Computer simulation of complex of lysine dendrimer with molecules of therapeutic KED peptide

I.M.NEELOV, M.Y.ILYASH, V.V.BEZRODNYI, E.I.FATULLAEV , S.E.MIKTANIUK,
ITMO University,
Kronverkskiy pr. 49, Saint-Petersburg, 197101, Russian Federation
RUSSIAN FEDERATION
i.neelov@mail.ru http://www.ifmo.ru/ru/viewperson/1627/neelov_igor_mihaylovich.htm

Abstract: - Lysine dendrimers could be used for drug and gene delivery to different target cells and organs. Delivery of other bioactive molecules by dendrimers, for example, short regulatory peptides consisting of several aminoacid residues is also possible. The goal of present paper is to study the possibility of complex formation between lysine dendrimer of 2nd generation and 8 or 16 molecules of KED peptide and compare results for these two cases. Two systems containing one dendrimer of second generation and 8 or 16 oppositely charged molecules of KED peptide in water with explicit counterions were studied by the method of molecular dynamics with full atomic details. It was obtained that dendrimer of 2nd generation can adsorb not more than 10-11 molecules of KED peptide simultaneously.

Key-Words: - Lysine dendrimers, peptides, molecular dynamics simulation

1 Introduction

Dendrimers are highly branched molecules with regular spherically symmetric branching from central core [1]. Different types of dendrimers are widely used in drug and other molecules delivery to different target cells [2] as well as for other biomedical applications. Lysine dendrimers are important class of dendrimers based on lysine aminoacid residue which has positively charged amine group [3]. Due to this reason lysine dendrimers could be used as antibacterial or antiviral agents and could make complexes with oppositely charged molecules for example DNA, RNA and some therapeutic peptides. Hydrogen bonds between dendrimers and peptide molecules and hydrophobic interactions between their nonpolar groups are also important for creation of such complexes. Therapeutic KED peptide (Lys-Glu-Asp) was selected for our study as a representative of regulatory peptides [4-5].

The goal of p-resent paper is to study the possibility of complex formation between molecule of lysine dendrimer of second generation and several molecules of therapeutic KED peptide using molecular dynamics simulation method and find how many molecules of peptides could be in such complex.

2 Model and calculation method

2.1 Molecular dynamics method

Molecular dynamics (MD) method is currently the main method for simulation of polymer and biopolymer systems. The method consists in numerical solution of the classical Newton equations of motion for all atoms of the all molecules in the system. MD was used for study of many specific molecules using both coarse-grained and detailed full-atomic models [6]. The potential energy of these models usually includes contribution from valence bonds, valence angles and dihedral angle energies as well as van der Waals and electrostatic energies [7]. The definition of parameters set adequately describing the test molecule properties (force-field) is challenging and requires the experimental data for these molecules, quantum chemical calculations as well as iterative procedures and a very large amount of computer time. Due to this reason several standard computer programs, in which these parameters are defined for a fairly wide range of molecules become widely used in recent years. Currently the most popular molecular modeling packages are GROMACS, AMBER, CHARMM, and some others. Our simulation was performed by molecular dynamics method using the GROMACS 4.5.6 software and one of the most modern AMBER_99SB-ildn force fields [6,7].

2.2 Full atomic model

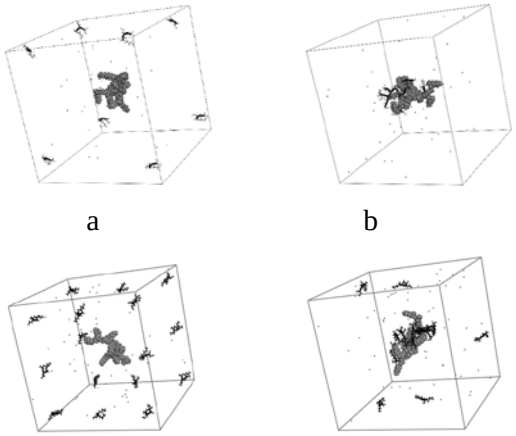
We use model with full atomic details including all hydrogen atoms to study two systems consisting of

one lysine dendrimer of second generation with positively charged NH3+ groups and 8 or 16 molecules of KED peptide (total charge equal -1 for each peptide), water molecules and counter-ions in a cubic cell with periodic boundary conditions. The initial conformation for peptide with internal rotation angles of fi = –135º, psi = 135º, theta = 180º was prepared by Avogadro molecular editor. The structures were optimized in vacuum using molecular mechanics of AMBER force field. Further energy minimizations and simulations were performed using the GROMACS 4.5.6 software package and AMBER_99SB-ildn force fields. The potential energy of this force field consists of valence bonds and angles deformation energy, internal rotation angles, van der Waals and electrostatic interactions. The simulation approach used in this paper for lysine dendrimer and peptides has been described by us earlier in papers on simulation by molecular and Brownian dynamics methods of linear polyelectrolytes [8-14] and non-charged [15-21] polymers as well as of dendrimers and hyperbranched polymers or polymer brushes [22-37]. In several papers we used for simulation of similar branched polymers the numerical calculations on the base of method of self-consistent field (SCF) and scaling approach [38-41]. In present paper the molecular dynamics simulation was performed in NPT ensemble (at temperature 300 K and pressure 1 ATM) using PME algorithm for calculation of electrostatic interactions.

3 Results and discussion

3.1 Snapshots

Snapshots of systems consisting of dendrimer of second generation, peptides, ions and water during simulation are shown on Fig. 1 (water molecules are not shown for clarity). Atoms of dendrimer molecule is shown as beads with diameter equal to their van der Waals radii. Peptides are shown with thick lines drawn along the backbone of each peptide. For both systems it is clearly seen that at the beginning of simulation (see fig. 1a and fig.1c) peptide molecules are far from dendrimer. In the end of simulation for the first system consisting of dendrimer and 8 peptide molecules (fig. 1b) all peptide molecules become adsorbed on dendrimer surface. For second system consisting of the same dendrimer and 16 peptide molecules the situation is a little bit different: at the end of simulation only 10-11 peptides are adsorbed on dendrimers while other stay free (see fig.1d). It means that dendrimer of 2nd generation used in this simulation is not big enough to attract all peptide molecules and 10-11 peptide molecules is the biggest amount which could be adsorbed by such dendrimer.

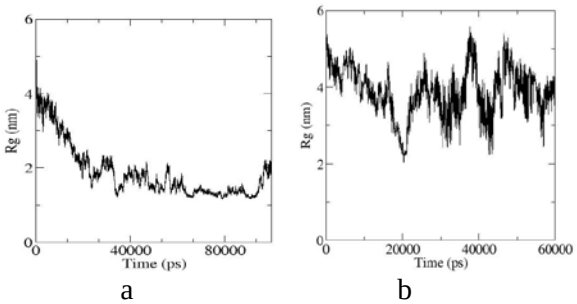


a b c d

Fig. 1. Stages of the complex formation (initial and final) between: dendrimer and 8 molecules of KED peptide at time: t = 0 (a); t = 100 ns (b); dendrimer and 16 molecules of KED peptide at time: t = 0 (c); t = 100 ns (d).

3.2 Complex formation

To characterize the size of the subsystems consisting of dendrimer and peptide molecules during the equilibration the mean squared gyration radius Rg(t) was used. This function was calculated using g_gyrate function of GROMACS software. The gyration radius Rg at the beginning of calculation decrease when peptide molecules approach to dendrimer during formation of complex (see fig.2) and go to plateau value after complex formation.



a b

Fig. 2. Time dependence Rg(t) for: (a) dendrimer G2+8 molecules of KED peptides, (b) dendrimer G2+16 molecules of KED peptides.

From fig. 2a it can be seen that complexes of dendrimer with 8 molecules of KED peptide forms within 40 ns. After this time the complex size Rg fluctuates slightly, but its average value practically

does not change with time. Complex of dendrimer and 16 KED peptides (see fig.2b) become close to dynamic equilibrium state after 20 ns. Significant fluctuations in fig. 2b occur due to that fact that not all peptide molecules are adsorbed by dendrimers and number of such molecules in second system fluctuates with time.

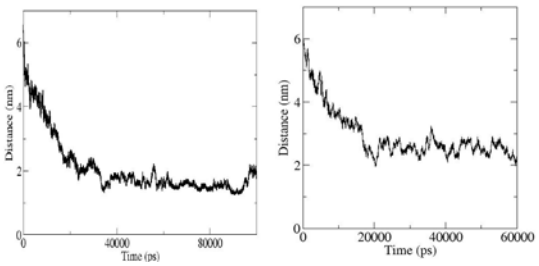


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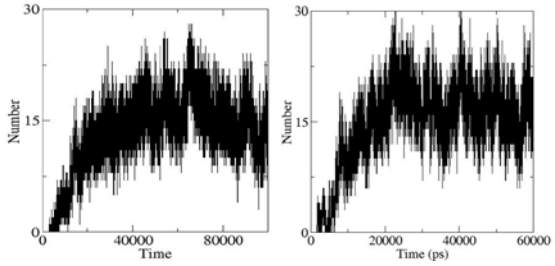
Fig. 3. Time dependence for distance: between (a) dendrimer G2 and 8 molecules of KED peptides, (b) dendrimer G2 and 16 molecules of KED peptides.

The distance between dendrimer and peptide molecules (fig.3a) decrease during about 40 ns in the first system (G2+8KED) and during 20 ns (fig.3b) in the second one (G2+16KED). After these times distance practically does not change in both cases. Therefore, we can assume that the complex between dendrimer and 8 KED peptides forms during first 40ns and become close to equilibrium state after this time, whereas the complex between dendrimer in second system could not adsorb all 16 peptides simultaneously. At the same time average number of peptide in complex after equilibration is nearly constant because there is dynamical equilibrium between adsorded and desorbed peptides.

Another quantity that can characterize the rate of complex formation is the total number of hydrogen bonds (N) between dendrimer and peptide molecules as function of time. This function was calculated using g_hbonds function of GROMACS and are shown on Fig. 4.



a b

Fig. 4. Time dependence of hydrogen bond (Hbonds) number between (a) G2 dendrimer and 8 molecules of KED peptide, (b) G2 dendrimer and 16 molecules of KED peptide during complex formation

The data demonstrates that the number of hydrogen bonds between dendrimer and peptide molecules increases during initial complex formation and then reaches equilibrium plateau after 40 ns in first case (see fig.4a) and after 20 ns in the second one (see fig.4b). It correlates with the character of time dependences of the inertia radius in fig. 2 and istances beten dendrimer and peptides in fig.3.

3.2 Equilibrium characteristics of complex

The sizes Rg of complexes and dendrimer in equilibrium state are evaluated by mean square of inertia radius averaged through the time t after equilibration. These values are given in table 1.

Table 1. Size Rg, main components of inertia tensor Rg11, Rg22 and Rg33 and axial ratio Rg33/Rg11

System	R_g^{11}, **(nm)**	R_g^{22}, **(nm)**	R_g^{33}, **(nm)**	R_g, **(nm)**	R_g^{33}/R_g^{11}
G2+8KED	0,85	1,35	1.43,	1,52	1,68
G2	0,59	0,89	0,95	1,00	1,61
G2+16KED	2,24	3,37	3,73	3,90	1,67

In equilibrium state the sizes of the complex consisting of G2 dendrimer and 8 molecules of KED peptides (see Table 1) is about 1.5 times greater than size of dendrimer itself. It is quite natural, since it correlates with the molecular weight of the complexes which increases in comparison to the molecular weight of the individual dendrimer.

The shape of both complexes can be characterized by their tensor of inertia main components (Rg_{11}, Rg_{22} and Rg_{33}) from Table 1. The rough evaluation of anisotropy of dendrimer and complex could be done using axial ratio of largest and smallest eigenvalues of inertia tensor - Rg_{33} / Rg_{11}. This ratio is equal 1.61 for dendrimer. The addition of 8 peptide almost did not change this ratio Rg_{33} / Rg_{11}=1.68, In second system (G2+16KED) the ratio is equal 1,67 for both dendrimer and complex. Information about the internal structure of the equilibrium complex could be obtained also using radial density distribution of atoms relatively center of inertia both for the complex itself and for its individual components (dendrimer and peptides).

These radial distribution functions (not normalized) are shown on Fig. 5. They were calculated using g_rdf function of GROMACS. The data demonstrates that in both systems (G2+8KED) and

(G2+16KED) the atoms of dendrimer (curve 2) are located mainly in the center of the complexes.

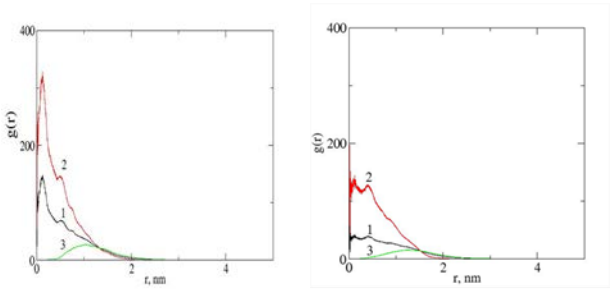


Fig. 5. Radial distribution p(r) for: (a) dendrimer G2 and 8 molecules of KED peptide, (b) dendrimer G2 and 16 molecules of KED peptide. Distribution curves for: all atoms (1); dendrimer atoms (2); peptide atoms of complex (3).

Atoms of peptides (curves 1) in both systems are located mostly on the surface of dendrimer, and do not penetrate into inner part of dendrimer (see curves3 in Fig. 5). Distributions of all atoms belonging to complexes (black curves) are between distributions for dendrimer atoms (red curves) and peptide atoms (green curves).

4 Conclusion

In this paper we demonstrated the formation of dendrimer-peptides complexes between lysine dendrimer of 2nd generation and 8 or 16 KED peptides. We have shown that in first system all 8 peptide molecules are adsorbed on dendrimer while in second system only10-11 molecules will be adsorbed. This information could be very useful for practical use of dendrimers for delivery of regulator peptides to different target cells and organs.

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