ESRF	**Experiment title:** High-resolution structural study of self-assembled Liposome-DNA-Metal Complexes	**Experiment number**: SC-1504
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Report:

The cationic liposomes (CL) complexed with DNA have been shown to be promising nonviral delivery systems for gene therapy applications. This is because they are able to mimic certain characteristics of natural viruses in their ability to act as efficient chemical carriers of extracellular DNA across outer cell membranes and nuclear membranes (transfection). The use of nonviral rather than viral methods for gene delivery has several advantages, including nonimmunicity and the potential for transferring and expressing (transfecting) large pieces of DNA into cells. In the frame of this wide general interests, we started recently with an extended project focused on the study of the self-assembled association of neutral liposomes (L), DNA and divalent metal cations (Me^{2+}) in ternary L-DNA-Me^{2+} complexes [1-4].

In the first part of this experiment we have developed the study, started with the experiment SC-1139, at higher resolution on different variety of L-DNA-Me^{2+} complexes, i.e L=DLPC, DPPC and Me = Mn^{2+}, Mg^{2+}, Ca^{2+}, Fe^{2+}. The goal of this study is twofold: (*i*) to test whether the fusogenic action of the metal cations is equally active in promoting and stabilizing triple complexes L-DNA-Me^{2+} when different neutral lipids and DNA molecules are used and (*ii*) to test to which extent the structure and phase-symmetry of the pure lipids in water solution reflect into the structure of the self-assembled complexes. The latter is a particularly important aspect connected with structure-transfection efficiency relationships. A systematic series of SAXS measurements on DPPC-DNA-Me^{2+} (3:4:n) complexes prepared from different metal cations (Me^{2+}= Mg^{2+}, Mn^{2+}, Co^{2+}, Fe^{2+}) was performed as a function of the metal ion moles (*n*) and the temperature. The Figure 1 shows the synchrotron XRD patterns of DPPC-DNA-Ca^{2+} triple complex at different molar ratio. In this experiment we sonicated a solution of DNA from calf-thymus for 5 minutes inducing a DNA fragmentation whose length distribution, detected by gel electrophoresis, is about 200 bp. It was possible to observe a broad diffraction peak at q=1.3nm^{-1} with d_{DNA} ~ 48 Å due to the DNA interaxial spacing. The lamellar spacing for $L_{\beta'}^{c}$ is d_c =78 Å and for $L_{\beta'}$ is d = 62 Å . The results show the coexistence between the triple complex with globules of multilamellar vesicles of the neutral lipid in the L_β phase.

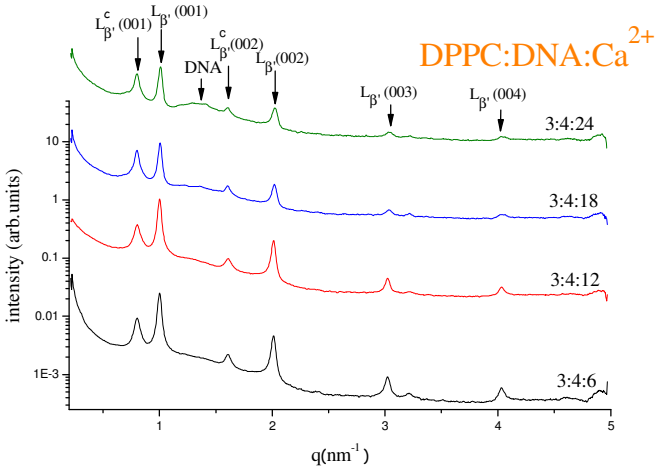
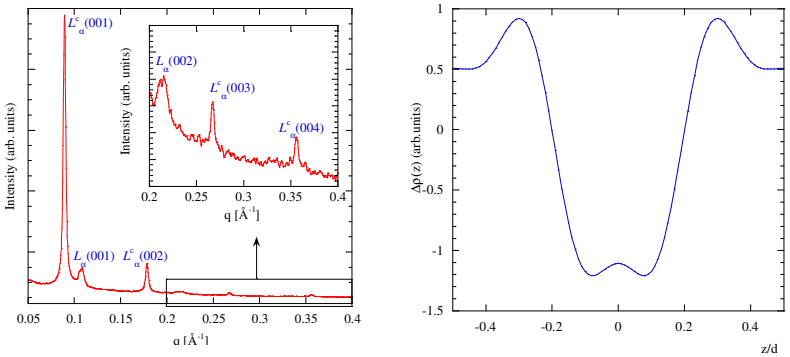


Fig.1

Similar results were observed for the complexes prepared with the neutral lipid DLPC. In Fig. 2 a representative example of the diffraction pattern of DLPC:DNA:Mn^{2+} (3:4:12) is reported (A), together with the electron density profile along the normal to the bilayers (B). The two peaks of electron density correspond to phospholipid headgroups, while the minimum at the centre of the membrane correlates with terminal hydrocarbon chain region. The distance between the centers of the density maxima gives the phosphate-phosphate group d_{HH} = 42.4 Å, the water-layer thickness d_w =*d*- d_{HH} =70.6 - 42.4 =28.2 Å. All the above investigated complexes exhibit the same structure and symmetry of the pure lipid parent phase (i.e. the L_α phase) irrespective of the nature of the cation and DNA. The L_α^c phase of these complexes consists of an ordered multilamellar assembly similar to that found in CL-DNA complexes, where the hydrated DNA helices are sandwiched between the liposome bilayers.



A B

Fig. 2

In the second part of the experiment we carried out a structural study on DOPC-DNA-Me^{2+} complexes using high SAXS resolution in order to evaluate the elastic properties of the membranes. We expect that consensation of DNA within the bilayers reduces the flexibility of the lamellae in the complex and strongly reduces the undulatory fluctuations. The elaboration data is in progress.

References

[1] O. Francescangeli, V. Stanic, L. Gobbi, P. Bruni, M. Incussi, G. Tosi, S. Bernstorff *Physical Review E* **67**, 011904/1 (2003).

[2] O. Francescangeli, M. Pisani, V. Stanic, P. Bruni, M. Iacussi *Recent Res. Devel. Macromol.* **7**, 247 (2003).

[3] O. Francescangeli, M. Pisani, V. Stanic, P. Bruni, T.M. Weiss *Europhysics Letters*, **67**, 4, 15 (2004)

[4] M. Pisani, P. Bruni, Conti, E. Giorgini and O. Francescangeli, in press on *Molecular Crystals Liquid Crystals*