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Neoglycolipids Micelle-like Structures as a Basis for Drug Delivery Systems

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Abstract

Targeted drug delivery is one of the most promising tasks of nanomedicine, as this is a real way to increase the effectiveness of therapeutic effects against many diseases. In this regard, the development of new inexpensive highly effective stimulating and non-immunogenic drug delivery systems (DDS) is of great importance. In this work new molecular candidates were proposed and studied for the creation of such systems based on the use of new compounds, neoglycolipids. It is shown that these compounds are capable of self-association in aqueous solutions and can serve as potential carriers of drug compounds with targeted delivery determined by their terminal groups (in particular, glycans). The processes of their associates formation and features of their structure are investigated. The results show that these self-organizing nanoscale systems can be used as a basis for developing new drug delivery systems.

Keywords: neoglycolipids, micelle-like structures, small-angle X-ray scattering, molecular dynamics simulation

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1. Introduction

For the last decades research in the area of targeted drug delivery has increased significantly[1-5]. However, the available systems that are currently on the market - liposomes and micelles (basic and PEG surface-modified) - have several drawbacks, including low (or extensively high if PEG-coated) stability[6], opsonization[7], poor cellular internalization of drugs[8]. Furthermore, those systems act using the passive

mechanism (i.e. rely on the enhanced permeability and retention effect), which is low-effective and has toxicity as a side effect[9]. Thus, further researchaimed at development of new molecular tools for creating high-effective drug delivery systems (DDS) is required.

Thestudied neoglycolipids(known also as Function-Spacer-Lipid, FSLs [15]) are synthetic analogs of natural glycolipids, consisting of a hydrophobic DE lipid residue, a spacer (CMG2 or Ad), and a carbohydrate (tri- or tetrasaccharide). The structures of neoglycolipids are presented in Fig.1. Being amphiphilic molecules, neoglycolipids are prone to spontaneous self-assembly in liquid media, forming structures that can potentially be used as carriers of drugs. The goal of this work is to study the structure and properties of neoglycolipid self-assembling micelle-like structures in terms of their possible use as new drug delivery systems.

2. Materials and methods

2.1. Materials

Neoglycolipid solutions in PBS(ph 7.4) buffer.

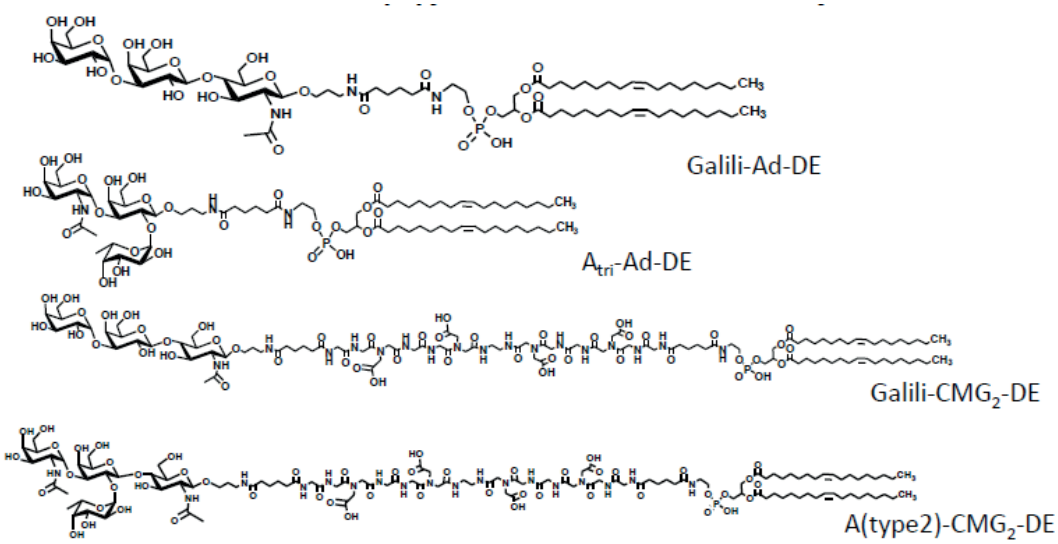

Figure 1: Chemical structures of the studied neoglycolipids.

2.2. Methods

2.2.1. Atomic force microscopy

For AFM imaging, the sample (100 μM, 10 μl) was deposited onto a freshly cleaved mica surface. After 15 min incubation at room temperature, the mica was rinsed three times with 100 μlMilli-Q water in order to remove salts and unbound sample molecules, followed by drying in desiccator for 24 hours. After that, the sample was put in the AFM for imaging.

An atomic force microscope (Smena,NT-MDT, Russia) with Ethalon silicon probes (NT-MDT, Russia) with resonance frequency of 240 kHz were used for AFM imaging. For data processing and presentation the unique scientific setup "System for probe-optical 3D correlative microscopy" IBCh RAS (http://ckp-rf.ru/usu/486825/) wasused. Equipment is provided by the IBCH core facility (CKP IBCH, supported by Russian Ministry of Education and Science, grant RFMEFI62117X0018).

2.2.2. Dynamic light scattering

Data on the size and size distribution of sample self-assembled structures in liquid were obtained by dynamic light scattering. All the measurements were performed on a Brookhaven Instruments 90Plus particle size analyzer (Brookhaven Instruments Corporation, USA). Sample solution (2 ml, 100 μM) was sonicated for 15 s, and in 10 minutes, measurements were taken.

2.2.3. Small-angle X-ray scattering

Sample solution (50μl, 3 mg/ml) was sonicated for 15 s, and in 10 minutes, measurements were taken. Each sample was measured 20 times. All measurements were performed on a synchrotron beamline P12 (EMBL, Hamburg). Spectra were processed using ATSAS software package.

2.2.4. Molecular dynamics simulations

All-atom structure of single neoglycolipid molecule was built using Avogadro software [10]. Coarse-grained (CG) model and topology were obtained from all-atom structure using PyCGTOOLsoftware[11]. Mapping of atoms onto CG beads was made for MARTINI force field, following recommendations from original MARTINI paper[12] i.e. on average four heavy atoms are represented by a single CG bead. In order to preserve the

geometry of small ring compounds, a two-(or three-) to-one mapping was applied for ring strucrures.

The next step was preparation of the starting structure for simulation. It was done as follows: 600 neoglycolipid molecules were randomly placed into cubic simulation box (20 nm side) with subsequent solvatation with CG water molecules and addition of Na^+ and Cl^- ions to achieve ionic strength of 0.15 M.All simulations and subsequent analyses were carried out using Gromacs 5.1.4 molecular dynamics package[13]. Simulation results were visualized using VMD software[14].

3. Results and discussion

3.1. Atomic force microscopy

In order to obtain data on the shape and size of self-assembled structures, atomic force microscopy was used. Results are shown in Fig. 2.

These results show evidence that all neoglycolipids self-assemble into ellipsoidal shape structures with height size in range of 6-10 nm and lateral size in range of 40-60 nm. However, lateral sizes broadening is one of the most common AFM artifact arising from difference in sample size and radius of curvature of the AFM tip, which makes lateral sizes unreliable. Because of that, lateral sizes were not taken into account and particle sizes were determined from height values.

3.2. Dynamic light scattering

In order to obtain information on particle size/size distribution under physiological conditions, dynamic light scattering method was used. Results are shown in Fig. 3.

DLS data shows that under physiological conditions particles with sizes in range 8-13 nm, and these results are in good agreement with AFM data, considering that DLS measures hydrodynamic size.

3.3. Small-angle X-ray scattering

Data on structure of self-assembled particles was obtained from Small-angle X-ray scattering method. After spectra processing electron density mapping was obtained which revealed that particles have micelle-like structure (Fig.4).

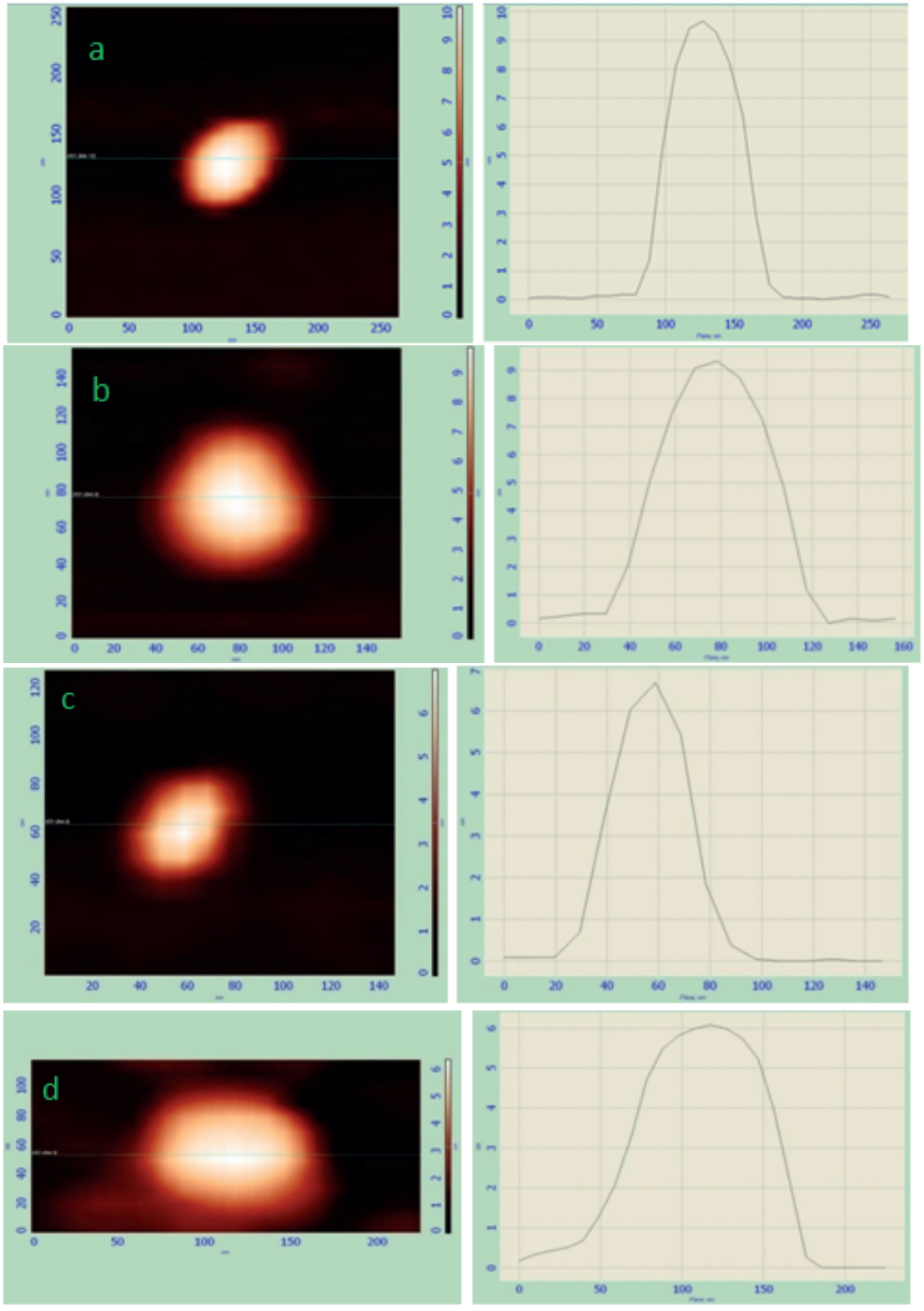

Figure 2: AFM images (left) and corresponding profiles (right) of neoglycolipids self-assembled structures. a, Galili-Ad-DE; b, Atri-Ad-DE; c, A(type2)-CMG_2-DE; d, Galili-CMG_2-DE; see Fig.1 for the structures.

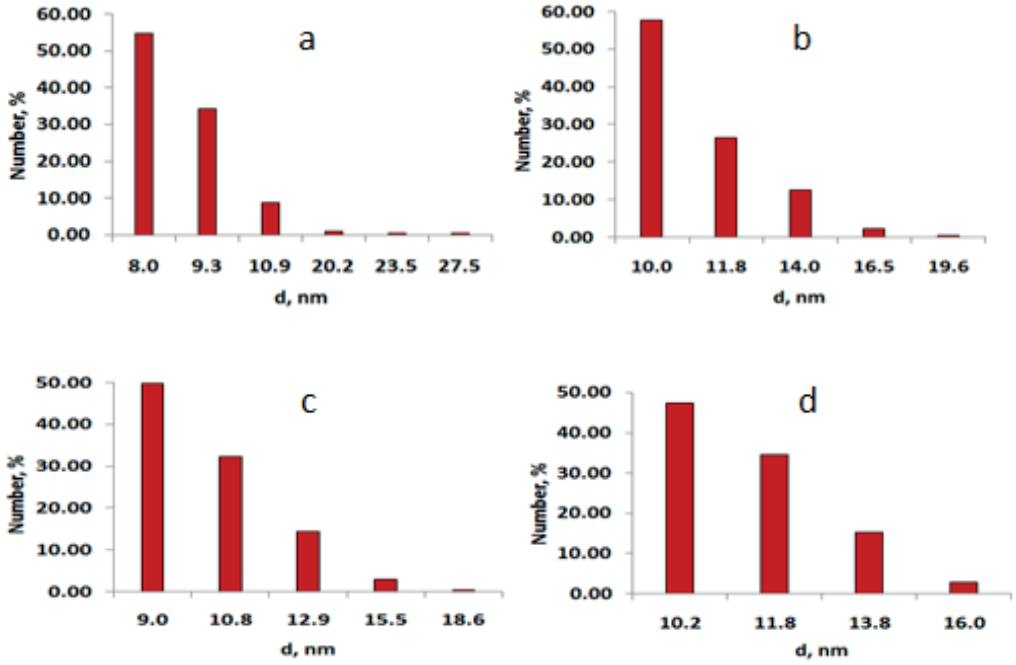

Figure 3: DLS particles size distributions. a, Galili-Ad-DE; b, Atri-Ad-DE; c, A(type2)-CMG_2-DE; d, Galili-CMG_2-DE.

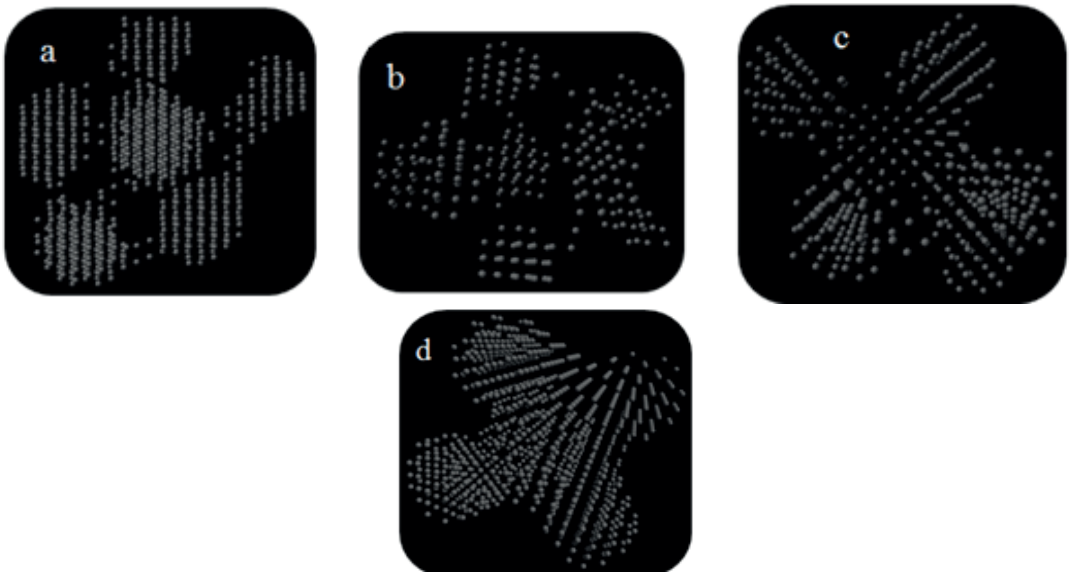

Figure 4: Electron density, obtained after processing SAXS spectra. a, Galili-Ad-DE; b, Atri-Ad-DE; c, A(type2)-CMG_2-DE; d, Galili-CMG_2-DE.

It should be noted that the structure reconstruction is carried out on the base of electron density distribution, that is, the areas that look like voids are in reality only areas with a minimum electron density, in our case - hydrophobic regions of molecules.

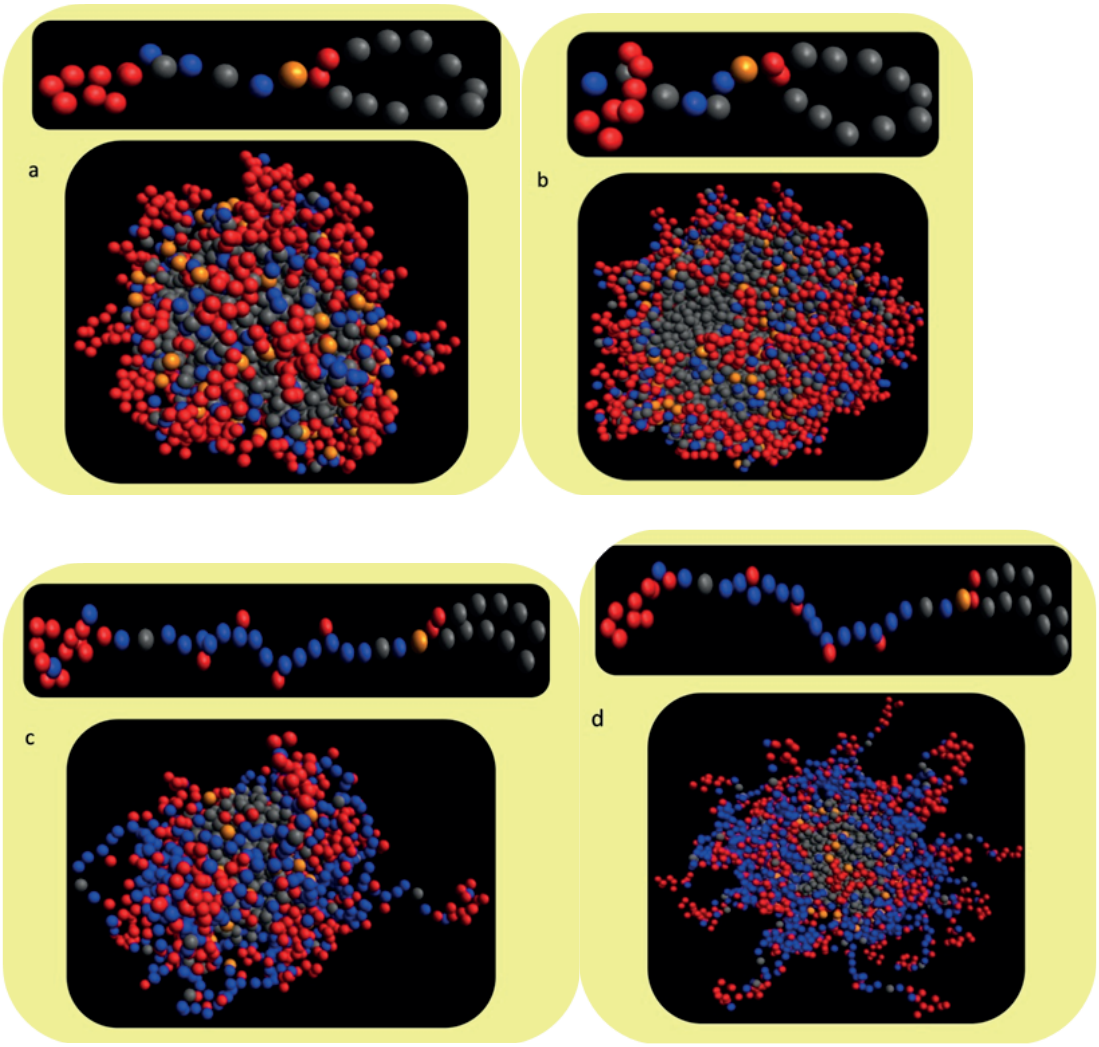

Figure 5: Coarse-grained representation of a single neoglycolipid molecule (top) and self-assembly result (bottom). a, Galili-Ad-DE; b, Atri-Ad-DE; c, A(type2)-CMG_2-DE; d, Galili-CMG_2-DE.

3.4. Molecular dynamics simulations

Since surface properties are crucial for developing high-effective drug delivery system, more detailed structural data including localization of functional group and spacer is required. This data was obtained from molecular dynamics simulations of self-assembly process. Results are shown in Fig. 5.

It was revealed that particles consist of a hydrophobic core, and a hydrophilic spacer fulfills a shielding role. It is particularly important to note that the functional parts of the molecules (saccharides) are on the surface and are available for binding to the receptor. Moreover, molecular dynamics provided information on aggregation number of each micelle-like particle: 77 molecules for Galili-Ad-DE, 156 molecules for Atri-Ad-DE, 25 molecules for A(type2)-CMG_2-DE, 59 molecules for Galili-CMG_2-DE.

4. Conclusion

Information on the shape and size of particles formed by each type of molecule was obtained, and their structure was determined. It was revealed that all particles have shape of an ellipsoid and size in range of 7-13 nm. The structure of the associates was determined and it was shown that they are all micelles-like particles, whose hydrophobic core is formed by the lipid tails of molecules, while the functional part (glycan) is locked on the surface. A computer simulation confirmed particles' micelle-like structure, and the detailed arrangement of the spacer and the functional part. Thehydrophobic core suggests a possibility of inclusion of small drug (prodrug) inside, whereas glycan moiety – as a vector for delivery of the vehicle to organs/cells due to lectin specific glycan recognition [16].

Acknowledgements

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