Computer Simulation of Lysine Dendrimers and their Interactions with Amyloid Peptides.

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Abstract: - Computer simulation of lysine dendrimers and their interactions with short amyloid peptides were performed by molecular dynamic method. The size, anisotropy and structure of these dendrimers at different temperatures were calculated. It was shown that behaviour of lysine dendrimer itself is different from behaviour of many other dendrimers. It was also demonstrated that stack of eight amyloid peptides (Ab17-22) is stable but peptide dendrimer destroys this stack and adsorbs the peptides. The structure of dendrimer-peptide complex was studied. The mechanism of destruction of amyloid fibrils by positively charged dendrimers is suggested. These process probably could be used in future for curing Alzheimer's disease.

Key-Words: - dendrimers, dendrimer-peptide complex, molecular dynamics simulation.

1 Introduction

Most of dendrimers are almost perfect spherical tree-like macromolecules with well-defined chemical structure. They were synthesized more than 30 years ago [1-5]. Their spherical symmetry, compactness and softness make them intermediate chemical compounds between colloids and linear polymers. Dendrimers could be used in many industrial and biomedical applications, for example, as drug and gene delivery vehicles and as antibacterial, antiviral, and antiamyloid agents [6-8]. Peptide dendrimers is an important class of biocompatible dendrimers consisting of different aminoacid residues. The simplest poly-L-lysine (PLL) dendrimers studied in this paper consist of lysine residues only. There are many publications about synthesis [2,9-13] and biomedical applications [14-18] of lysine dendrimers, but only a few papers contain information about their spatial structure in water obtained by experimental [19-20] and computational [21-25] methods.

It is well known that excessive formation of amyloid peptides and their aggregation in the brain is associated with Alzheimer's disease and other neurodegenerative disorders. Therefore, in the past two decades, much attention was paid to the experimental and theoretical study of amyloid peptides and their aggregates (amyloid fibrils).

There are also many papers about simulation of short amyloid peptides (see, for example, recent review of Nguyen and Derreumaux [26] and their references.

In several papers of Klajnert et al [27-29] it was shown that branched polyelectrolytes and in particular dendrimers with positively charged groups may inhibit the formation of amyloid fibrils from fragments of amyloid peptide (Ab1-28) or even completely destroy existing mature fibrils.

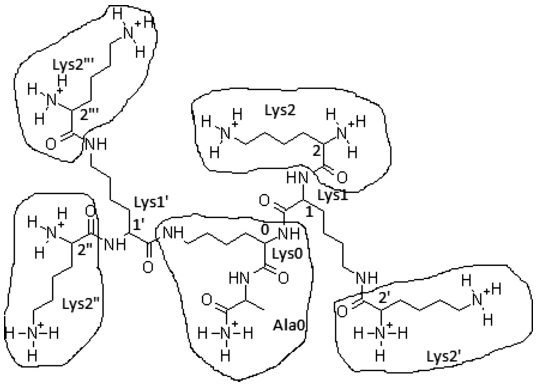
In this paper, the method of molecular dynamics simulation is applied for the stugy the lysine dendrimers and their complexation with short Ab17-22 amyloid peptides.

The chemical structure of PLL dendrimers has asymmetry of the branching, i.e. the two linear fragments (spacers) originating from each branching point of each generation have different lengths (see Fig. 1).

The contour length of the long spacer is near 2.5 times larger than that of the short one. In our recent work [22], we have used molecular dynamics simulation to study the spatial structure of PLL dendrimers of different generation numbers *G*

(G = 1-5) in a dilute water solution at room temperature.

(a)



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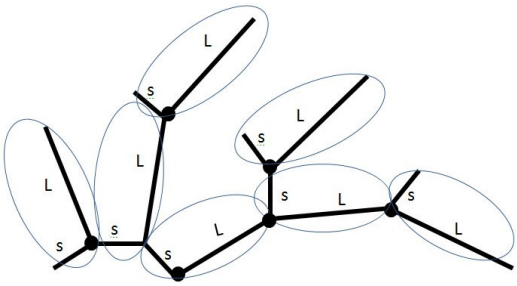

Fig.1 (a) Chemical structure of lysine dendrimer of generation G=1 and (b) its sketch picture demonstrating anisotropy of branching (where "s" are short and "l" are "long" spacers).

It was shown that the global characteristics of the PLL dendrimers, such as, the size and shape as well as the density profile are similar to those of dendrimers with the symmetric branching. In the present work, we pay particular attention to the structural properties of PLL dendrimers, which are important for applications.

In our previous paper we studied structural properties of polylysine dendrimers of 1-5 generations at single temperature T=300K by using MD simulation [22]. In other recent papers [23,24] we studied the temperature dependences of global structural charactreristics and local dynamics of second and fourth generation PLL dendrimers using MD simulation and NMR.

The goal of the present paper is to study additional structural properties of PLL dendrimers. as well as to study interactions of PLL dendrimers with amyloid peprides.

In part 2 of the paper we desribe model and simulation method, results of simulation of dendrimers at different temperatures and their interactions with amyloid peptides. Part 3 contains conclusion and part 4 - acknowledgment.

2 Molecular Dynamics Simulation

2.1 Model and simulation method

Method of molecular dynamics (MD) was for the first time applied to polymer systems by Balabaev et al.[30]. Metod MD as well as metod of Brownian dynamics was applied later to both linear polymers [31-35], polyelectrolytes [36-39] and dendrimers [40-43]. The detailed description of the full-atomic model of the polylysine dendrimer used in this work and simulation method, including the preparation and equilibration of the system have been given in our previous papers [22]. In brief, two dendrimers of second (G = 2) or fourth (G=4) generations with protonated NH_3^+ terminal groups were studied in the dilute water solution. Some parameters of the dendrimers are shown in Table 1. One dendrimer molecule was placed in the cubic simulation box containing 6975 or 19808 water molecules (TIP3P model) correspondingly. The number of Cl^- counterions was equal to the number of charged NH_3^+ terminal groups in the dendrimers (16 and 64 correspondingly). The periodical boundary conditions and Amber99sb-ildn force field were used.Calculations were performed in an NPT ensemble. Trajectories for five temperatures (T = 283, 300, 323, 343, and 363 K) were obtained. The Gromacs-4.5.5 package was used for simulation. First, 50 ns of each MD simulation were used for the equilibration of the system and final 150ns for calculations of the equilibrium characteristics of systems.

Molecular dynamics method was applied also for the modeling amyloid and dendrimer-amyloid systems. We used in our simulation full-atomic model of dendrimer and amyloids, force field Amber99-SB-ildn and simulation package Gromacs. First of all we considered a stack of eight amyloid peptides (Ab17-22, each of which had a small negative charge (-1)), in water, and checked how stable is this ordered aggregate. In the next step we added to our amyloids the lysine dendrimer of second generation with 16 positively charged terminal NH_3^+ groups. Both systems were placed in cubic water box with periodical boundary conditions at a temperature of T = 300K. Counter ions (Cl^-) were added in both cases to provide electrical neutrality of the systems. All systems were modeled for 160 ns. The initial part of the trajectory (50ns) was used for equilibration (complex formation). Subsequent part of the trajrectory was used to calculate the equilibrium characteristics of the

complex (for example, size, anisotropy and distribution functions).

Table1. Main characteristics of PLL dendrimers. *G* is the number of generations, N_{in} is the number of inner spacers, N_t is the number of terminal segments, N_w is the number of water molecules in the simulation cells.

G	*M, g/mol*	N_{in}	N_t	N_w
2	2028	15	16	6975
4	8229	63	64	19808

2.2 The structural properties

Typical snapshots of simulated dendrimers are shown in Fig. 2. It can be seen that PLL dendrimers have an open structure with large pores.

(*a*) (*b*)

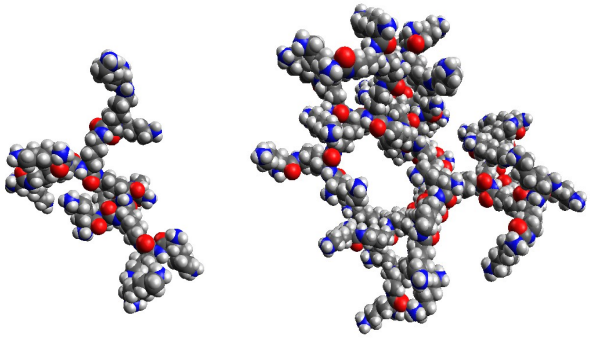

Fig. 2. The snapshots poly-L-lysine dendrimers of G=2 (a) and G=4 (b) generations

The dendrimer size could be characterized by the mean-squared radius of gyration

$$\left\langle R_g^2 \right\rangle = \frac{1}{M}\left\langle \sum_i^{N_a} m_i r_i^2 \right\rangle , \qquad (1)$$

where r_i is the distance to *i*th atom from the center of the mass of the dendrimer, m_i is the mass of *i*th atom, N_a is the number of atoms in a dendrimer and *M* is the total mass of dendrimer. The averaging in Eq. (1) is performed over the simulation time. It was obtained [23, 24] that the mean squared values of $R_g = \sqrt{\left\langle R_g^2 \right\rangle}$ are equal to 1.15 nm and 1.85 nm for $G = 2$ and $G = 4$ dendrimers, correspondingly, and practically do not change in the temperature interval studied (Fig. 3). Such behavior is not usual for dendrimers. In particular, for solutions of POPAM in chloroform and carbosilane dendrimers in chloroform the 10-15% change of R_g was observed in the temperature range corresponding to the liquid phase of the solvent (220 – 320K). The distributions of R_g for the PLL dendrimer at different temperatures (Fig. 4**)** are rather narrow and also insensitive to the temperature. This means that PLL dendrimers are rather rigid macromolecules.

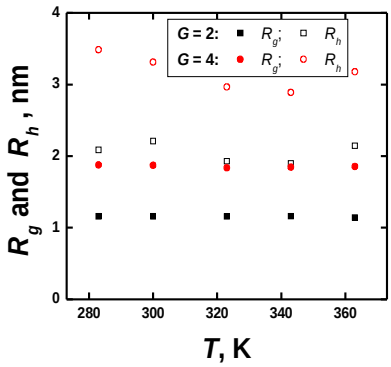

Fig. 3. The gyration R_g and hydrodynamic R_h radii for G = 2 and G = 4 PLL dendrimers at different temperatures

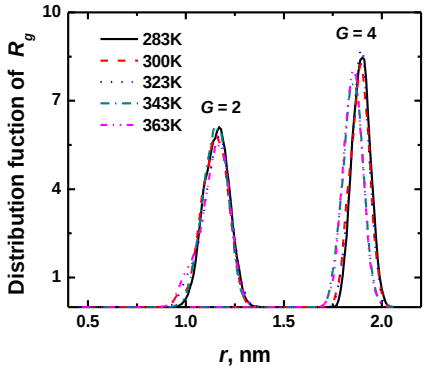

Fig. 4. Distribution of R_g of PLL dendrimers for G = 2 and 4 at different temperatures.

The internal structure of a dendrimer could be characterized by the overall radial distribution function of the dendrimer atoms relative to its center of mass (radial density profile)

$$\rho(r) = \frac{m_{shell}(r)}{V_{shell}(r)} , \qquad (2)$$

as well as the radial distribution of branching points

$$\rho_{br}(r) = \frac{m_{br}(r)}{V_{shell}(r)} ; \qquad (3)$$

and radial distribution of terminal NH_3 groups (N atoms)

$$\rho_{term}(r) = \frac{m_{term}(r)}{V_{shell}(r)}, \qquad (4)$$

where m_{shell}, m_{br} and m_{term} are the mass of all atoms, C atoms and N atoms correspondingly in a spherical shell at the distance r from the dendrimer center of mass. V_{shell} is the shell volume.

Radial density profiles of all dendrimers (see [24]), C atoms in branching points (Fig. 5a), and N atoms in terminal groups (Fig. 5b) show that the internal structure of dendrimers is also weakly sensitive to the temperature.

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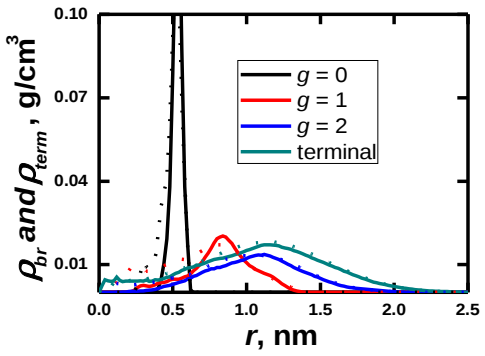
a)



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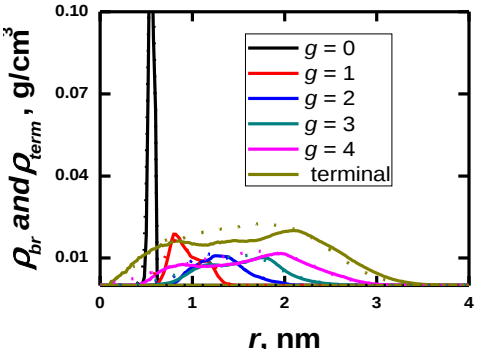
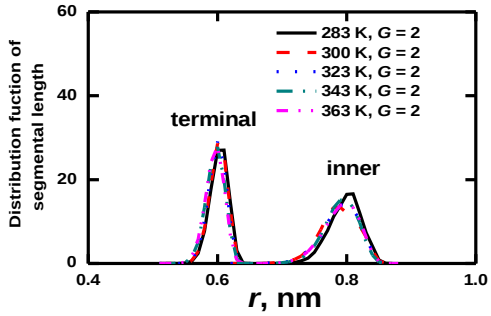


Fig. 5. The radial density profile of C atoms of branching points of different generation shells (ρ_{br}) and nitrogens in terminal NH_3 groups(ρ_{term}) for G =2 (a) and G=4 (b) PLL dendrimers at 283K (solid lines) and 363K (dotted lines).

To understand the origin of the PLL dendrimers rigidity we calculated the distribution of end-to-end distances for internal spacers and terminal segments (Fig. 6) and the distribution of branching angles (Fig. 7). Short spacers and short terminal segments are rigid because they consist of only three chemical bonds and contain rather rigid plane peptide bond. Long spacers and terminal segments consist of seven and five chemical bonds correspondingly and due to the rotation around these additional bonds being more flexible. Nevertheless, Fig. 6 shows that the distribution of the end-to-end distances of long inner spacers and long terminal segments are rather narrow and their average end-to-end length are close to their maximum values, i.e. both inner spacers and terminal segments are almost completely extended. All these distributions (Fig. 6) are also practically insensitive to the temperature

(a)



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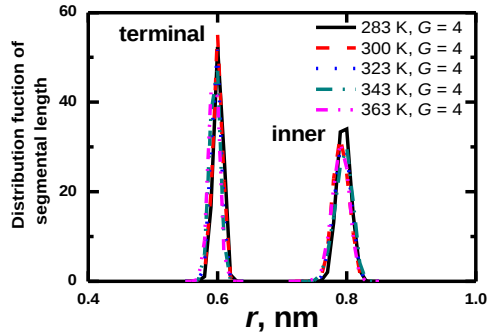
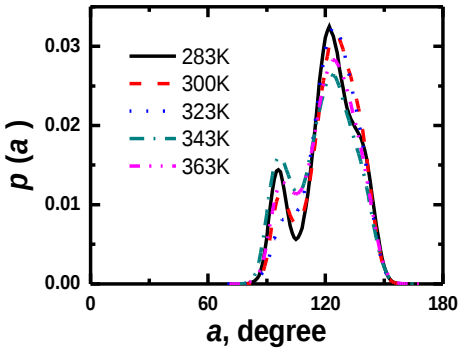


Fig. 6. Distribution of the end-to-end distances for long inner spacers and terminal segments at different temperatures for G = 2 (a) and G = 4 (b) dendrimers.

The distributions of different branching angles between two short spacers, between short and long spacers and between two long spacers in a G = 4 lysine dendrimer at different temperatures is shown on Fig.7a,b and c correspondingly. It is seen that angles between two short spacers are distributed

in the rather narrow interval about 60° (between 90° and 150°) while other two types of angles in wider but still also resticted interval about 120^0 and their distributions do not change with the temperature.

(a)



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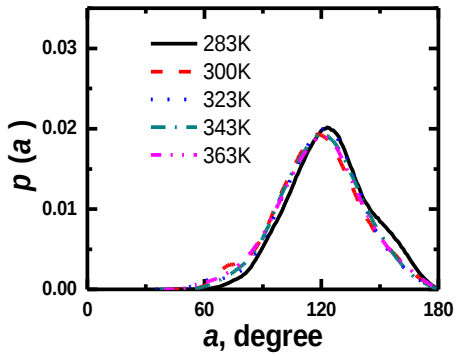

(c)

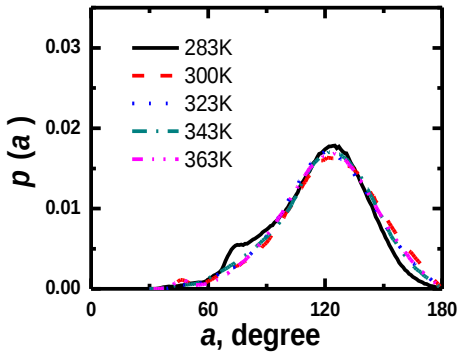

Fig. 7. Distribution of angles between spacers in i and i+1 subgenerations: (a) between short spacers, (b) between short and long spacers, (c) between long spacers in a G4 lysine dendrimer at temperatures T = 283-363 K.

All these data confirm that PLL dendrimers are rather rigid systems. At the same time, the radial density profile [24] and Fig. 5 show that the internal structure of PLL dendrimers corresponds to the hard core – loose shell model. This means that there is a back-folding of dendrimer branches into the macromolecule interior. What is the mechanism of this back-folding in PLL dendrimers?

In PLL dendrimers the stiff spacers can be considered as long effective "bonds" and the angles between them as effective "valence" angles. The only possible mechanism of the structural rearrangements and of the back-folding in this case is the rotations around these effective "bonds", similar to internal rotations in linear polymers with rigid chemical bonds and fixed valence angles. Such a mechanism of the conformational rearrangements (rotation around these effective bonds) should lead to two effects. The first one is that the long and short spacers or terminal segments rotate together. Therefore, we have to expect a similarity of the orientational mobility of long and short spacers (in contrast to dendrimers with flexible spacers or flexible angles between spacers where short and long spacers originating from the same branching point can change their orientation independently from each other). Another possible effect is an increase of the correlations between the reorientations of spacers belonging to different subgenerations as comparing with flexible dendrimers.

2.3 Dendrimer-peptide system

2.3.1 Stability of free fibril and formation of complex with dendrimer

In the first stage we studied the stack consisting of eight amyloid peptides Ab17-22. For this system we obtained that the stack is stable at least for the duration of our computer modeling (160ns). Then we studied the system consisting of the dendrimer and 8 amyloid peptides. We have found that during the first 40 ns of our simulation all peptides were completely adsorbed by the oppositely charged dendrimer. Thus the addition of dendrimer with positively charged ends could lead to the destruction of stable stack structure (fragment of amyloid fibril) composed of amyloid peptides.

2.3.2 The structure of dendrimer-peptide complex

After first 40ns we obtained the dendrimer-peptide complex which is stable until the end of our simulation (160ns). We studied the equlibrium structure of this complex using second part of our trajectory. To characterize the size and anysotropy

of our system we calculated gyration tensor and its principle components for dendrimer and for complex. The distribution functions of radius of gyration Rg and components allows us to compare the sizes of the complex and two subsystems and its shape anisotropy. Figure 8 shows the distribution functions of Rg (thick lines) in dendrimer in water and Figure 9 for dendrimer-peptide complex in water, respectively. Comparison of distributions Rg indicates that the dendrimer radius of gyration in the range from 1.0 nm to 1.3 nm, while in the complex Rg varies from 1.2 nm to 1.8 nm. Thus not only complex is larger than the dendrimer, but also about twice (0.6 nm compared to 0.3 nm) fluctuations dimensions than in a single dendrimer in water. Comparison of the all three components of the inertia tensor (thinner lines in Figure 8 and Figure 9) gives information about the form of a separate and dendrimer complex. In dendrimer distribution for the two components are close to largest one, so the form is close to a disk-like shape, while in the complex all three components are different and its shape can be described only by general ellipsoid.

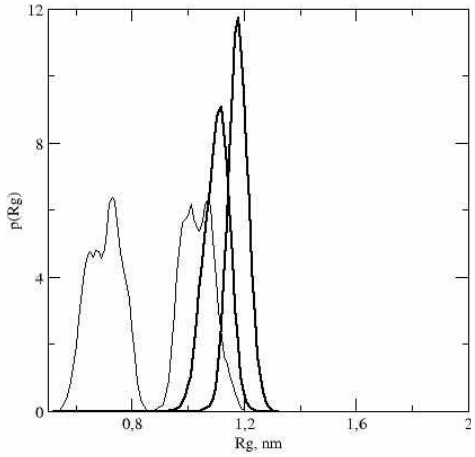

Fig. 8. The distribution functions of the radius of gyration of the dendrimer. Thick line is for the radius of gyration and more subtle lines are for the three principal components of the inertia tensor.

Figure 10 demonstrates the distribution functions for all atoms including dendrimer and peptides (thick lines), for the atoms of the dendrimer complex (thin curves) and for atoms peptides (average thickness of the curves) on dendrimer.

An important characteristic of complex is the radial distribution function of their terminal charges relative to the center of the dendrimer (Figure 11).

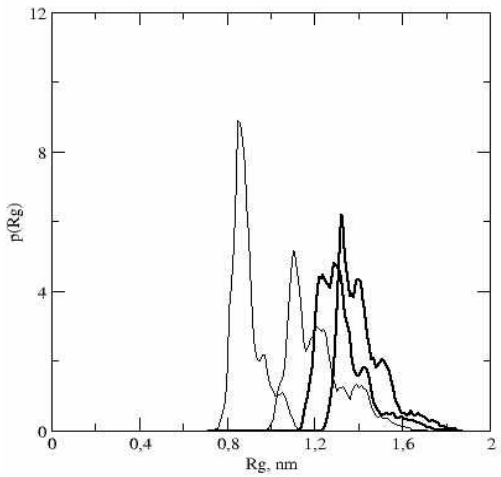

Fig. 9. Same as Fig.8, but for complex of dendrimer with 8 short amyloid peptides.

The resulting distribution of the atoms for peptides was shifted to larger distances r from the center of the dendrimer compared to the distribution for the atoms of the dendrimer itself. From a comparison of these functions we can conclude that the peptides are mostly on the surface of the dendrimer (high r). At the same time because the distributions overlap, the individual peptides can go inside dendrimer also, but part of such peptides is not large. It means that they spend on average less time inside dendrimer than on its surface.

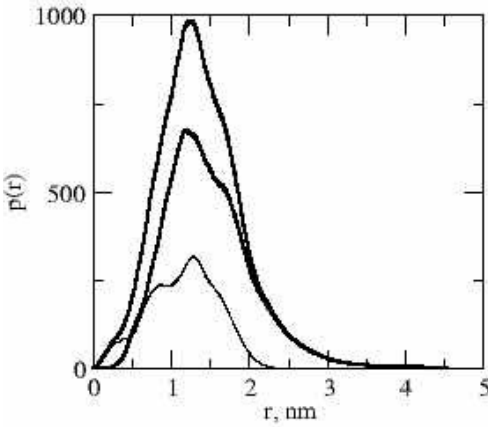

Fig. 10. The radial distribution function p (r) relative to the center of the dendrimer (for all atoms (thick line), for the dendrimer (thin lines) and for peptide (average thickness) where r - distance from the center of the dendrimer.

As we mentioned before in the lysine dendrimer of the third generation there are 16 positively charged terminal NH_3^+ groups. In the complex there are also 8 additional negative charges (-1 from each peptide). The distribution of positive

charges is slightly shifted to higher distances (Figure 11, a thin line) than the distribution of negative charges (the line of medium thickness).

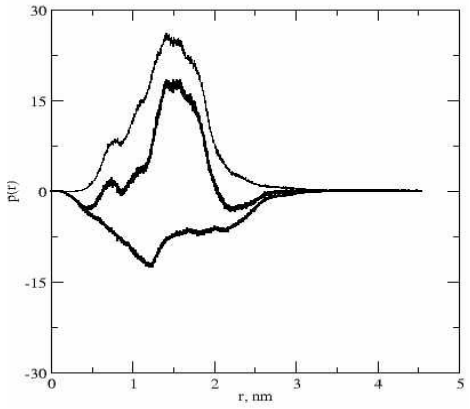

Fig.11. The radial distribution function of the charges relative to the center of the dendrimer. The thin line (top) - a positive charge, the average thickness of the line (at the bottom) - a negative charge, and a thick line (in the middle) - the total charge on the hybrid

At the same time distribution of positive charges is more narrow. This leads to the fact that the radial distribution function of the total charge of the complex (thick line) has a small negative values for both small and at large distances from the center of the dendrimer, while at intermediate values of r, the net charge is positive. It means that the negatively charged part of peptides could penetrate inside dendrimer while most of peptide atoms stay adsorbed on surface of dendrimer.

3 Conclusion

Molecular dynamic simulations of second and fourth generation poly-L-lysine dendrimers in water at different temperatures have been performed. Simulation shows that the size and density profile of dendrimers practically does not change with the temperature. These insensitivity is likely due to the rigidity of chemical structure of lysine dendrimer (rigidity of spacers between neighboring branching points). The internal structure of PLL dendrimers corresponds to the dense core - loose shell model, i.e. the back-folding of dendrimer branches is observed. Conformation rearrangements occur by means of the rotations around stiff spacers, which can be considered as effective bonds.

For peptide and dendrimer-peptide systems we demonstrated the stability of the stacks of amyloid peptides (fragments of amyloid fibrils), consisting of eight short amyloid peptides Ab17-22. We also demonstrated that the addition of lysine dendrimer with positively charged ends leads to rapid and irreversible binding of the negatively charged peptides to positively charged lysine dendrimer. We also calculated the structure of the complex formed by the dendrimer and amyloid peptides and, in particular, the size, anisotropy and the mutual arrangement of components in the complex.

We plan to study in next papers the behaviour of biodegradable lysine dendrimers and more general peptide dendrimers (containing other aminoacid residues) as well as interaction of these dendrimers with amyloid peptides.

4 Acknowledgment

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