Front. Chem. China 2007, 2(4): 378–382
DOI 10.1007/s11458-007-0071-z

RESEARCH ARTICLE

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Studies on synthesis and molecular dynamics simulation of dendrimers containing amino acids and peptides

Abstract A series of poly(ether-amide) dendrimers with amino acids and peptides as the peripheral functional groups was synthesized, and their structures were confirmed by nuclear magnetic resonance (NMR) and electrospray ionization-mass spectrometry (ESI-MS) spectrometry. Molecular dynamics simulation of the peptide dendrimers in solution was performed, indicating that, the prior conformations of the dendrimers were atom number dependent, i.e., with the increases of the atom number, the prior conformations were more spherical. Also, the amino acid α-C atom radial distribution indicated that, with larger peripheral groups, more back-folding of the dendrimers occurred.

Keywords peptide dendrimer, synthesis, conformation, molecular dynamics

1 Introduction

Since Vögtle et al. reported the first synthesis of dendrimers [1], there have been many studies regarding the dendrimer applications in preparation of novel materials catalysts and drug delivery systems and in gene therapy area, etc. [2–4]. Nowadays, the dendriemer research has been no longer focused on the synthesis of molecules with large algebra generation and molecular weight, but in dendritic linkage of different functional groups to prepare new dendrimers with diverse natures. Tam described the poly-lysine type dendrimers as the first peptide dendrimers in 1988 [5]. The peptide dendrimers can be classified into two types [6], for one the peptide fragments are linked to a non-peptide dendritic vector, and for the other a peptide is directly utilized as the dendritic scaffold. Recently, there have been many researches on the application of peptide dendrimers in vaccines, diagnostic reagents, immuno suppressants, antibiotics and drug carriers [7,8].

Gennes et al. [9,10] reported the molecular modeling of dendrimers in 1983 and 1996, respectively. Murat and Grest [10] first used molecular dynamics method to inspect the structures of dendritic molecules in detail. Further study showed that the use of molecular dynamics simulation of the compounds were consistent with the experimental results (such as molecular size and hydrogen bond, etc.), proving the reliability of this method [11]. Now the molecular modeling of dendrimers has attracted wide attention because of the improvement of calculation methods and conditions.

We report herewith the synthesis and molecular dynamics simulations of seven peptide dendrimers with a polyether-amide type dendrimers as the core, as well as with Arg-Gly-Asp (RGD) tripeptide, Gly-Asp (GD) dipeptide and four kinds of amino acid as the functional groups, respectively. Additionally, we will explore the influence of size and type of different functional groups on the structures of peptide dendrimers. The RGD is the receptor binding sites of human fibronectin. The RGD containing peptides can regulate many physiological processes, such as: cell adhesion, signal transduction and identification, platelet aggregation and the transportation of tumor cells, etc. [12].

2 Experimental

2.1 Instruments and reagents

All the reagents and solvents were analytically pure and commercially available. Gel silica used for column chromatography and thin-layer chromatography was bought from Qingdao Ocean Chemical Plant (chemical pure). Compound **1** was prepared according to a documental method [13], nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ on a Unity INVOA 400 spectrometer with tetramethylsilane (TMS) as the internal standard. Mass spectrometry (MS) spectra were performed on an Agillent 1964B spectrometer.

Translated from *Acta Chimica Sinica*, 2007, 65(1): 21–26 [译自: 化学通报]

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2.2 Synthesis and exosyndrome of dendrtic polypeptidic compounds

He et al. [14] previously synthesized compounds **2a**, **2e** and **2f** using DCC/HoBt. However, this method has some defects, such as the reaction time is long and DCU is difficult to eliminate completely. In the present research, thionyl chloride was used to synthesize **2a**−**2f**. For instance, in the synthesis of **2a**, the reaction time was shortened to about 2 h, and the yield rosed from 60% to 80%, and the work-up procedure was simple. The general procedure for the syntheses (with the synthesis of compound **2a** as an example): compound **1a** (424 mg, 1 mmol) and $SOCl_2$ (2 mL) reacted for 0.5 h at 50°C under nitrogen atmosphere, then $SOCl_2$ was removed under diminished pressure. A solution of glycine benzyl ester tosylate (1.69 g, 4 mmol) in dichloromethane and $N(Et)_3$ (10 mL) were added. The mixture was stirred for 1 h at rt, then concentrated under diminished pressure. Purification of the residue by flash chromatograpy with dichloromethane-MeOH ($V : V = 80 : 1 - 10 : 1$) as the eluent afforded **2a**.

2.2.1 The synthesis of compound **2a**

Compound **2a** was prepared with the general procedure as described above and obtained as colorless oil (812 mg, 81%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.43−2.51 (m, 8 H), 3.35−3.42 (s, 8 H), 3.63−3.67 (m, 8 H), 4.06−4.08 (m, 8 H), 5.15 (s, 8 H), 7.33−7.37 (m, 20 H); MS found *m/z* 1,013.3, calcd. for $C_{53}H_{64}N_4O_{16}$ 1,012.4.

2.2.2 The synthesis of compound **2b**

Compound **2b** was prepared with the general procedure as described above and obtained as colorless oil (920 mg, 86%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.52−1.65 (m, 12 H), 2.50−2.56 (m, 8 H), 3.44−3.51 (s, 8 H), 3.69−3.75 (m, 8 H), 4.11−4.14 (m, 4 H), 5.10 (s, 8 H), 7.28−7.32 (m, 20 H); MS found *m/z* 1,069.8, calcd. for $C_{57}H_{72}N_4O_{16}$ 1,068.5.

2.2.3 The synthesis of compound **2c**

Compound **2c** was prepared with the general procedure as described above and obtained as colorless oil (1,204 mg, 75%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.48−2.57 (m, 8 H), 2.61−2.76 (m, 8 H), 2.80−3.01 (m, 8 H), 3.40 (s, 8 H), 3.62−3.67 (m, 8 H), 4.50−4.56 (m, 4 H), 5.13 (s, 8 H), 5.17 (s, 8 H), 7.26−7.29 (m, 40 H); MS found *m/z* 1,605.8, calcd. for $C_{89}H_{96}N_4O_{24}$ 1,604.6.

2.2.4 The synthesis of compound **2d**

Compound **2d** was prepared with the general procedure as described above and obtained as white wax (968 mg, 79%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.44−2.47 (m, 8 H), 2.81−2.86 (m, 8 H), 3.10−3.15 (m, 8 H), 3.42(s, 8 H), 3.65−3.68 (m, 8 H), 3.70−3.78 (m, 12 H), 4.80−4.86 (m, 4 H), 6.84−6.99 (m, 4 H), 7.16−7.54 (m, 16 H); MS found *m/z* 1,225.3, calcd. for $C_{65}H_{76}N_8O_{16}$ 1,224.5.

2.2.5 The synthesis of compound **2e**

Compound **2e** was prepared with the general procedure as described above and obtained as nankin wax (1,613 mg, 88%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.66−2.69 (m, 8 H), 2.86−2.94 (m, 4 H), 3.00−3.08 (m, 4 H), 3.37 (s, 8 H), 3.72−3.79 (m, 8 H), 4.02−4.03 (m, 8 H), 4.55−4.57 (m, 4 H), 5.11−5.18 (m, 16 H), 7.25−7.38 (m, 40 H); MS found *m/z* 1,833.7, calcd. for $C_{97}H_{108}N_8O_{28}$ 1,832.7.

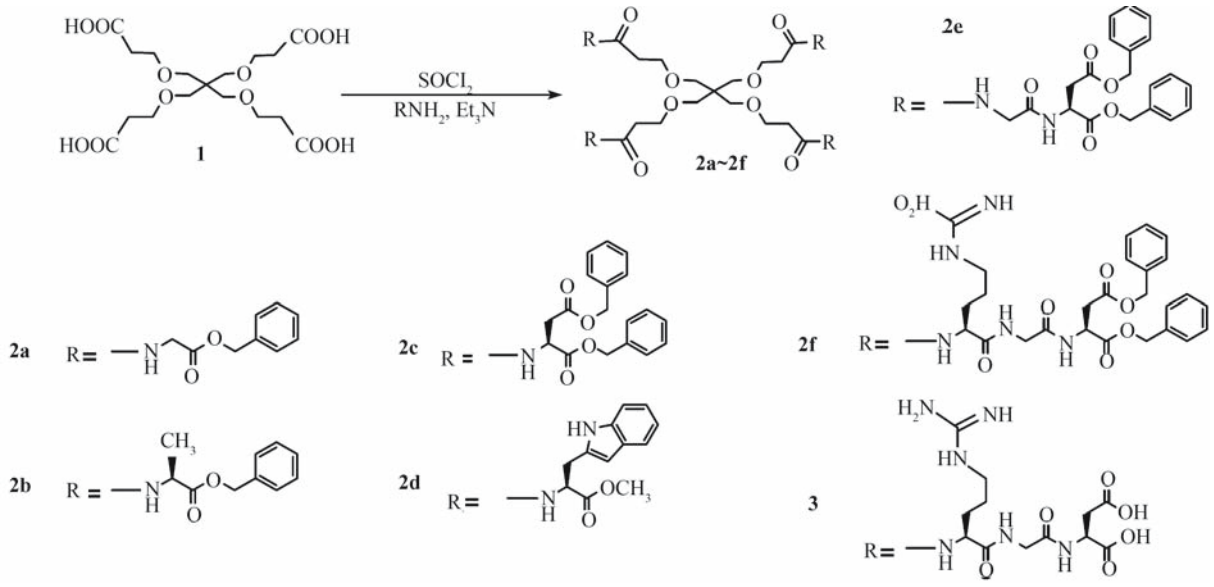

Fig. 1 Synthetic routs of **2a**−**2f** and **3**

2.2.6 The synthesis of compound **2f**

Compound **2f** was prepared with the general procedure as described above and obtained as yellow wax (1,801 mg, 70%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.55−1.68 (m, 16 H), 2.60−2.63 (m, 8 H), 2.87−2.93 (m, 4 H), 2.96−3.02 (m, 4 H), 3.26 (s, 8 H), 3.52−3.59 (m, 8 H), 3.68−3.73 (m, 16 H), 4.42−4.50 (m, 4 H), 4.78−4.85 (m, 4 H), 5.11–5.16 (m, 16 H), 7.24−7.42 (m, 40 H); MS found *m/z* 2,577.9, calcd. for $C_{121}H_{148}N_{24}O_{40}$ 2,577.0.

2.2.7 The synthesis of compound **3**

To a solution of **2f** (258 mg, 0.1 mmol) in MeOH (5 mL) was added 1% Pd/C (10 mg), and the catalytic hydrogenation was carried out for 24 h at rt, then the reaction mixture was filtered, and the filtrate was concentrated under diminished pressure, giving **3** as yellow wax (105 mg, 60%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.55−1.68 (m, 16 H), 2.60−2.63 (m, 8 H), 2.87−2.93 (m, 4 H), 2.96−3.02 (m, 4 H), 3.26 (s, 8 H), 3.52~3.59 (m, 8 H), 3.68−3.73 (m, 16 H), 4.42−4.50 (m, 4 H), 4.78−4.85 (m, 4 H), 5.11−5.16 (m, 16 H), 7.24−7.42 (m, 40 H); MS found *m/z* 2,577.9, calcd. for $C_{121}H_{148}N_{24}O_{40}$ 2,577.0.

3 Molecular dynamics simulation

3.1 Simulation method

The starting structures for the simulations were built up by use of Hyperchem program [15]. The properties and simulation parameters of the peptide dendrimers, such as molecular weight, atom numbers and periodic boundary conditions, were listed in Table 1. The following growing steps were considered to build the original conformation of the dendrimers: (1) building of the dendritic core; (2) building of the first glysine unit at each branch; (3) conversion of the glysine residues into the correspondent amino acid residues; (4) repeating the (2) and (3) steps until all the amino acid residues were attached; (5) conversion of all the terminal COOH groups into the corresponding esters and protection of the side chain functional groups. For each of these growing steps a long-time molecular dynamic (MD) simulation was performed. An analogous procedure was used by Murat and Grest [10].

Table 1 Properties and simulation parameters of peptide dendrimers

Compound	M_w	Atom number	Box vector /nm	Water number	Length of simulation /ps
2a	1,012.4	137	25	468	200
2b	1,068.5	149	28	674	200
2c	1,604.6	213	30	827	200
2d	1,224.5	165	25	466	200
2e	1,832.7	241	28	617	200
2f	2,577.0	333	32	951	200
3	1,737.8	229	30	814	200

The MD simulations were performed with the GROMACS 3.2.1 program [17]. The authors chose to use the OPLS all-atoms force field [16], bonds to hydrogen atoms were kept at fixed lengths by the LINCS and RATTLE algorithm, and the BERENDSEN algorithm was utilized at a fixed temperature (298.15 K) and pressure (101,325 Pa). Data were collected every 0.1 ps, and the analysis was performed only on the last 100 ps of the simulation. Solvent-accessible surface areas and excluded volumes were computed by the use of the Connolly algorithms as implemented in the TINKER package. In these calculations hydrogen atoms were not considered. The probe radius was set to 0.14 nm. All other analyses were performed by programs developed by the authors.

3.2 Result and discussion

Solvent-accessible surface areas and excluded volumes of compounds **2f** and **3** were shown in Fig. 2, and the total potential energy of all the compounds were shown in Fig. 3. The data collection and analysis were performed only on the last 100 ps of the simulation, and the results were listed in Table 2. The radial distribution of the α-C atoms of amino acids were shown in Fig. 4 (for compounds **2e**, **2f** and **3**, the calculation was performed with the α-C atoms of the N-terminal amino acid). Compounds **2a**−**2d** and **3** had relatively narrow distribution of the α-C atoms and symmetry structures, suggesting that the functional groups may locate at the surface of the molecules. However, compound **2e** and **2f** had a wider distribution of the α-C atoms and asymmetry structures, indicating that their conformations were relatively complicated and partly coil-like structure of these molecules maybe exist.

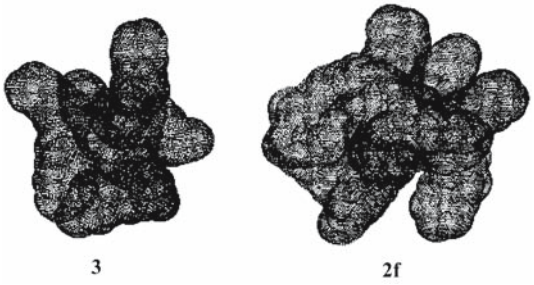

Fig. 2 Solvent accessible surface area of **3** and **2f**

As shown in Fig. 6, the average radius of gyration (R_G) and the average distance of the α-C atom of the amino acid residues from the mass centre of the dendrimer ($R_C{}^{\alpha}$) were closely related to the atom numbers. An almost linear relationship was present for both the R_G and $R_C{}^{\alpha}$. Our result was basically in agreement with the studies of Gennes and Hervet [9]. However, the R_G and $R_C{}^{\alpha}$ of compound **3** were different from those of the other molecules, because there were many peripheral polar groups located at the surface of molecules, which could make a strong interaction with the solvent molecules. The ratios of the average moments of inertia along the principal axes *x*, *y* and *z* as a function of the atom numbers

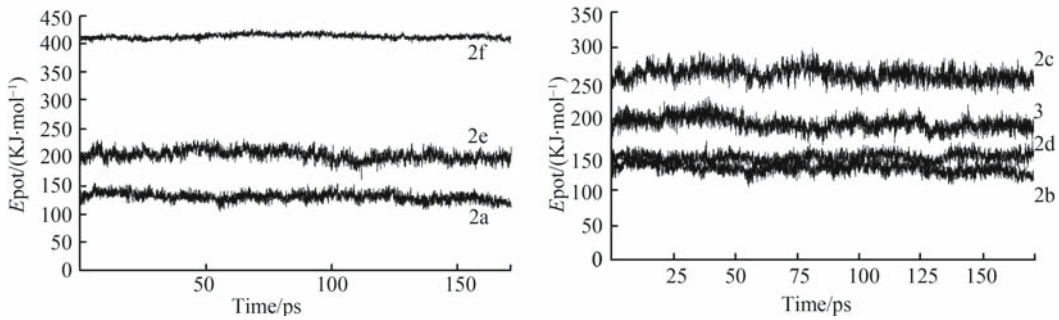

Fig. 3 Time evolution of the total potential energy for the peptide dendrimers

Table 2 Calculated average properties from the MD simulations for the peptide dendrimers

	2a	2b	2c	2d	2e	2f	3
R_G [nm][a]	0.736±0.04	0.776±0.04	0.917±0.02	0.772±0.04	1.009±0.03	1.233±0.01	1.091±0.01
R_C^{α} dist. [nm][b]	0.704±0.16	0.738±0.15	0.858±0.19	0.739±0.15	0.951±0.21	1.158±0.27	1.064±0.11
I_x [dalton × 10^{-6} nm][c]	45.2±8.6	62.5±10.6	107.6±16.2	69.7±11.6	151.8±20.1	463.2±33.6	596.5±24.6
I_y [dalton × 10^{-6} nm][c]	58.2±10.1	85.3±15.1	147.6±18.8	99.6±17.0	199.1±28.5	624.3±36.1	661.9±36.7
I_z [dalton × 10^{-6} nm][c]	79.5±14.2	111.8±16.8	180.1±22.1	123.5±19.6	229.8±26.1	659.0±38.7	720.9±33.5
I_z/I_x[d]	1.76±0.3	1.78±0.3	1.67±0.2	1.77±0.3	1.51±0.2	1.42±0.1	1.21±0.1
I_z/I_y[d]	1.37±0.2	1.31±0.2	1.22±0.2	1.24±0.2	1.15±0.2	1.06±0.1	1.09±0.1
E_{pot} [kJ mol^{-1}][e]	130.8±9.2	145.4±9.8	268.8±10.5	151.5±8.4	219.5±12.5	412.3±16.2	204.2±7.3

Note: [a] R_G is the radial distribution of the α-C atom; [b] R_C^{α} is the distance of the α-C atom from the mass centre of the dendrimer; [c] I_x, I_y, I_z are the moments of inertia along the principal axes sorted in ascending order; [d] I_z/I_x, I_z/I_y are the ratio of the moments of inertia *Iz* and *Ix*, and *Iz* and *Iy*; [e] E_{pot} is the total potential energy.

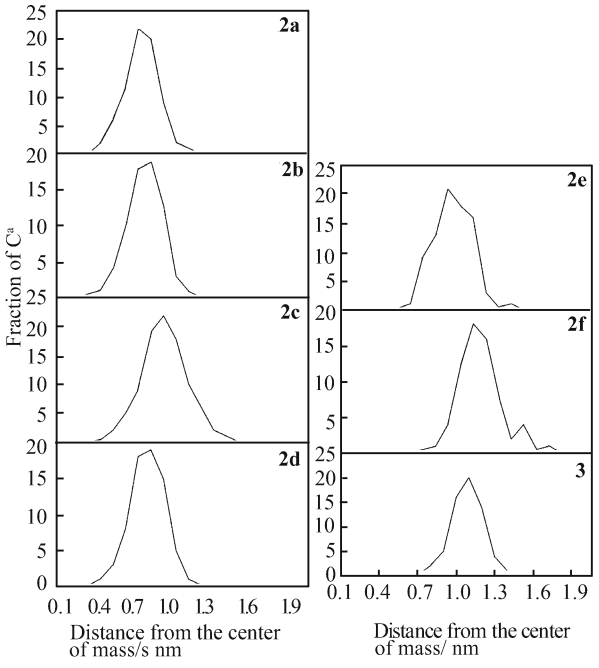

Fig. 4 Radial distribution of the α-C atom

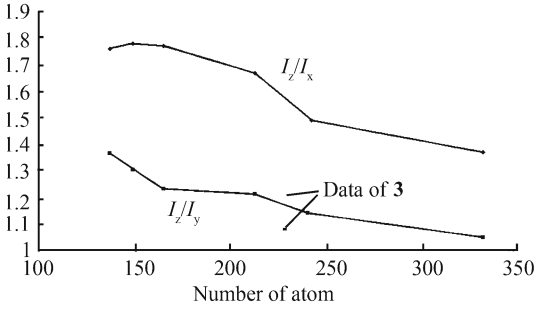

Fig. 5 Relative values I_z/I_x, I_z/I_y of the three principal moments of inertia as a function of the number of atoms

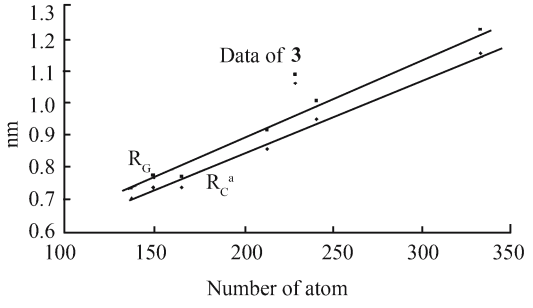

Fig. 6 Average radius of gyration and average distance of the α-C atom as a function of the number of atoms

were shown in Fig. 5. It was found that these ratios were close to one for large molecules, indicating that the dendrimers were close to spheres.

The average solvent-accessible surface area and excluded volume observed in the performed simulations were shown in Table 3, the per-atom contributions were also reported. With the increase of the atom number, an almost linear decrease of the solvent accessible surface area per atom was

Table 3 Calculated average solvent accessible areas (SASA) and excluded volumes (EV) from the MD simulations for the peptide dendrimers

	2a	2b	2c	2d	2e	2f	3
SASA (nm^2)	5.433 0	5.855 0	8.272 0	6.451 0	9.038 0	1.148 9	1.050 9
EV (nm^3)	0.651 3	0.674 1	0.958 4	0.744 1	1.070 1	1.465 2	1.287 9
				Value per atom			
SASA (nm^2)	3.97×10^{-2}	3.93×10^{-2}	3.88×10^{-2}	3.91×10^{-2}	3.75×10^{-2}	3.45×10^{-2}	4.59×10^{-2}
EV (nm^3)	4.75×10^{-3}	4.52×10^{-3}	4.50×10^{-3}	4.51×10^{-3}	4.44×10^{-3}	4.40×10^{-3}	5.62×10^{-3}

observed. However, the excluded volume per atom was almost unchanged for the different dendrimers. This analysis was consistent with a more dense packing of the residues for higher molecules.

These molecular dynamics simulation results revealed that the size and nature of the peripheral functional groups played an important role in the conformations of dendrimers in solution. Because of the conformation flexibility of the peptide dendrimers in solution, different types and sizes of peripheral groups had obvious effects on the dendrimer conformations. Further studies on the effect of different peripheral groups, solvent and generations to the conformations of the dendrimers were ongoing.

Acknowledgements This work was supported by the National Natural Science Foundation of China. (Grant No. 20472055).

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