Available online at www.sciencedirect.com
SCIENCE DIRECT®
Chemical Physics Letters 366 (2002) 200–204
CHEMICAL PHYSICS LETTERS
www.elsevier.com/locate/cplett

A new approach for the study of cationic lipid–DNA complexes by energy dispersive X-ray diffraction

G. Caracciolo [a], R. Caminiti [b,*], F. Natali [c], A. Congiu Castellano [a]

[a] *Dipartimento di Fisica and INFM, Università 'La Sapienza', IT-00185 Rome, Italy*
[b] *Dipartimento di Chimica and INFM, Università 'La Sapienza', IT-00185 Rome, Italy*
[c] *INFM-OGG, Institute Laue-Langevin, 38042 Grenoble Cedex 9, France*

Received 31 May 2002; in final form 6 September 2002

Abstract

X-ray diffraction (XRD) studies on the cationic liposomes (CL)–DNA complexes currently used in gene delivery have provided detailed structural informations on these compact, ordered self-assemblies sheding light on the poorly understood structure–activity relationship. Among these, the experiments carried out by using a synchrotron radiation source have showed an experimental resolution remarkably better than that achieved one by traditional in house apparatuses. Here we show a new experimental approach for the study of CL–DNA complexes, based on the employment of silicon wafers as substrates, which allows to obtain high-resolution structural informations by energy dispersive X-ray diffraction (EDXD).

1. Introduction

Cationic lipid–DNA complexes (lipoplexes) are currently known as the most useful carriers of genes in nonviral gene delivery applications and are extensively used in clinical trials worldwide [23]. Although the transfection efficiencies of CL–DNA vectors are lower than that achieved with virus-based strategies, they are less immunogenic and have a significant ability to package large transgenes.

The addition of linear DNA to a binary mixtures of suitable cationic and neutral lipids leads to a topological transition: liposomes and DNA condensates into optically birefringent liquid-crystalline globules as shown by polarized microscopy. These macromolecular self-assemblies can deliver extracellular DNA into the cells by an electrostatic binding to their anionic surface. The key role is played by the cationic lipids which serve as the condensing agents of the negatively charged DNA [2]. Anyway, it is well established that also important are the neutral helper lipids (HL) [3] which determine the morphologies of the composite condensates [4] and significantly affect the DNA transfection efficiency [5]. However, even if the transfection efficiency of these systems is

* Corresponding author. Fax: +39-06-490631.
E-mail address: r.caminiti@caspur.it (R. Caminiti).

0009-2614/02/$ - see front matter
PII: S0009-2614(02)01454-9

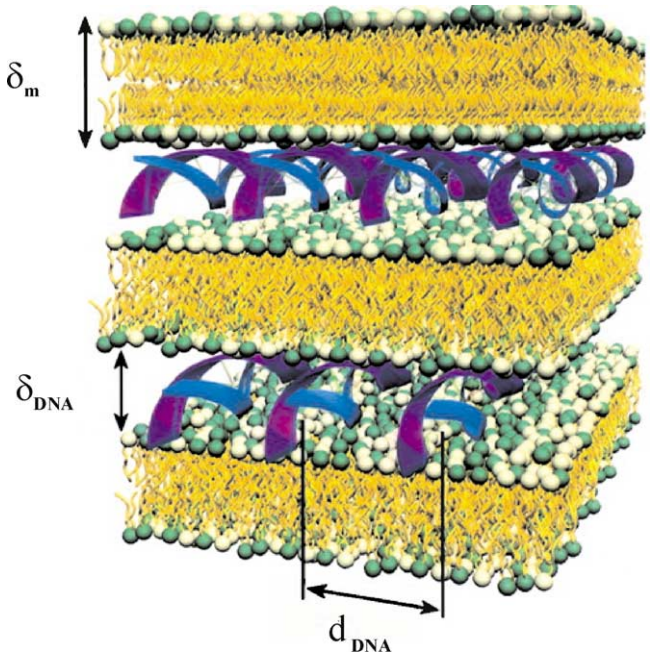

Fig. 1. Lamellar L_{α}^{c} phase of CL–DNA complexes. It is a periodic multilayer structure with DNA adsorbed between cationic membranes. The repeat distance is $d = \delta_{m} + \delta_{DNA}$ whereas the interaxial distance is d_{DNA}. Adapted from [I. Koltover, T. Salditt, J.O. Radler, C.R. Safinya, Science 281 (1998) 78].

influenced by many physico-chemical properties [6], it is well recognized that the final structure of the CL–DNA complexes modulate their biological activity. In addition, it is strongly believed that the supramolecular structure is critical to the mechanism of how cationic liposomes promote intracellular delivery of DNA and the systematic knowledge of their structure–activity relationship has been the subject of many theoretical and experimental studies. Anyway, despite the extensive work of the last years, it remains a crucial issue that needs to be investigated. Starting from the firstly hypothesized 'bead on string' structure [7], a considerable breakthrough towards determining the supramolecular order of the self-assembled lipoplexes was provided by synchrotron X-ray diffraction studies [8] unambiguously revealing the existence of two different phases: the more commonly occuring self-assembly is a multilayer structure (L_{α}^{c}) comprising DNA monolayers coated between lipid bilayers (Fig. 1; adapted from the Letter entitled 'An Inverse Hexagonal Phase of Cationic Liposome–DNA Complexes Related to DNA Release and Delivery' by I. Koltover, T. Salditt, J.O. Radler, C.R. Safinya, Science 281 (1998) 78) whereby an inverted hexagonal (H_{II}^{C}) phase has also been observed with DNA strands confined in inverted lipid micelles. In particular, synchrotron XRD experiments have provided high-resolution measurements because of the unique characteristics of this radiation source whereas lower-resolution data have been collected in-house by using traditional small-angle diffraction apparatuses [9]. For this reason, what is strongly needed is some way to enhance the low-resolution of the SAXS data collected in-house [10] simultaneously obtaining useful informations about their repeatability without beam-time limitations assigned by sinchrotron facilities.

In this Letter we show that high-resolution XRD experiments can be equally carried out by using in-house apparatuses. The innovation here proposed is based on the employment of silicon wafers as substrates for the CL–DNA complexes combined with an appropriate preparation of the samples, which allows us to obtain highly oriented

multilayers significantly improving the experimental resolution of our XRD scans with respect to the low-resolution ones previously collected in our laboratory [10].

The general idea of using silicon wafers in order to obtain highly oriented multilayers is not new. The evaluation of the high degree of lipid orientation, which can be achieved by the use of silicon wafers has been extensively monitored by measurements of the mosaic spread of the sample, as elsewhere reported [11]. The most convenient technique for evaluating the mosaic spread is wide angle neutron scattering since it allows simultaneous measurements of water content and mosaicity of the sample. This valid procedure has been successfully employed for studying the molecular motions of lipid bilayers and the dynamics of proteins using incoherent quasi-elastic neutron scattering [12].

However, to our knowledge, it has never been used for the structural characterization of CL–DNA complexes currently used in gene delivery applications.

2. Experimental

2.1. Cationic liposomes preparation

Lamellar CL–DNA complexes were formed by mixing SUV, prepared by sonicating the mixtures of cationic lipid dioleoyl trimethylammonium propane (DOTAP) (MW = 698.6) and helper lipid dioleoyl phosphatidylcoline (DOPC) (MW = 705) choosing the weight fraction of DOPC $\Phi =$ (weight of DOPC/total lipid weight) $= 0.5$, with linear DNA (MW(bp) = 649) as elsewhere described [10]. Both lipid and DNA were purchased from Sigma (St. Louis, MO). We have used DOPC because this neutral lipid is known to favor planar lipid bilayers and the DOTAP/DOPC mixture was well characterized by synchrotron XRD. Then thin layers were deposited onto the oriented surface (1 1 0) of silicon wafers [13]. The wafers had a thickness of 150 μm and a diameter $d = 50.8 \pm 0.3$ mm. The formation of ordered multilayers was promoted by drying the samples in a closed chamber under gentle nitrogen flux and, laterly, by storing them at $T = 40$ °C for several hours. The total process lasted about two days. Since DNA carries two negative charges/bp whereas each DOTAP molecule has one positive charge head group, the complex is stoichiometrically neutral only when the numbers of DNA bases and DOTAP molecules are equal or when $\rho = \rho^{\mathrm{ISO}} = (\text{weight DOTAP/weight DNA}) = 2 \times \mathrm{MW}(\mathrm{DOTAP})/\mathrm{MW}(\mathrm{bp}) = 2.2$. Varying the amount of DNA into the complex different values of ρ can be obtained leading the complexes to different DNA packing regimes [14]. We have chosen three different values of ρ ($\rho = 1$; $\rho^{\mathrm{iso}} = 2.2$; $\rho = 4$) in order to extend all our conclusions to all the mentioned DNA packing regimes.

2.2. X-ray Diffraction

Our X-Ray experiments were carried out at the Chemistry Department by using an Energy Dispersive X-Ray Diffraction (EDXD) apparatus elsewhere described [15,16]. An incident polychromatic X-ray radiation is used and the diffracted beam is energy resolved by a solid-state detector located at a suitable scattering angle. The diffractometer operates in vertical θ/θ geometry and is equipped with an X-ray generator (W target), a collimating system, step motors, and a solid-state detector connected via an electronic chain to a multichannel analyzer. The X-ray source is a standard Seifert tube operating at 50 kV and 45 mA whose Bremsstrahlung radiation is used whereas the detecting system is composed of an EG&G liquid-nitrogen-cooled ultrapure Ge solid-state detector connected to a PC through ADCAM hardware. Both the X-ray tube and the detector can rotate around their common center where the sample is placed. After a preliminary set of measurements, two scattering angles, $\theta = 0.3°$ and $\theta = 0.45°$, were selected to investigate the interesting range of the reciprocal space. The uncertainty associated to θ is $\Delta\theta = 0.001°$ and it directly affects the uncertainty Δq associated to the transfer momentum q ($q = \mathrm{cost} E \sin\theta$; $\mathrm{cost} = 1.01354\ \text{Å}^{-1}\ \mathrm{keV}^{-1}$). Although the long acquisition time is an unfavorable aspect of this technique, no structural modifications and damage occur [17].

3. Results and discussion

All the XRD scans reported in Fig. 2 show two sharp peaks at $q_{001} = 0.105$ Å^{-1} and $q_{002} = 0.210$ Å^{-1}. They correspond to the 001 and 002 Bragg reflections of an ordered multilamellar structure with a periodicity $d = \delta_m + \delta_{DNA} \cong 59.8$ Å, where δ_m is the thickness of lipid bilayer and $\delta_{DNA} \cong 20$ Å is the thickness of a DNA monolayer, which has lost its thin hydration layer. Since δ_{DNA} is expected not to vary as a function of ρ, the thickness of lipid bilayer $\delta_m = d - \delta_{DNA} \cong 39.8$ Å is calculated. Since the multilamellar repeat distance does not vary as a function of ρ, DNA remains tightly bound to lipid bilayers. The uncertainty associated to d is simply calculated starting from Δq using the Bragg relation.

The additional middle peak (for $\rho = 1$) is observed at $q = 0.177$ Å^{-1} and it corresponds to an interhelical spacing between the DNA chains of $d_{DNA} = 35.5$ Å. It arises from the diffraction of the 1D lattice of DNA chains coated between the lipid bilayers. The middle peak moves to lower q increasing ρ and we have obtained $q = 0.157$ Å^{-1} and $q = 0.132$ Å^{-1}s for $\rho^{iso} = 2.2$ and $\rho = 4$, respectively, corresponding to the distances $d_{DNA} = 40.1$ Å and $d_{DNA} = 47.7$ Å.

At the isoelectric point both lipid and DNA are incorporated into the complex and the cationic lipid group heads exactly neutralize the phosphate groups on the DNA backbone whereby, the average interhelical spacing d_{DNA} shows, as expected, the existence of two different packing regimes: the first one where the complexes are positive and the other one where the complexes are negative. The former are strongly influenced by the interbilayer repulsions that set the finite amount of lipid that the complex can accommodate whereas the latter are influenced by inter-DNA repulsions [10]. The complexes are one-phase in the region of the isoelectric point ($\rho \cong \rho^{ISO}$), coexisting with DNA at lower ρ and with lipid at higher ρ. The DNA packing density marked by our EDXD data confirm, as expected, the model in which the CLs and DNA condense into a multilayer structure with DNA sandwiched between the bilayers. Our results are absolutely in line with the previous findings on the same complex except for the periodicity distance of the multilamellar structure d, which results about 5 Å shorter. This effect is simply interpretable in terms of the described preparation of the samples, which strongly stabi-

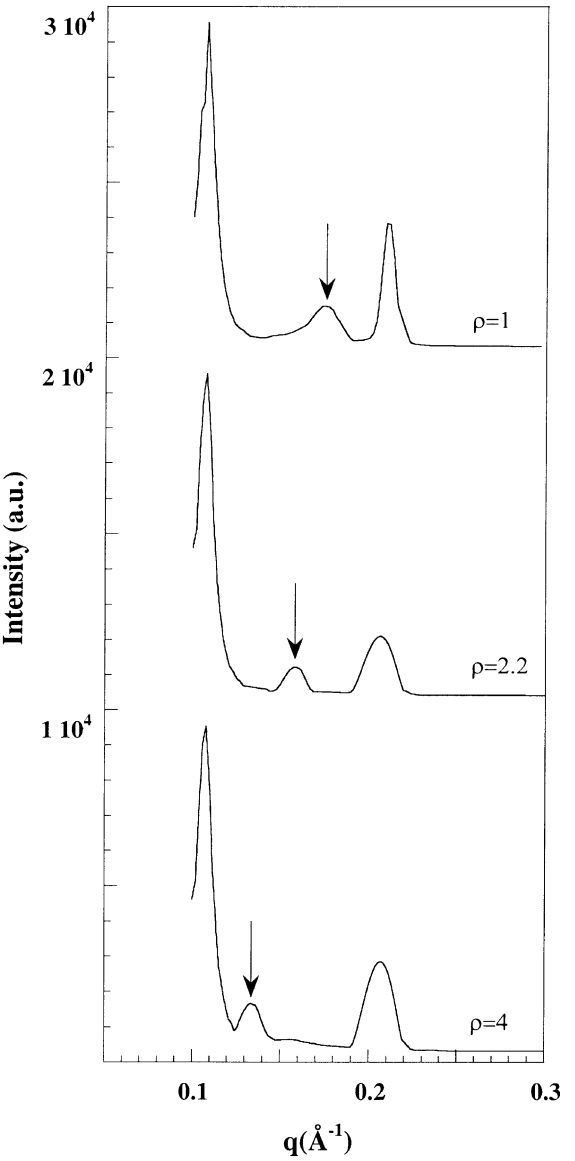

Fig. 2. EDXD scans collected at $\theta = 0.3$ and $\theta = 0.45°$. The two fixed peaks are the Bragg reflections of an ordered multilamellar structure with a periodicity d. The intermediate peak (marked by arrows) is due to the interaxial spacing d_{DNA} and shows different packing regimes as a function of the cationic lipid/DNA weight ratio ρ.

lizes the structure forcing it to loose its hydration water.

Anyway, what is really remarkable is the comparison between the resolution of these findings and the previously achieved ones as elsewhere reported [10]. They clearly show that the obtained highly ordered structure of the complexes, enables us to use our EDXD apparatus to quantify the DNA packing density and to investigate the internal microscopic structure as carely as never before by our energy dispersive X-ray diffractometer.

Indeed, the proposed experimental approach has allowed us to succeed in achieving the same structural informations and to collect high-resolution XRD scans with the same features than synchrotron ones [18]. Furthermore, this procedure has involved a sensible reduction of the acquisition times, which have been brought down from 10,000 s to only 1000 s for each EDXD collected scan. Since an extensive work will be required to have a complete understanding of the various possible self-assembled structures and to relate these structures to their biological function, our approach allows to characterize the most used lipoplexes in a systematic way.

Overmore, aside from the biomedical gene delivery applications, the highly oriented L_{α}^{c} phase represents an interesting model system to investigate the DNA–DNA interactions and the DNA condensation on a surface.

Acknowledgements

This work was supported in part by grants from Italian MIUR (Ministero dell'Università e della Ricerca), contract no. 9902018391. We thank Dr. D. Pozzi and Dr. M. Friello for providing samples and the useful discussions.

References

[1] D.D. Lasic, Adv. Drug. Deliver. Rev. 20 (1996) 221.
[2] I. Koltover, K. Wagner, C.R. Safinya, Proc. Natl. Acad. Sci. USA 97 (2000) 14046.
[3] S.W. Hui, M. Langner, Y.L. Zhao, E. Hurley, K. Chan, Biophys. J. 71 (1996) 590.
[4] K.W.C. Mok, P.R. Cullis, Biophys. J. 73 (1997) 2534.
[5] S. Simões, V. Slepushkin, R. Gaspar, M.C. Pedroso de Lima, N. Düzgünes, Gene Ther. 5 (1998) 955.
[6] M.C. Pedroso de Lima, S. Simões, P. Pires, H. Faneca, N. Düzgünes, Adv. Drug. Deliver. Rev. 47 (2001) 277.
[7] P.L. Felgner, T.R. Gadek, M. Holm, R. Roman, H.W. Chan, M. Wenz, J.P. Northrop, G.M. Ringold, M. Danielsen, Proc. Natl. Acad. Sci. USA 84 (1987) 7413.
[8] J. Radler, I. Koltover, T. Salditt, C.R. Safinya, Science 275 (1997) 810.
[9] I. Koltover, T. Salditt, C.R. Safinya, Biophys. J. 77 (1999) 915.
[10] G. Caracciolo, R. Caminiti, D. Pozzi, M. Friello, F. Boffi, A. Congiu Castellano, Chem. Phys. Lett. 351 (2002) 222.
[11] S. Konig, W. Pfeiffer, T. Bayerl, D. Richter, E. Sackmann, J. Phys. II France II (1992) 1589.
[12] F. Natali, A. Gliozzi, R. Rolandi, P. Cavatorta, A. Deriu, A. Fasano, P. Riccio, Phys. B 301 (1–2) (2001) 145.
[13] M. Käsbauer, T.M. Bayerl, Biochemistry 38 (1999) 15258.
[14] D. Harries, S. May, W.M. Gelbart, A. Ben-Shaul, Biophys. J. 75 (1998) 159.
[15] V. Rossi Albertini, L. Bencivenni, R. Caminiti, F. Cilloco, C. Sadun, J. Macromol. Sci.-Phys. 835 (1996) 199.
[16] R. Caminiti, C. Sadun, V. Rossi Alberini, F. Colloco, R. Felici, Presented at XXV National Congress of Chemical Physics, Cagliari, Italy (1991). Patent No. 01261984 (1996).
[17] G. Caracciolo, G. Amiconi, L. Bencivenni, G. Boumis, R. Caminiti, E. Finocchiaro, B. Maras, C. Paolinelli, A. Congiu Castellano, Eur. Biophys. J. 30 (2001) 163.
[18] C.R. Safinya, Curr. Opin. Struct. Biol. 11 (2001) 440.