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Effect of "helper lipid" on lipoplex electrostatics

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Abstract

Lipoplexes, which are complexes between cationic liposomes (L^+) and nucleic acids, are commonly used as a nucleic acid delivery system in vitro and in vivo. This study aimed to better characterize cationic liposome and lipoplex electrostatics, which seems to play a major role in the formation and the performance of lipoplexes in vitro and in vivo. We characterized lipoplexes based on two commonly used monocationic lipids, DOTAP and DMRIE, and one polycationic lipid, DOSPA—each with and without helper lipid (cholesterol or DOPE). Electrical surface potential (Ψ_0) and surface pH were determined using several surface pH-sensitive fluorophores attached either to a one-chain lipid (4-heptadecyl hydroxycoumarin (C17HC)) or to the primary amino group of the two-chain lipids (1,2-dioleyl-*sn*-glycero-3-phosphoethanolamine-*N*-carboxyfluorescein (CFPE) and 1,2-dioleyl-*sn*-glycero-3-phosphoethanolamine-*N*-7-hydroxycoumarin) (HC-DOPE). Zeta potentials of the DOTAP-based cationic liposomes and lipoplexes were compared with Ψ_0 determined using C17HC. The location and relatively low sensitivity of fluorescein to pH changes explains why CFPE is the least efficient in quantifying the differences between the various cationic liposomes and lipoplexes used in this study. The fact that, for all cationic liposomes studied, those containing DOPE as helper lipid have the least positive Ψ_0 indicates neutralization of the cationic charge by the negatively-charged phosphodiester of the DOPE. Zeta potential is much less positively charged than Ψ_0 determined by C17HC. The electrostatics affects size changes that occurred to the cationic liposomes upon lipoplex formation. The largest size increase (based on static light scattering measurements) for all formulations occurred at DNA^-/L^+ charge ratios 0.5–1. Comparing the use of the one-chain C17HC and the two-chain HC-DOPE for monitoring lipoplex electrostatics reveals that both are suitable, as long as there is no serum (or other lipidic assemblies) present in the medium; in the latter case, only the two-chain HC-DOPE gives reliable results. Increasing NaCl concentrations decrease surface potential. Neutralization by DNA is reduced in a NaCl-concentration-dependent manner.

Keywords: Cationic lipid; DNA; Surface potential; Hydroxycoumarin; Carboxyfluorescein; Ionic strength

Abbreviations: Chol, cholesterol; CFPE, 1,2-dioleyl-*sn*-glycero-3-phosphoethanolamine-*N*-(carboxyfluorescein); C17HC, 4-heptadecyl-7-hydroxycoumarin; DC-Chol, 3β-[*N*-(*N*′,*N*′-dimethyl-aminoethane)-carbamoyl]-cholesterol; DMRIE, *N*-(1-(2,3-dimyristyloxypropyl)-*N*,*N*-dimethyl-(2-hydroxyethyl) ammonium bromide; DNA^-/L^+, mole charge ratio of DNA negatively-charged phosphate to positively-charged lipid; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DOSPA, 2,3-dioleyloxy-*N*-[2(sperminecarboxamido)-ethyl]-*N*,*N*-dimethyl-1-propanaminium trifluoro acetate; DOTAP, *N*-(1-(2,3-dioleoyloxy)propyl)-*N*,*N*,*N*-trimethylammonium chloride; HC-DOPE, 1,2-dioleyl-*sn*-glycero-3-phosphoethanolamine-*N*-(7-hydroxycoumarin); HEPES, *N*-(2-hydroxyethyl)-piperazine-*N*′-(2-ethanesulfonic acid); L^+, positively charged lipid; LUV, large (≥100 nm) unilamellar vesicles; ODN, oligonucleotide; TMADPH, 1-(4-trimethyl-ammoniumphenyl)-6-phenyl-1,3,5-hexatriene; UHV, unsized heterogeneous vesicles

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1. Introduction

Delivery of nucleic acids into live cells in vitro and in vivo has gained increased interest as a novel modality to obtain transgene expression or to specifically inhibit expression of a gene by either antisense oligonucleotides (ODN) or by small inhibitory RNA (siRNA). Intensive efforts have been focused on developing a well-characterized "synthetic" delivery system for nucleic acids, which is efficient and harmless, in order to find alternatives that will overcome the drawbacks of viral delivery [1]. Cationic liposomes that complex with nucleic acids (DNA, ODN, RNA) to form lipoplexes have been shown to be promising candidates [1–3], although they are still much less efficient than viral vectors [4–6]. Improving our understanding of the structure–activity relationships of lipoplexes in gene delivery in cell culture and in vivo may aid in optimizing lipoplex performance.

In many (but not all) cases, the inclusion of "helper" lipids in the cationic liposomes improves lipoplex efficiency [7]. Helper lipids affect lipoplex electrostatics as well as the way the lipid self-assembles (micellar, lamellar, hexagonal, vesicular, etc.), the level of hydration, and DNA secondary and tertiary structure [8–10]. For in vitro transfection a favorable helper lipid is 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), which enables better matching of charge density of the lipid surface to DNA helices, facilitates counterion release from the lipid surface by DNA [11,12], and decreases lipid hydration [9,13]. On the other hand, for in vivo transfection, cholesterol is a much better helper lipid [14–17]. In general, it seems that in order to obtain efficient transfection in vitro [7,18] and in vivo [2,19,20], the lipoplex should remain cationic and should have membrane defects leading to size instability [10,18].

It is now obvious that electrostatics plays a major role in lipoplex formation and in various steps of the transfection process. Lipoplexes are formed spontaneously by electrostatic neutralization. The driving force for such complex formation is the removal of small counterions (cations from the DNA surface and anions from the cationic liposome surface) [14,15]. Counterion release depends on the liposome lipid composition, i.e., the type of cationic lipid and the type of neutral (helper) lipid present in the assembly [12,18], and on the medium composition (reviewed in [2]).

The commonly used measure of electrostatics is zeta potential, which is the electrical potential at the plane of shear of the liposomes or lipoplexes, i.e., the "border" between the fixed and diffuse ion layers [21]. This plane is further away from the plane of the quaternary amino group of the cationic lipid.

Fluorescent probes, which monitor electrical surface potential [22] and are evenly distributed over all liposome lipid bilayer(s), are a better tool to look at the intimate interaction between liposome and nucleic acid, as was demonstrated with the probe 4-heptadecyl-7-hydroxycoumarin (C17HC) previously [11,12,18]. However, C17HC suffers from a major drawback related to being a single-chain amphiphile, being desorbed and equilibrated between all lipid-based assemblies present, including cell membranes and serum and lipoproteins [22]. To overcome this obstacle, we used two additional fluorescent phospholipids containing a fluorophore in their headgroups, 1,2-dioleyl-*sn*-glycero-3-phosphoethanolamine-*N*-(carboxyfluorescein) (CFPE) and 1,2-dioleyl-*sn*-glycero-3-phosphoethanolamine-*N*-(7-hydroxycoumarin) (HC-DOPE). In addition to electrostatics, we also studied lipoplex instability. This was followed by change in degree of exposure of 1-(4-trimethylammoniumphenyl)-6-phenyl)-1,3,5-hexatriene (TMADPH)-labeled cationic liposomes to water, monitoring membrane defects [13].

2. Materials and methods

2.1. Materials

2.1.1. Lipids and fluorescent probes

DOTAP, DOPE, DOPC, and CFPE were purchased from Avanti Polar Lipids (Alabaster, AL). Cholesterol (purity 99%) was obtained from Sigma (St. Louis, MO). DC-Chol was a generous gift of Dr. L. Huang (Department of Pharmacology and Pharmaceutical Science, University of Pittsburgh, Pittsburgh, PA). *N*-(1-(2,3-dimyristoyloxypropyl)-*N*,*N*-dimethyl-(2-hydroxyethyl) ammonium bromide (DMRIE) and 2,3-dioleyloxy-*N*-[2(sperminecarboxamido)-ethyl]-*N*,*N*-dimethyl-1-propanaminium trifluoro acetate (DOSPA) were synthesized by Vical Inc. (San Diego, CA). Fluorescent probes C17HC and TMADPH were purchased from Molecular Probes (Eugene, OR). HC-DOPE was synthesized as described in International Patent Publication #WO 01/59156, 8/16/2001. All lipids and fluorescent probes were $\geq$98% purity based on TLC analysis [23].

All others chemicals were of analytical grade or better.

2.1.2. DNA preparation

An *Escherichia coli* containing the plasmid S16 hGH (size 4.8 kbp) was kindly supplied by Dr. O. Meyuhas of our department [24]. The plasmid S16 hGH was grown and isolated using a QIAGEN Mega Plasmid Kit (QIAGEN, Hilden, Germany). After the plasmid purification, DNA was dissolved in 20 mM HEPES (pH 7.4) with 1 mM EDTA, and its concentration was quantified by organic phosphate determination [25]. This concentration is equivalent to the DNA negative charge concentration [26]. Plasmid purity was determined using agarose gel (1%) electrophoresis [27] and ratio of absorbance at 260 nm to that at 280 nm. Agarose gel (1%) electrophoresis showed that the plasmid DNA was mostly in its supercoiled form (>70%), free of chromosomal DNA or RNA. In the different DNA batches, the A_{260}/A_{280} ratios were about 1.8–1.9, indicating lack of contamination by proteins [28].