Complexes of Lysine Dendrimers of 2nd Generation with Semax and Epithalon Peptides. Molecular Dynamics Simulation

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Abstract: - Dendrimers are widely used as vehicles for drug and gene delivery. In present study complexes of lysine dendrimer of second generation and two types of therapeutic regulatory peptides (Semax and Epithalon) were investigated. Two systems containing lysine dendrimer and 16 oppositely charged model peptides (Semax or Epithalon) were simulated using full atomic models and molecular dynamics method. Both complex formation and equilibrium properties of complexes were studied. It was obtained that dendrimer forms complexes with both peptides very quickly and the sizes and the structures of complexes are very close to each other. These or similar complexes could be used in future for delivery of different therapeutics peptides to patients body.

Key-Words: - Lysine dendrimer, Semax, Epithalon, Complex, Molecular dynamics simulation

1 Introduction

Today the use of dendrimers in industrial and biomedical applications is very wide. They are used as drug and gene delivery systems, as a branched carrier for multiple antigen peptides (MAPs), as antiviral and antibacterial agents. They are also promising anti-amyloid agents for treatment of neurodegenerative diseases (Alzheimer's, Parkinson's and etc.).

Dendrimers are the macromolecules with regular star-like branched structure. Dendrimers usually have shape close to spherical one, constant size and a great number of terminal groups available for functionalization. It makes possible the creation of well-characterized complexes with other compounds (for example, with peptides).

Lysine dendrimers (Fig. 1) consist of lysine monomers (a natural amino acid residues) that makes them less toxic than most of synthetic drug delivery systems.

The goal of this study was to check whether lysine dendrimer forms a complex with Semax and Epithalon peptides (Tab.1).

Both of the chosen peptides are regulatory therapeutic synthetic peptides. Semax is one of the few synthetic regulatory peptides that have found its application in therapy as a nootropic and neuroprotective agent. As for Epithalon, one of the most important properties of this peptide is its ability to activate the telomerase enzyme in patients' body.

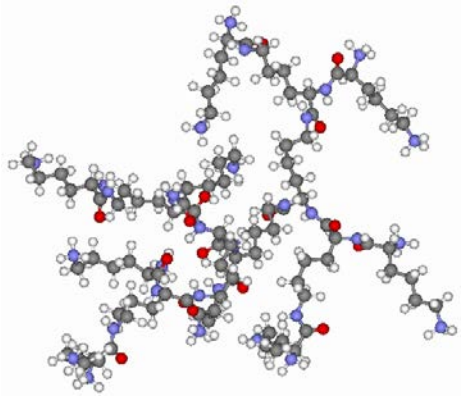

Fig. 1. Structure of a lysine dendrimer of 2nd generation

Table 1. Characteristics of peptides

Peptides	Amino acid sequence	MM, Da
Semax	MET-GLU-HIS-PHE-PRO-GLY-PRO	863
Epithalon	ALA-GLU-ASP-GLY	390

2 Problem Formulation

The goal of this work is to check possibility of complex formation by lysine dendrimer and therapeutic peptides Semax and Epithalon. This question will be studied using molecular dynamics simulation method and full atomic models.

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:

$$F_i = m_i \frac{d^2 r_i(t)}{dt^2} \quad (1)$$

MD is used for detailed study of many specific molecules using both detailed full-atomic models as well as more general coarse-grained models. The potential energy of these models usually include valence bonds, valence angles and dihedral angle energies as well as van der Waals and electrostatic energies. 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 machine time. These calculations can be made only by large groups of specialists. Due to this reason several packages of 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 package [1] and one of the most modern AMBER_99SB-ildn force fields [2].

2.2 Model and Calculation Method

Modeling was performed using the molecular dynamics method for systems consisting of one lysine dendrimer of second generation with 16 positively charged NH_3^+ end groups, 16 Semax peptides, 16 Epithalon peptides, water molecules and chlorine counterions in a cubic cell with periodic boundary conditions. The initial conformation for peptide with internal rotation angles of $\varphi = -135^o$, $\psi = 135^o$, $\theta = 180^o$ was modelled by Avogadro chemical 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 procedure of molecular dynamics simulation used for lysine dendrimers and polyelectolytes has been described earlier in [3-31]. In all calculations the normal conditions (temperature 300 K, pressure 1 ATM) were used. Computing resources on supercomputers "Lomonosov" were provided by supercomputer centre of Moscow State University [32].

3 Problem Solution

Complex formation will be evaluated using different standard characteristics of subsystem containing dendrimers and peptides including size of this subsystem, its anisotropy of shape, number of hydrogen bonds between dendrimer and peptides and number of ion pairs between positively charged dendrimer ends (NH_3+) and negatively charged COO- groups of peptides.

The size of dendrimer and complexes at time *t* was evaluated by the mean square radius of gyration $R_g(t)$ which is defined from:

$$R_g^2(t) = \frac{1}{M} \times \left[\sum_{i=1}^{N} m_i \times \left| r_i(t) - R \right|^2 \right] \quad (2)$$

where R – is the center of mass of subsystem, r_i и m_i – coordinates and masses of i-atom correspondingly, N – is the total number of atoms in subsystem, M is the total mass of dendrimer. This function was calculated using *g_gyrate* function of GROMACS software.

Radial distribution of density p(r) of atoms in dendrimer and complexes as well as distribution of ion pairs were calculated using g_rdf function of the GROMACS package

To calculate the coefficient of translational mobility of dendrimer and complexes, the time dependence of the mean square displacements of the centers of inertia (MSD) of corresponding sub-system, were calculated.

MSD was calculated using g_msd function of GROMACS.

$$\left\langle \sum_t \Delta r^2(t+k\Delta t) \right\rangle = \left\langle \sum_t (r(t+k\Delta t) - r(t))^2 \right\rangle = 6Dt$$

4 Results and discussion

2.1 Dendrimer-peptide Complex Formation

Snapshots of systems consisting of dendrimer, Semax or Epithalon peptides, ions and water during simulation are shown on Fig. 2 (water molecules are not shown for clarity). It is clearly seen that at the beginning of process in cases of 16 Semax and 16 Epithalon (Fig. 2, a, d) peptide molecules are rather far from dendrimer. After 30 ns (Fig. 2, b, e) some part of peptide molecules are already adsorbed on the surface of dendrimer, and in the end after 160 ns (Fig. 2, c, f) all peptide molecules in the systems are on its surface. Atoms of dendrimer molecule is shown as beads with diameter equal to their van der Waals radii. Valence bonds of various peptides are shown with lines of different colors (backbone of each peptide is shown by thick line of the same color as valence bonds).

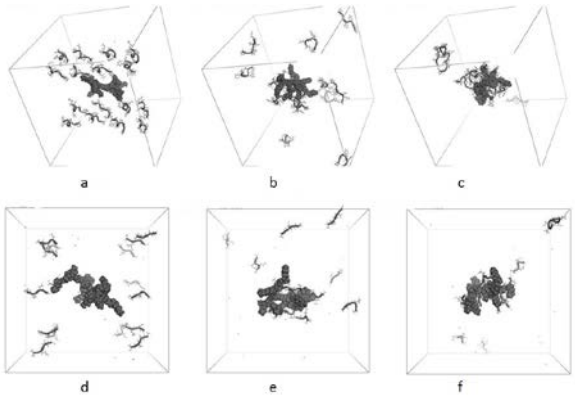

Fig. 2. Snapshots of the dendrimer with 16 Semax peptides at different time moments t = 0 (a); t = 20 ns (b); t = 160 ns (c) and of the dendrimer with 16 Epithalon peptides at different time moments t = 0 (d); t = 20 ns (e); t = 160 ns (f)

The time dependence of gyration radius R_g at the beginning of calculation describes the process of equilibrium establishment during complex formation (Fig. 3). From Fig. 3 it can be seen that both complexes forms within 20 ns. After that the complexes sizes R_g fluctuate slightly, but their average value practically does not change with time. Therefore, we can assume that the systems are in equilibrium state.

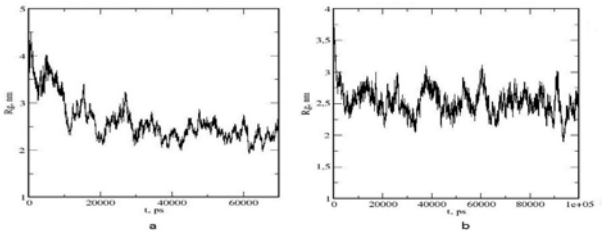

Fig. 3. System of dendrimer G2 and 16 Semax peptides (a) and of dendrimer G2 and 16 Epithalon peptides (b)

The total number of hydrogen bonds (N) between dendrimer and peptides can characterize complex formation. The dependence of this value on time is shown on Fig. 4 and demonstrates how the number of contacts between dendrimer and peptides increases complex formation. This value was calculated using g_hbonds function from package of GROMACS. The average number of hydrogen bonds was equal to 19 for G2+16 Semax and equal to 20 for G2+16 Epithalon.

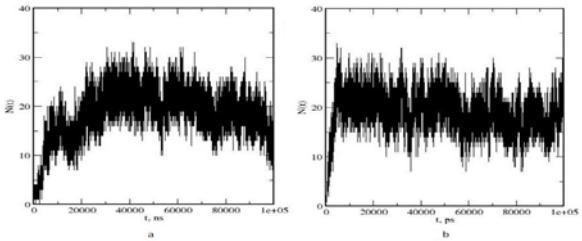

Fig. 4. Time dependence of dendrimer-peptides hydrogen bond number (N) during dendrimer-peptides complex formation: 1 – G2 and 16 Semax; 2 – G2 and 16 Epithalon

2.2 Modelling of Equillibrium State of Dendrimer-peptide Complexes

In this study the mean square radius of gyration Rg of the dendrimer and two complexes (G2 and 16 Semax peptides, G2 and 16 Epithalon peptides) was calculated. It was obtained that the value of Rg of both complexes was nearly twice larger than the size of a dendrimer itself (see Tab.2). The shape of both complexes can be characterized by their tensor of inertia main component ratio (R_g^{11}, R_g^{22}, R_g^{33}), that are in Tab. 2. For example, in the simplest case, anisotropy can be characterized by ratio R_g^{33} / R_g^{11}.

Table 2. Eigenvalues R_g^{11}, R_g^{22}, R_g^{33} of tensor of inertia in dendrimer and dendrimer - peptide complex

System	Rg^{11}	Rg^{22}	Rg^{33}	Rg,	Rg

	, nm	, nm	, nm	nm	$^{33}/_{Rg_{11}}$
G2	0.64	0.97	1.08	1.12	1.69
G2+16Semax	1.36	1.88	1.97	2.30	1.46
G2+16Epithalon	1.76	2.08	2.26	2.44	1.28

Information about the internal structure of the equilibrium complex could be obtained using radial density distribution of different groups of atoms relatively center of inertia for the complexes and for their individual components (Fig. 5).

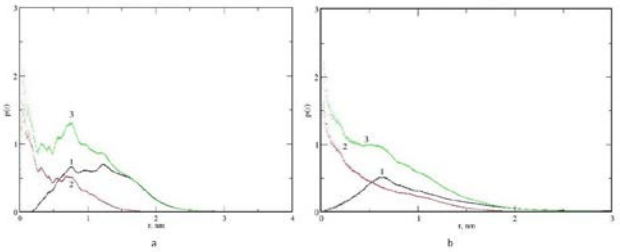

Fig. 5. Radial distribution p(r) density of complexes G2 and 16 Semax (a); G2 and 16 Epithalon (b). Distribution curves: peptide atoms (1); dendrimer atoms (2); all atoms of complex (3)

The data demonstrates that in both subsystems dendrimer (curve 2) is located in the center of the complex and peptides (curve 1) are mainly on the surface of complex. At the same time, some fraction of peptides could slightly penetrate into outer part of dendrimer.

The other characteristic of interaction between dendrimer and peptides (1) in equilibrium dendrimer-peptide complex is the distribution of ion pairs number between their oppositely charged groups. Fig. 6 shows the dependence of ion pairs number on the corresponding distance between pairs of charges of dendrimer and peptides in our complex.

It is seen that there is very sharp peak in both cases, at the distance corresponding to the direct contact between positively charged groups (NH_3^+) of dendrimer and negatively charged groups (COO^-) of the glutamic acid in peptides (Fig 6, curves 1). At the same time, NH_3^+ groups of dendrimer form much fewer ion pairs with ions (Fig 6, curves 2).

To evaluate the translational mobility of our complex, the time dependence of the mean square

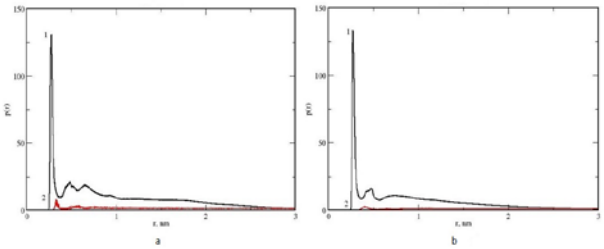

Fig. 6. Radial distribution function of ion pairs: a — complex of G2+16 Semax; b – complex of G2+16 Epithalon. Curves on figure: 1 - NH_3^+ groups of dendrimer and peptides COO^-; 2 - NH_3^+ groups of dendrimer and ions

displacement of the center of inertia (MSD), was calculated. MSD was calculated using g_msd function of GROMACS. Coefficient of translational diffusion of the complex with Semax was obtained from the slope of this time dependence and was equal to $(0.12 \pm 0.03) \times 10^5$ sm^2/s. Coefficient of translational diffusion of the complex with Epithalon was also obtained from the slope of this time dependence and was equal to $(0.21 \pm 0.03) \times 10^5$ sm^2/s.

2.3 Modelling of the Disruption of Dendrimer-peptide Complexes

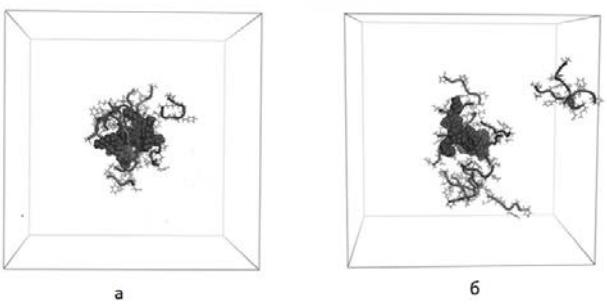

Fig7. Snapshots of the dendrimer with 16 Semax peptides at different time moments *t* before a) and after b) switching off charges of dendrimers

A change in the properties of the medium, for example, pH, can lead to a significant decrease, and even complete nullification of the positive charge of the dendrimer. Here the behavior of the previously studied dendrimer complex of the 2nd generation dendrimer with 16 Semax peptides is simulated after the complete switching off all positive dendrimer charges.

Instantaneous snapshots of dendrimer were taken, before and after switching off dendrimer charges (Fig.7). It is clearly seen from these figures that at the beginning of the calculation (Fig.7a) all peptide molecules are on or very near the surface of

the dendrimer. After 5 ns (Fig.7, b), some of the peptide molecules have already left the surface of the dendrimer. However, the destruction of the complex occurs rather slow (see increase of Rg of complex and distance between dendrimer and peptides after switching off charges of dendrimer in Fig.7) due to the remaining hydrophobic interactions between the atoms of the dendrimer and the peptides.

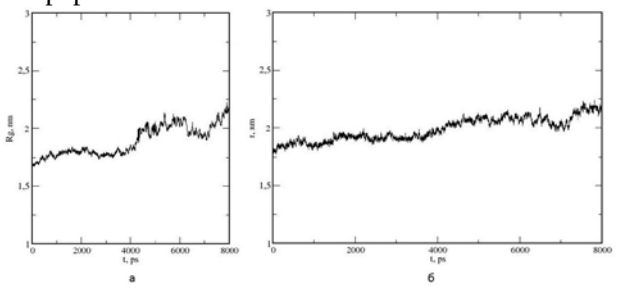

Fig7. Dependence of the of size *Rg* of complex (a) and the distance *r* between the centers of the G2 dendrimer and 16 peptides (b) on the time *t* after switching of charges of dendrimer.

4 Conclusion

The process of complexes formation by lysine dendrimer and therapeutic model peptides (Semax and Epithalon) and the equilibrium structures of these complexes were investigated by the method of molecular dynamics simulation. It was shown that formation of dendrimer-peptide complexes occurs very quickly. The equilibrium size and the anisotropy of both complexes were rather close to each other. The radial distribution function of atoms in all complexes shows that dendrimer atoms are mainly inside the complex, while most of peptide atoms are on its surface. It was demonstrated that there are strong electrostatic interactions between dendrimer and peptides in both complexes. Switching off these interactions leads to the destruction of the complex and the release of peptides from it.

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