2. Overlay of experimental titration curves (shown as symbols) with computational titration curves (shown as lines). The salt concentration for experiments $C_s = 0\text{M}$ (circles) and $C_s = 0.1\text{M}$ (triangles). Salt concentrations for computational curves $C_s = 0\text{M}$ (dot-dashed line), 0.01M (solid line), 0.1M (dashed line). pK_0 was assumed to 10.0.