Structure and function of self-assembled liposome-DNA-metal complexes for gene delivery

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Introduction

Complexes composed of cationic liposomes (CLs) and DNA exhibit great potential in *gene therapy* (GT), an innovative technique for correcting defective genes responsible for disease development. Realization of the full potential of the GT will depend, in a major way, on the future development of safe and efficient nonviral gene delivery reagents. Cationic lipid-DNA complexes [1] are presently the most diffuse DNA carriers in nonviral gene delivery applications and are extensively used in clinical trials worldwide because of their ability to mimic natural viruses as chemical carriers of extracellular DNA across outer-cell and nuclear membranes (transfection). However, their transfection efficiency is still low compared to that of viral vector and they are unstable in the presence of serum, which creates difficulties for *in vivo* applications. Also, CLs are frequently toxic for the cells. Complexes composed exclusively of neutral (zwitterionic) lipids offer an alternative to CLs, in that they exhibit lower inherent cytotoxicity and much longer circulation lifetimes. These main drawbacks are presently stimulating scientists to look for new synthetic gene vectors with a focus to understand the structure-function relationships, where the ultimate goal is to enable a design-based approach to gene delivery. Within this frame, we have recently started the study of new complexes formed by the self-assembled association of neutral liposomoes (L), DNA and bivalent metal cations in water solutions [2].

Methods

SAXS and WAXS measurements were carried out at the ID02 beamline of the ESRF (Grenoble, France). A monochromatic beam was used (λ=0.995 Å). We investigated the q range 0.04 $Å^{-1}$ - 0.5 $Å^{-1}$ with resolution of $5{\cdot}10^{-3}$ $Å^{-1}$ (FWHM).

Results and Discussion

We have studied L-DNA-Me^{2+} complexes having different microstructures reflecting the structure and phase symmetry of the parent pure lipids, prepared from different neutral liposomes (DOPC, DLPC, DPPC and DOPE), DNA (from calf thymus, salmon sperma and λ-phage) and bivalent metal cations (Mn^{2+}, Mg^{2+}, Ca^{2+}, Co^{2+}, Fe^{2+}). The supramolecular packing forms lyotropic liquid crystals that display richness of phase behaviour and structures [2,3]. We found that the DOPC-DNA-Me^{2+} complexes exhibit a novel liquid-crystalline L_a^c phase consisting of the multilamellar aggregation of stacked alternating lipid bilayers and hydrated DNA monolayers (inset of Fig. 1). More recently, we have demonstrated the self-assembly of the L_a^c phase in the ternary complexes DOPC-DNA-Me^{2+} (Me = Ca, Mn) using supercoiled plasmid (the conformation *physiologicaly active*) instead of linear DNA [4]. Fig.1 shows a representative SAXS pattern of the DOPC-DNA-Me^{2+} complex at 3:4:12 molar ratios. L_a^c and L_a refer to the lamellar structure of the complex and of the unbound lipid, respectively.

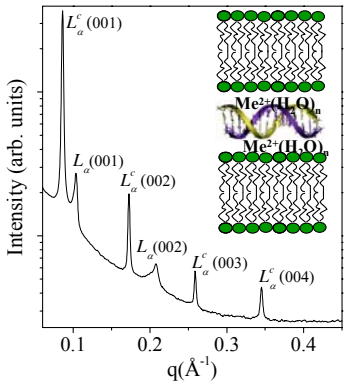

Fig. 1. SAXS pattern of the DOPC-DNA-Mn^{2+} complex. Inset: schematic picture of the lamellar strcture of the L_a^c phase.

Preliminary tests have been carried out to probe the transfer capacity of these systems. We have attempted *in vitro* transfections on mouse fibroplast NIH 3T3 cell lines, using DOPC-DNA(plasmid)-Ca^{2+} as DNA vectors. The results are encouraging since they unquestionably show the capability of these complexes to transfect DNA (Fig. 2) [4].

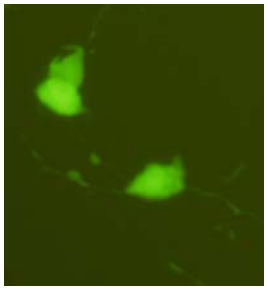

Fig. 2. Fluorescence micrograph of mouse fibroplast NIH 3T3 cell lines transfected with pGreenLantern.

Based on these results, the efficient encapsulation of DNA in these ternary neutral complexes may represent an important alternative to the current systemic gene approaches.

Acknowledgments

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References

[1] J.O. Rädler *at al*, *Science* **275**, 810 (1997).
[2] O. Francescangeli *et al*., *Phys. Rev. E* **67**, 011904 (2003).
[3] O. Francescangeli *et al*. *Europhys. Lett*. **67**, 669 (2004).
[4] P. Bruni *et al*., *Appl. Phys. Lett*. **88**, 073901(2006).