	Experiment title: Structure and supramolecular organization of a new class of self-assembled Liposome-DMA-Me^{2+} complexes for gene transfer.	**Experiment number**: SC2283
Beamline: ID02	**Date of experiment**: from: 14/09/2007 to: 17/09/2007	**Date of report**: 19/03/2008
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Report:

The purpose of gene therapy (GT) is the transfer of extracellular genetic material into appropriate cells of a patient, providing therapeutic effects. Realization of the full potential of the GT will depend, in a major way, on the future development of safe and efficient gene delivery reagents. The final goal of the present research is the development of vectors that lead to a significant increase of the transfection efficiency.Within this frame, in recent years we have extensively studied as potential transfecting agents a new class of complexes formed by the *self-assembled* association of neutral liposomes (Ls), DNA and bivalent metal cations in water solutions.[1-7] Complexes composed exclusively of neutral (zwitterionic) lipids offer an alternative to most widely used cationic lipsomes (CLs), in that they exhibit lower inherent cytotoxicity and much longer circulation lifetimes.

It is well known that transfection process is enhanced by positively charged complexes because this promotes adhesive interaction with the cell's plasma membrane[8] To this purpose, in this experiment we have carried out an extensive x-ray diffraction (XRD) study focused to characterize the nanoscale structure of a new class of lipids derived from diacylglicerol having have a crown ether on the hydrophilic head, with ifferent size and linkage to hydrophobic portion. This kind of molecules has been demonstrated to be able to catch metal ion in aqueous solution when included in a lipid membrane.[9]

We have studied a series of mixtures of dioleoylphosphatidylcholine (DOPC) or dioleoylphosphatidylethenolamine (DOPE) and lipids (VF10-VF20-VF22) having different crown ether on the hydrophylic headgroup. VF10 is a dyacylglicerol structure based on lipid with oleic acid in the fatty acid portion and having an ethyloxymethyl-12-crown-4 mojety in the hydrophylic portion of the molecule; VF20 is a cholesterol-based lipid leading in position 3 of its skeleton a O-[2-(2-pyridil-carbonilamino)-phenylaminocarbonyl] residue as chelating mojety and VF22 is a bis-2-picolylamide of malonic acid featured by a satured linear C18 chain in 2-position.These mixtures have been investigated both as pure lipids in aquesous dispersion and in water solutions of metal (II) cloride and DNA.

In Figure 1 we show a representative example of the XRD results obtained from a preliminary elaboration of the experimental data. Mixtures of DOPC and VF10 in $MnCl_2$ aqueous solution have proved to complex DNA giving rise to supramolecular aggregates showing lamellar and cubic phase. Similar structures have been observed by our group in recent mixtures of [DOPE-PEG(350)/DOPE]-DNA-Ca^{2+}. In particular, we have found that metal cations (Mn^{2+} or Ca^{2+}) in aqueous dispersions of mixtures of dioleoylphosphatidylethanolamine (DOPE) and poly(ethyleneglycol)-functionalized DOPE (DOPE-PEG(350)) induce, above a certain amount of the PEG-lipid component, a phase transition from the inverted hexagonal phase H_{II} to the bicontinuous inverted cubic phase Q^{224} with space group *Pn3m*. [10] We observed this cubic phase in the presence of DNA.

Cubic phases, which are much rarer than lamellar phases, play an important role in membrane fusion, control of functions of membrane proteins, ultrastructural organization inside cells, and crystallization of the membrane proteins. Owing to their 3D nanostructure with hydrophobic and hydrophilic domains, cubic LC phases also find application in pharmaceutical drug delivery and exhibit great potential for controlled drug release applications.[11]

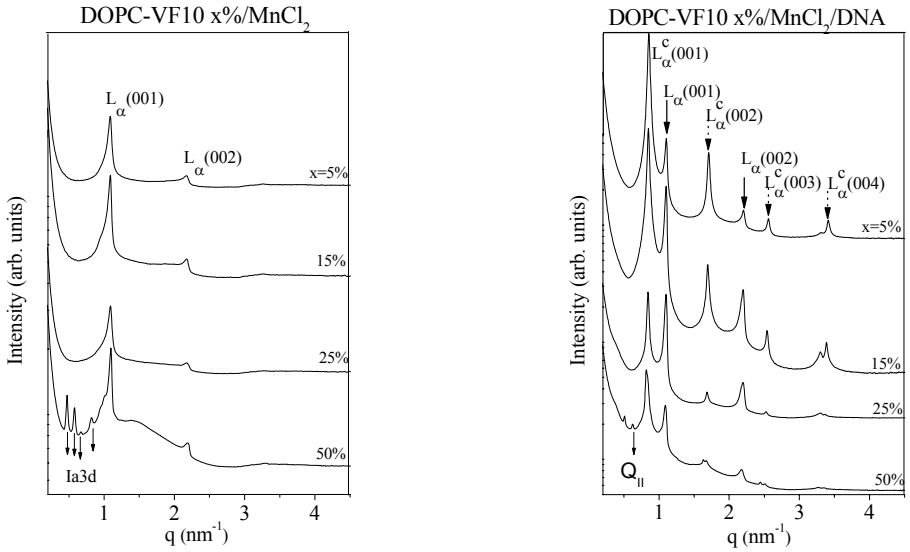

Figure 1. XRD patterns of aqueous suspension of DOPC/VF10-DNA-$MnCl_2$ complexes at various amounts of the synthetic vector VF10. (A) Lipids in $MnCl_2$ aqueous solution. (B) Solutions of lipids, DNA and $MnCl_2$. L_α and L_α^c are the lamellar structures of the lipid and the complex respectively. Q_{II} is the bicontinuous cubic phase

We are presently studying the structure and mesomorphic behavior of the supramolecolar aggregates that are formed in a self-assembled fashion when aqueous suspension of the synthesized lipids (pure or in association with helper) are properly mixed with DNA and aqueous solution of metal (II) chloride. The next step will consist of probing the ability of these complexes as DNA delivery agents both *in vitro* and *in vivo* transfection tests carried out on appropriate cell lines. The goal is to create a useful alternative to the actually used synthetic vectors.

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