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Dendrimers complexed with HIV-1 peptides interact with liposomes and lipid monolayers

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ABSTRACT

Aims: We have investigated the effect of surface charge of model lipid membranes on their interactions with dendriplexes formed by HIV-derived peptides and 2 types of positively charged carbosilane dendrimers (CBD).
Methods: Interaction of dendriplexes with lipid membranes was measured by fluorescence anisotropy, dynamic light scattering and Langmuir–Blodgett techniques. The morphology of the complexes was examined by transmission electron microscopy.
Results: All dendriplexes independent of the type of peptide interacted with model lipid membranes. Negatively charged vesicles composed of a mixture of DMPC/DPPG interacted more strongly, and it was accompanied by an increase in anisotropy of the fluorescent probe localized in polar domain of lipid bilayers. There was also an increase in surface pressure of the lipid monolayers. Mixing negatively charged liposomes with dendriplexes increased liposome size and made their surface charges more positive.
Conclusions: HIV-peptide/dendrimer complexes interact with model lipid membranes depending on their surface charge. Carbosilane dendrimers can be useful as non-viral carriers for delivering HIV-peptides into cells.

1. Introduction

Human immunodeficiency virus (HIV) is a retrovirus responsible for the acquired immunodeficiency syndrome (AIDS) [1,2]. Antiretroviral therapy (ART) can greatly improve quality of life and decrease viral load, but several limitations of current approaches must be addressed to give safe and effective therapy [3]. The focus must now be on the development of new preventive and therapeutic vaccines, and their effective delivery into the target cells. The application of dendritic cells (DCs) as a vaccine adjuvant is one way to improve deterioration of immune functioning in HIV-1-infected individuals [4,5]. For therapeutic purposes, the antigen (HIV-derived peptide) should be transported through the cell membrane into the cytoplasm. A method to load DCs with antigens is therefore urgently needed, since it requires an efficient carrier to deliver antigen across the plasma membrane. Different methods have so far included the use of liposomes, nanoparticles, polymeric micelles and nanogels [6–13]. In this context, dendrimers offer an alternative approach [14,15].

Dendrimers are hyperbranched, multifunctional and completely defined macromolecules that are already known to have high therapeutic potential in delivering biomolecules [5,6,15]. Among various types of cationic dendrimers of different generation, the carbosilane dendrimers (CBD) of second generation are of special interest. These dendrimers show good toxicity profile up to concentration of 5 μM and antibacterial properties [16]. CBDs were primarily synthesized for delivery of siRNA and other oligonucleotides. The negatively charged siRNA can interact electrostatically with cationic dendrimers so the stable complexes are formed. In addition, due to certain neutralization of the siRNA negative charge this complex can strongly interact with negatively charged cell surface in contrast with naked siRNA, that is repulsed from the cell surface. In our recent works we studied the mechanisms of interaction with model lipid membranes (liposomes and monolayers) of two types of CBDs containing 16 amino groups at the surface, but differing in the composition of their branched core: first one, BDBR0011, contained

Abbreviations: ART, antiretroviral therapy; CBD, cationic carbosilane dendrimer; DC, dendritic cells; DLS, dynamic light scattering; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DPPG, dipalmitoylphosphatidylglycerol; HIV, human immunodeficiency virus; LUVs, large unilamellar vesicles; PALS, phase analysis light scattering; PB, phosphate-buffer; TEM, transmission electron microscopy

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carbon silicon bonds (CBD-CS) and the second one, NN16, had oxygen silicon bonds (CBD-OS). While CBD-CS are stable in aqueous solution CBD-OS are slowly hydrolyzed.

The study of the interaction of dendriplexes based on CBDs and HIV-1 derived peptides has not been reported so far. In this work we therefore studied the interaction of dendriplexes based on CBDs and three different peptides that are prospective for development of artificial immune system against HIV-1: P24, Gp160 and Nef. We explored the nature and mechanism of interaction of the HIV-peptide/dendrimer complex with artificial lipid membranes composed of zwitterionic or negatively charged phospholipids [18–20]. We showed, that these complexes interact with DMPC and DMPC/DPPG membranes most probably by being incorporated into their polar domains. The presence of dendriplexes markedly changed some parameters of the DMPC/DPPG lipid membranes in comparison with DMPC membranes. In contrast with dendriplexes, we have shown that non-complexed peptides did not interact with lipid membranes. The results indicate the importance of electrostatic interactions between dendriplexes and lipid membranes. The analysis of the interaction of dendriplexes with model membranes can help develop the strategy for immunotherapy of HIV-1 infection using DCs loaded with synthetic HIV-derived peptides, which can be delivered by CBD dendrimers.

2. Materials and methods

2.1. Dendrimers

Second generation of cationic carbosilane dendrimers with carbon silicon bonds BDBR0011 (CBD-CS) and oxygen silicon bonds NN16 (CBD-OS) has been used. Both CBDs contain 16 cationic surface terminal groups and have following composition: CBD-CS, $C_{128}H_{316}I_{16}N_{16}O_8Si_{13}$, Mw = 4603.6 g/mol; CBD-OS, $C_{144}H_{348}I_{16}N_{16}Si_{13}$, Mw = 4699.99 g/mol. Dendrimers were synthesized in the Departamento de Quimica Inorganica, Universidad de Alca, Spain [21–24], the structures of CBDs being shown in Fig. 1.

2.2. HIV-derived peptides

HIV-derived peptides were prepared by Eurogentec (Belgium)

- Peptide derived from Nef sequence, HIV-HXB2 location Nef (172–191): NHGMDDPEREV-LEWRFDSRLAFCOOH, length 20 amino-acids, charge (−3);
- Peptide derived from Gag-P24 sequence, HIV-HXB2 location P24 (71e80): NH-DTINEEAAEW-COOH, length 10 amino-acids, charge (−4);
- Peptide derived from envelope Gp160 sequence, HIVHXB2 location Gp160 (634e648): NH-EIDNYTNTIYTLLEE-COOH, length 15 amino-acids, charge (−4)

2.3. Phospholipids

DMPC-1,2-dimyristoyl-sn-glycero-3-phosphocholine and DPPG-dipalmitoylphosphatidylglycerol were synthesized by Avanti Polar Lipids Inc. (USA). Other reagents were purchased from Sigma-Aldrich Company (USA).

2.4. Preparation of dendriplexes

Dendriplexes were formed by adding dendrimers into peptide solutions at a molar ratio of 1:3 (peptide:dendrimer). CBD dendrimers and HIV-derived peptides were dissolved in 10 mM Na-phosphate buffer at pH 7.4. The mixture was vortexed and incubated for 10 min at room temperature (approx. 20 °C).

2.5. Preparation of liposomes

To prepare the liposomes, the DMPC or DMPC/DPPG mixtures (9:1 *w*/*w*) were dissolved in chloroform and placed into a round glass flask under vacuum to evaporate the solvent. The dry lipid film was hydrated with buffer to yield a lipid concentration of 0.3 mg/ml,

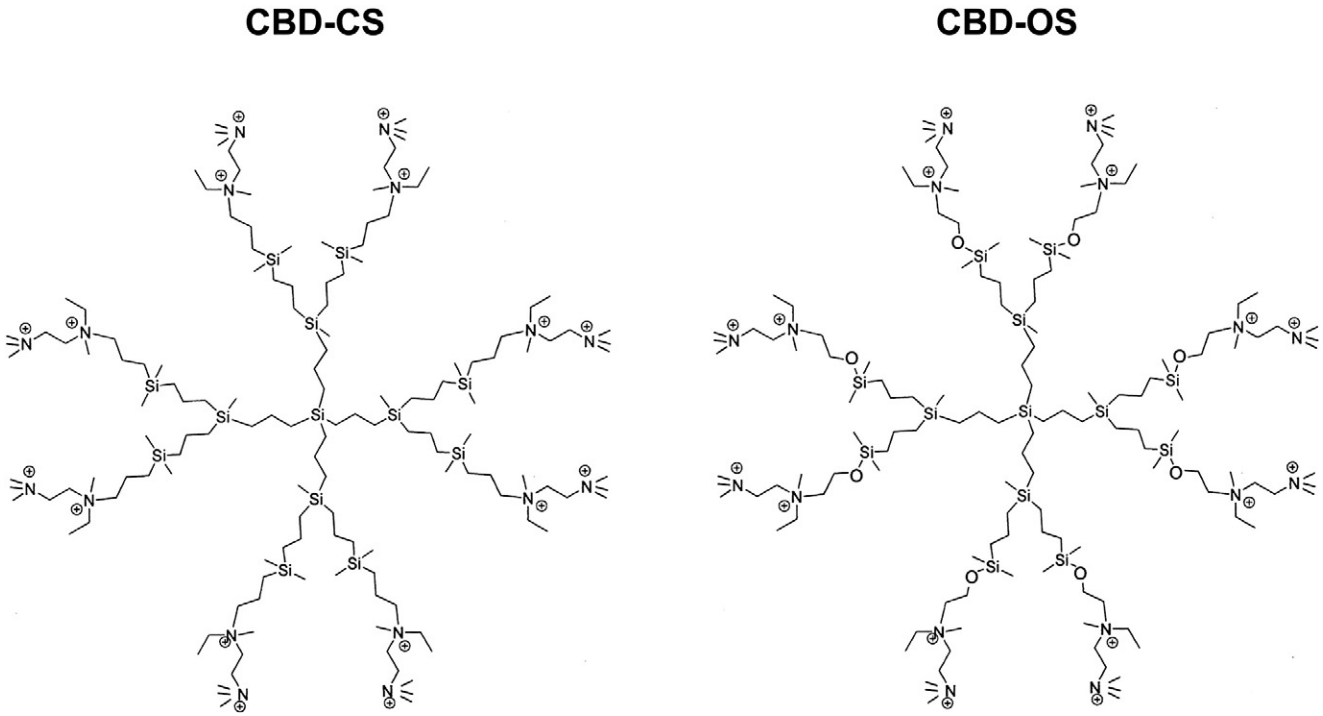

Fig. 1. Molecular structure of the carbosilane dendrimers of 2nd generation. With Si – C bonds—CBD-CS (BDBR0011); with Si – O bonds—CBD-OS (NN16).

which was continuously shaken. The lipid solution was extruded 15–17 times through Millipore polycarbonate filters (100 nm pore size) using Avanti extruder (Avanti Polar Lipids, USA) to get large unilamellar vesicles (LUVs). The temperature of samples during extrusion was kept at approx. 37 °C, which is well above the main phase-transition temperature of the lipids (~24 °C).

2.6. Measurement of particle size

The hydrodynamic diameter of vesicles was measured using a dynamic light-scattering technique using Zetasizer Nano-ZS (Malvern Instruments, UK). Samples were placed in plastic Malvern cells (DTS0012) in 10 mM Na-phosphate buffer at pH 7.4 (filtered twice through 0.22 μm filters). The refraction factor was assumed to be 1.33 at a detection angle of 90°, a wavelength of 633 nm, and the sample temperature during measurements was 25 °C [25,26]. Particle size was measured from the average of 11–15 cycles. Malvern software was used to analyze the data.

2.7. Measurement of zeta potential

The electrophoretic mobility of the samples in an applied electric field was measured in Malvern capillary plastic cells (DTS1061) using Zetasizer Nano-ZS. The zeta potential was calculated directly from the Helmholtz–Smoluchowski equation with the Malvern software [27,28]. HIV-derived peptides alone or complexed with CBD dendrimers were added to 1 ml liposome suspension to obtain dendriplex/lipid molar ratios of 1:300, 1:150, 1:100, 1:50, 1:30, 1:15, 1:10, 1:7.5, and 1:5. Samples were prepared in 10 mM Na-phosphate buffer at pH 7.4 (filtered twice through 0.22 μm filters), and measured at 25 °C. Zeta potentials were averaged from 11 to 13 measurements.

2.8. Fluorescence spectroscopy

Fluorescence anisotropy was measured with a Perkin-Elmer (U.K.) LS-50B spectrofluorimeter. Two fluorescent probes were used to monitor membrane fluidity. l,6-Diphenyl-l,3,5-hexatriene (DPH) was located in the hydrophobic region of the lipid membrane, while 1-(4-(trimethylamino)phenyl)-6-phenylhexa-1,3,5-triene (TMA-DPH) probes were near the hydrophilic part of the lipid bilayer. The molar ratio of phospholipid to probe was 300:1. Excitation at 348 nm and emission at 426 nm wavelengths were used to analyze the probe mobility in the bilayer [29,30]. For both probes the slits widths used were 5 nm and 3 nm for excitation and emission monochromators, respectively. The measurements were performed at 37 °C, i.e. well above the main phase-transition temperature of lipids. Steady-state fluorescence anisotropy values (r) were calculated using Perkin-Elmer software from Jablonski's equation:

$$r = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH})$$

where I_{VV} and I_{VH} are the vertical and horizontal fluorescence intensities, respectively, to the vertical polarization of the excitation light beam. $G = I_{HV}/I_{HH}$ (grating correction factor) corrects the polarizing effects of the monochromator.

2.9. Monolayer technique

A Langmuir–Blodgett method was applied for monolayer experiments. A small Teflon cell of circular shape (volume 4.5 ml) was used. Appropriate amounts of lipid dissolved in chloroform at 0.3 mg/ml were added to the surface of 10 mM Na-phosphate buffer at pH 7.4, to reach a surface pressure close to 30 mN/m that corresponds to the condensed state of the monolayer. Wilhelmy method has been used to measure surface pressure by means of PS4 sensor (NIMA Technology, UK) [31]. When surface pressure reached 30 mN/m and stabilized, peptides or peptide/dendrimer complexes were added to the water subphase (under the monolayer) through a special channel in a concentration of 0.25 μM, and the surface pressure was registered until steady-state value was reached.

2.10. Transmission electron microscopy

The effect of peptide/dendrimer complexes on the morphology of liposomes was examined by transmission electron microscopy (TEM; JEOL-10, JEOL Ltd., Tokyo, Japan). Dendriplexes were added to liposomes suspended in 10 mM Na-phosphate buffer at pH 7.4 with a molar ratio of 1:25 (dendriplex to lipid). The resultant complexes were placed on a carbon surface of a 200-mesh copper grid (Ted Pella, Inc., USA) 10 min and drained with blotting paper. Samples were negatively stained with 2% (w/v) uranyl acetate (Sigma) for 2 min. A magnification of 50,000–100,000× proved best for examining liposomes interacting with LUVs with peptide/dendrimer complexes.

2.11. Statistical analysis

Origin 8 software (Microcal Software Inc., Northampton, MA, USA) was used for exponential curve-fitting and statistical analysis. Data were obtained from a minimum 3 independent experiments and presented as mean ± SD (standard deviation).

3. Results and discussion

3.1. Fluorescence anisotropy

Liposomes of different lipid composition, DMPC and DMPC/DPPG (9:1 w/w) were used in order to model the interactions between HIV-peptide/dendrimer complexes (dendriplexes) with cell membrane. The 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC) was chosen as synthetic analog of lecithin, the main component of lipid membrane matrix of living cells. The dipalmitoylphosphatidylglycerol (DPPG) was used to prepare negatively charged membrane which can simulate the charge of living cell. However, due to the presence of proteins in the cell membrane and the affinity of dendrimers to proteins [16,22,24] the interaction of dendriplexes with biological membranes will be more complex than with the model membranes. The effect of dendriplexes on model lipid bilayers was measured by fluorescence anisotropy using DPH and TMA-DPH fluorescent probes. This gave data on membrane fluidity that reflected the effect of the proteins or dendriplexes on the order of the lipids in the bilayer's hydrophobic and polar part, respectively. The DPH probe has cylindrical symmetry around its long axis in the lipid bilayer [32], and is aligned with the axis parallel to the acyl chains of the lipid [33].

The TMA-DPH probe is located in the head group region of the lipid membrane [34]. Fig. 2 shows the effect of peptides or peptide/dendrimer complexes on the fluorescence anisotropy of liposomes formed with DMPC or DMPC/DPPG (9:1 w/w) phospholipids labelled with TMA-DPH (A) or DPH (B) probes. An increased concentration of dendriplexes significantly increased TMA-DPH anisotropy for negatively charged liposomes (DMPC/DPPG). The effect was stronger for CBD-CS based dendriplexes in comparison with CBD-OS. However, the fluorescent anisotropy of zwiterionic DMPC liposomes was not significantly changed by dendriplexes. The naked peptides did not affect membrane fluorescence anisotropy either for neutral or negatively charged liposomes. Increase in anisotropy in the presence of dendriplexes was attributed to a decrease in membrane fluidity, which may be due to some chelation as a result of interaction of positively charged dendriplexes with negatively charged membranes. Negatively charged peptides are repulsed from the negatively charged surface of DMPC–DMPG liposomes, or weakly interact with zwitterionic DMPC liposomes; thus they do not affect the TMA-DPH fluorescence anisotropy (Fig. 2A).

The effect of peptides and dendriplexes on DPH fluorescence anisotropy is not uniform. There was a small decrease in anisotropy for both DMPC and DMPC–DPPG liposomes for some peptides, as well as a decrease of fluorescence anisotropy for dendriplexes composed of CBD-CS dendrimers for negatively charged lipids. Little or no increase of anisotropy was seen with zwitterionic DMPC liposomes. The data suggest a weaker effect of dendriplexes on the hydrophobic domain of the membrane (Fig. 2B).

3.2. Zeta potential and size of liposome/dendriplex systems

To correlate the interaction between dendriplexes and vesicles of different lipid composition observed using fluorescence anisotropy, DLS and zeta potential experiments were conducted. Zeta potential measurements are important to control the electrical charge properties of nanoparticles. Analyzing the electrical charge characteristics allows better understanding the interactions between dendriplexes and liposomes and therefore determining the charge behavior of the samples. The changes in zeta size (hydrodynamic diameter) of liposomes at their interaction with peptides or peptide/dendrimer complexes are given in Fig. 3. The hydrodynamic diameters of the DMPC or DMPC/DPPG liposomes in controls were 111 ± 1.7 and 104 ± 1.6 nm, respectively, in a good agreement with the diameter of polycarbonate filter pores used for liposome preparation as well as with our previous studies [17]. With increased concentration of peptides, the size of both types of LUVs was practically unchanged (Fig. 3A), but the size of liposomes in the presence of dendriplexes significantly increased, especially with dendriplexes containing CBD-OS dendrimers. The hydrodynamic diameter of DMPC liposomes in the presence of the highest CBD-OS dendriplex/lipid molar ratio (0.2:1) increased to 190 ± 17.5 nm (P24), 153 ± 17 nm (Nef) and 165.5 ± 11.8 nm (GP160). For DMPC/DPPG liposomes, the hydrodynamic diameter increased to 235 ± 13 nm (P24), 217 ± 27 nm (Nef) and 199 ± 10.3 nm (GP160) at the same molar ratio (Fig. 3C).

For dendriplexes composed of CBD-CS dendrimers, the difference between liposome size with or without dendriplexes was less obvious; DMPC liposomes size increased to 124 ± 2.6 nm (P24), 127 ± 3.0 nm (Nef) and 129.9 ± 5.5 nm (GP160). For DMPC/DPPG liposomes, the particle sizes changed to 137 ± 3.6 nm (P24), 145.6 ± 9 nm (Nef) and 140.8 ± 4 nm (GP160) (Fig. 3B).

It is likely that in the case of naked peptides, the negatively charged thin layer is formed at the liposome surface, which does not contribute significantly to the particle diameter. In contrast, dendriplexes can induce aggregation of vesicles. This effect is more pronounced for

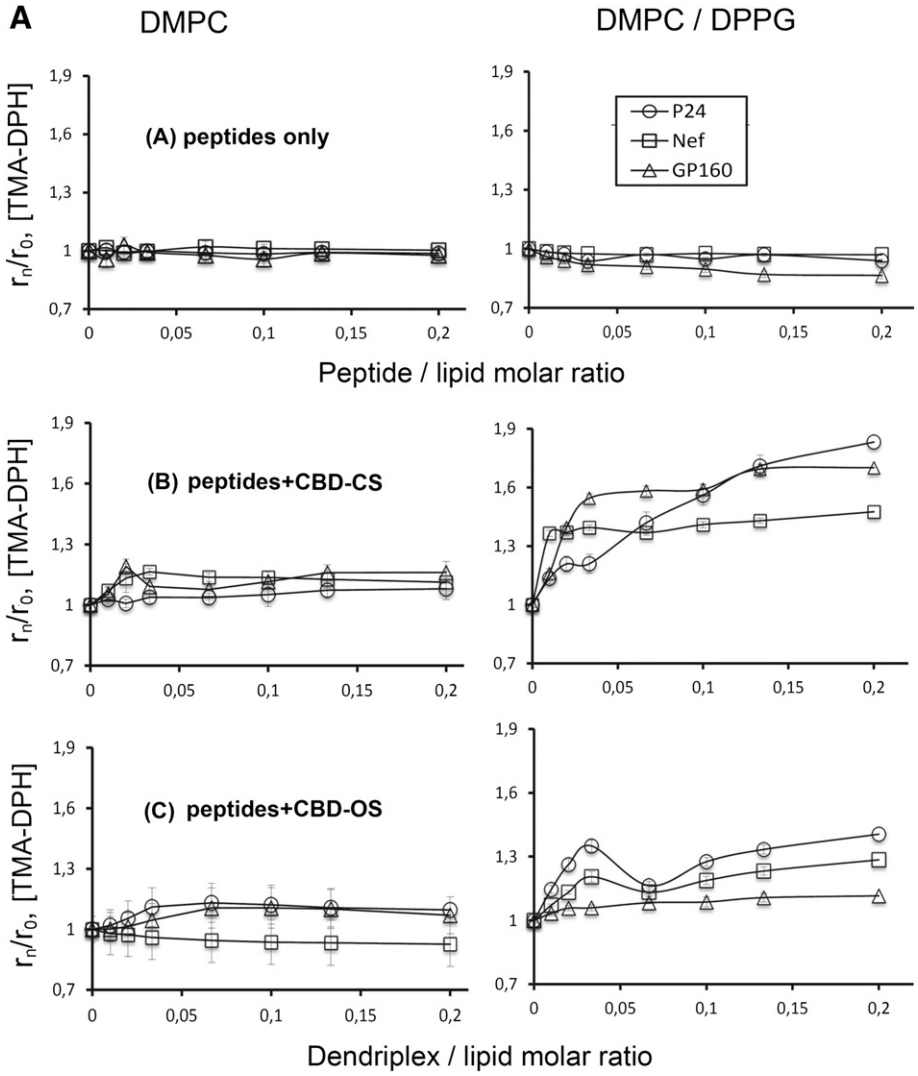

Fig. 2. Fluorescent anisotropy of TMA-DPH or DPH probes in liposomal membranes containing DMPC or DMPC/DPPG (9:1) *w*/*w* phospholipids. Molar ratio of probe to lipid = 1:300. (A)—TMA-DPH fluorescence anisotropy estimates membrane fluidity in the membrane interface region; (B)—DPH fluorescence anisotropy estimates membrane fluidity in the hydrophobic interior region of the membrane.

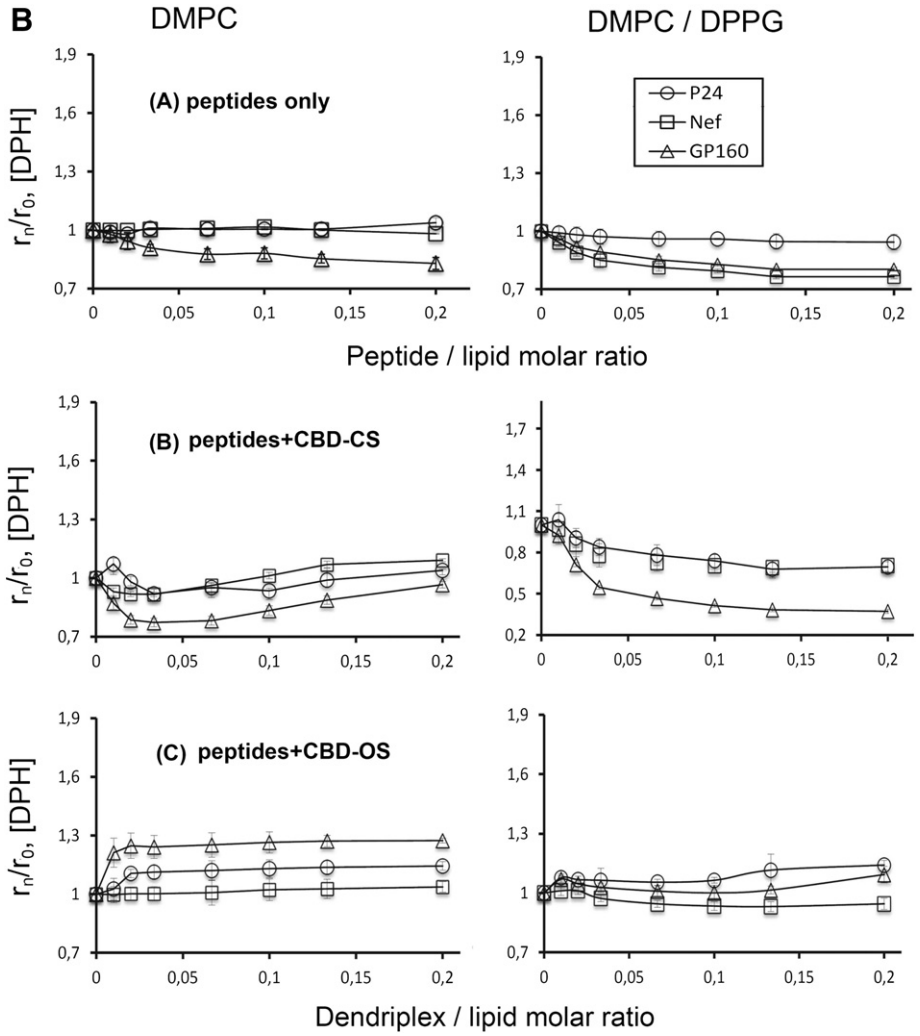

Fig. 2 (*continued*).

negatively charged liposomes. Considering that peptide:dendrimer molar ratio was 1:3, the dendriplexes are positively charged. Therefore they will strongly interact with negatively charged liposomes and will induce their aggregation.

Interactions between liposomes and peptides or dendriplexes were also characterized by measurements of zeta potential. Addition of peptides alone to a suspension of DMPC liposomes decreased the zeta potential of LUVs from -5.73 ± 1.3 mV to -20 ± 3 mV (P24), -29 ± 5 mV (Nef) and -25 ± 6 mV (GP160), whereas the zeta potential of DMPC/DPPG liposomes was unaltered in the presence of peptides (Fig. 4A). Addition of dendriplexes to a liposome suspension had the opposite effect.

All tested dendriplexes changed the zeta potential from negative to positive values. After addition of dendriplexes containing the CBD-CS dendrimer at a dendriplex/lipid molar ratio 0.1:1, the zeta potential of liposomes composed of DMPC increased from -3.6 ± 1.84 mV to 16.7 ± 1.5 mV (P24), 16 ± 1.1 mV (Nef) and 17 ± 1.6 mV (GP160) (Fig. 4B). Peptide/dendrimer complexes formed with the CBD-OS dendrimer changed the liposome surface charge from -4.07 ± 0.74 mV to 21 ± 0.9 mV (P24), 21.2 ± 2 mV (Nef) and 22.7 ± 1.5 mV (GP160) (Fig. 4C). For LUVs prepared using DMPC/DPPG, the difference between surface charge of control liposomes and liposomes treated by dendriplexes was more pronounced than for DMPC LUVs.

The presence of CBD-CS dendriplexes increased the zeta potential of DMPC/DPPG liposomes from -31.4 ± 1.07 mV to 20.2 ± 1.7 mV (P24), 21 ± 1.6 mV (Nef) and 22 ± 1 mV (GP160) (Fig. 4B). Addition of CBD-OS dendriplexes changed particle surface potential from -30.7 ± 1.0 mV to 26.6 ± 1.2 mV for P24, 27 ± 1.4 mV for Nef and 29 ± 1.7 mV for GP160 (Fig. 4C).

Summarizing the results in Figs. 3 and 4, we can conclude that all the HIV-derived peptides (not complexed with dendrimers) had no effect on the size of DMPC liposomes, whereas the zeta potential decreased. The decrease of zeta potential is a proof of adsorption of peptides to a liposome surface. However, as we mentioned above the peptide shell is too thin to affect the liposome size. For DMPC/DPPG liposomes, peptides alone produced no significant changes in the parameters measured. This result is obvious and is connected with repulsion of the peptides from negatively charged surface of liposomes. In contrast with the effect of peptides, adding peptide/dendrimer complexes shifted the zeta potential of DMPC and DMPC/DPPG liposomes towards positive values, as anticipated considering the positive surface charge of the dendriplexes. The hydrodynamic diameter of negatively charged DMPC/DPPG liposomes significantly increased in the presence of dendriplexes containing both dendrimers, which suggests formation of larger aggregates of liposome–dendriplexes due to strong adsorption of positively charged dendriplexes onto negatively charged liposome surfaces. We previously showed [7] that the presence of non-complexed carbosilane dendrimers in a suspension of LUVs composed of DMPC did not significantly change vesicle size, but vesicles formed by DMPC/DPPG had a more pronounced difference. For vesicles prepared from pure

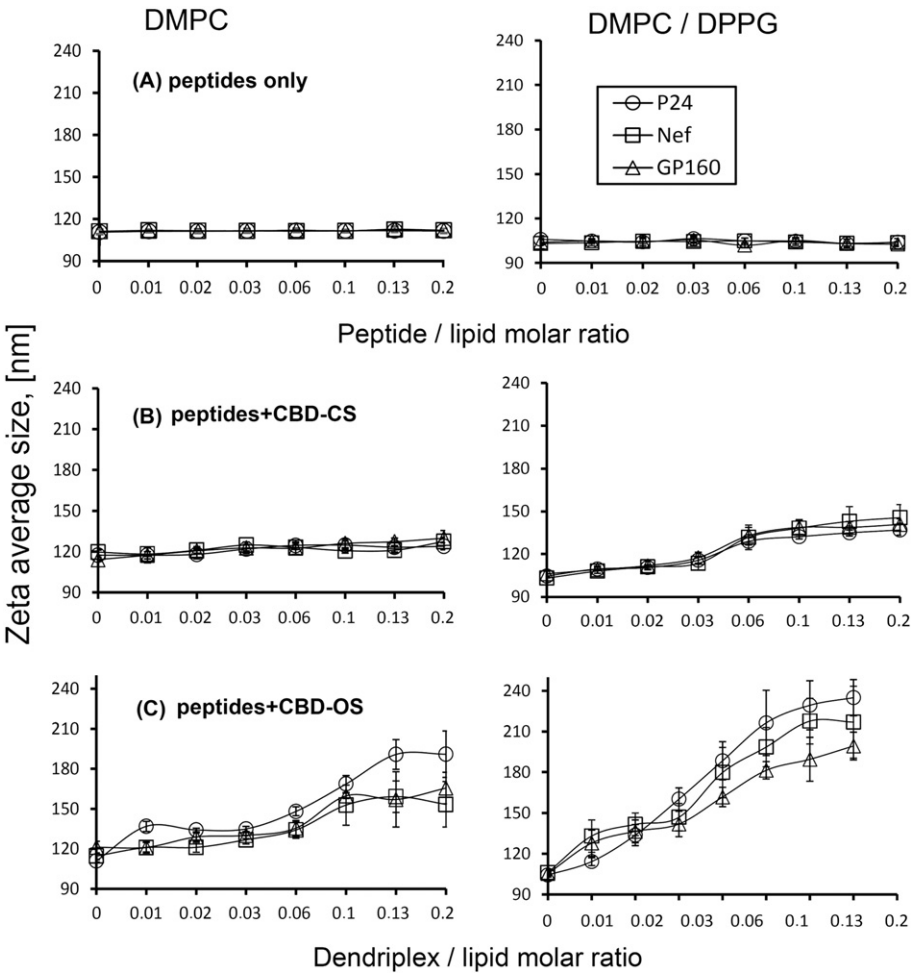

Fig. 3. Average zeta size of DMPC (left panels) and DMPC/DPPG (9:1), *w/w* (right panels) LUVs exposed to HIV-derived peptides (A) and their complexes with carbosilane dendrimers, CBD-CS (B); CBD-OS (C).

DPPG, the mean diameter increased markedly, which suggests that both the carbosilane dendrimers examined interacted mostly with negatively charged components of the lipid membrane, reflecting the importance of lipid composition in membrane-dendrimer interactions [7]. However, interactions between LUVs and peptides complexed with CBD-OS had similar character to those of CBD-CS. CBD-OS caused more pronounced changes in the measured parameters for both the DMPC and DMPC/DPPG lipid vesicles.

It is interesting to compare the effect of dendriplexes composed either of peptides or siRNA on the zeta potential. As we mentioned above, the dendriplexes composed of siRNA and CBD-CS resulted in the decrease of zeta potential, but those based on CBD-OS resulted in its increase to more positive values. This has been attributed to different rearrangements of siRNA at the liposome surface (see Ionov et al. 2012). In contrast with siRNA-based dendriplexes those composed of HIV-1 derived peptides uniformly increase zeta potential independently on the dendrimer structure. We can speculate that peptides more strongly interact with dendrimers due to their negative charge and, additionally, neutral amino acids can partially penetrate into dendrimer core. Thus, the peptide-based dendriplexes represent more compact particles that do not allow substantial rearrangements of the peptides and dendrimers after interaction with vesicles.

3.3. Monolayer measurements

To explore the mechanisms of interactions between lipid membrane and HIV-peptide/dendrimer complexes, the monolayer technique was used. The popular approach to describe a monolayer system is to analyze its surface pressure–area isotherm [35]. Monolayer compression leads to changes in the lipid packing, which can be measured by the surface pressure of the film [36]. We prepared monolayers composed of a DMPC or DMPC/DPPG mixture (9:1) *w/w* at the air-water interface. Na-phosphate buffer (10 mM, pH 7.4) was used as a subphase. The surface pressure of monolayers was set at 30 mN/m to correspond with the condensed state of the membrane.

Changes in surface pressure with or without peptides/peptide complexes and dendrimers are shown in Fig. 5. A significant increase in surface pressure occurred in all cases of lipid formulations treated with dendriplexes, but not with peptides. Dendriplexes formed using CBD-OS dendrimers induced more visible changes in surface pressure than dendriplexes with CBD-CS dendrimers (Fig. 5). Dendriplexes with CBD-OS interacted more strongly with uncharged than negative monolayers.

These results indicate that analyzed dendriplexes interact with DMPC and DMPC/DPPG lipid monolayers. Most probably the dendriplexes become incorporated into the polar domain of the monolayer and caused its additional condensation, which is the main reason for an increase surface pressure. This is supported by data from fluorescence anisotropy. Uncomplexed peptides interacted weakly with lipid monolayers, which agrees with the weak fluidity changes in the fluorescence measurements.

3.4. The morphology of liposome–dendriplex complexes studied by TEM

The effect of dendriplexes on liposome morphology was examined by TEM. Due to similarities in the peptides and dendriplexes used, we

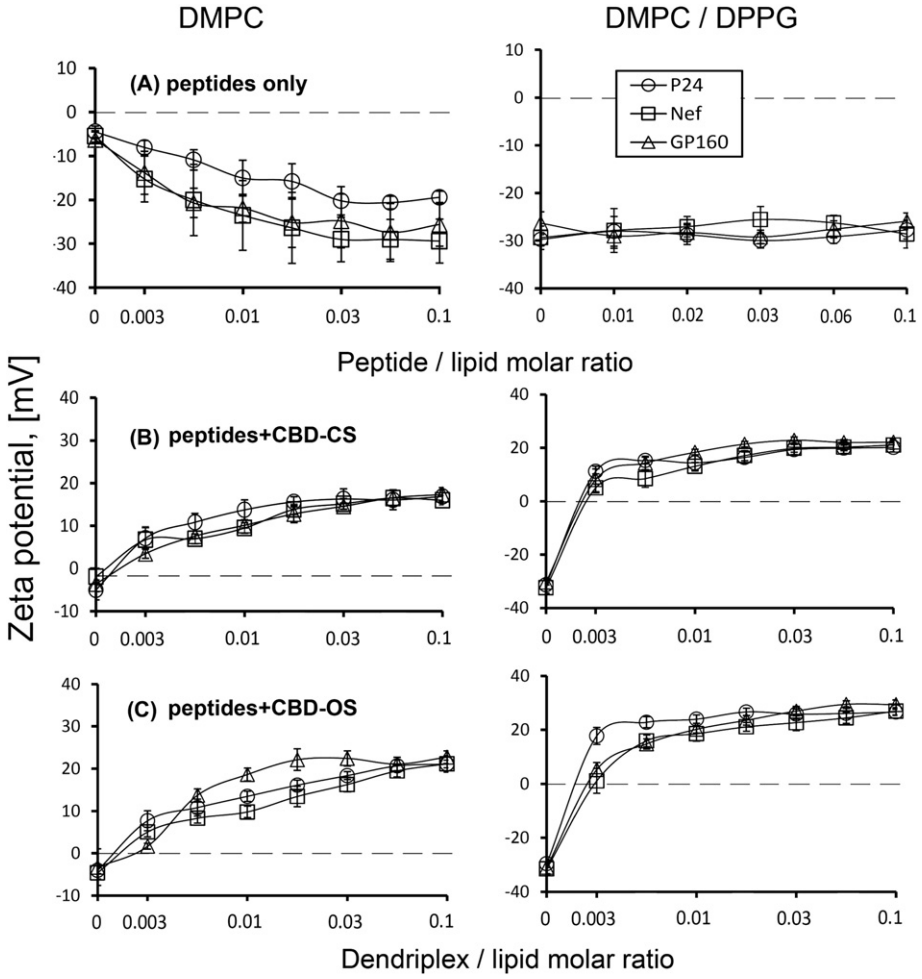

Fig. 4. Zeta potential of DMPC (left panels) and DMPC/DPPG (right panels) LUVs exposed to HIV-derived peptides (A) and its complexes with carbosilane dendrimers, CBD-CS (B); CBD-OS (C).

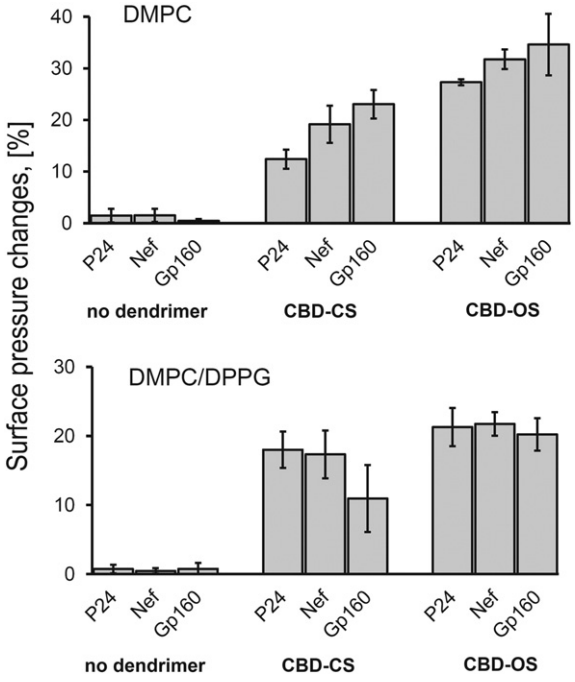

Fig. 5. Changes of surface pressure of monolayers formed by DMPC and DMPC/DPPG lipid systems in the presence of peptides or peptide/dendrimer complexes at 0.25 μM in the water subphase.

chose only one dendrimer (CBD-CS) and one peptide (P24) for detailed study of the morphology of dendriplexes and their complexes with lipid vesicles. The structures of complexed (lower panel) and non-complexed (upper panel) lipid vesicles shown in Fig. 6 indicate the ability of dendriplexes to interact with lipid membranes.

In all cases the size of liposomes varied from 100 to 160 nm, confirming the correctness of vesicles isolation. DMPC and DMPC/DPPG liposomes incubated with peptide/dendrimer complexes revealed large structural variations that were of different shape and morphology compared with untreated liposomes, which suggests that dendriplexes interact with both types of liposome formulation. In the case of DMPC/DPPG liposomes, all dendriplexes were complexed with LUVs, whereas the liposomes composed of DMPC and the peptide/dendrimer complexes not attached to LUVs can clearly be seen (Fig. 6). These findings confirm the results obtained by fluorescence and zeta techniques, and add further evidence for the stronger interaction of dendriplexes with the negatively charged lipid membranes, which can be explained by the electrostatic nature of interaction between dendriplexes and lipid membrane.

By means of steady-state fluorescence anisotropy method we showed that interaction of both CBDs with neutral and negatively charged phospholipids resulted in an increase of fluorescence anisotropy of the probes localized in hydrophobic and polar parts of the bilayer. This has been explained by dendrimer-induced ordering of the lipid bilayer. However, CBD-CS interacted more strongly with hydrophobic part and weaker with hydrophilic part of bilayers, while opposite tendency was observed for CBD-OS dendrimers. Both dendrimers interacted more strongly with negatively charged liposomes in

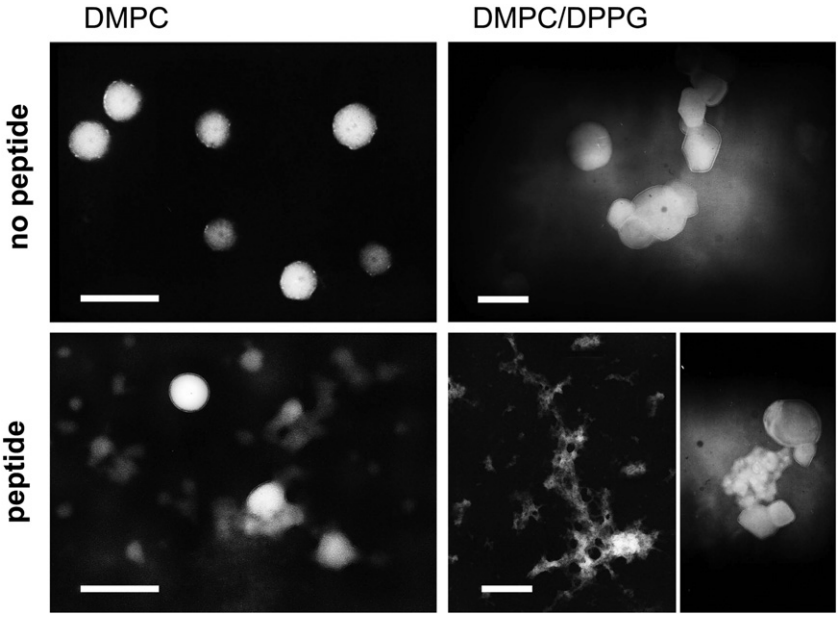

Fig. 6. Electron micrographs of DMPC or DMPC/DPPG lipid vesicles (upper panel) and their mixture with peptide/dendrimer complex [p24/CBD-CS] peptide/lipid ratio 0.02 (lower panel). Dendriplexes were formed in 10 mM Na-phosphate buffer at pH 7.4 and immediately mixed with liposome suspensions. Magnification 100,000×; bar = 200 nm. To obtain greater contrast, the color of the microphotographs has been reversed.

comparison with neutral ones. These data correlated well with experiments on lipid monolayers. CBD-OS dendrimers increased more strongly surface pressure of monolayers in comparison with CBD-CS. It has been suggested that cationic CBD can penetrate into lipid monolayers or create ordered domains at the surface of lipid layer. The possibility of penetration of CBD-CS inside the bilayer has been supported also by NMR data, which revealed interaction of CBD-CS with acyl chains of phospholipids. It has been suggested that dendrimer core can also be involved in these interactions. The mechanisms of CBD–membrane interactions are, however, not fully understood yet [17]. Further, we studied also interaction of CBD modified by siRNA with unilamellar vesicles composed of DMPC or a mixture of DMPC and DPPG as well as DPPG alone. Similarly to naked CBD those modified by siRNA interacted more strongly with negatively charged vesicles. This interaction has been accompanied by increase of average size of vesicles, which indicate formation of larger aggregates. The measurement of zeta potential, however, showed different effect of CBD-CS and CBD-OS on the vesicle properties. While CBD-CS–siRNA complexes resulted in a decrease of zeta potential to more negative values, the complexes based on CBD-OS exhibited an increase of zeta potential to more positive values [7]. A possible explanation of this effect is due to different localization of dendrimers in lipid bilayers. CBD-CS dendrimers are incorporated more deeply into lipid bilayer interior, while CBD-OS preferably interact with polar part of the membrane. This results in different localization of siRNA (see Ionov et al. 2012 for detailed explanation). The obtained results indicate that CBD has great potential for siRNA delivery. In analogy with above mentioned studies, we propose that CBD can be effective carriers also for delivery of selected HIV-1 peptides to DCs.

According to Scopus database, our work is the first study of the interaction of carbosilane dendrimer–short peptide complexes with model lipid membranes. Recently there was, however, attempt to use molecular dynamic simulation method to study the mechanisms of interaction of the dendrimer-short peptide complexes with lipid membranes. The peptide consisted of three histidines (H) with the pH-sensitive property and an arginine (R). It has been shown that dendrimers are adsorbed or penetrate through the negatively charged, asymmetrical bilayer depending on the pH environment [37]. These model studies should be considered as an additional approvement of the strategy indicated in this work on the high perspective of peptide based dendriplexes as possible carriers of nanostructured vaccines.

4. Conclusions

We have demonstrated that HIV–peptides complexed with carbosilane dendrimers interact with model lipid membrane. The results give an example of the importance of electrostatic interactions between peptide/polymer complexes and lipid systems. The strong interaction of dendriplexes with negatively charged lipid membranes is due to electrostatic interaction of positively charged dendriplexes and negatively charged lipids, whereas interaction of dendriplexes with vesicles composed of zwitterionic lipids is due to disturbances in the hydrophobic domain of the membrane. The results obtained suggest that the carbosilane dendrimers studied can be considered as carriers for delivering HIV-peptides to target cells.

Conflict of interest

The authors do not have commercial or other associations that might pose a conflict of interest.

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