Polymorphism of DNA-anionic Liposome Complexes Reveals Hierarchy of Ion-mediated Interactions

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Gene therapy using either viral or synthetic vectors is currently one of the most promising strategies for developing cures for many hereditary and acquired diseases. Protocols have been approved for cancer, hemophilia, cystic fibrosis, neuromuscular disorders, and others. Although synthetic nonviral systems such as cationic liposomes generally transfect less efficiently than viruses, they have a number of advantages such as high DNA packaging capacity and low immunogenicity. Since their introduction in 1987, cationic lipid-DNA complexes (CL-DNA) have emerged as one of the major non-viral DNA delivery platforms. CL-DNA complexes have been used in gene therapy for a broad range of cell types as well as delivery systems for cancer vaccines.

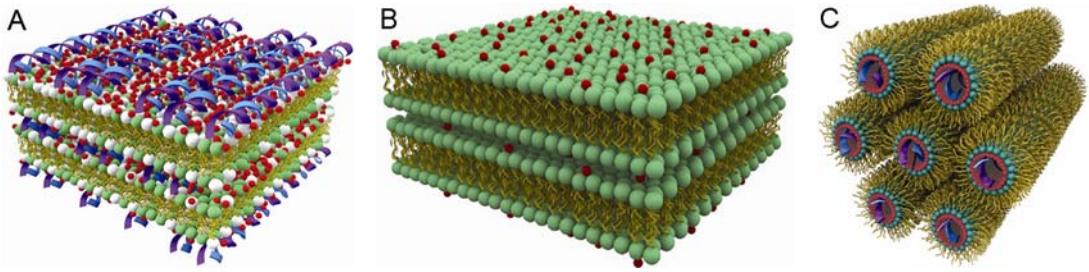
Anionic lipids (AL) occur naturally in eukaryotic cell membranes, and DNA delivery systems based on anionic lipids have recently been examined as an alternative to cationic lipids due to their low cytotoxicity. Anionic lipids can be complexed with anionic DNA via interaction with multivalent cations such as Ca^{2+}, and have been shown to transfer oligonucleotides into hippocampal neurons and bacterial cells. An outstanding problem of this approach is the inefficient association between the anionic lipids and DNA molecules, which is attributed to their like-charge electrostatic repulsion.

Rational design of AL-DNA vectors requires a coherent understanding of their structures and interactions. A useful starting point is the physics governing the analogous CL-DNA complexes that has emerged in the last few years. The addition of DNA to cationic lipid mixtures induces a topological transition from liposomes into condensed multi-lamellar self-assemblies, where parallel DNA chains are confined between lipid sheets. By lowering the membrane's bending rigidity or by changing its spontaneous curvature, an inverted hexagonal phase with an enhanced tendency for membrane fusion can be formed, in which DNA chains coated by lipid monolayers are packed into a 2D columnar hexagonal array. In these self-assembled complexes, the cationic lipid head groups neutralize the phosphate groups on the DNA chains, effectively releasing the counterions previously bound electrostatically to lipids and DNA, thus gaining translational entropy in the bulk. The pioneering studies have shown that physical parameters, such as self-assembled nanostructure and membrane charge density, are crucial elements in transfection efficiency.

In this work, the structure and interactions of AL-DNA complexes in the presence of different divalent cations have been systematically investigated using confocal microscopy and synchrotron small angle X-ray scattering (SAXS) on beam lines 4-2 at SSRL and 34ID at the Advanced Photon Source. AL-DNA complexes are governed by more complex interactions than that for their CL-DNA analogues. While cationic membranes are attracted to DNA mainly via entropic forces due to counterion release, anionic membranes require multivalent cations to mediate attractions to anionic DNA through direct electrostatic "bridging" interactions. Further, the addition of multivalent ions can mediate strong attractions between different combinations of membranes and DNA and induce the formation of not just condensed DNA-membrane complexes, but also potentially condensed membrane complexes and condensed DNA complexes, both of which have no analog in CL-

DNA systems. Finally, divalent cations can also coordinate non-electrostatically with lipid molecules and modify membrane structure.

We find from the SAXS measurements that at low membrane charge densities, AL-DNA complexes self-assemble into a lamellar structure, with alternating layers of like-charged DNA and anionic membranes bound together with divalent cations (A). As the membrane charge density is increased, we find a new phase with no analog in CL-DNA systems: DNA is systematically expelled from the complex, and the divalent ions mediate attractions between anionic membrane sheets to form a lipid lamellar stack (B). Divalent ions differ in their tendency to coordinate non-electrostatically with lipids. Zn^{2+} ions are known to have strong non-electrostatic interactions with lipids, involving significant dehydration of the lipid headgroups, while others such as Mg^{2+} have a much smaller effect. The SAXS data show that as the global Zn^{2+} concentration is increased, both lamellar phases are destabilized. The system instead forms an inverted hexagonal phase, comprised of a hexagonal array of divalent cation coated DNA strands wrapped in turn by anionic membrane monolayers (C). While Zn^{2+} is known to adhere to both lipids and DNA, we suggest that the change in AL-DNA structure is primarily due to a cation-induced change in the membrane spontaneous curvature c_0. Using simple theoretical considerations, we show that the expected cross-over between the lamellar phase and hexagonal phase occurs at a critical c_0 close to the experimentally observed values.



Schematic pictures of (A) Condensed DNA-ion-membrane lamellar structure with alternating layers of DNA and anionic membranes 'glued' together by divalent cations; (B) Condensed ion-membrane lamellar structure in which charged membranes stacks are held together by divalent cations; and (C) 2-D inverted hexagonal structure in which hexagonal arrays of divalent cations coated DNA strands wrapped in the anionic membrane monolayer tubes.

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