Molecular Dynamics Studies on Folic Acid and Fluorescein-Derivatized PAMAM Dendrimers

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ABSTRACT

Molecular dynamics simulations on PAMAM dendrimers were performed to design and optimize the condition for cancer cell targeting and imaging. Folic acid and fluorescein-conjugated dendrimers with a primary amine surface group (without capping) and with a carboxyl group showed local branch aggregation, implying less solubility in water. Simulations indicated that folic acid conjugates on the acetamide surface tend to extend out, while most folic acids on the hydroxyl surface are buried in the dendrimer, predicting less potential interaction with folate receptors on the surface of cells. These results strongly suggest that the acetamide capping group for folic acid and fluorescein-conjugated dendrimers optimal for targeting, a finding supported by cellular targeting experiments using flow cytometry and 3-D confocal microscopy. Our computer simulation results have informed nanomolecular synthesis, and have led to structures which exhibit desired biologic activity.

Keyword: Molecular Dynamics Simulation, Dendrimer, Folic Acid, Fluorescein.

1. INTRODUCTION

Polyamidoamine (PAMAM) dendrimers have been used for biomedical application extensively because of its biocompatibility and well-defined nano-structure. They can be synthesized in large quantities and are uniform in structure. Our group has successfully used PAMAM dendrimers in a gene delivery system and is developing them as delivery systems for cancer therapeutic agents.

Since a Corey-Pauling-Koltun (CPK) model building was used to predict the dendrimer diameters [1], atomistic molecular dynamics (MD) simulations have been performed to provide the 3D structures of specific dendrimers [2, 3] and to suggest possible interactions in dendrimer formation [4], following coarse-grain MD studies on dendrimers [5]. Recently, more exhaustive atomistic MD simulations on dendrimers were carried out for solution behavior [6], potential usage as catalyst supports [7], effect of repeat unit flexibility [8], and several nanoscale applications [9].

In this study, we used the atomistic MD simulations to predict which surface derivative group is better for cellular targeting when a PAMAM dendrimer is modified by fluoresceins (imaging moiety) and folic acids (cancer cell targeting moiety). The major difference between previous PAMAM dendrimer simulations and ours is that we used protonated primary amine groups and deprotonated carboxyl groups, which play an essential role in biological phenomena. To reduce the strong electrostatic interactions in the models, we used a distance-dependent dielectric constant for all simulations.

2. SIMULATION CONDITIONS

Model building and MD simulations were performed on an Onyx workstation (Silicon Graphics, Inc., Mountain View, CA) using the Insight II software package (Molecular Simulations Inc., San Diego, CA). A generation 5 PAMAM dendrimer (EDA core) was first built and simulated with the consistent valence force field (CVFF) [10] for 50 ps at 295 °K, after standard minimization and annealing processes. The primary amines of a PAMAM dendrimer were all protonated to simulate a pH 7 condition. A distance-dependent dielectric constant was used to shield electrical charges. We used the final configuration of this amino-surfaced simulation for fluorescein (FI) and folic acid (FA) attachment and surface modification modeling.

Randomly selected surface amines from this configuration were modeled to attach to FI and FA, respectively, resulting in 16 FI and 3 FA moieties in one dendrimer. The remaining surface amines were derivatized with a carboxyl group, giving the dendrimer a negatively charged surface, or with either an acetamide or a 2,3-dihydroxypropyl group, leading to non-charged dendrimer surfaces. These three surface group models, in addition to the uncapped protonated amine surface model, were then simulated for 100 ps at 310 °K after minimization and annealing. Equilibration occurred about 30 ps in all simulations based on a flattening out of potential energy to stabilizing and the root-mean-square (RMS) variation of the dendrimers from their initial structures after annealing. All linkers used in the conjugation and capping were identical to those used in the synthesis. Carboxyl groups of surface capping, FI and FA were all deprotonated to

simulate a pH 7 condition. All data used for evaluation were collected from simulations between 50 and 100 ps to insure equilibrated states.

3. RESULTS AND DISCUSSION

The structure of generation 5 PAMAM dendrimer after 50 ps simulation is shown in Figure 1 A. With its protonated amine surface, the PAMAM dendrimer presents a relatively open and extended structure due to charge-repulsive interactions. Figure 1 B shows which is FI and FA-attached PAMAM (G5-FI-FA) with amine surface before MD simulation. All folic acids stretch out of the dendrimer. Both acetamide and hydroxyl-capped G5-FI-FA have the same initial FI and FA configurations as amine-surfaced G5-FI-FA, with the main surface groups (primary amines) changed to acetamide or 2,3-dihydroxypropyl groups (structure not shown).

Figure 2 presents the final configurations of G5-FI-FA with different surface groups after MD simulations. Local branch aggregation is observed in the G5-FI-FA dendrimer with a protonated amine surface, indicating potential solubility problems due to the possible intermolecular branch aggregation. This is in addition to the known problem of non-specific charge interaction between positively-charged dendrimers and negatively-charged cell surfaces. When the surface is derivatized with a carboxyl group and the surface charge changed from positive to negative, we still observe the local branch aggregation (Figure 2B). Therefore, intermolecular aggregation and precipitation at high dendrimer concentration can be predicted in G5-FI-FA dendrimer with a carboxyl surface. If intermolecular aggregation occurs, the folic acid availability of the aggregates will be limited. As for one G5-FI-FA dendrimer molecule with a carboxyl group, folic acids seem to loosely hang around the dendrimer branches while they sit close to the branches with an amine surface group. With the open structure of G5-FI-FA dendrimer with a carboxyl group, the folic acid near the surface of dendrimer have a good chance to interact with receptors. After surface derivatization of the amino groups of G5-FI-FA with 2,3-dihydroxypropyl and acetamide groups leading to a neutral surface, formerly charge-repulsive stretched branches of amine-surface dendrimer relax back (Figure 2C and 2D). Judging from other simulation results on smaller dendrimers (not shown here) and from the argument of a vacuum being a poor solvent [7], the resulting overall structures are more compact than would actually arise with explicit water molecules (not computationally feasible at this time due to software limitations). G5-FI-FA with a 2,3-dihydroxypropyl surface-derivatized group shows much greater surface crowding because the number of hydroxyl groups is twice that of acetamide or amide groups. Some of the folate moieties in G5-FI-FA with a hydroxyl group seem to be buried inside the dendrimer, with a reduced

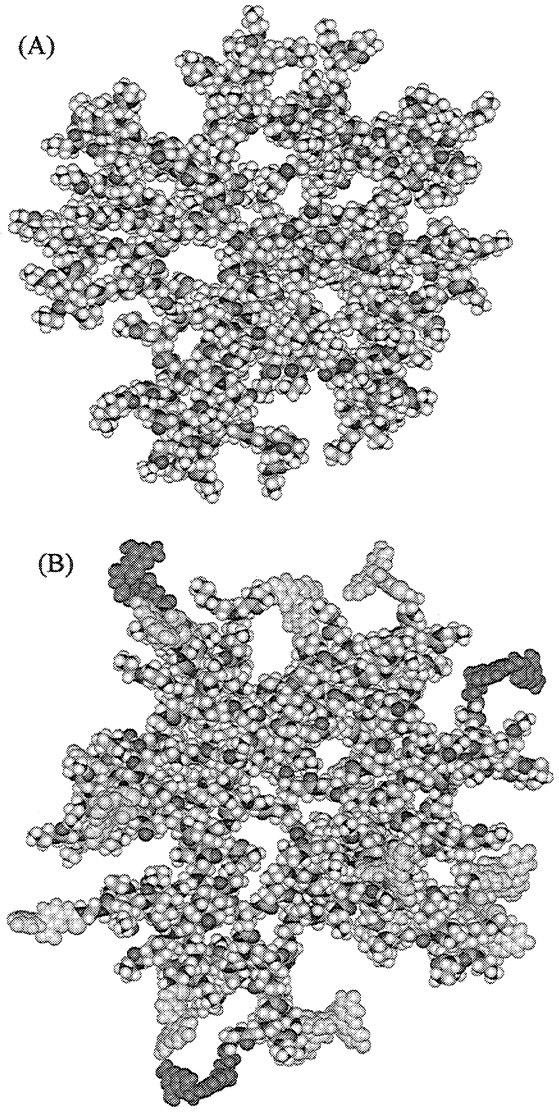

Figure 1: (A) Final configuration of generation 5 PAMAM dendrimer after MD simulation (50 ps). (B) The initial configuration of FI and FA-derivatized PAMAM using configuration (A) without any further surface derivatizing reactions. Primary amines on dendrimer surfaces of (A) and (B) are all protonated. Colored-by-atom parts are underlying G5 PAMAM dendritic structure, with dark gray and bright gray sections FA and FI, respectively.

chance of receptor interaction. On the other hand, folic acids in G5-FI-FA with an acetamide group tend to extend in various directions from the surface of the dendrimer, increasing the likelihood of interaction with folic acid receptors on cells.

Computational Nanoscience 2001, (www.cr.org), ISBN 0-9708275-3-9.

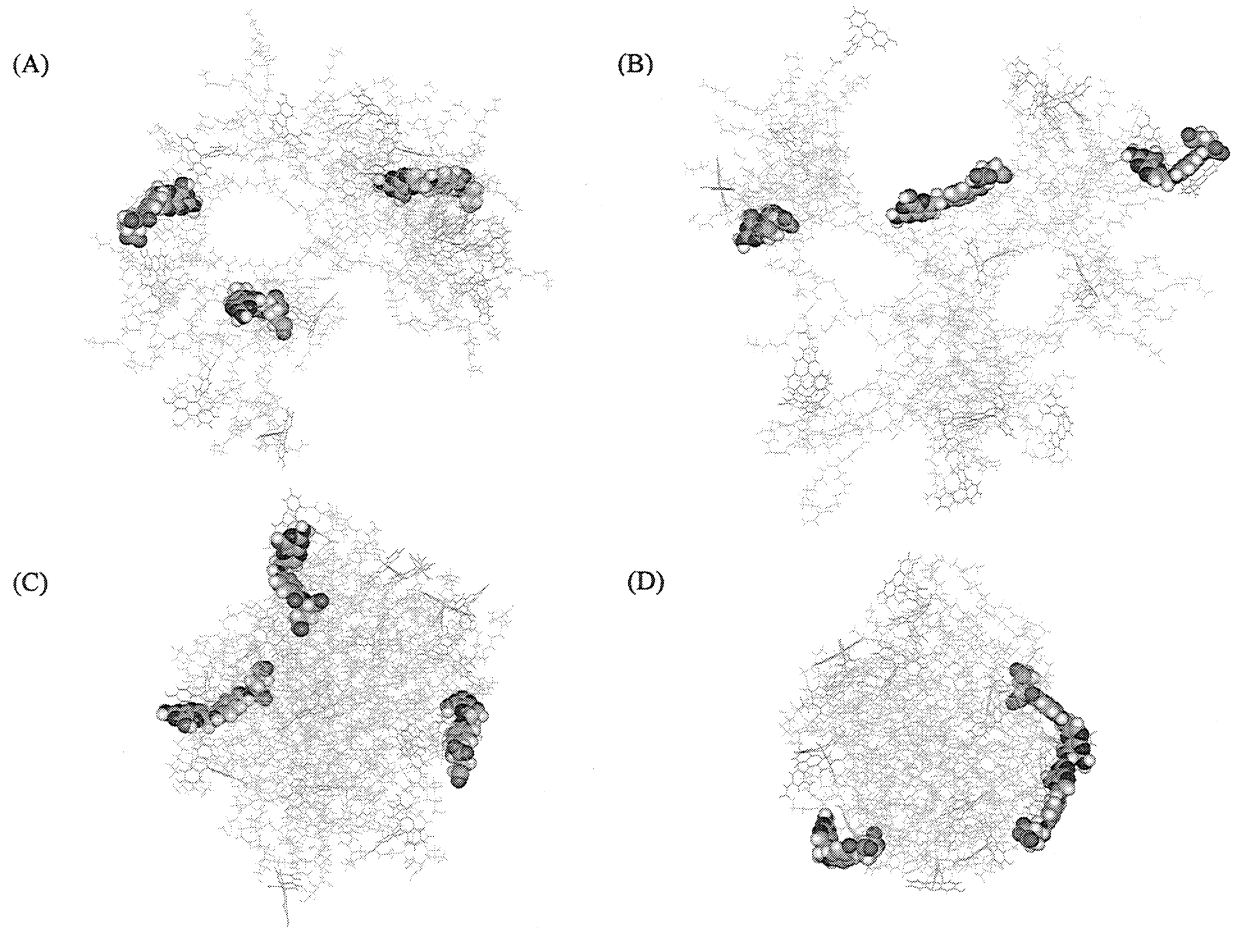

Figure 2: Final configuration of FI and FA-derivatized PAMAM dendrimer after MD simulation (A) without surface derivatization (primary amine surface), (B) with carboxylic group capping, (C) with hydroxyl group capping, and (D) with acetamide group capping. Light gray parts are underlying G5 PAMAM dendritic structure with dark gray section FI, and CPK rendering parts present FA .

For quantitative characterization, we measured two radial distances of a folic acid in a dendrimer: one from the center of mass of the G5-FI-FA dendrimer to the folic acid/dendrimer-attached point and the other to the center of the pteridinyl ring of a given folate group. The average radial distances of the pteridinyl ring of folic acid in dendrimers (mean folic acid distances) are shown in Table 1, along with the mean radii of gyration of G5-FI-FA dendrimers. The mean folic acid distance of G5-FI-FA with an amino group is smaller than its radius of gyration, suggesting poor folic acid accessibility to the receptor in G5-FI-FA with amine surface over the 50 to 100 ps simulation period. Mean radius of gyration of G5-FI-FA with a carboxyl group is greater than that of G5-FI-FA with an amine group, probably because of increased charge repulsion coming from the surface carboxyl group and carboxyl groups in the FI and FA. In the case of G5-FI-FA with a carboxyl group, the mean radius of gyration and mean folic acid distance are very similar, with increasing folic acid accessibility to the receptors. The mean radius of gyration of G5-FI-FA with a hydroxyl group is slightly larger than that of G5-FI-FA with an acetamide. Both surface groups show larger mean folic acid distances than radii of gyration, suggesting a good probability of folic acid accessibility. Please note that the difference between mean folic acid distance and radius of gyration in the G5-FI-FA with an acetamide group is 1.5 Å larger than that in the G5-FI-FA with a hydroxyl, though within the standard deviation. Our study of individual folic acids in dendrimers found them localized at one of the minimal energy states during 50 to 100 ps. Based on the distances from the center of mass to 1) the dendrimer/folate-attached point and 2) the pteridinyl ring, we can determine whether the whole folate moiety is accessible to the receptor or not. One out of three folic acids in G5-FI-FA dendrimer with a carboxyl group is far outside of the radius of gyration, implying good accessibility to the receptors. Considering the open structure of G5-FI-FA dendrimer with a carboxyl group, folic acids around the radius of gyration may also have

Table 1: The average values of the radial distance of folic acid in G5-FI-FA dendrimers with different surface groups.

Surface group	Mean[1] R_G (Å)	Mean[1] folic acid distance[2] (Å)	Individual folic acid distances[3] (Å)	
			dendrimer attached point	center of the pteridinyl ring
Amino group	29.5 ± 0.2	22.9 ± 5.3	27.2 ± 1.1	23.4 ± 1.3
			37.8 ± 0.8	29.0 ± 1.7
			24.6 ± 0.6	16.5 ± 1.4
Carboxyl group	34.3 ± 0.2	34.9 ± 4.8	32.4 ± 0.9	30.7 ± 1.6
			34.2 ± 1.4	33.1 ± 1.6
			46.8 ± 1.5	41.0 ± 2.2
Hydroxyl group	21.8 ± 0.1	27.2 ± 2.4	20.6 ± 0.6	30.2 ± 1.1
			17.0 ± 0.4	26.9 ± 0.3
			25.3 ± 0.5	24.6 ± 0.4
Acetamide group	19.8 ± 0.1	26.7± 2.8	25.8 ± 1.1	23.4 ± 0.4
			18.7 ± 0.8	26.7 ± 0.5
			22.3 ± 0.6	30.0 ± 0.7

[1] mean values are calculated from simulation time between 50 ps and 100 ps.
[2] mean folic acid distances are calculated from the center of mass to the center of the pteridinyl ring of folic acid groups.
[3] radial distances are calculated from the center of mass.

access to the receptors, unless intermolecular aggregation buries them in the middle of aggregates. Since the aggregation is likely at higher concentration, we can predict limited availability of folic acids at higher concentration. Even though all pteridinyl ring distances in a G5-FI-FA dendrimer with a hydroxyl group are larger than the dendrimer's radius of gyration, some of the other ends of the folic acids are within the radius of gyration. Some or significant portion of two out of three folic acids is buried in the G5-FI-FA with a hydroxyl group during 50 to 100 ps, reducing the accessibility to the whole folic acid. The remaining folic acid also seems to be crawling peripherally. On the other hand, only one out of three folic acids in G5-FI-FA with an acetamide group is partially buried in the dendrimer, while one folic acid stretches out of the dendrimer, possibly leading to a good ligand/receptor interaction with minimal steric hindrance by surrounding atoms. These results strongly suggest that the acetamide capping group for folic acid and fluorescein-conjugated dendrimers are optimal for targeting, a finding supported by cellular targeting experiments using flow cytometry and 3-D confocal microscopy.

ACKNOWLEDGMENT

We appreciate the assistance of the Pittsburgh Supercomputing Center in providing access to their graphics laboratory equipment for simulation and video production and for consultations with PSC staff members. This project has been funded in whole or in part with Federal Funds from the National Cancer Institute, National Institutes of Health, under Contract #NOI-CO-97111 and from the Defense Advanced Research Project Agency, DSO, Contract MDA972-1-007.

REFERENCES

1. Donald A. Tomalia, A. M. N., William A. Goddard III (1990) *Angew. Chem. Int. Ed. Engl.* **29,** 138-175.
2. Frank Morgenroth, C. K., Klaus Mullen (1997) *Journal of Material Chemistry* **7,** 1207-1211.
3. Srinivas Uppuluri, S. E. K., Donald A. Tomalia, Petar R. Dvornic (1998) *Macromolecules* **31,** 4498-4510.
4. S. De Backer, Y. P., W. Verheijen, M. Smet, K. Desmedt, W. Dehaen, F. C. De Schryver (1998) *Journal of Physical Chemistry* **102,** 5451-5455.
5. Michael Murat, G. S. G. (1996) *Macromolecules* **29,** 1278-1285.
6. Andre M. Striegel, R. D. P., J. L. Willett (1999) *Analytical Chemistry* **71,** 978-986.
7. Kevin J. Naidoo, S. J. H., and John R. Moss (1999) *Macromolecules* **32,** 331-341.
8. C. B. Gorman, J. C. S. (2000) *Polymer* **41,** 675-683.
9. Tahir Cagin, G. W., Ryan Martin, Nicholas Breen, William A. Goddard III (2000) *Nanotechnology* **11,** 77-84.
10. S. Lifson, A. T. H., P. Dauber (1979) *Journal of the American Chemical Society* **101,** 5111-5121.