Interaction of cationic phosphorus dendrimers with lipid membranes

ABSTRACT

Large unilamellar liposomes and multilamellar vesicles consisting of DMPC interacted with cationic phosphorus-containing dendrimers CPDs G3 and G4. DSC and ζ-potential measurements have shown that liposomal-dendrimeric molecular recognition occurs due to the interaction between the complementary surface groups. Calorimetric studies indicate that the enthalpy of the transition of the lipids that interact with CPDs is dependent on the dendrimers generation. These results can be used in order to rationally design mixed modulatory liposomal locked-in dendrimeric, drug delivery nano systems.

Keywords: Dendrimer; Lipid membrane; Dendrimer/membrane interaction.

INTRODUCTION

Dendrimers represent the fourth and most recent category of macromolecular architecture [1]. Unlike linear polymers they have a well-defined structure that leads to low MW polydispersity index values. Dendrimers have attracted much interest since their discovery due to the specific structure which makes them suitable for a variety of biomedical applications [2-5]. They are small in size, while their low polydispersity can contribute to the reproducibility of their pharmacokinetic behavior [6]. The use of dendrimers as modulators of the release rate of a drug incorporated into liposomes and the possible alterations of the drug bioavailability seems to be an attractive field for research [7]. In the present work, we especially focus on interactions between dendrimers and model lipid membranes. The findings from this study could prove helpful to rationally design new advanced liposomal drug delivery systems for bioactive molecules by combining dendrimeric and liposomal technologies. We tested cationic phosphorus containing dendrimers for lipid membranes interaction.
CPDs differ from the molecules described previously by Supattapone et al., [8]. They have a hydrophilic surface and a hydrophobic backbone which allows for efficient membrane penetration [9]. Here we show that CPDs were able to change the properties of DMPC lipid membranes.

EXPERIMENTAL

Materials

Phosphorus dendrimers were synthesized by the Laboratoire de Chimie de Coordination du CNRS [10,11]. The main characteristics and synthesis of CPDs were described earlier [12-14] CPDs - G3, C_{62}H_{110}N_{18}Cl_{18}O_{42}P_{45}S_{42} (generation 3, 48 surface cationic end groups, Mw: 16280; diameter: 4.1 nm) and CPDs - G4, C_{1296}H_{2256}N_{375}Cl_{96}O_{90}P_{93}S_{90} (generation 4, 96 surface cationic end groups, Mw: 33702; diameter: 5 nm), [10,11] are presented on Figure 1. Phospholipid: 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), was purchased from Avanti Polar Lipids and used without further purification. All other reagents used were of analytical grade and purchased from Sigma-Aldrich Chemical Company.

Differential scanning calorimetry (DSC)

The DSC method was chosen in order to investigate changes in lipid membranes thermal properties upon the interaction with dendrimers. All scans were performed on a DSC 822e calorimeter (Mettler Toledo, Switzerland) calibrated using pure indium (T_m = 156.6 °C). The appropriate amounts of DMPC and dendrimers CPDs-G3 or G4 were co-dissolved in chloroform–methanol (2:1, v/v). The solvent was evaporated under a stream of nitrogen and then samples were placed under vacuum to remove any traces of solvent. 2-3 mg of dried residue was accurately weighed in aluminum crucibles of 40 μl capacity. 10 mM Hepes, pH-7.4 buffer was added to the dry lipid/dendrimer film and hydrated to give a ratio of sample/buffer, 1:10 (w/v). An empty pan was used as a reference [15]. Enthalpies and characteristic temperatures were calculated using Mettler-Toledo STARe software.

Liposome preparation

Large unilamellar vesicles (LUV) composed of DMPC were prepared using the thin-film hydration method. Briefly, appropriate amounts of lipid solutions in chloroform were placed in a round bottom flask and the thin lipidic film was formed by slow removal of the solvent under argon atmosphere. The remaining solvent traces were removed under 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, allowed to equilibrate for 30 min, under argon 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 1000-μl Hamilton gastight syringes. Exposure to light was minimized throughout the liposome preparation process [16].

Measurement of vesicle size and zeta (ζ) potential

The vesicle size distribution (z-average mean) and ζ-potential of empty and dendrimer loaded liposomes were measured at room temperature using dynamic light scattering in a photon correlation spectrometer (Zetasizer Nano, Malvern Instruments, Malvern UK) based on photon correlation spectroscopy [17]. The data were analysed using Malvern software. As the sizes of liposomes were always large enough compared with the Debye–Hückel parameters, the ζ – potentials were calculated directly from the Helmholtz–Smoluchowski equation (by the zetasizer).
RESULTS AND DISCUSSION

The DSC heating parameters of DMPC dispersions (lipid bilayers) in Hepes buffer 10 mM pH 7.4 containing increasing concentrations of CPDs, obtained at a scan rate of 2 °C/min, are given in Table 1. DMPC lipid bilayers exhibit two endothermic transitions upon heating: the pre-transition (PT) and the chain melting main transition (MT) [18].

The pretransition (PT) is observed at 15.56°C and the enthalpy, ΔH, associated with this transition is 3.04 kJ/mol. The chain melting transition is observed at temperature Tm = 24.71 °C and the enthalpy, ΔH, associated with it is 22.87 kJ/mol. The values of these thermal parameters are in a good agreement with those reported in the literature [18,19].

We previously reported [16] that phosphorus cationic dendrimers interact weakly with neutral DMPC membranes for dendrimer:lipid ratios from 2 to 20% (mol). In present DSC studies dendrimers were incorporated into membranes during preparation of samples (see experimental section for details). Table 1, shows that the thermally-induced transition of membrane lipids is affected by the presence of dendrimers (DSC peaks are shifted towards lower enthalpies) and this perturbation is concentration-dependent. With increased dendrimer: lipid ratios from 0.1 to 1% (mol) both CPDs G3 and G4 induced changes in a pretransition peak. For a concentration of 0.3 mol% following values were found: ΔH was changed from 3.04 ± 0.02 to 0.33 ± 0.08 kJ/mol-1 for CPD G3 and from 3.04 ± 0.02 to 0.45 ± 0.22 kJ/mol-1 for CPD G4.

Melting temperatures Tm were changed from 15.56 ± 0.07 oC to 12.12 ± 0.01 oC for CPD G3 and from 15.56 ± 0.07 to 12.48 ± 0.16 for CPD G4. The half width of the peaks ½ΔTp changed from 1.99 ± 0.06 oC to 1.46 ± 0.01 oC for CPD G3 and from 1.99 ± 0.06 to 1.60± 0.34 for CPD G4. The pretransition peak was fully abolished for dendrimer:lipid of 1% (mol) for CPD G4 and for dendrimer:lipid of 0.5% (mol) for CPD G3 (Table 1). This suggests an interaction of dendrimers with the DMPC membranes polar head groups.

Thermal analysis of the main transition peaks in the presence of CPD G3 and CPD G4 (Table 1) shows that Tm decreased by about 5.37 °C for CPD G3 and about 3.48 °C for CPD G4 (dendrimer:lipid ratio 1 mol%) in comparison with lipid blank curve. Main transition enthalpy ΔH decreased by about 15.51 kJ/mol for CPDs G3 and 8.58 kJ/mol for CPD G4. The half width of the peaks ½ΔTp increased by about 3.15 oC for CPD G3 and 1.57 oC for CPD G4 (for the same concentration, 1 mol%) in respect to the corresponding values of the pure DMPC lipid bilayers. For lipid:dendrimer ratio of 1% (mol) the main transition peak was fully abolished for both dendrimers, (Table 1).
The effect of cationic CPDs G3 and G4 dendrimers on zeta potential of DMPC liposomes is presented in Table 2. The zeta potentials of the control liposomes (without additives) were 1.13 ± 0.18 - 2.06 ± 0.21 mV. When dendrimers were present in dendrimer/lipid, molar ratios ranging from 0 to 65%, both formulations exhibited higher and positive ζ-potential values, ranging from 1.13 ± 0.18 to 29.64 ± 0.50 mV (CPD G3) and from 2.06 ± 0.21 to 42.25 ± 0.22 mV (CPD G4). The increase of ζ-potential was dependent on dendrimers concentrations.

Table 2. Changes of DMPC liposomal ζ-potential values following interaction with CPD G3 and CPD G4.

<table>
<thead>
<tr>
<th>CPD/DMPC (mol%)</th>
<th>CPD G3</th>
<th>CPD G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.13±0.18</td>
<td>2.07±0.20</td>
</tr>
<tr>
<td>1.96</td>
<td>3.72±0.57</td>
<td>5.73±0.49</td>
</tr>
<tr>
<td>9.09</td>
<td>4.82±0.67</td>
<td>6.9±0.28</td>
</tr>
<tr>
<td>16.6</td>
<td>5.75±0.72</td>
<td>12.72±1.46</td>
</tr>
<tr>
<td>28.6</td>
<td>10.26±0.55</td>
<td>17.07±0.42</td>
</tr>
<tr>
<td>44.4</td>
<td>15.52±0.24</td>
<td>24.55±0.83</td>
</tr>
<tr>
<td>54.5</td>
<td>20.23±0.51</td>
<td>25.66±1.31</td>
</tr>
<tr>
<td>66</td>
<td>29.64±0.50</td>
<td>42.25±0.21</td>
</tr>
</tbody>
</table>

Dendrimer-vesicle complexes were produced by incubating dendrimers and LUVs in corresponding lipid/CPD molar ratios. All measurements were carried out using 10 mM PBS (pH-7.4). Results represent mean±SEM obtained from 3 independent experiments.
CONCLUSION

The interaction of cationic phosphorus dendrimers of generation 3 and 4 – with model lipid bilayers consisting of DMPC and dispersed in aqueous Hepes buffer solution (10 mM, pH 7.4) was studied with DSC and ζ - size/potential techniques in order to rationally design advanced drug delivery systems, to estimate the interaction of CPDs with human cells and to reveal the difference between interactions of different CPDs generations (G3 and G4). Calorimetric studies indicate that the effect of CPD G3 and CPD G4 dendrimers on the DMPC bilayer was different. In the presence of dendrimers (from 0.1 to 1 mol %) significant changes of the main transition enthalpy and phase transition temperature values were observed. Presence of dendrimers altered the thermotropic behavior of samples in a concentration-dependent manner. Obtained results indicate an increased fluidity of the lipid–dendrimer complexes with rising dendrimer/lipid molar ratios. The decrease in the transition enthalpy corresponds to the relaxation of the rotamers of the hydrocarbon tails of the lipids as a consequence of the insertion of the dendrimers into the membrane.

The effect of the CPD dendrimers on the zeta potential of the liposomes was studied. Upon addition of both dendrimers the liposomes formulations produced positively charged species ranging from 29.64 to 42.25 mV. The results of this study provide information on membrane integrity and physical properties under phosphorus dendrimers action that are essential for the rational design of dendrimer-lipidic drug delivery systems.

ACKNOWLEDGEMENT

Authors thank COST – European Cooperation in Science and Technology for financial support (COST TD0802-05354). Studies were also funded by project “Biological Properties and Biomedical Applications of Dendrimers” operated within Foundation for Polish Science TEAM programme cofinanced by the European Regional Development Found.

REFERENCES


