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A mechanistic view of lipid membrane disrupting effect of PAMAM dendrimers

Szilvia Berényi[a,*,1], Judith Mihály[a,2], András Wacha[a,2], Orsolya Tőke[b], Attila Bóta[a,2]

[a] Dept. of Biological Nanochemistry, Institute of Molecular Pharmacology, Research Centre of Natural Sciences, Hungarian Academy of Sciences, Magyar Tudósok körútja 2. 1117 Budapest, Hungary
[b] Dept. of Structural Chemistry, Institute of Organic Chemistry, Research Centre of Natural Sciences, Hungarian Academy of Sciences, Magyar Tudósok körútja 2. 1117 Budapest, Hungary

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ABSTRACT

The effect of 5th generation polyamidoamine (PAMAM G5) dendrimers on multilamellar dipalmitoylphosphocholine (DPPC) vesicles was investigated. PAMAM was added in two different concentrations to the lipids (10^{-3} and 10^{-2} dendrimer/lipid molar ratios). The thermal behavior of the evolved systems was characterized by DSC; while the structure and the morphology were investigated with small- and wide-angel X-ray scattering (SWAXS), freeze-fracture electron microscopy (FFTEM) and phosphorus-31 nuclear magnetic resonance (^{31}P NMR) spectroscopy, respectively. IR spectroscopy was used to study the molecular interactions between PAMAM and DPPC. The obtained results show that the dendrimers added in 10^{-3} molar ratio to the lipids generate minor perturbations in the multilamellar structure and thermal character of liposomes, while added in 10^{-2} molar ratio dendrimers cause major disturbance in the vesicular system. The terminal amino groups of the dendrimers are in strong interaction with the phosphate headgroups and through this binding dendrimers disrupt the regular multilamellar structure of DPPC. Besides highly swollen, fragmented bilayers, small vesicles are formed.

1. Introduction

Dendrimers are highly branched, globular macromolecules with numerous terminal groups and internal cavities. The branches are repeated in a radial concentric way and each concentric layer is called a generation (G). The higher the number of generations is the more terminal groups and bigger size the dendrimer has. The pharmacological and biomedical applicability of dendrimers has been extensively studied and reviewed lately, yet it is still a progressive field of research [1–7]. Dendrimers are ideal nanocarriers for drugs, because not only the drug itself, but also targeting and imaging molecules can be attached to the same macromolecule. Toxicity, however can limit their use, especially in the case of higher generation, positively charged dendrimers [8–11].

The interaction between cell membranes and nanocarriers, such as dendrimers, is very important, because in the most cases carriers have to get across the lipid bilayer without disrupting it. Previous studies based on atomic force microscopy (AFM) [12–17], isothermal titration calorimetry (ITC), fluorescence correlated spectroscopy (FCS) [17,18], in vitro experiments [13,19,20] and molecular dynamics simulations (MD) [14,17,21–24] showed that cationic dendrimers disrupt lipid bilayers by forming holes on the bilayer surface and may remove lipids from it. The degree of the disruption depends on the size and charge of the dendrimer. Acetylated and small generation (G3) dendrimers attach to the lipid surface without destabilizing it. Hole formation caused by positively charged dendrimers up to G5 has been found to be reversible. This is an important question regarding drug delivery and cytotoxicity, because dendrimers should penetrate into cells without causing cell injury. Most of the above mentioned studies were based on supported lipid bilayers.

Liposomes are widely used as model systems of biological membranes, since both contain lipid bilayers as their basic structural unit [12]. This way, investigating the effect of foreign molecules on the structure and thermal behavior of liposomes, we can obtain basic information on the physicochemical level of toxicity. Karoonuthaisiri et al. investigated the effect of different generation

* Corresponding author. Tel.: +36 14631598.
E-mail address: berenyi.szilvia@mail.bme.hu (S. Berényi).
[1] MTA-BME "Lendület" Chemical Nanosensors Research Group, Department of Inorganic and Analytical Chemistry, Budapest University of Technology and Economics, Szent Gellért tér 4, 111 Budapest, Hungary.
[2] Dept. of Biological Nanochemistry, Institute of Materials and Environmental Chemistry, Research Centre of Natural Sciences, Hungarian Academy of Sciences, Magyar Tudósok körútja 2. 1117 Budapest, Hungary.

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PAMAM dendrimers on the permeability of unilamellar liposomes composed of different lipids [25]. The results of their experiments indicate that PAMAM dendrimers effectively disrupt the lipid bilayer, when membranes contain also non-bilayer forming lipids (e.g. dioleylphosphoethanolamine/DOPE), while they found minor effect on membranes composed only from phosphocholine lipids. Åkesson and coworkers also used unilamellar liposomes as model system and investigated the effect of G6 PAMAM dendrimers ($d \approx 6.7$ nm) with dynamic light scattering, cryo-TEM and small-angle X-ray scattering (SAXS) [26]. They showed that dendrimers attach to the surface of the vesicles and can bridge neighboring vesicles until liposomes collapse in lamellar phase in which dendrimers are located between the layers. Later they investigated the effect of dendrimers on giant unilamellar vesicles (GUVs) by fluorescent microscopy and on supported lipid bilayers by quartz crystal microbalance with dissipation monitoring (QCM-D) and neutron reflectivity [27–29]. Interestingly they have found that the dendrimers enhance the permeability of the membranes for small molecules without hole formation or passive translocation across the membrane. Leaning on the results a model was interpreted in which the dendrimers are intercalated in the bilayer headgroup region and a large-scale roughness is generated as the bilayer changes curvature to follow the shape of the dendrimers.

Differential scanning calorimetry (DSC), small- and wide-angle X-ray scattering (SWAXS), infrared spectroscopy (IR) and freeze-fracture electron microscopy (FFTEM) are prevalent methods to characterize liposomes and to monitor the changes in their structure caused by other molecules [30–33]. Still there are only a few papers in which these methods are used in order to investigate the interactions between dendrimers and liposomes [34–36]. Klajnert et al. used DSC to investigate the effect of hydrophilic and hydrophobic dendrimers on liposomes. It was established that hydrophilic dendrimers would be located near to lipid headgroups, while hydrophobic dendrimers interact with the alkyl chains of the lipids and may cause loss of integrity in the membrane [34]. Gardikis et al. examined the incorporation of G4 and G3.5 polyamidoamine (PAMAM) dendrimers ($d_{G4} \approx 4.5$ nm, $d_{G3.5} \approx 4$ nm) in dipalmitoylphosphatidylcholine (DPPC) bilayers using DSC and Raman spectroscopy. They found that the maximum percentage of PAMAM dendrimers that can incorporate into multilamellar liposomes is 5 mol% for G4 and 3.5 mol% for G3.5 [35]. Wrobel et al. investigated the effect of positively charged phosphorous-containing dendrimers on unilamellar liposomes by measuring fluorescence anisotropy and DSC and showed that dendrimers interact with both hydrophobic and hydrophilic parts of the bilayer [36]. Smith and coworkers investigated the effect of G5 and G7 PAMAM dendrimers on multilamellar vesicles by solid-state NMR techniques. They have found that dendrimers have higher impact on alkyl chain region than on headgroups, also they used partially hydrated or highly concentrated fully hydrated membranes [37].

In this study the interaction between positively charged fifth generation (G5) PAMAM dendrimers and DPPC based liposomes were investigated in order to reveal what happens between dendrimers and lipids when the size of the dendrimer is not big enough to form a liposome-coated dendrimer complex. Mecke and coworkers have made thermodynamic calculations and proposed that G7 PAMAM dendrimers ($d \approx 8$ nm) are big enough to have a closed lipid bilayer wrapping them [14]. Although the diameter of G5 ($d \approx 5.5$ nm) dendrimers is too small for this, they still remove lipids from PC membranes. We aimed to understand this interaction in depth.

For a comprehensive characterization we have combined DSC, SWAXS, FFTEM, phosphorus-31 nuclear magnetic resonance (^{31}P NMR) and infrared spectroscopy. Thermal characterization with DSC provides information on the lipid membrane phase transition temperatures and changes in enthalpy. Structural information can be obtained using X-ray scattering techniques as one can deduce the lamellarity from a SAXS pattern of a liposomal system and establish the characteristic distances between lamellae, while WAXS patterns provide information about subcells in the alkyl chain region [31,38]. FFTEM enables direct visualization of lipid structures when examining a replica made on the freeze-fractured surface of the sample. ^{31}P NMR spectroscopy can give information of the phase behavior of the lipids as the shape of the signal is characteristic for the different isotropic and anisotropic phases. [39,40] Infrared spectroscopy is used to study the structure and organization of lipid bilayers. Changes in the frequencies and widths of vibrational bands and/or splitting of the spectral features provide information on the interactions between functional groups of lipids and macromolecules [32,33].

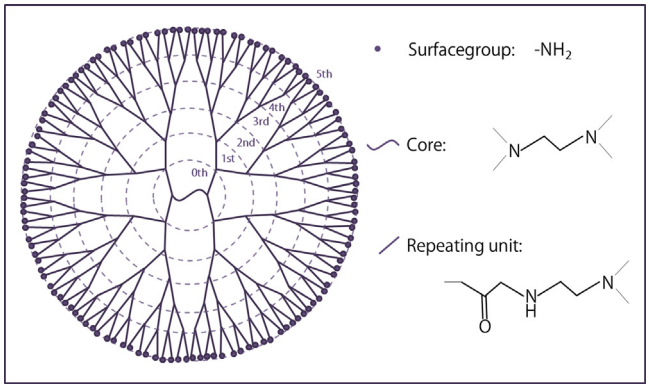

Fig. 1. Schematic structure of 5th generation polyamidoamine dendrimer with ethylenediamine core.

2. Materials and methods

PAMAM G5 (PAMAM) dendrimer (with ethylenediamine core, MW: 28,824 g/mol, $d \approx 5.5$ nm, Fig. 1) in 5 wt% methanol solution was purchased from Sigma–Aldrich. Highly purified synthetic 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from NOF Corporation. All materials were used without further purification.

Mixtures of PAMAM dendrimers and DPPC were prepared at 10^{-3} and 10^{-2} dendrimer/lipid molar ratio. The methanol solution of the dendrimer was added to the DPPC, and then pure chloroform was added until all of the lipids were dissolved. The mixture was dried under vacuum. Millipore water was added to the dried films, so that the lipid/water ratio was 20 wt%. Hydration was followed by heating up to 60 °C, cooling down to 4 °C, reheating up to 60 °C and vortexing intensively. This procedure was repeated several times to achieve homogeneous dispersions.

2.1. Differential scanning calorimetry

Samples were examined with a Setaram μDSC3 evo apparatus. All samples were scanned three times from 25 °C to 70 °C. Scanning rate was 1 °C/min first time, then 0.5 °C/min during the heating and 1 °C/min during cooling period. All measurements were repeated after 30 min incubation at 25 °C. As a reference an empty sample holder was used. The sample quantity used for DSC measurements was about 10 mg in each case.

2.2. Small- and wide-angle X-ray scattering

Small- and wide-angle X-ray scattering measurements were performed using a modified compact Kratky-type camera with