Molecular Dynamics Simulation for the Interaction of Lysine Dendrimers with Therapeutic Peptides. New Advances and New Methods.

IGOR NEELOV
ITMO University
197101, St. Petersburg, Kronverksky pr. 49
Institute of Macromolecule Compounds RAS
199004, St. Petersburg, Bolshoy pr. 31
RUSSIAN FEDERATION
i.neelov@mail.ru

ELENA POPOVA
ITMO University
197101, St. Petersburg, Kronverksky pr. 49
Research Institute of Hygiene, Occupational Pathology and Human Ecology
188663, Leningradskaya obl., Vsevolozhsky dist., Kuzmolovsky, Capitolovo Station, building 93
RUSSIAN FEDERATION
arabka2008@mail.ru

DILOROM KHAMIDOVA
Tajik National University
734025, Dushanbe, Rudaky pr. 17
TAJIKISTAN
deya757@mail.ru

IRINA I. TARASENKO
Institute of Macromolecule Compounds RAS
199004, St. Petersburg, Bolshoy pr. 31
RUSSIAN FEDERATION
itarasenko@list.ru

Abstract: - Lysine dendrimers are often used for drug and other molecules delivery (e.g., DNA, peptides, and polysaccharides) to different target cells. In present study complexes of lysine dendrimers of second and third generations and two types of therapeutic regulatory peptides (Semax and Epithalon) were investigated. Our simulation demonstrates that the lysine dendrimer form complexes with these therapeutics peptides. It was shown that two types of interactions acts in complex formation in all three cases – electrostatic and hydrophobic. It was also demonstrated, that electrostatic interactions between dendrimer and peptides in all complexes are stronger than hydrophobic. Structures of these complexes were investigated. It was also shown that switching off electrostatic interactions leads to the destruction of the complex and the release of peptides from it.

Key-Words: - lysine dendrimers, Semax, Epithalon, computer simulation, molecular dynamics method

1 Introduction

Dendrimers are regularly branched molecules which have a spherical shape and many terminal groups available for modification. Lysine dendrimers consist of natural lysine aminoacid residues (Fig. 1). Due to this reason, lysine dendrimers are usually not as toxic as other dendrimers and could be made biodegradable.

Due to the property of dendrimers to have great number of terminal groups available for

functionalization, it makes possible the creation of well-characterized complexes with other compounds such as peptides.

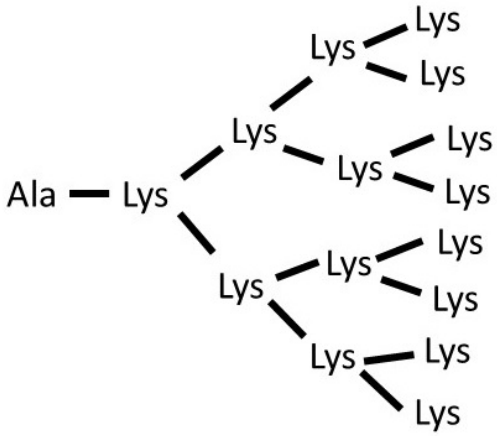

Fig. 1 Structure of lysine dendrimer

The goal of this study was to check whether lysine dendrimers of 2nd and 3rd generation forms a complex with Semax and Epithalon peptides, and to study the destruction of these complexes.

Both of the chosen peptides are regulatory therapeutic synthetic peptides. Semax is one of the few synthetic regulatory peptides that, after all the fundamental research, have found its application in therapy as a nootropic and neuroprotective agent. Its structure is shown in Table 1. Semax peptide is used for acute ischemic stroke prevention, during traumatic brain injury treatment, recovery of a patient after a stroke, in the case of optic nerve disease and glaucoma optic neuropathy.

Epithalon is a regulatory tetrapeptide with the amino acid sequence shown in Table 1, synthesized to mimic the peptide drug “epithalamin” extracted from the pineal gland of animals. As for Epithalon, one of the most important properties of this peptide is its ability to activate the telomerase enzyme in patients’ body and to prolong human cells life. The most well-known pharmacological properties of Epithalon are the following: regulation of the neuroendocrine system, the increase of hypothalamus sensitivity to endogenous hormonal effects, normalization of gonadotroponah hormones, uric acid and cholesterol, strengthening of the immune system, inhibition of spontaneous and induced carcinogenesis, improvement of rheological properties of blood, reduction of the formation of blood clots.

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 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 [1-30]. In all calculations the normal conditions (temperature 300 K, pressure 1 ATM) were used.

2.1 Characterization of Complexes

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:

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

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.
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.

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

3 Results and Discussion

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 a 2nd generation 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.

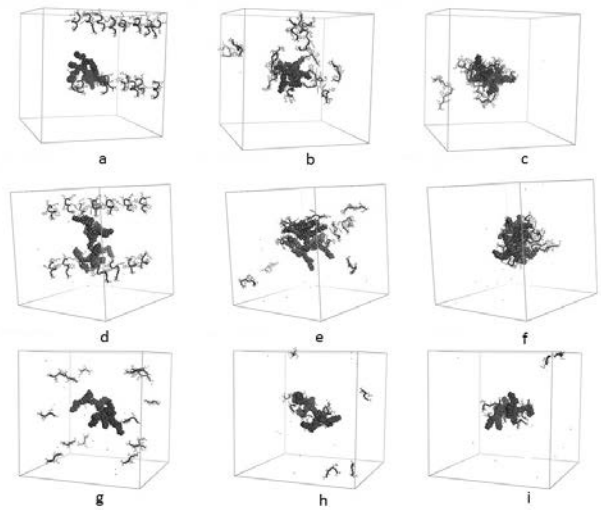

Fig. 2. Snapshots of the G2 dendrimer with Semax peptides at different time moments t = 0 (a); t = 20 ns (b); t = 160 ns (c); G3 dendrimer with 16 Semax peptides at different time moments t = 0 (d); t = 20 ns (e); t = 160 ns (f); G2 dendrimer with Epithalon peptides at different time moments t = 0 (g); t = 20 ns (h); t = 160 ns (i)

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 colours (backbone of each peptide is shown by thick line of the same colour as valence bonds).

3.1 Dendrimer-Peptides Complex Formation

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 with G2 dendrimer forms within 20 ns. Complex of G3 dendrimer and 16 Semax forms only after 40 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.

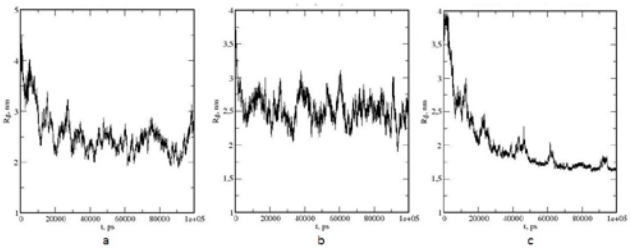

Fig. 3. System of dendrimer G2 and 16 Semax peptides (a); of dendrimer G2 and 16 Epithalon peptides (b); of dendrimer G3 and 16 Semax peptides (c)

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.

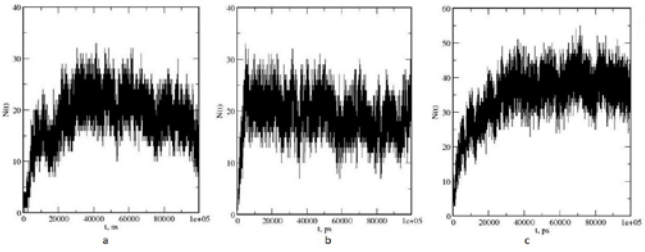

Fig. 4. Time dependence of dendrimer-peptides hydrogen bond number (N) during dendrimer-peptides complex formation: 1 – G2 and 16 Semax; 2 – G2 and 16 Epithalon; 3 – G3 and 16 Semax

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, equal to 39 for G3+16 Semax and equal to 20 for G2+16 Epithalon.

3.2 Modelling of Equillibrium State of Dendrimer-Peptide Complexes

The mean square radius of gyration Rg of the dendrimers (G2 and G3) and three complexes (G2 and 16 Semax peptides, G3 and 16 Semax peptides, G2 and 16 Epithalon peptides) was calculated. It was obtained that the value of Rg of the complex of G2 and 16 Semax was nearly twice larger than the size of a dendrimer itself (see Tab.2). The same result was obtained for the complex of G2 and 16 Epithalon. In case of G3 and 16 Semax the size of a complex was only 1.15 times larger than the size of a dendrimer. The shape of all three 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}, nm	Rg^{22}, nm	Rg^{33}, nm	Rg, nm	Rg^{33}/Rg^{11}
G2	0.64	0.97	1.08	1.12	1.69
G2+16 Semax	1.36	1.88	1.97	2.30	1.46
G3	0.98	1.22	1.32	1.44	1.34
G3+16 Semax	1.24	1.34	1.51	1.66	1.22
G2+16 Epithalon	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 centre of inertia both for the complexes themselves and for their individual components (Fig. 5).

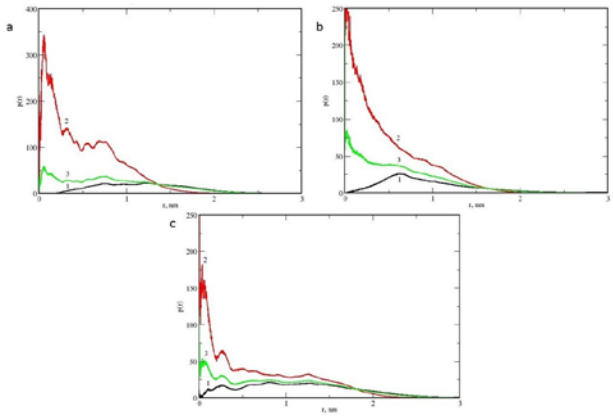

Fig. 5. Radial distribution p(r) density of complexes G2 and 16 Semax (a); G2 and 16 Epithalon (b); G3 and 16 Semax (c). Distribution curves: peptide atoms (1); dendrimer atoms (2); all atoms of complex (3)

The data demonstrates that in all cases dendrimers (curve 2) are 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 all 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 displacement of the centre of inertia (MSD), was calculated. MSD was calculated using g_msd function of GROMACS.

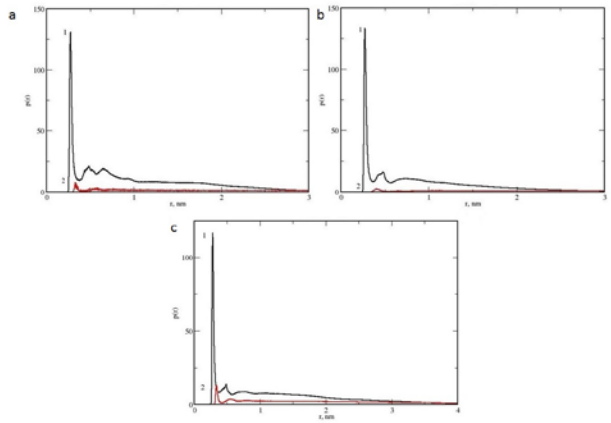

Fig. 6. Function of ion pairs radial distribution: a – G2 and 16 Semax, b - G2 and 16 Epithalon, c - G3 and 16 Semax. Curves: 1 - NH_3^+ groups of dendrimer and COO^- groups of peptides; 2 - NH_3^+ groups of dendrimer and ions

Coefficient of translational diffusion of the complex of G2 with 16 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. For complex of G3 with 16 Semax it was equal to $(0.10 \pm 0.05) \times 10^5$ 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. It was greater than for dendrimers with Semax peptides due to smaller size of Epitalon peptides.

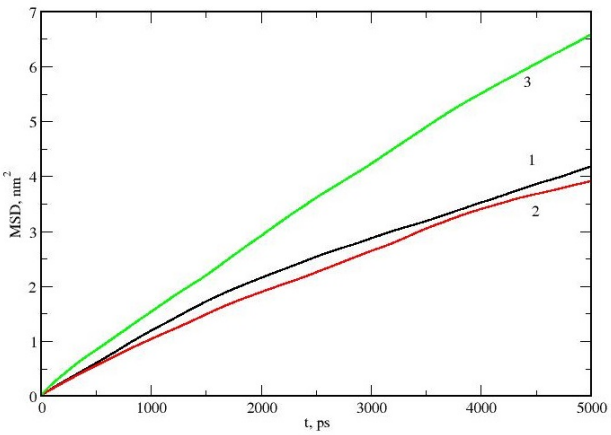

Fig. 7. Mean square displacements of the centres of inertia: complex of G2 and 16 Semax (1); G3 and 16 Semax (2); G2 and 16 Epithalon (3)

3.3 Modelling of the Disruption of Dendrimer-peptide Complexes

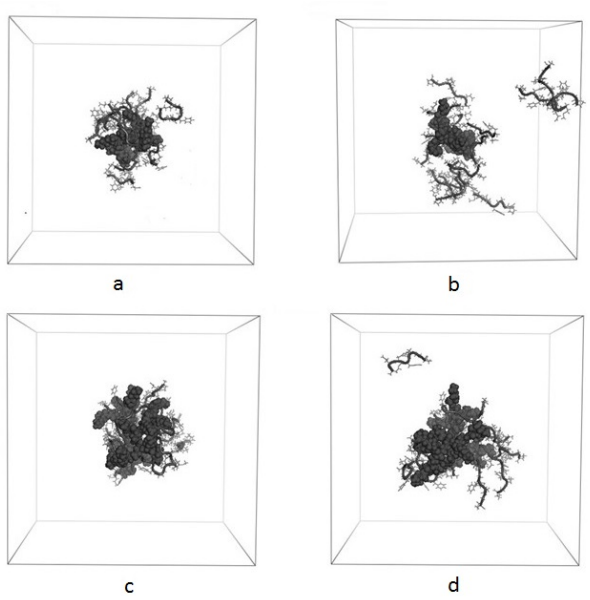

Fig. 8. Snapshots of the G2 (a, b) and G3 (c, d) dendrimers with 16 Semax peptides at different time moments *t* before and after switching off charges of dendrimers

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 behaviour of the previously studied dendrimer complexes of the 2nd and 3rd generation dendrimers 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.8). It is clearly seen from these figures that at the beginning of the calculation (Fig.8a) all peptide molecules are on or very near the surface of the dendrimer in both cases. After 5 ns (Fig.8b), some of the peptide molecules have already left the surface of the dendrimers. However, the destruction of the complex occurs rather slow (see increase of Rg of complex and distance between dendrimers and peptides after switching off charges of dendrimer in Fig.9) due to the remaining hydrophobic interactions between the atoms of the dendrimer and the peptides.

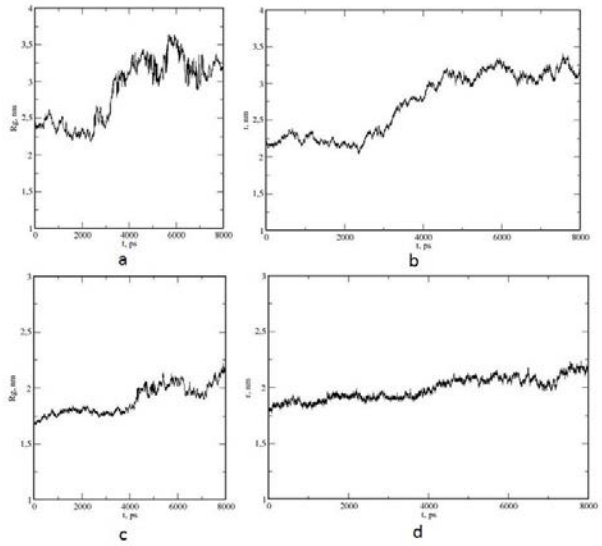

Fig. 9. Dependence of size *Rg* of complexes of dendrimers G2 (a) and G3 (c) with 16 Semax; the distance *r* between the centres of the G2 (b) and G3 (d) dendrimers and peptides on the time *t* after switching of charges of dendrimer.

4 Conclusion

The process of complexes formation by lysine dendrimers of second and third generation 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 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 all complexes. Switching off these interactions leads to the destruction of the complexes and the release of peptides from it.

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