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Report "Towards Effective Non-viral Gene Therapy: SAXS Studies on Novel Cationic Lipid-DNA Complexes

Gene therapy refers to the use of nucleic acids as a potential therapeutic treatment of a variety of diseases, including cancer and inherited disorders such as cystic fibrosis and cardiovascular disease. It involves replacing nonfunctional segments of DNA with functional DNA. Free DNA cannot passively and efficiently cross cellular membranes without the assistance of a delivery agent or vector. These facilitation processes are known as transfection and may be viral or nonviral. Unfortunately, positive results using viruses as the vehicle to carry the therapeutic gene have been overshadowed by pathogenic effects and a few deaths. This has given impetus to studies on nonviral deliveries as an alternative and this is the motivation for this experiment.

While promising, cationic lipid-mediated gene delivery can still benefit from improvements in lipid design and lipid-DNA (lipoplex) formulation. The putative mechanism of cellular lipoplex uptake is believed to occur by endocytosis, where the key influential factors are lipoplex size and morphology; lamellar and inverted hexagonal. Lamellar lipoplexes offer superior protection to the DNA cargo, while the inverted hexagonal phase best facilitates endosomal escape. Ideally, the initial lipoplex packaging would have the lamellar phase upon uptake, followed by a phase transition to hexagonal, facilitating cargo release into the cytosol. The cationic lipid structure defines its molecular packing parameter, S, which in turn controls the lipid phase transition. A molar weighted average packing parameter (Smix) for the overall cationic and neutral co-lipid mixture within a lipoplex formulation is predictive of a lamellar ($S<1$) or hexagonal ($S>1$) phase lipoplex.

Aims

Pyridinium-based cationic lipids represent a class of non-viral vectors that have shown promise in gene delivery. The objective of this study was to test the influence of lipid shape on lipoplex phase structure through small-angle x-ray diffraction (SAXD), and correlate shape with transfection efficiency. Lipoplexes co-formulated with pyridinium lipid, (16:0)(11:1) having a calculated shape parameter, S, of 1.08 and the commercial cationic vector, EPC (S=0.94) were predicted to undergo a packing transition from lamellar to hexagonal as the ratio of (16:0)(11:1) / EPC is increased. A fixed amount of neutral co-lipid was employed (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine, DOPE: S=1.01; or cholesterol, Chol: S=1.20). Given that cholesterol is a higher-S lipid than DOPE, the lipoplex phase transition from lamellar to hexagonal was anticipated to occur at a lower ratio of (16:0)(11:1) to EPC.

Methods

Liposomes were prepared from pyridinium-based cationic lipids in combination with EPC and co-lipid, DOPE or cholesterol. Lipoplexes were then formulated by incubating the liposomes with plasmid DNA at various N/P (+/-) molar charge ratios, and subsequently characterized by gel retardation, DNAse I degradation, biocompatibility and β-galactosidase (β-gal) transfection assays using Chinese Hamster Ovarian (CHO-K1) cells. Lastly, lipoplexes at N/P molar charge ratio 3 (only) were analysed by SAXS/SAXRD using the facilities of BM26 (DUBBLE). SAXS/SAXRD data were collected for ??? samples of the liposomes* and lipoplexes**.

Results

The SAXS/SAXRD results revealed that the (16:0)(11:1)/EPC/DOPE-DNA lipoplex formulations underwent a lamellar to hexagonal packing transition when the (16:0)(11:1)/EPC

molar ratio was increased from 1:2 to 1:1, where the Smix increased from 1.01 to 1.04, as anticipated. However, (16:0)(11:1)/EPC/Chol-DNA lipoplex formulations underwent a lamellar to hexagonal transition when the (16:0)(11:1)/EPC molar ratio increased from 1:1 to 2:1; when Smix increased from 1.11 to 1.13. For both DOPE and Chol containing lipoplexes, the greatest transfection was found at (16:0)(11:1) to EPC ratios below 2:1, at an N/P molar charge ratio of 3.

Conclusion

The lamellar to hexagonal packing transition, as determined by SAXRD, for the lipid-DNA lipoplexes composed of the (16:0)(11:1)/EPC mixture occurred as predicted by our Smix calculations when DOPE was employed as co-lipid. The same was not observed when cholesterol was the co-lipid. Finally, superior lipoplex transfection correlates with lamellar packing.

*liposome - a nanosized lipid sphere composed from a phospholipid bilayer enclosing a water droplet, which can carry drugs or other substances into the tissues.
**lipoplex – a complex formed upon combining plasmid DNA a liposome.

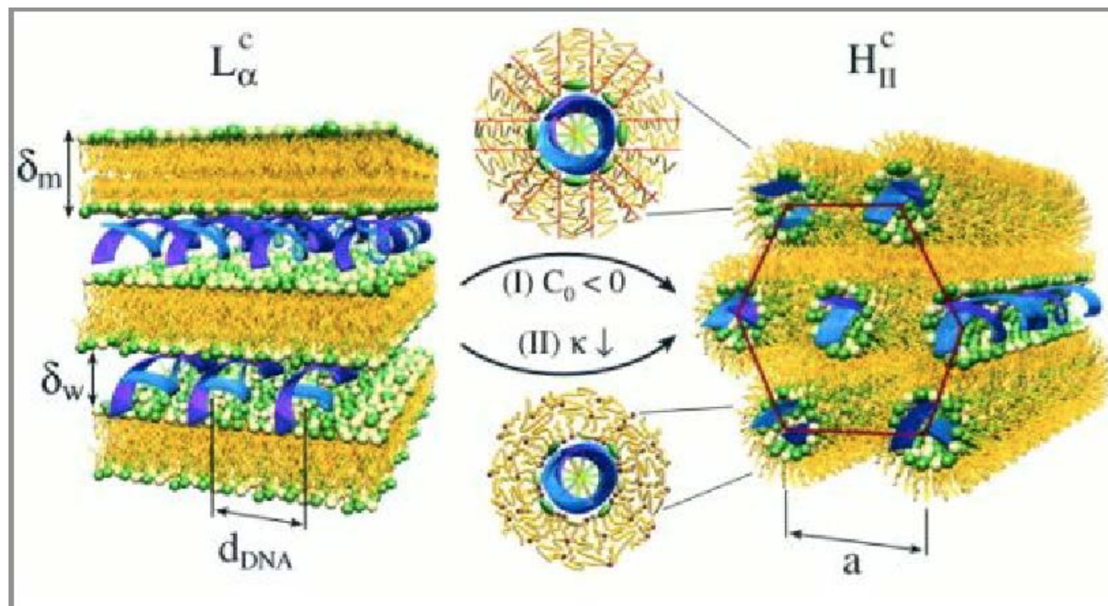

Figure 1 [left] *Conceptual depictions of cationic liposome-DNA complex (lipoplex). DNA helices are blue. The formation of a layered multilamellar lipoplex (left) or a hexagonal lipoplex (right) depends on the type of cationic lipid used (Koltover, I et al Science, 281, 1998, 78.)*