Dendritic nanoparticles (PAMAM) used as an optimal mechanism for transport and controlled drug delivery

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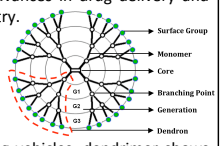
Numerous efforts have been focused on the development of drug carrier systems able to enhance drug therapeutic efficacy. Polyamidoamine (PAMAM) dendrimers have been widely considered for pharmaceutical industry as an optimal mechanism for transport and controlled drug delivery. Properties such as biocompatibility, water solubility, versatility in modifying their functional groups, and responsiveness of their conformational properties to an aqueous environment become these macromolecules appropriate for such uses. Amine-terminated PAMAM dendrimers are able to solubilize different families of hydrophobic drugs, but the cationic charges on dendrimer surface may disturb the cell membrane. Therefore, acetylation is a convenient strategy to neutralize the peripheral amine groups and improve dendrimer biocompatibility[1]. The aim of this work is to analyze the structural properties that determine the encapsulation of drug-like molecules into PAMAM-G_5 and PAMAM-G_5-Acetylated dendrimers using Dexamethasone 21-phosphate (Dp21) as a model drug. For this purpose, PAMAM dendrimers and Dp21 were parameterized using the new CGenFF force field. Then, full atomistic molecular dynamics simulations, in aqueous solutions at different pH conditions, were employed to position Dp21 into internal cavities of dendrimer and characterize the host-guest chemistry of acetylated dendrimer/Dp21 and cationic dendrimer/Dp21 complexes. Our results show that the orientation of Dp21 molecules in the dendrimer cavities depends on the quaternization degree of tertiary amine groups of dendrimer and the protonation ratio of phosphate groups of Dp21. These results provide a new insight for the design and optimization of biocompatible dendrimer-based drug delivery systems.

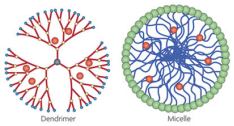
Introduction

Nanotechnology has brought dramatic advances in drug delivery and revolutionalized the pharmaceutical industry.



Dendrimers are outstanding candidates to act as drug carriers due to their well-defined nanostructures and chemical versatility.

As compared to traditional polymeric drug vehicles, dendrimer shows important advantages: **(1)** Dendrimer has controllable sizes and regular shapes. **(2)** Dendrimer has high density and a defined number of surface functionalities. **(3)** Dendrimers allow solubilizing organic substrates. These are similar to a stable monomolecular micelle.



(4) Dendrimers have a well-defined globular structure with low polydispersity. **(5)** Dendrimer can easily penetrate through the cell membrane.

In general, these properties confer a high dendrimers potential to be used in the biomedical and pharmaceutical industry.

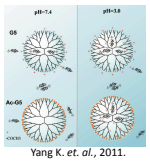
Polyamidoamine dendrimer (Tomalia, 1985) is the most investigated dendrimer in the biomedical field. However, the **cytotoxicity of dendrimer** is an issue that limits the clinical applications of most **PAMAM** dendrimer-based drug formulations. Depending of pH, the cationic charges on dendrimer surface may disturb the cell membrane. Therefore, the neutralization of dendrimer surface charge by **Acetylation** has been proposed as a necessary step to improve dendrimer biocompatibility.

The purpose of this study is evaluate the structural properties that determine the encapsulation of Dexamethasone 21-phosphate (Dp21) into fully acetylated and cationic G5 PAMAM dendrimer, at different pHs (3.0 and 7.4); and then, correlate the theoretical results with experimental data published by Yang K. *et. al.*, 2011[1].

• Dexamethasone 21-phosphate (Dp21) is an amphiphilic drug used as antiinflammatory and immunosuppressant.

Methods

Parametrization: The force field parameters for acetylated and cationic G_5 PAMAM dendrimer were prepared using Paratool[2] plugin implemented in VMD[3]. Dp21 was parameterized using the new CGenFF[4] force field.



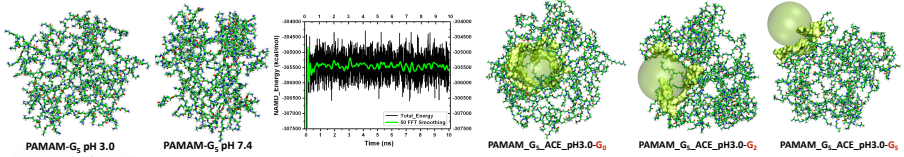
Yang K. *et. al.*, 2011.

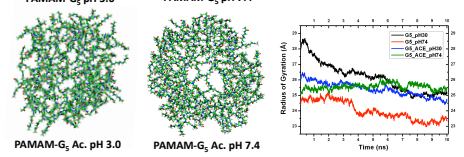
Relaxation Dynamics and Generation of Cavities: The initial configurations of each system (G_5 and G_5-Ac., at pH 3.0 and 7.4) were optimized using energy minimization followed by an equilibration and relaxation with MD simulation at 310°K by 10 ns under NPT ensemble (NAMD2.7[5]). Later, through the script *bubble.tcl*[6] three cavities were generated in each dendrimer (Core, G_2 and Surface) for the subsequent encapsulation of Dp21.

Docking and Molecular Simulation: The Dp21 optimized structure was docked into each dendrimer using AutoDock4.0[7]. The lowest energy conformations were used as the starting point for molecular dynamics simulation (5 ns.) of the different complexes dendrimer-Dp21. Each complex was analyzed at molecular and energetic level, allowing to identify specific interactions that determine the affinity dendrimer-drug.

Results and Discussion

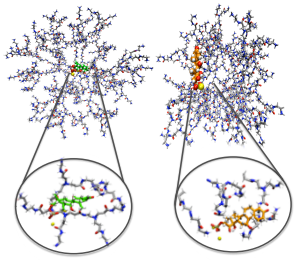
Molecular Dynamics of Dendrimers:





Final conformations show that all the dendrimers shrink and form ellipsoidal spheroids, being PAMAM G_5-Ac. more compact than PAMAM G_5. The values of $\mathbf{R_g}$ compare favorably with experimental data obtained from SANS and SAXS[8]. Therefore, acetylated and cationic dendrimers present a reliable structure, with cavities of 12Å that allow the encapsulation of drugs.

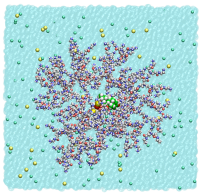
Docking Dendrimers-Dp21:



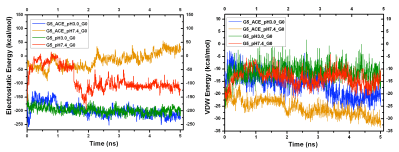
The energy values show that acetylated dendrimers are able to encapsulate Dp21 only at acidic conditions, while cationic dendrimers can host Dp21 at both acidic and neutral conditions.

PAMAM ACE pH3.0	PAMAM ACE pH7.4
G_0 = - 5.56	G_0 = - 1.33
G_2 = - 4.40	G_2 = - 1.04
G_5 = - 2.12	G_5 = - 0.46
PAMAM pH3.0	**PAMAM pH7.4**
G_0 = - 7.56	G_0 = - 8.25
G_2 = - 7.35	G_2 = - 7.50
G_5 = - 6.73	G_5 = - 7.76

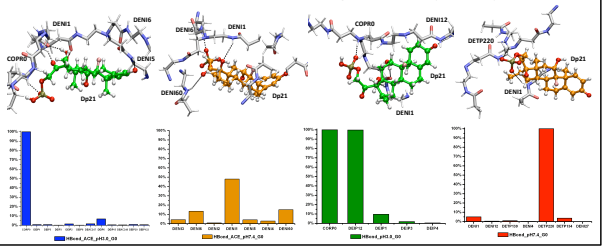
Molecular Dynamics of Complexes Dendrimer-Dp21:



Dendrimer-Dp21 complex in an explicit water box (TIP3P), neutralized with 0.15 M NaCl.



The interaction of Dp21 in the dendrimer cavities depends on the protonation degree of amine groups. Tertiary and primary amines interact with Dp21 through hydrogen bonding, electrostatic and van der waals interactions, showing better affinity at acid pH.



Conclusions

- Theoretical analysis are correlated with experimental data, confirming that acetylated dendrimer only encapsulates Dp21 at acidic conditions, whereas nonacetylated dendrimer forms inclusions with Dp21 at acidic and neutral environments.
- The structural characterization of dendrimer-drug complexes using molecular dynamics provides optimal insight to evaluate the encapsulation of new drugs into PAMAM functionalized dendrimers.
- The dendrimer-based drug delivery systems provide an attractive platform to load and release conventional drug molecules, improving the pharmacodynamic and pharmacokinetic behaviors of these drugs.

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