

**ABOUT NON-BILAYER PHASES IN THE MECHANISM OF
LIPOPLEX-MEDIATED GENE DELIVERY**

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We have applied cationic liposomes, consisting of an equimolar mixture of pyridinium amphiphile SAINT-2 and the phospholipid DOPE, as carriers for oligonucleotides (ODNs) and plasmids, and investigated the mechanism of lipoplex-mediated gene and ODN delivery *in vitro*. Cryo-electron microscopy and small angle X-ray scattering revealed that PEG-lipids stabilize the lamellar phase of the lipoplexes. As a result, the internalized lipoplexes, including their cargo, remain trapped in the endosomal/lysosomal pathway. Acyl chain-length dependent transfer of the PEGylated lipid derivatives from the complex enables adaptation of the hexagonal phase. This is imperative for release of ODNs and plasmids across the endosomal membrane into the cytosol, and nuclear homing. Thus delivery resumed when the complexes had been coated with exchangeable PEG-lipid derivatives, the kinetics of which was dependent on the length of the acyl chains (PEG-ceramide and SAINT-PEGs).

To investigate the significance of DOPE's preference to adopt (in isolation) a hexagonal phase in the overall mechanism of lipoplex-mediated transfection, the properties and transfection efficiencies of mixed cationic lipid SAINT-2/DOPE lipoplexes were compared to those containing lamellar phase forming dipalmitoylphosphatidyl-ethanolamine (DPPE). Following an interaction with anionic vesicles, to simulate lipoplex-endosomal membrane interaction, SAINT-2/DOPE lipoplexes show a perfect hexagonal phase, whereas pure lamellar phase SAINT-2/DPPE lipoplexes form a mixed lamellar-hexagonal phase upon such interactions. This transition to the hexagonal phase is crucial for anionic lipid-induced dissociation of DNA or oligonucleotides from the lipoplexes, as emphasized by the absence of significant release when such a transition is frustrated at a variety of conditions. However, the efficiency of nucleic acid release from either complex does not correlate with the two-three-fold higher transfection efficiency or nuclear delivery of ODNs observed when SAINT-2/DOPE lipoplexes were incubated with cells. Interestingly, rupture of endosomes following a cellular incubation with ODN-containing SAINT-2/DPPE complexes dramatically improved nuclear ODN delivery up to a level, which was similar to that observed for SAINT-2/DOPE complexes. Our data demonstrate that although formation of the hexagonal phase in lipoplexes is a prerequisite for nucleic acid release from the complex, it appears highly critical

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for accomplishing efficient translocation of nucleic acids across the endosomal membrane into the cytosol for transport to the nucleus.

Delivered antisense ODNs bind extensively to the RNA matrix in the cell nuclei, thereby interacting with target mRNA and causing its subsequent degradation. The latter is essential in downregulating receptor expression and functional impediment of the receptor, as revealed by studies of the expression and function of the 5-HT_{1A} receptor in neuronal cells.