Supramolecular structure and phase behavior of self-assembled liposome-DNA-metal complexes for gene transfer

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Gene therapy is an innovative technique for correcting the defective genes responsible for disease development. The goal is to achieve the transfer of extracellular genetic material into somatic cells and thereby provide therapeutic effects. Realisation of the full potential of gene therapy will depend in a major way on future developments of safe and efficient *nonviral* gene delivery (NGD) agents. Cationic lipid-DNA complexes are presently the most diffuse DNA carriers in NGD applications and are extensively used in clinical trials. However, their transfection efficiency is still relatively low compared to that of viral vectors and they are also unstable in the presence of serum, which creates problems for *in vivo* applications. Furthermore, cationic liposomes are frequently toxic for the cells. These drawbacks are stimulating scientists to look for new *synthetic* gene vectors with a focus on understanding the structure-function relationships, where the ultimate goal is to enable a design-based approach to gene delivery.

We have recently started to study new ternary complexes formed by the self-assembled association of neutral liposomes, DNA and bivalent metal cations in water solution. Complexes composed of neutral lipids offer a promising alternative to cationic liposomes as they exhibit lower inherent cytotoxicity and longer circulation lifetimes. The supramolecular packing of these Liposome-DNA-Metal^{2+} complexes forms lyotropic liquid crystals that display richness of phase behaviour and supramolecular architecture[1-4]. X-ray diffraction (XRD) measurements at ID02 have demonstrated the self-assembled formation of the lamellar $L\alpha$ phase in DOPC-DNA(plasmid)-Metal^{2+} complexes[5-6]. This is a novel liquid crystal phase consisting of the multilamellar aggregation of stacked alternating lipid bilayers and DNA monolayers, which are mutually bound by the metal cations. Biological tests carried out to probe the transfection capacity of these systems have unquestionably demonstrate the capability of these complexes to transfect DNA[5]. More recently, we have started the study of the structure and phase behaviour of ternary water solutions of DOPE/DOPE-PEG mixture, DNA and bivalent metal cations. In fact, liposomes containing lipids with covalently attached PEG have proven to be more effective DNA carriers for gene therapy applications. This is because the presence of grafted PEG creates a steric barrier that inhibits opsonization and prevents the interaction of lipoplexes (lipid-DNA or amphiphile-DNA complexes) with serum proteins and macrophages, therefore prolonging their circulation lifetime in blood. Recent synchrotron XRD measurements performed at ID02 at low concentration of the PEG-lipid component have shown the self-assembled formation of ternary [DOPE/DOPE:PEG(350)]-DNA-M^{2+} complexes that exhibit the inverted hexagonal phase, H_{II}^{c}, in which the DNA strands fill the water space inside the cylindrical cavities of the DOPE/DOPE:PEG(350) hexagonal lattice. This result represents the first experimental evidence of a self-assembled formation of an inverted hexagonal complex structure in aqueous dispersions of DNA, metal cations and liposomes made of mixtures of pure and PEG-lipids. In addition, these experiments have shown that high concentrations of the PEG-lipid component destabilize the inverted hexagonal phase towards either a lamellar phase, in the absence of metal cations, or a novel inverted cubic phase (Q^{224} with space group *Pn3m*), in the presence of metal cations

In this talk a comprehensive overview of the main scientific results obtained on these materials over the last few years will be reported, with a focus on the structural and phase behaviour aspects.

References

[1] O. Francescangeli, V. Staniç, L. Gobbi, P. Bruni, M. Iacussi, G. Tosi, S. Bernstorff, *Phys. Rev. E* **67**, 011904 (2003).
[2] O. Francescangeli, M. Pisani, V. Staniç, P. Bruni, T. Weiss, *Europhys. Lett.* **67**, 669 (2004).
[3] M. Pisani, P. Bruni, G. Caracciolo, R. Caminiti, and O. Francescangeli, *Journal of Physical Chemistry B* **110**, 13203 (2006).
[4] M. Pisani, V. Fino, P. Bruni, E. Di Cola, and O. Francescangeli, *Journal of Physical Chemistry B Letters* **112**, 5276 (2008).
[5] P. Bruni, M. Pisani, A. Amici, C. Marchini, M. Montani, and O. Francescangeli, *Applied Physics Letters* **88**, 073901/1 (2006).
[6] P. Bruni, M. Pisani, A. Amici, C. Marchini , M. Montani, and O. Francescangeli**,** *ESRF Highlights 2006*, edited by G. Adams (Grenoble, France, 2007), 35-37.