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The Carbosilane Dendrimers Affect the Size and Zeta Potential of Large Unilamellar Vesicles

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Abstract: The interaction of cationic carbosilane dendrimers NN16 and BDBR0011 with large unilamellar vesicles (LUVs) composed of neutral dimyristoylphosphatidylcholine (DMPC), negatively charged dipalmitoylphosphatidylglycerol (DPPG), and their mixture DMPC/DPPG in a molar ratio 7 : 3 was studied. Dendrimers can be used as potential drug carriers and the LUVs are considered a model of the plasma membrane of the cell. Using the dynamic light scattering method the average size and zeta potential of the dendrimer-LUVs suspension as a function of dendrimer concentration were measured. The interaction of dendrimers with DMPC LUVs had no significant effect on the LUVs average size, however, aggregates of LUVs-dendrimer complexes were observed for negatively charged vesicles. Dendrimers induced an increase of the zeta potential from the negative to positive values for all LUVs studied. But, for negatively charged LUVs, the dendrimer concentration, at which the surface charge polarity changes from negative to positive values, was shifted to the higher dendrimer concentrations. This suggests an electrostatic nature of the dendrimer-vesicle interactions.

Keywords: Carbosilane dendrimers, Large unillamelar vesicles, Surface charge, Zeta potential, Dynamic light scattering

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1. Introduction

Dendrimers and lipid vesicles represent the most extensively studied categories of particles that could be used as carriers of bioactive molecules. With careful tailoring of their physicochemical characteristics and thermodynamic parameters, these carriers can alter the ADME profile (Absorption, Distribution, Metabolism and Excretion) of candidate molecules leading to more potent and less toxic therapeutic agents [1 5]. An attractive approach for delivery of drugs to cells is the use of dendrimers as targeted delivery vehicles [6 10]. Cationic dendrimers interact efficiently with nucleic acids, forming dendrimer/nucleic acid complexes in their interiors or by bonding the acids to the surface groups. Carbosilane dendrimers (CBD) are presently one of the most intensely studied classes of dendrimers [11 13]. CBDs possess branches made of carbon-silicon bonds which are water-stable (Fig. 1A). On the other hand, CBDs with oxygen-silicon bonds (Fig. 1B) are

slowly hydrolysed in aqueous solutions. This property allows for gradual release of drugs from dendrimers [14]. CBDs can make stable complexes with nucleic acids, thus protecting them from binding to the proteins. It is important for a gene-therapy drug administration into the bloodstream to protect DNA/RNA from sequestration and degradation [15]. The second generation of these types of dendrimers shows good toxicity profiles in a primary cell culture and erythrocytes up to the concentrations of 5 M [16]. There is also evidence on some antibacterial properties of CBDs, which depend on the dendrimer generation and the type of bacteria. The most efficient antibacterial properties were observed for the first and second dendrimer generation [17]. Here, we have studied the interaction of two types of cationic CBDs with large unilamellar vesicles (LUVs) composed of phospholipids of different charge. The aim of this work was to explore the mechanism of interaction of CBDs with lipid membranes by measuring the zeta potential which is sensitive to the changes of the surface charge. The obtained results can later be used to customize the dendrimers for a given drug delivery system.

2. Experimental

2.1. Materials

Two types of water-soluble cationic carbosilane dendrimers (CBDs) were synthesized in the Departamento de Quimica Inorganica, Universidad de Alcala. The main characteristics and synthesis of CBDs were described earlier [14, 16, 18]. CBD - NN16, $C_{128}H_{316}I_{16}N_{16}O_8Si_{13}^{16}$, Mw = 4 603.56 g/mol and CBD - BDBR0011 $C_{144}H_{348}I_{16}N_{16}Si_{13}^{16}$, Mw = 4 699.99 g/mol, are presented in Fig. 1. Phospholipids: 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), dipalmitoylphosphatidylglycerol (DPPG) were purchased from Avanti Polar Lipids Inc (USA). All other reagents used were of analytical grade and purchased from Sigma-Aldrich (USA).

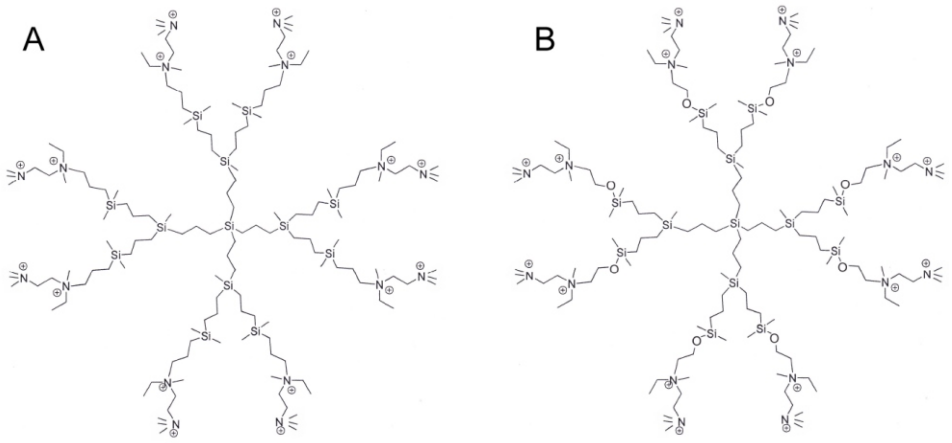

Fig. 1. Molecular structure of the carbosilane dendrimers of the 2nd generation. A – with Si-C bonds – BDBR0011; B – with Si-O bonds – NN16.

2.2. Liposome preparation

Large unilamellar vesicles (LUVs) composed of DMPC or DMPC/DPPG (molar ratio 7 : 3) or DPPG were prepared using an extrusion method. Briefly, appropriate amounts of lipid solutions in chloroform were placed in a round bottom flask and a thin lipid film was formed by slow removal of the solvent under nitrogen atmosphere. The remaining solvent traces were removed under the vacuum using a rotary evaporator over a water bath at 37 C for 30 min. The resulting lipid film on the wall of the flask was hydrated with an appropriate volume of buffer resulting in a final lipid concentration of 5 mg/ml. The mixture was vortexed for 5 min with glass beads, and allowed to equilibrate for 30 min under nitrogen atmosphere at 37 C (above the gel-liquid crystal transition temperature of the lipid mixture). Subsequently the liposome suspension was forced to pass at least 15 times through a polycarbonate membrane of 100 nm porosity (Nuclepore, T-E), mounted in a mini-extruder (Avanti Polar Lipids) fitted with two 1 ml Hamilton gastight syringes. Exposure to light was minimized throughout the liposome preparation process [19].

2.3. Measurement of vesicle size

The particle size and size distribution (*z*-average mean) were measured using dynamic light scattering in a photon correlation spectrometer (Zetasizer Nano-ZS, Malvern Instruments, UK) [20]. The refraction factor was assumed 1.33 while the detection angle was 90 and the wavelength was 633 nm. Samples were prepared and measured at 25 C in 10 mM PBS, pH 7.4, filtered with 22 nm filter. The electrophoretic mobility of the dynamic light scattering samples was determined from the 12 cycles in Malvern plastic cells. The data were analyzed using Malvern software.

2.4. Measurement of zeta potential

The particles charge measurements were conducted using phase analysis light scattering with Zetasizer Nano-ZS, Malvern Instruments, UK. The electrophoretic mobility of the dynamic light scattering samples of an applied electric field was measured in Malvern capillary plastic cells (DTS1061). Samples were prepared and measured at 25 C in 10 mM PBS, pH 7.4, filtered with 22 nm filter. Nine zeta potential measurements were collected for each dispersion, and the results were averaged. The zeta potentials were calculated directly from the Helmholtz-Smoluchowski equation using the Malvern software [21].

2.5. Statistical analysis

Statistical analysis and exponential curve fitting were performed using Origin 8 (Microcal Software Inc., Northampton, MA) software. The results are expressed as the means standard error of the mean (S.E.M.).

3. Results and discussion

3.1. Average particles size

The interaction of cationic dendrimers with LUVs of different lipid composition: (a)-DMPC; (b)-DMPC/DPPG (molar ratio 7 : 3); (c)-DPPG was studied. The above-mentioned composition allowed us to vary the surface charge of the LUVs, which plays an important role in the interaction of the bilayer with cationic dendrimers. Changes in the

average size of the vesicles in the presence of various concentrations of either BDBR0011 or NN16 dendrimers are shown in Fig. 2. For LUVs composed of DMPC a slight increase in the vesicle mean diameter was observed when the dendrimers were present in the suspension (Fig. 2). The vesicle mean diameter maintained approximately the same features of the pure LUVs thus evidencing a negligible dendrimer – neutral DMPC membrane interaction.

For vesicles formed from DMPC/DPPG the difference in the mean diameter of dendrimer-loaded and empty vesicles were larger and statistically significant for CBD-BDBR011, but not for CBD-NN16. In the case of vesicles prepared from pure DPPG the average size increased dramatically already at low dendrimer concentrations. This suggests that dendrimer-vesicle complexes tended to form aggregates, and that CBD-BDBR001 and CBD-NN16 interacted strongly with the negatively charged lipid membranes. This means that lipid composition plays an important role in the membrane-dendrimer interactions which can be accomplished in different ways: without affecting the vesicle size (neutral liposomes) or by formation of relatively large aggregates (negatively charged liposomes). The significant increase of the mean diameter of the DMPC/ DPPG vesicles from their initial values suggests that the drug-loaded vesicles aggregated. Since aggregation may be used as a measure of a vesicle physical stability, we may conclude that the interaction of BDBR001 and NN16 with negatively charged LUVs bilayers did modify their physical stability. However, the vesicle mean size was practically not modified (NN16) or it only slightly increased (BDBR001) when the DMPC/DPPG vesicles were used (Fig. 2). Thus, differences in the liposome/dendrimer interactions are based on different surface charge of LUVs. Cationic dendrimers are bound stronger to LUVs which contain negatively charged lipids.

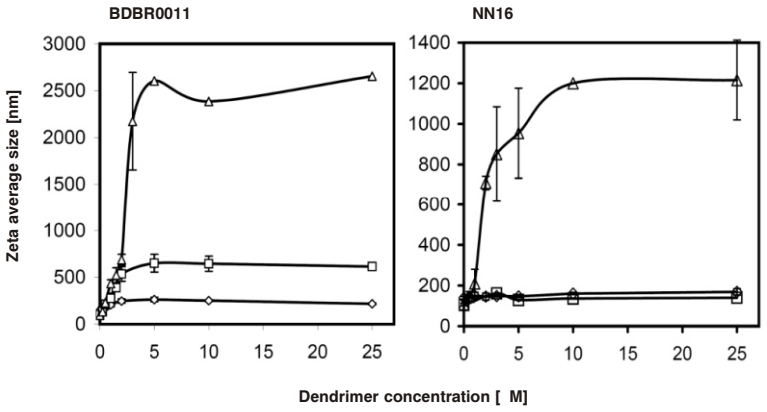

Fig. 2. Average size of the LUVs (concentration 5 mM) composed of: ◇ DMPC; □ DMPC+DPPG (Molar ratio 7 : 3); DPPG, in a presence of increased concentrations of either BDBR0011 or NN16 dendrimers. Lipids/Dendrimer molar ratios were: 200 : 1; 100 : 1; 50 : 1; 33 : 1; 25 : 1; 10 : 1; 5 : 1; 2 : 1. PBS 10, pH 7.4 at 25 C. The results represent the mean SEM obtained from 3 independent experiments.

3.2. Zeta potential measurements

The effect of cationic BDBR0011 and NN16 dendrimers on zeta potential of DMPC, DMPC/DPPG (Molar ratio 7 : 3) or DPPG LUVs is presented in Fig. 3. The zeta potentials of the control vesicles (without additives) were from 0.98 mV to 6.40 mV for the neutral vesicles and 13.58 mV to 20.35 mV for the negatively charged vesicles.

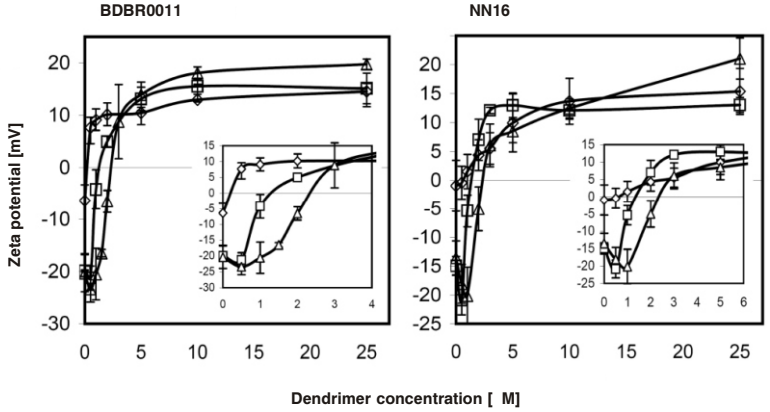

Fig. 3. The plot of zeta potential of the LUVs (0.5 mM) composed of: ◇ DMPC; □ DMPC+DPPG (Molar ratio 7 : 3); DPPG, as a function of BDBR0011 or NN16 dendrimer concentration. Lipids/Dendrimers molar ratios were: 100 : 1; 50 : 1; 25 : 1; 10 : 1; 5 : 1; 2 : 1. PBS 10 mM, pH 7.4 at 25 C. The results represent the mean SEM obtained from 3 independent experiments. Insets changes of zeta potential at low concentrations of dendrimers.

It is clear from the insets in Fig. 3 that the raise of zeta potential to the positive values took place already at low dendrimer concentrations. After the initial sharp potential increase, the saturation took place for dendrimer concentrations higher than 10 M for BDBR0011 and 5 M for NN16, suggesting a slightly stronger charge neutralization of the liposome surface by NN16 dendrimers in comparison with BDBR0011. Nevertheless, the final value of potential change caused by both dendrimers in the saturation region was not statistically significant. The interaction of dendrimers with the liposome surface was rather strong and their ability to change zeta potential in the saturation region practically did not depend on the liposome zeta potential, i.e. on the surface charge. However, for negatively charged vesicles the dendrimer concentration, at which the surface charge polarity changes from negative to positive values, was shifted to the higher dendrimer concentrations (Table 1). This suggests an electrostatic nature of the dendrimer-liposome interactions. The plots presented in Fig. 3 have the shape of Langmuir isotherms. Thus, it is likely that the dendrimers bound the liposome surface independently. Strong electrostatic interaction of cationic poly(amidoamine) (PAMAM) dendrimers with negatively charged membranes was reported recently. PAMAM also induced aggregation of the small unilamellar vesicles composed of anionic lipids [22].

Table 1. Zeta-potential of LUVs (mV) of various compositions as a function of molar ratios of the lipids to either BDBR0011 or NN16 dendrimers. Dendrimer-vesicle complexes were produced by incubating dendrimers and LUVs at the corresponding lipid/CBD molar ratios. All measurements were carried out using 10 mM PBS (pH 7.4). The results represent the mean ± SEM obtained from 3 independent experiments.

Molar ratio Lipid/ CBD	CBD-BDBR0011			CBD-NN16		
	DMPC	DMPC /DPPG	DPPG	DMPC	DMPC /DPPG	DPPG
1 : 0	-6.4±3.1	-19.9±0.2	-20.4±3.5	-0.98±4.42	-15.0±1.6	-13.6±3.0
100 : 1	7.6±2.0	-21.3±2.3	-23.4±2.5	-0.45±2.89	-20.9±2.5	-18.1±3.4
50 : 1	9.1±2.1	-4.2±3.8	-20.5±5.0	1.5±3.0	-5.4±2.8	-20.1±5.0
25 : 1	10.1±2.2	4.9±1.0	-6.5±2.1	4.4±2.7	7.0±3.5	-5.0±3.8
10 : 1	10.4±0.4	13.1±2.2	8.8±7.1	9.9±5.0	13.0±2.1	8.5±2.0
5 : 1	13.0±2.9	15.5±1.5	13.9±2.4	13.7±3.9	12.1±1.5	12.5±1.7
2 : 1	14.6±1.5	15.1±2.9	18.1±1.2	15.4±4.0	13.0±1.3	21.1±3.6

4. Conclusion

This work was aimed mainly at the characterization of the influence of cationic carbosilane dendrimers on the size and zeta potential of the vesicles of various lipid composition. The study of the dendrimer interaction with the lipid membranes was carried out using DMPC, DPPG or DMPC/DPPG mixtures. Results showed that either BDBR0011 or NN16 dendrimers had no significant effect on the size of LUVs composed of DMPC. These data imply that CPDs-dendrimers probably interact with DMPC membranes in a manner of random contacts, whereas in the case of negatively charged DPPG mixtures, both dendrimers prefer electrostatic interaction with the membrane surface, which induces formation of large liposome aggregates. The zeta potential of the LUVs was changed when cationic dendrimers were present. All the vesicle formulations produce positively charged species and this effect is dependent on the dendrimers concentration.

Acknowledgments

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