Computer Simulation of Complex Formation by LysArgHis and LysHisArg Peptide Dendrimers with Ovagen Peptide Molecules

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Abstract: - Ovagen is a short bioactive peptide that normalizes liver and gastrointestinal tract function. In this work, we study complexes of Ovagen peptide molecules with the KRH dendrimer containing arginine-histidine (RH) spacers and the KHR dendrimer containing histidine-arginine (HR) spacers. These dendrimersë can be used as a nanocontainer for the delivery of oppositely charged bioactive molecules, including peptides. The amino acid residues in the spacer (H and R) of each dendrimer are different, and the charge of the H residue depends on pH. Therefore, we performed molecular dynamics simulations of the complexation of 16 Ovagen molecules with the dendrimer at two different pH values: a) KRH at pH>7 with fully uncharged histidines (H) and b) KRHp at pH<5 with fully protonated (Hp) histidines; c) KHR at pH>7 with fully uncharged histidines (H) and d) KHpR at pH<5 with fully protonated (Hp) histidines in aqueous solution with explicit counterions. It was found that dendrimers with protonated histidines (KHpR and KRHp) could carry more Ovagen peptide molecules than dendrimers with neutral histidines (KHR and KRH), respectively. Moreover, Ovagen peptide molecules penetrate deeper into the dendrimer center in KHpR and KRHp dendrimers than in KHR and KRH dendrimers.

Key-Words: - Peptide dendrimers, medicinal oligopeptides, complexes, molecular dynamics simulation

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1 Introduction

Ovagen is a short bioactive tripeptide (Glu-Asp-Leu, EFL), obtained by hydrolysis from animal proteins. It normalizes liver and gastrointestinal tract function. However, their clinical application is complicated by rapid degradation in the body and low bioavailability. To solve this problem the delivery systems capable of protecting the peptide from destruction. Dendrimers can be used for the delivery of oppositely charged biological and bioactive molecules, including peptides. Classical dendrimers are macromolecules with a regular star-shaped structure originating from a single core [1]. The number of branching points between the core and the end segments determines the number of generations G. Dendrimer molecules of a given generation are monodisperse. The number of their ends, available for functionalization, increases very rapidly with increasing dendrimer G [2]. Dendrimers can carry a large electric charge distributed throughout the entire volume of the dendrimer or only on its surface.These properties determine the practical interest in the use of dendrimers in industry [3] and biomedicine [4]. Dendrimers can be used as nanocontainers for the drug delivery [5,6]. Dendrimers were applied primarily to increase the solubility of hydrophobic drugs and protect them during delivery, as well as to protect healthy cells from exposure to toxic drugs. Application of polyamidoamine and polypropylene mine for delivery of genetic material was described in [7], for delivery of DNA in [8] and for delivery of siRNA using PAMAM in [9] and using PAMAM and PPI in [10]. Peptide dendrimers based on natural monomers, such as amino acid residues, are a good alternative to synthetic c dendrimers. The first to be synthesized were simple lysine dendrimers, consisting of lysine monomers only [11]. The repeating unit of such a dendrimer is a single (branched) lysine residue (Lys). Experimental studies of the sizes of lysine dendrimers of generation G=1-10 were carried out in dimethylformamide [12] and in aqueous solutions for G=3, [13] and G=1-5 [14]. Dendrimers were examined using molecular dynamics computer simulations. The dependences of

the sizes and other structural and dynamic characteristics of these dendrimers on the number of generations (G) contained in them were studied earlier for G=2-5 [15], G=1-6 [16], G=2, 4 [17], G=1-5 [18] and G=1-5 [19]. In the last paper, the main attention was paid to the comparison of lysine and PAMAM dendrimers of the same generations. More complicated peptide dendrimers containing lysine octa-branched core and linear peptides consisting of 9-16 amino acids attached to their ends were synthesized first in 1988 [20] for application as multiple antigen peptides (MAPs). Synthesis of similar dendrimers with 24-residue peptides attached to dendrimer ends as described in [21]. Lysine dendrimers with one additional amino acid between branching points were described in, [14], for G=1-5 and in, [22], for G=2. Dendrimers with one or more other amino acid residues attached to their ends were also described. In particular, dendrimers with arginine and histidine were studied for applications in gene delivery and as antibacterial, antiviral, and antiamiloid agents. In particular, PAMAM dendrimers of generation G=4 with terminal lysine or terminal arginine were studied in [23]. Arginine functionalized peptide dendrimer of generations G=5 and G=6 was investigated in [24]. Arginine-, lysine- and leucine-bearing polyethylenimine (PEI) dendrimers were studied in [25]. Lysine dendrimer of generation G=6 with lysine, arginine, and histidine ends was used in [26]. PAMAM of generation G=4 with arginine and histidine ends was studied in, [27]. Dendritic polyglycerolamine with arginine and histidine end groups was investigated in, [28]. Properties of different dendrimers with aminoacid residues were compared in, [29]. However, there was practically no work on lysine dendrimers in which other amino acid residues were inserted in the internal generations of the dendrimer (with the exception of papers, [14], for G=5 and, [22], for G=2).

Recently, new types of lysine-based dendrimers in which the internal part of the dendrimer was functionalized. These dendrimers were successfully synthesized and studied by NMR method for dendrimers with double glycine and double lysine spacers, [30], double arginine spacers, [31] and double histidine (with protonated and non-protonated imidazole) spacers, [32]. The dendrimers were also tested as a carriers for delivery of genetic material with double glycine and double lysine spacers, [33] and with double lysine, arginine and histidine spacers, [34]. In this case, additional linear spacers were inserted into the dendrimers between all adjacent branch points, which consisted of double amino acid residues of lysine or glycine, [30] and [33], arginine, [31] and [34], and protonated histidine, [32] and [34]. The repeating units in the dendrimer were, respectively, Lys2Lys/Lys2Gly, Lys2Arg, and Lys2His.

Peptide dendrimers based on second-generation lysines with spacer inserts between their branch points have also been studied using computer modeling methods (2Lys or 2Gly, [35], 2Arg, [36], and 2His, [37]). However, there were no studies on dendrimers with spacers containing only highly hydrophobic amino acid spacers in the internal generations of the dendrimer. In this article, we close this gap. The insertion of such groups should increase the capabilities of peptide dendrimers for the transport of hydrophobic drugs.

It is well known that many dendrimers are amphiphilic. In this case, the inner region of the dendrimer is usually more hydrophobic and can be used to transport hydrophobic drug molecules. Improved solubility of the dendrimer, as well as its complex with hydrophobic drugs, is provided by hydrophilic monomers, which are located at the periphery of the dendrimer and are often positively charged. In our recent papers we also studied dendrimers with double histidine spacers, [38] and with histidine-arginine/arginine-histidine spacers, [39] which could change their hydrophobicity with pH.

2 Model and Method

In this work, we used an all-atom model of a peptide dendrimer consisting of LysArgHis (KRH) repeating element and a peptide dendrimer consisting of LysHisArg (KHR) repeating elements. One KHR/KRH dendrimer with neutral histidines (H) in each spacer or one KHpR/KRHp dendrimer with protonated histidine (Hp) in each spacer and 16 molecules of the bioactive Ovagen peptide (Glu-Asp-Leu, EDL) were placed in a 9 nm periodic cubic cell filled with water and counterions. The study was carried out using a computer simulation approach [40-45]. Molecular dynamics (MD) method with the AMBER-99SB-ILDN force field and the TIP3P water model in the Gromacs package was used. Before the start of the simulation, the energy of both systems was` minimized. Further minimization of the energy of the entire system in an aqueous solution was done before MD simulation. Electrostatic interactions were calculated using the PME method. The calculation of the main MD trajectory was carried out in the NPT ensemble. The constant

temperature was ensured using a Nose-Hoover thermostat, and constant pressure was maintained using a Parrinello-Raman barostat. MD simulation was carried out for 500 ns. First 250 ns were used for equilibration of systems and study of complex formation and further 250ns for calculation of averaged values of all charactteristics in stable complex.

3 Results and Discussion

At the beginning of all calculations, the dendrimer molecule was located in the center of the cubic cell, and all 16 peptides were on its periphery in all studied systems (not shown). At the end of the simulation, for dendrimers with neutral histidines (KRH in Fig. 1a and KHR in Fig. 1c), only 14 of 16 peptide molecules were attached to the dendrimer. At the same time, for systems containing dendrimers with protonated histidines (KRHp in Fig. 1b and KHpR in Fig. 1d), all 16 of 16 peptide molecules were adsorbed on the dendrimer. (The average numbers of adsorbed peptide molecules for all systems are given in Table 1).

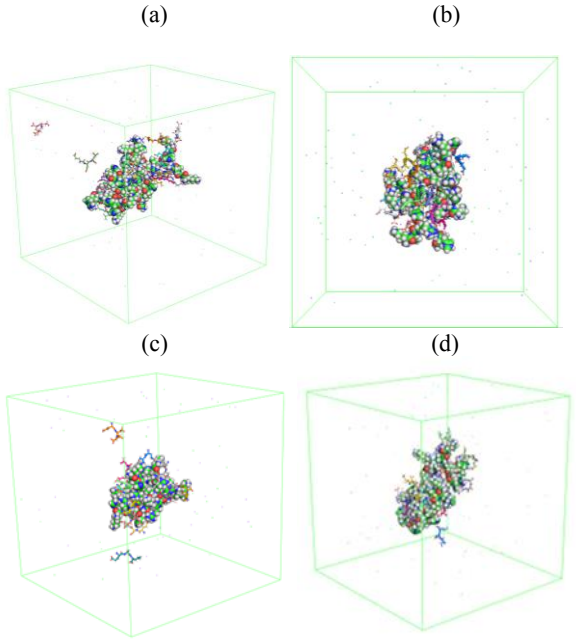

Fig.1. The snapshots of the systems: a) KRH - 16EDL, b) KRHp-16EDL, c) KHR-16EDL, d) KHpR-16EDL at the end of first half of simulation, at time t = 250 ns.

At the beginning of the simulation, the initial distance between the dendrimer and each peptide molecule is large. To quantitatively describe the process of bringing the dendrimer and peptides closer together due to electrostatic interactions, the root-mean-square distances d between the center of the mass of dendrimer and each of the 16 tetrapeptide molecules were calculated as function of time t (see Fig. 2). From this picture it is clear that initial distance is close to 4 nm in all systems. Then it constantly decreases during the first 20-50 ns of MD simulation and reaches a plateau. This behavior indicates the establishment of dynamic equilibrium in the system during first 50-100 ns. After this time the instant distance d between the dendrimer and peptide molecules fluctuates but its average value is close to 2nm and remains practically unchanged.

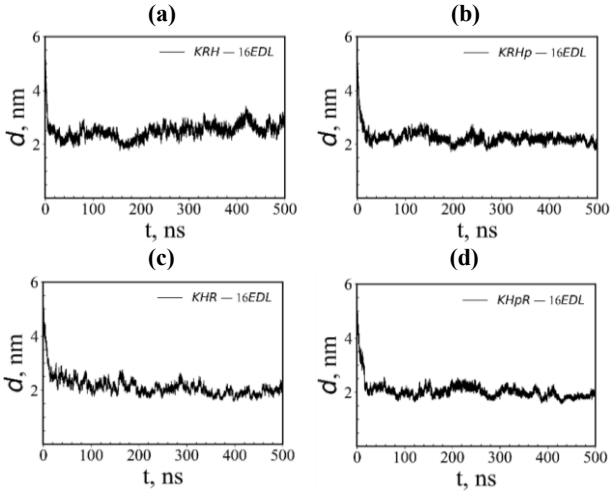

Fig. 2. Time dependences of distance $d(t)$ between the center of mass of the dendrimer and peptides in complexes of the dendrimer with neutral histidines: a) and c) and with protonated histidines: b) and d).

The instant number of hydrogen bonds between the dendrimer and the peptide molecules indicates how tightly the peptides are bound to the dendrimer. From Fig. 3, it follows that the initial number of hydrogen bonds (before the peptides approach the dendrimer) is zero, then it quickly increases within 30-50 ns after the first contact of the dendrimer with the peptide molecules.

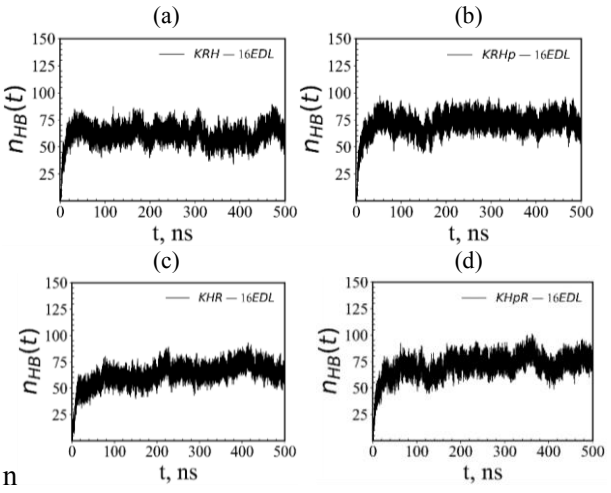

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Fig. 3. Dependences of hydrogen bond numbers n_{HB} (t) between dendrimer and tetrapeptides on time t in

complexes of the dendrimer with neutral histidines: a) and c) and with protonated histidines: b) and d).

At long times, the instant value goes to plateau while fluctuating between 50 and 70 for complexes with KRH/KHR dendrimer (Fig. 3a and Fig.3c) and between 60 and 90 for complexes with KRHp/KHpR dendrimer (Fig. 3b and 3d) but the average value for each system (see Table 1) practically does not change with time during second half of MD simulation (between 250 and 500 ns). Higher average numbers of hydrogen bonds for dendrimers with protonated histidines confirm the complexation of more peptide molecules by them.

Another characteristic demonstrating the formation of the complex of a dendrimer with peptides is its radius of gyration. The dependence of this value on time is shown in Fig. 4. It confirms that stable complex formation in all systems occurs during first 100-250 ns, as demonstrated in Fig.1, Fig.2 and Fig.3. But this value does not indicate how many peptides are in the complex. Moreover, Rg can either increase with the increase of number of peptides in the complex (if the peptides are near the surface of the dendrimer) or decrease (if the peptides penetrate deep into the dendrimer).

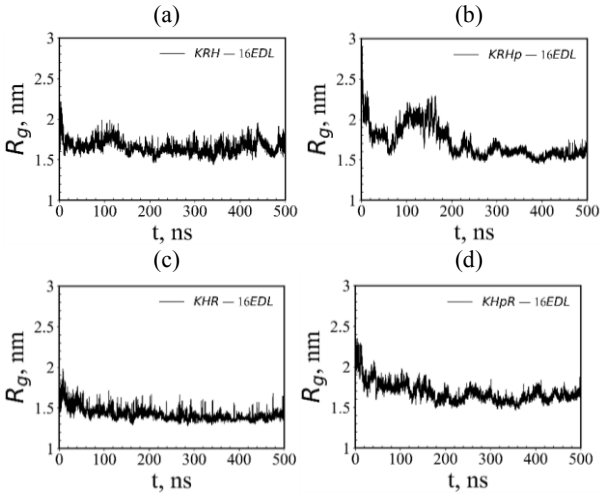

Fig.4. Time dependences of the gyration radius Rg(t) in complexes of the dendrimer with neutral histidines: a) and c) and with protonated histidines: b) and d).

We calculated also the time dependence of the instantaneous number of peptide molecules *n* in the complex on time *t* and its average value at long simulation times after reaching a plateau.. Time dependences are shown in Fig. 5 and average values in Table 1. The number of peptides in the complex is zero until the first contact of one of the peptides with the dendrimer. Then it quickly grows to a maximum value in the first 20-50 ns, and after that, it reaches a plateau and fluctuates mainly around average value of approximately *n*=14 (Fig. 5a and Fig.5c for KRH and KHR dendrimers with neutral histidines) and close to *n*=16 (Fig. 5b and Fig.5d) for KRHp and KHpR dendrimers with protonated histdines. (For average values see Table1).

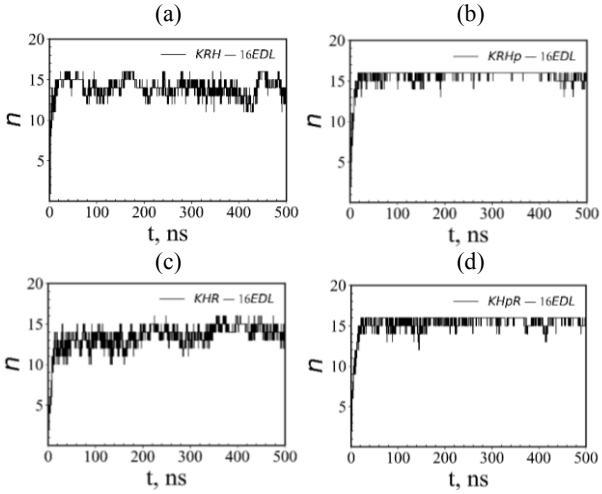

Fig.5. Time dependences of number of peptides *n(t)* in complexes of the dendrimer with neutral histidines: a) and c) and with protonated histidines: b) and d)

After all the characteristics of both complexes reach a plateau (which certainly occurs in the first 250 ns), any equilibrium characteristics can be calculated by averaging them over the second half (250 -500 ns) of the MD trajectory (see Fig.6 and Table 1).

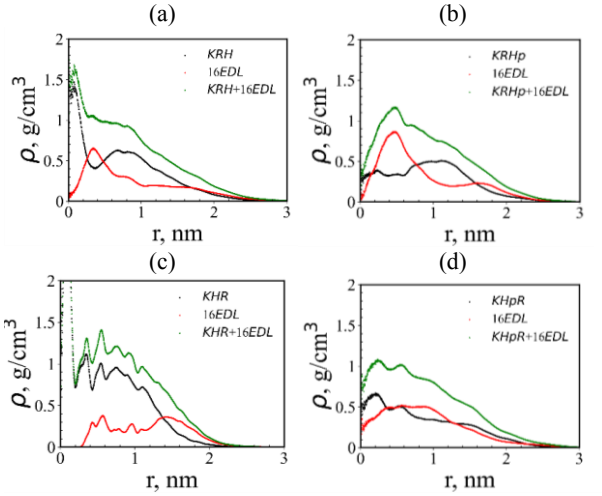

Fig. 6. Radial distribution function from the center of mass of the dendrimer (density profile) in complexes of the dendrimer with neutral histidines: a) and c) and with protonated histidines: b) and d).

The radial density of atoms of the dendrimer, peptides, and all atoms in the system relative to the center of inertia of the dendrimer provides the most complete information about the internal structure of the complex. For a dendrimer with uncharged

histidines (Fig. 6a and Fig. 6c, black curves), the dendrimer atoms make up the majority near its center of mass. It means that the dendrimer with uncharged histidines has rather dense core and the size of complex is small. Due to this reason, tripeptide atoms can not penetrate into its center (i.e., to r<0.5 nm), and the density of tripeptide atoms (red curve) has a maximum located at a distance of about 0.6 nm from the center of the dendrimer. The density of all atoms of complex (green curve) is equal to the sum of density of dendrimer and tetrapeptide atoms. Atoms of a dendrimer with protonated (Hp) histidines (Fig. 6b and Fig. 6d, black curve) distributed in a wider interval of distances from dendrimer centers (r=0) due to strong electrostatic repulsion between additionally charged histidines (Hp). In last case, the dendrimer is swollen, and tetrapeptides (red curve) could penetrate deeply into the dendrimer. The negative charges (in Glu and Asp residues of peptide molecules) could also interact with of additional positive charges of protonated histidines in dendrimer. That's why the density of peptide (red curve) has maximum close to the center of mass (near r=0.4nm) of dendrimer, while dendrimer atoms (black curves) are partly displaced from its center and have median density at a distance of about r = 1 nm from the center of inertia of the protonated dendrimer.

Table 1. Average values of distances between the centers of dendrimers and peptides $<d>$, the number of hydrogen bonds $<n_{HB}>$ between dendrimers and peptides, the size $<R_g>$ of complexes, and the number of peptides $<n>$ in complexes according to the local criterion,

System	$<d>$	$<n_{Hb}>$	$<n>$	$<R_g>$
KRH+16EDL	2.62	61	14	1.64
KRHp+16EDL	2.17	74	16	1.58
KHR+16ED	1.98	68	14	1.38
KHpR+16ED	1.93	75	16	1.63

These average values are in good agreement with results of calculation of corresponding instant values, obtained in part 3 of the paper.

4 Conclusion

In the present paper, molecular dynamics simulation was used to study interactions of molecules of OVAGEN peptide with lysine dendrimer of second generations containing stimuli-responsive arginine-histidine (RH) and histidine-arginine (HR) linear spacers between all neighboring lysine branch points. We demonstrated the complexation of OVAGEN molecules with a dendrimer at two different pHs: a) pH>7 with fully uncharged histidines (H) and b) pH<5 with fully protonated (Hp) histidines in aqueous solution. It was found that the dendrimer with protonated histidines forms a complex containing a larger number of Ovagen tripeptide molecules. Dendrimers with protonated histidines form more stable complexes with Ovagen peptides compared to neutral analogs, demonstrating increased binding capacity. Protonation also leads to structural changes in dendrimers, promoting deeper penetration of tripeptides into center of dendrimer.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

The authors equally contributed in the present research, at all stages from the formulation of the problem to the final findings and solution.

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Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.