Modeling of polypeptide- and polymer-conjugated nanoparticles for drug delivery application

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ABSTRACT

G4 polyamidoamine (PAMAM) dendrimers grafted with histidine-rich peptides or polyethylene glycol (PEG) were simulated using coarse-grained (CG) models. In the simulations of dendrimers without conjugates, the size, shape, and internal structure of dendrimers agree well with those from experiments at different pH. Simulations of dendrimers grafted with peptides and PEG show that the size and grafting density of conjugates significantly modulate the conformation and internal structure (dense core or dense shell) of dendrimers, implying important possible effects of the conjugate methodology on encapsulation efficiency and cytotoxicity, as observed or proposed in experiment.

Keywords: molecular dynamics simulation, drug delivery, nanoparticle, and conjugation

1 INTRODUCTION

PAMAM dendrimers, which consist of a central core, repeated building blocks, and surface terminal groups, have shown great potential for biomedical applications such as drug delivery and antitumor therapeutics because of their controlled mass, uniform structure, surface functionality, and good water solubility. Ligands such as drugs and imaging molecules can be attached to the dendrimer, and those complexes can be targeted to the specific cancer cell. To achieve this, high solubility, transfection efficiency, and long circulating lifetime are required, and hence the dendrimer surface has been often modified.[1] (Figure 1)

Experimentally, dendrimer terminal groups have been grafted with peptides and polymers. Choi et al. found that the arginine-conjugated PAMAM dendrimers (PAMAM-Arg) show much higher gene-transfection efficiency than do the unmodified PAMAM dendrimers.[2] Also, different numbers of histidine, which is neutral at pH 7 and cationic at pH 5, were conjugated, leading to even higher efficiency of the gene transfection. For the delivery to the cell nucleus, dendrimers are carried by the lysosome at pH ~5; thus, the effects of peptide conjugation on cytotoxicity and transfection efficiency at different pH need to be understood. For PEG conjugation, Yang et al. showed that grafting longer PEGs onto dendrimers improve their encapsulation ability, but dendrimers grafted with the longest PEG (Mw = 5000) showed reduced encapsulation.[3] This nonmonotonic dependence of encapsulation on the length of the PEG has been interpreted as an indication that long PEG chains (Mw = 5000) may induce intermolecular aggregation of PEGylated dendrimers. To understand these, we here report coarse-grained (CG) molecular dynamics (MD) simulations of dendrimers grafted with peptides and PEGs.

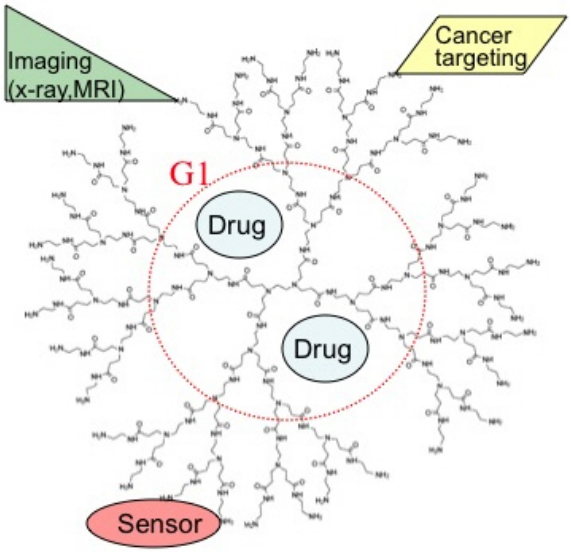

Figure 1. Structure of G3 PAMAM dendrimer for drug delivery application.

2 METHODS

All simulations and analyses were performed using the GROMACS4.5.3 simulation package [4] with the "MARTINI" CG force field (FF).[5] All 64 surface-terminals of a G4 dendrimer are linked to the amino acid conjugates, -Arg, -His-Arg, -His-His-Arg, and –His-His-His-Arg. At pH 7, primary amines of the dendrimer surface are protonated, while both the tertiary amines of the dendrimer interior and the primary amines of the dendrimer surface are protonated at pH 5. For PEG conjugation, 8, 16, 32, and 60 copies of PEG with Mw of 550 (PEG550) or 5000 (PEG5000) were attached to the surface terminals of

G4 dendrimers. These complexes were solvated with enough counterions for electroneutrality. For the Lennard-Jones and Coulomb potentials, a cutoff of 12 Å was applied. Simulations were performed for 1μs with a time step of 20 fs, and the last 100 ns was used for analyses.Fonts and Spacing

Times or Times Roman is the recommended typeface for the main text using 10-point type. The smallest allowed type size for all text, figures, captions, references and within figures is 10-points. See Table 1 for a compete summary of Font formats. Single (1.0) line spacing is recommended for the main text.

3 RESULTS AND DISCUSSION

3.1 Peptide conjugation

Simulations show that the Arg-grafted dendrimer is slightly larger than the unmodified dendrimer. The increased particle size and net charges of the Arg-grafted dendrimer may slightly increase cytotoxicity, as observed in experiment. As more His residues are conjugated to the Arg-grafted dendrimer, the net charges and particle size significantly increase at pH 5, but not at pH 7 where His is neutral, suggesting that the increase surface charges and larger size may increase cytotoxicity of the dendrimer at pH 5. Also, increased conjugation with His residues induces a dense-core structure at pH 7 but does not change the dense-shell structure of the unmodified dendrimer at pH 5. These results indicate that increased His conjugation may result in a reduced free volume within the dendrimer core at pH 7, and this could reduce encapsulation efficiency. These findings regarding the size and internal structure of the peptide-conjugated dendrimer, which possibly modulate cytotoxicity and efficiency of transfection and encapsulation.

3.2 PEG conjugation

Dendrimers grafted with larger PEG's at higher grafting densities showed larger R_g and denser dendrimer shell structure, consistent with experiment. However, experiments also showed a decrease in solubility when the PEG size was increased further to PEG5000. To explain this nonmonotonic effect, it has been hypothesized that long PEG chains induce interparticle aggregation, whereby PEG chains can penetrate into other dendrimers and thus suppress uptake of a hydrophobic compound. However, our simulations showed no interparticle aggregation. In particular, we found that long PEG5000 chains at high grafting density self-penetrate into the attached dendrimer, occupying the dendrimer's vacant interior that would otherwise be available for encapsulating hydrophobic compounds. These results suggest that the observed lower uptake with PEG5000 might be induced by self-penetration of PEG chains into their own dendrimers, not by the interparticle aggregation.

4 CONCLUSIONS

CG simulations of dendrimers grafted with peptides and PEGs at different sizes and grafting densities show the effects of those conjugates on encapsulation efficiency and cytotoxicity. The conjugation of His residues densifies the inner cavity of the dendrimer core at pH 7, leaving less room for other agents, and thus likely to lower drug encapsulation efficiency. Also, long PEG chains at high grafting density self-penetrate into the dendrimer core, while short ones do not, which helps explain the reduced encapsulation of hydrophobic compounds, which was observed but not explained by experiments.

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