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How lipid hydration and temperature affect the structure of DC-Chol–DOPE/DNA lipoplexes

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Abstract

Effect of lipid hydration on the structure of lamellar lipoplexes made of the cationic lipid 3-[*N*-(*N*,*N*-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol), the neutral 'helper' lipid dioleoylphosphatidylethanolamine (DOPE) and calf-thymus DNA was investigated by synchrotron small angle X-ray diffraction (SAXD). Here, we show that lipid hydration is the key factor regulating the equilibrium structure of lipoplexes. Thermotropic behavior was also investigated between 5 and 65 °C. Both the membrane thickness and the water layer thickness were found to decrease linearly as a function of temperature while the one dimensional DNA rod lattice between lipid bilayers was found to enlarge. Structural results were interpreted in terms of recently proposed theoretical models.

1. Introduction

In recent years, several new strategies have been proposed to develop non-viral transfection vectors of DNA for gene therapy. Cationic liposomes (CLs) complexed with DNA, currently named lipoplexes, have been shown to be promising nonviral delivery systems for gene therapy applications [1–4]. Although easy of production and capability of transferring large pieces of DNA represent the most relevant advantages with respect to viral vectors, their low transfection efficiency (TE) is the main concern that remains to be solved [5].

Among the current treatments of cancer, hypothermia and hyperthermia have been used in both humans and animals [6]. Hyperthermia has been found to enhance the effectiveness of several CL formulations. Very recently, many different liposomal formulations with temperature sensitivity have also been reported [7]. As a result, hyperthermia and liposomal drug delivery started to be used together in attempt to exploit their mutual interactions against cancer [8].

Although this combination therapy seems to hold great promise towards improving current TE, the molecular mechanisms by which it does so remain unclear.

Nowadays, despite the wealth of empirical data available, underlying interactions and physical mechanisms are not completely understood. This is unfortunate, since a more complete understanding of the process is needed to open novel possibilities also for therapeutic intervention [9].

Here we report, for the first time, a synchrotron small angle X-ray diffraction (SAXD) study on the effect of lipid hydration and temperature on the structural properties of DC-Chol/DOPE–DNA lipoplexes.

The exact role of hydration, i.e. the influence of bounded DNA on the hydration of lipid bilayer, is an important question to be addressed since lipid-DNA aggregates are so strongly packed that the amount of water entrapped within them should be rather limited [10]. According to standard procedures, liposomes are usually stored at 4 °C and typically used within 3 days. In the major part of works [10–12], lipoplexes are allowed to equilibrate to assure complex formation, equilibration times ranging between 20 min and a few hours. Unfortunately, a straightforward interpretation on the role of hydration on the structure of both liposomes and lipoplexes is still lacking. To shed light on this

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point DC-Chol–DOPE/DNA lipoplexes were prepared with different hydration schedules.

It has been hypothesized that varying temperature modifies the structural properties of the lipid membrane of lipoplexes by changing molecular organization of the headgroup region and elastic properties of the hydrophobic core.

Although DC-Chol/DOPE–DNA lipoplexes are the most efficient non-viral vectors in a wide variety of cell lines [12–14], no systematic structural investigation on the effect of lipid hydration and temperature on the membrane thickness, the interbilayer water layer thickness and the DNA packing density has been reported so far.

Aside from biomedical applications, DC-Chol/DOPE–DNA lipoplexes can be regarded as excellent model systems to evaluate the effect of physical chemical parameters on the DNA packing density within lamellar lipoplexes.

2. Experimental section

2.1. Samples preparation

Calf thymus Na-DNA was purchased from Sigma (St. Louis, MO). Calf thymus Na-DNA solution (10 mg/ml) was sonicated inducing a DNA fragmentation with length distribution between 500 and 1000 bp which was determined by gel electrophoresis.

DC-Chol and DOPE were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purifications. The liposome solutions were prepared solving appropriate amounts of DC-Chol and DOPE in chloroform, so that the molar ratio of neutral lipid in the bilayer was $\Phi_M = 0.5$. First, the solvent was evaporated under a stream of nitrogen and then under a vacuum for 12 h. Multilamellar vesicles (MLVs) were formed by hydrating the dry lipid film in Tris–HCl buffer solution (10^{-2} M, pH 7.4) to reach the desired final concentration of 100 mg/ml.

In the first case, lipoplexes were prepared by injecting appropriate amounts of sonicated DNA solution into lipid emulsions immediately after hydration of the lipid film (assumed as initial time, $t = 0$). Resulting DC-Chol–DOPE/DNA lipoplexes at charge ratios $\rho =$ (positive charge)/(negative charge) = 0.3, 0.6, 1.2, 2.4, 4.8 and 9.6 were characterized as a function of time.

In the second case, DC-Chol–DOPE liposomes were incubated at 30 °C for 36 h and then lipoplexes were formed by adding appropriate amounts of DNA solution to liposomal dispersions at the charge ratios $\rho = 0.3$, 0.6, 1.2, 2.4, 4.8 and 9.6.

2.2. Synchrotron SAXD measurements

Small angle X-ray diffraction (SAXD) experiments were carried out at the SAXS beamline, ELETTRA [15]. SAXD patterns were recorded on a SAXD camera with pinhole collimation covering a q-range ($q = 4\pi\sin(\theta)/\lambda$) of between 0.5 and 5.5 nm^{-1}. The angular calibration of the delay line detector was performed by using the diffraction pattern of silver behenate powder [$CH_3(CH_2)_{20}COOAg$] (the repeat unit is 5.838 nm). The lipid dispersions were kept in a thin-walled 1-mm-diameter Mark capillary held in a steel cuvette, which provides good thermal contact to the Peltier heating unit (Anton Paar, Graz, Austria). SAXD scans were carried out from 5 to 65 °C by increasing the temperature in 15 °C steps. The sample was held at each temperature for 5 min before the measurement was started, so that the system can be considered as being in thermal equilibrium. The exposure time for each sample at each temperature was 100 s. No evidence of sample degradation was observed in any of the samples at this exposure. The data have been normalized to the same primary beam intensity and corrected for detector efficiency, as well as the background has been subtracted.

2.3. Data analysis

Due to the bilayer nature of lipid membranes, the electron density profile (EDP) has a centre of symmetry in the middle of the bilayer. The electron density profile, $\Delta\rho$, along the normal to the bilayers, z, was then calculated as a Fourier sum of cosine terms

$$\Delta\rho = \frac{\rho(z) - \langle\rho\rangle}{\left[\langle\rho^2(z)\rangle - \langle\rho\rangle^2\right]^{1/2}} = \sum_{l=1}^{N} F_l \cos\left(2\pi l \frac{z}{d}\right) \quad (1)$$

where $\rho(z)$ is the electron density, $\langle\rho\rangle$ its average value, $N$is the highest order of the fundamental reflection observed in the XRD pattern, F_l is the form factor for the $(0\,0\,l)$ reflection, d is the thickness of the repeating unit including one lipid bilayer and one water layer. Each form factor F_l was calculated from the integrated intensity $I_l = F_l^2/C_l$ under the lth diffraction peak, where C_l is the Lorentz-polarization correction factor for unoriented samples. The previous equation determines the form factors except for the phase factor which must be ± 1 for symmetric bilayers. The phase problem, i.e. the choice of the best sign sequence for the structure factor, was solved as previously proposed [16]. The sign combination used to calculate the electron density profile is (----) relative to the structures factors F_1, F_2, F_3; F_5. F_4 is equal to zero due to systematic absence of the fourth-order BP in the SAXD pattern.

3. Results and discussion

In Fig. 1, we show SAXD patterns of negatively charged DC-Chol–DOPE/DNA lipoplexes ($\rho = 0.6$; $T = 5$ °C) formed immediately after hydration of lipid film resulting in the formation of MLVs ($t = 0$).

DC-Chol/DOPE–DNA lipoplexes exhibited a multilamellar structure consisting of a one-dimensional arrays of DNA chains intercalated between oppositely charged membrane bilayers. Four sharp Bragg reflections at q_{00n} for the alternating lipid bilayer-DNA monolayer structure were observed with lamellar periodicity $d = 2\pi/q_{001} = 6.47$ nm.

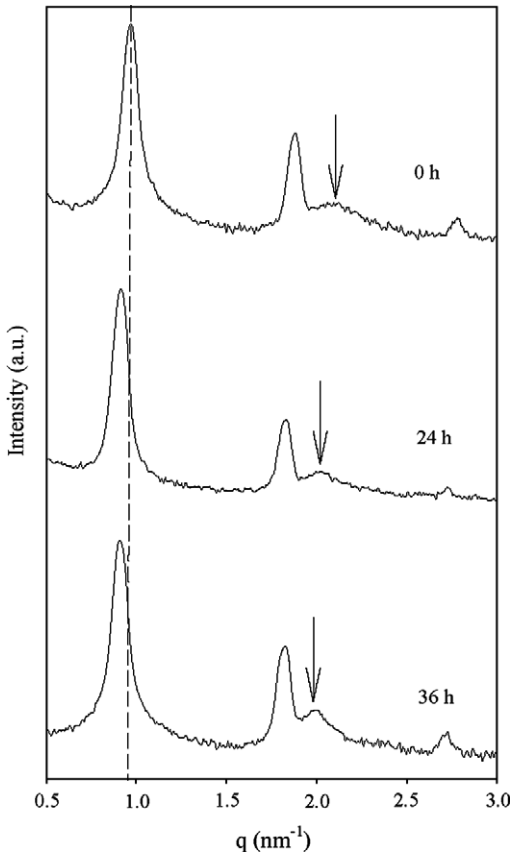

Fig. 1. SAXD patterns of DC-Chol–DOPE/DNA lipoplexes ($\rho = 0.6$; $T = 5$ °C) collected immediately after complex formation and then after 24 and 36 h of incubation. Dashed line indicates the shift of the first-order lamellar peak due to the enlargement of the lipid bilayer-DNA monolayer structure. The mobile DNA–DNA peak (indicated by arrows) moves toward smaller values of q as t increases. For clarity, the q-range is restricted to between 0.5 and 3 nm^{-1}.

The Bragg peak (BP) marked by arrow was due to the well-known smectic structure of DNA arrays with spacing $d_{DNA} = 2\pi/q_{DNA} = 3.00$ nm [17–20].

After complex formation ($t = 0$), DC-Chol–DOPE/DNA lipoplexes were let to equilibrate and SAXD patterns were collected as a function of time. Two representative SAXD patterns are also shown in Fig. 1 corresponding to 24 and 36 h of incubation of the complexes.

Fig. 1 shows that the diffraction BPs move to lower q-values as a function of increasing time, the lamellar repeat distance, d, moving from 6.47 to 6.89 nm. According to general definitions, the lamellar d-spacing can be separated into its structural components: the lipid bilayer thickness, d_B, and the thickness of the interbilayer water region, d_W. While the bilayer thickness gives fundamental information about the fluidity and the elastic properties of lipid bilayer, the thickness of the water spacing between adjacent bilayers is essential for evaluating interactions between membranes [16].

In principle, the observed increase of the repeat distance d could be ascribed to both d_B and d_W. Thus, a question arose if the swelling was the result of the enlargement of the water layer or of the lipid bilayer. In attempt to answer this question, EDPs along the normal to lipid bilayers were calculated from the SAXD patterns of Fig. 1.

Fig. 2 shows EDPs for DC-Chol–DOPE/DNA ($\rho = 0.6$, $T = 5$ °C) lipoplexes at $t = 0$ (continuous line), 24 h (dotted line) and 36 h (dashed line) of incubation. The large central minimum corresponds to the middle of the lipid bilayer while the two strong maxima correspond to the polar headgroups. DNA strands sandwiched between lipid bilayers are responsible for the increase in electron density at the edges of the EDPs. The distance between the maxima of EDP gives a reliable estimate of d_B, whereas the water layer thickness is defined as $d_W = d - d_B$. The values of d_B and d_W obtained from the EDPs of Fig. 2 are reported in Table 1. Our results showed that the membrane thickness and the thickness of the water layer increased monotonously (Table 1) as a function of time and reached final values at $t = 36$ h.

Our structural findings revealed that the increase of d depended not only on the adsorption of free water molecules but also on the development of pronounced bilayers undulations reflecting a more fluid lipid bilayer as hydration proceeds. Indeed, lipid bilayer responded to the water layer expansion by a simultaneous enlargement in membrane thickness as each lipid molecule required more space due to the increasing of motional freedom at higher levels of hydration.

Recent publications have shown that an enlargement of d_B within lamellar lipoplexes produces a denser packing of lipid molecules in the membrane plane resulting in a marked reduction of d_{DNA} [17]. Conversely, Table 1 shows a simultaneous enlargement of d_B and d_{DNA} as a function of increasing time.

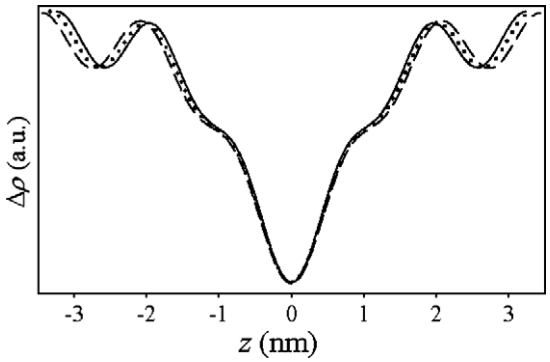

Fig. 2. Electron-density profiles along the normal to the bilayers of DC-Chol–DOPE/DNA ($\rho = 0.6$) lipoplexes at $t = 0$ h (continuous line), $t = 24$ h (dotted line) and $t = 36$ h (dashed line) of incubation.

Table 1
Structural parameters of DC-Chol–DOPE/DNA lipolexes ($\rho = 0.6$, $T = 5$ °C) as a function of incubation time, i.e. of increasing hydration

Time (h)	d (nm)	d_B (nm)	d_W (nm)	d_{DNA} (nm)
0	6.47	3.88	2.59	3.00
24	6.86	4.12	2.74	3.11
36	6.89	4.13	2.76	3.13

It probably occurs because, at $t = 0$, just formed lipoplexes are not equilibrium structures since lipid molecules are not fully hydrated. Upon hydration, the uptake of water molecules inside the lipoplex lets the volume of the interbilayer water region increase forcing the DNA molecules to separate each other (Fig. 3, side 1). For $t > 36$ h, no relevant structural changes could be detected in that the SAXD pattern did not vary anymore. Structure, as revealed by the SAXD pattern collected at $t = 36$ h, was therefore assumed as the equilibrium structure of the lipoplex.

DC-Chol–DOPE/DNA ($\rho = 0.6$, $T = 5$ °C) lipoplexes were also prepared following a different hydration schedule. After hydration of lipid film ($t = 0$), DC-Chol–DOPE liposomes were incubated at 30 °C for 36 h and then appropriate amounts of DNA solution were added. SAXD patterns (data not reported) collected immediately after complex formation ($t = 36$ h) showed a lamellar phase with repeat distance, d, of 6.90 nm and a DNA–DNA in plane distance, d_{DNA}, of 3.14 nm in excellent agreement with the above reported equilibrium values.

Thus, our structural results obtained following two different hydration schedules allowed us to identify lipid hydration as the key factor regulating the equilibrium structure of lipoplexes (Fig. 3).

Otherwise, evidence of very long time scale relaxation processes has been recently reported in the literature [21]. Thus, lipoplexes were stored for 3 months at $T = 4$ °C and SAXD experiments were replicated under identical experimental conditions. The structure of the lamellar phase was found to be exactly the same, i.e. superimposable SAXD patterns (data not reported) were collected. As a consequence, we concluded that the equilibrium state of the lipid/DNA carrier was likely to be unique.

In Fig. 4, we show SAXD patterns of fully hydrated DC-Chol–DOPE/DNA lipoplexes ($\rho = 0.6$) collected at increasing temperature, starting from 5 °C at the top and ascending in 15 °C steps to 65 °C at the bottom. The lamellar repeat distance, d, decreases as the temperature increases from 6.89 to 6.55 nm. To better elucidate the effect of temperature on the inner structure of DC-Chol–DOPE/DNA lipoplexes, we calculated the EDPs along the normal to the lipid bilayer. Table 2 shows the temperature dependence of the structural parameters as calculated from the EDPs.

As evident, d_B and d_W decrease as a function of increasing temperature, correspondingly from 4.13 to 3.93 nm and

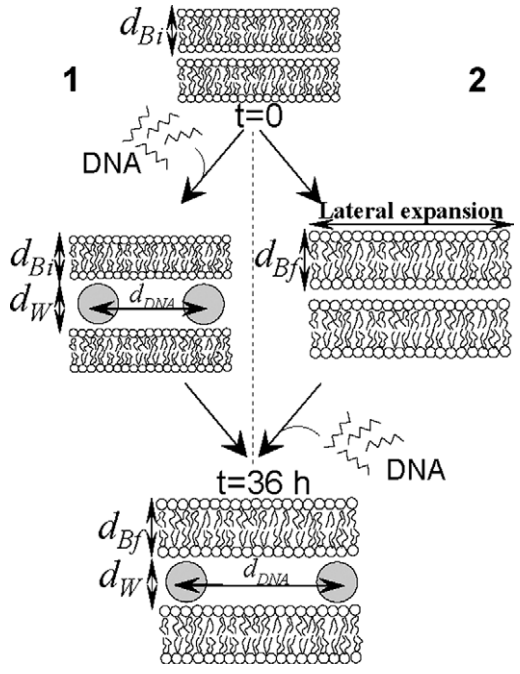

Fig. 3. Schematics of the process occurring, at the molecular level, upon incubation of lipoplexes. (Side 1) Lipoplexes were prepared by injecting DNA into lipid emulsions immediately after hydration of the lipid film ($t = 0$). Resulting lipoplexes were characterized as a function of time and reached equilibrium at $t = 36$ h. (Side 2) DC-Chol–DOPE liposomes were incubated at 30 °C for 36 h and then lipoplexes were formed by adding DNA to liposomal dispersions. Final structures, obtained following different hydration schedules were found to be the same.

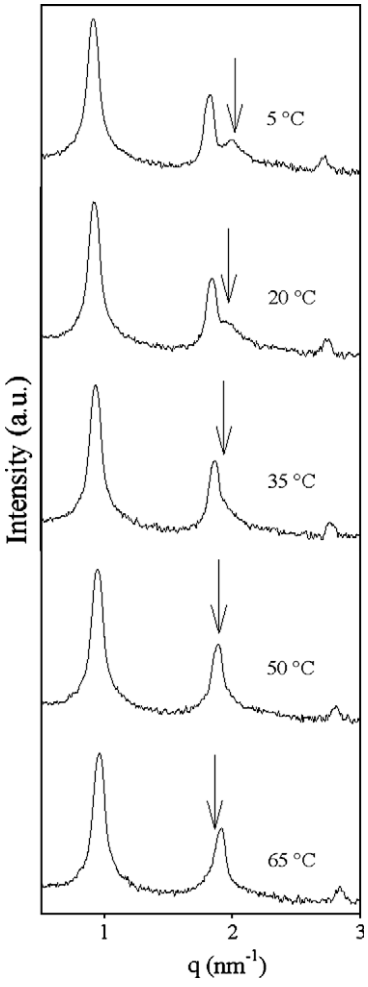

Fig. 4. SAXD patterns of DC-Chol–DOPE/DNA lipoplexes ($\rho = 0.6$) collected at increasing temperature, starting from 5 °C at the top and ascending in 15 °C steps to 65 °C at the bottom. The mobile DNA–DNA peak (indicated by arrows) moves toward smaller values of q as T increases. For clarity, the q-range is restricted to between 0.5 and 3 nm^{-1}.

Table 2
Temperature dependence of the structural parameters of fully hydrated DC-Chol–DOPE/DNA lipoplexes ($\rho = 0.6$, $T = 5$ °C) calculated from the EDPs

T (°C)	d (nm)	d_B (nm)	d_W (nm)	d_{DNA} (nm)
5	6.89	4.13	2.76	3.14
20	6.82	4.09	2.73	3.18
35	6.73	4.04	2.69	3.25
50	6.65	3.99	2.66	3.31
65	6.55	3.93	2.62	3.35

from 2.76 to 2.62 nm. The membrane thickness, d_B, decreases linearly with the temperature because a rise in temperature induces a transition from all-*trans* to a *trans–gauche* state thus reducing the ordering of the hydrocarbon chains with a decrease in the effective chain length [22,23].

Furthermore, reduced density thermal fluctuations diminish repulsive forces between bilayers and therefore reduce d_W [24].

The thermal thickness expansion coefficient, α_B, of DC-Chol–DOPE mixed bilayers, calculated according to Luzzati and Husson [25]

$$\alpha_B = \frac{\Delta d_B}{d_B}\frac{1}{\Delta T} \tag{2}$$

is given as a function of temperature in Table 3. The absolute value of α_B increases linearly as the temperature increases, starting from $-0.822 \times 10^{-3}\ K^{-1}$ ($T = 5$ °C) up to $-0.865 \times 10^{-3}\ K^{-1}$ ($T = 65$ °C), indicating a more fluid bilayer. It must also be noticed that the structural changes induced by temperature were found to be fully reversible in all cases: no hystereses effects in the SAXD patterns were observed after equilibration times of about 1000 s.

It is well-known [24, and references therein] that raising temperature induces an increase in interfacial area per each single lipid molecule resulting in a lateral bilayer expansion.

At the molecular level, the continuous enlargement in DNA–DNA spacing observed between 5 and 65 °C (Table 2) could be therefore interpreted in terms of the lateral expansion of the DC-Chol–DOPE membranes, i.e. in terms of the dilatation area of lipid headgroups resulting in a continuous reduction of surface charge density, σ, according to the following relation [17]:

$$\sigma = \frac{e(1-\Phi_W)}{A} \tag{3}$$

In order to quantify the temperature induced renormalization of surface charge density, information on the temperature dependence of the interfacial area would be therefore desirable [26,27]. Unfortunately, to our knowledge, no previous work has been devoted to investigate the thermotropic behavior of DC-Chol–DOPE mixed bilayers. Recently, some of us have proposed an electrostatic model [28] describing DNA–DNA interactions within lamellar lipoplexes. Afterward, the model has been successfully applied to estimate the thermotropic dilatation area of lipid headgroups within DOTAP–DOPC mixed bilayers [29]. The model yields DNA–DNA interaction force within lamellar lipoplexes

$$F = \frac{Z^2}{2\pi\varepsilon}\frac{e^2}{l^2}\left[\frac{2ld_{DNA}(1-\Phi_M)}{A} - 1\right]^2 \times \left[\frac{\left((d_{DNA})^2 + \xi_p^2\right)^{1/2}}{d_{DNA}} - 1\right] \tag{4}$$

where (Ze) is the charge of the cationic lipid headgroup, A is the average lipid headgroup area, σ_M is the molar ratio of neutral lipid in the bilayer, ξ_p is the DNA persistence length ($\xi_p \sim 500$ Å), ε is the dielectric constant of water ($\varepsilon \sim 80$) and $l = 1.7$ Å is the distance between two phosphate entities along the DNA axis.

By force minimization with respect to d_{DNA}, one finds a relation between A and d_{DNA}

$$A = 2ld_{DNA}(1-\Phi_M) \tag{5}$$

that, inserted into Eq. (3), yields the following relation between surface charge density of lipid membranes and DNA–DNA repeat spacing:

$$\sigma = \frac{(1-\Phi_W)}{(1-\Phi_M)}\frac{1}{2ld_{DNA}} \tag{6}$$

As evident, the observed enlargement of the DNA in plane rod lattice (Table 2) may be really interpreted in terms of the reduction of surface charge density induced by the dilatation area of lipid headgroups.

The temperature induced renormalization of surface charge density of DC-Chol–DOPE membranes can be therefore evaluated by inserting into Eq. (6) the DNA–DNA spacings, d_{DNA}, as calculated by the SAXD patterns of Fig. 4.

Fig. 5 shows the variation in DNA packing density as a function of σ that is in close agreement with the recently reported universal charge-condensation curve for lamellar lipoplexes [30].

It is well-recognized that differences in the physical properties of lipoplexes can depend upon whether liposomes interact with a small or a large amount of DNA [31,32]. Thus, the inner structure of mixed lipoplexes could depend on their net charge [33]. To clarify this matter, we replicated SAXD experiments on DC-Chol/DOPE–DNA lipoplexes both at the isoelectric point ($\rho = 1$) and in excess of positive charge ($\rho = 2$, 2.4, 4.8, 9.6, 18).

Table 4 shows that, at each temperature, the lamellar periodicity, d, of DC-Chol/DOPE–DNA lipoplexes did not vary as a function of ρ while d_{DNA} was adjusted by varying the relative percentage of CL and DNA in the

Table 3
Thermal thickness expansion coefficient of DC-Chol–DOPE bilayers

T (°C)	α_B ($10^{-3}\ K^{-1}$)
5	−0.822
20	−0.831
35	−0.842
50	−0.852
65	−0.865

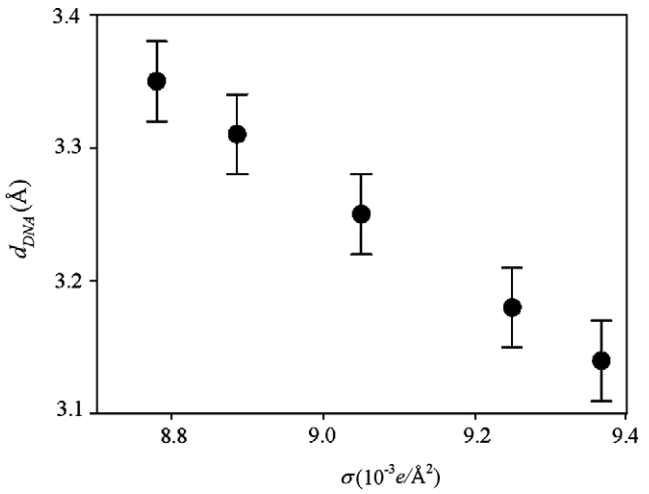

Fig. 5. DNA–DNA spacing of isoelectric DC-Chol–DOPE/DNA lipoplexes as a function of surface charge density, σ, as determined according to Eq. (6).

Table 4
Lamellar repeat distance, d, and DNA–DNA spacing, d_{DNA}, as a function of the charge ratio ρ at $T = 5$, 35 and 65 °C

ρ	$T = 5$ °C		$T = 35$ °C		$T = 65$ °C	
	d (nm)	d_{DNA} (nm)	d (nm)	d_{DNA} (nm)	d (nm)	d_{DNA} (nm)
0.6	6.89	3.14	6.73	3.2	6.55	3.33
1	6.90	3.14	6.70	3.24	6.54	3.35
2	6.87	3.18	6.71	3.45	6.55	3.52
4.8	6.89	3.31	6.73	3.60	6.54	3.78
9.6	6.90	3.33	6.70	3.61	6.53	3.82

complex. Above and beyond the isoelectric complexes, DC-Chol/DOPE–DNA lipoplexes regulate the interaxial spacing d_{DNA} in order to minimize the total free energy by separating the DNA strands. Finally, electrostatic interactions set a constraint on the excess lipid or DNA the complex can accommodate and two distinct plateau regimes are reached [33–35].

In the case of isoelectric complexes, the absolute DNA–DNA spacing can be calculated from the following relation [17]:

$$d_{DNA}^{iso} = \frac{A_D \rho_D}{d_B \rho_L} \times \frac{\rho^{iso}}{(1 - \Phi_W)} \quad (7)$$

where $\rho_D = 1.7$ g/cm^3, $\rho_L = 1.07$ g/cm^3 are densities of DNA and lipid, respectively, ρ^{iso} is the lipid-to-DNA weight ratio, $A_D = 190$ Å^2 is the cross-section area of DNA and Φ_W is the weight percentage of neutral lipid in the mixture. In Table 5, experimental DNA–DNA spacings and theoretical values calculated using Eq. (7) are reported. The very good agreement between experimental and theoretical values shows that the phase behavior of the DC-Chol/DOPE–DNA system looks like that of the most studied lamellar lipoplexes [31–34] and that the increase in the DNA spacing is just a function of the thinning of the bilayer thickness d_B.

Table 5
Comparison between experimental and theoretical DNA–DNA spacings

T (°C)	$(d_{DNA})_{exp}$ (nm)	$(d_{DNA})_{th}$ (nm)
5	3.14	3.19
20	3.18	3.22
35	3.24	3.27
50	3.31	3.30
65	3.35	3.35

Theoretical values were calculated according to Eq. (7).

4. Conclusions

We have investigated the effect of hydration and temperature on the inner structure of DC-Chol/DOPE–DNA lamellar lipoplexes. We have shown that following two different hydration schedules leads to the same structures suggesting that lipid hydration is the key factor regulating the equilibrium structure of DC-Chol/DOPE–DNA lipoplexes.

The membrane thickness, d_B, decreases linearly as a function of temperature due to the increasing disorder, i.e. the decrease in the aliphatic chain length, and due to the reduction of membrane flexibility induced by the simultaneous diminution of the interbilayer water region d_W.

DC-Chol/DOPE–DNA lipoplexes showed that, according to well-established models describing the phase behavior of lamellar lipoplexes [17,19,28], the thermotropic changes in the DNA packing density within DC-Chol/DOPE–DNA lipoplexes follow the area dilatation of the lipid headgroups.

References

[1] 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.
[2] P.L. Felgner, Sci. Am. 276 (1997) 102.
[3] D.D. Lasic, Liposomes in Gene Delivery, CRC Press, Boca Raton, FL, 1997.
[4] A.J. Lin, N.L. Slack, A. Ahmad, I. Koltover, C.X. George, C.E. Samuel, C.R. Safinya, J. Drug Target 8 (2000) 13.
[5] C.R. Safinya, Curr. Opin. Struct. Biol. 11 (2001) 440.
[6] G. Kong, G. Anyarambhatla, W. Petros, P.R.D. Braun, O.M. Colvin, D. Needham, M.W. Dewhirst, Cancer Res. 60 (2000) 6950.
[7] H. Mushiake, M. Aoe, W. Kazuhiro, A. Andou, N. Shimizu, Acta Med. Okayama 56 (2002) 35.
[8] A. Okita, H. Mushiake, K. Tsukuda, M. Aoe, M. Murakami, A. Andou, N. Shimizu, Oncol. Rep. 11 (2004) 1313.
[9] P.K.J. Kinnunen, J.M. Holopainen, Bioscience Rep. 6 (2000) 465.
[10] W. Pohle, C. Selle, D.R. Gauger, R. Zantl, F. Artzner, J.O. Rädler, Phys. Chem. Chem. Phys. 2 (2000) 4642.
[11] F. Artzner, R. Zantl, G. Rapp, J.O. Rädler, Phys. Rev. Lett. 81 (1998) 5015.
[12] L. Ciani, S. Ristori, A. Salvati, L. Calamai, G. Martini, Biochim. Biophys. Acta 1664 (2004) 70.
[13] M. Keller, M.R. Jorgensen, E. Perouzel, A.D. Miller, Biochemistry 42 (2003) 6067.
[14] P.S. Ajmani, J.A. Hughes, Neurochem. Res. 24 (1999) 699.
[15] H. Amenitsch, M. Rappolt, M. Kriechbaum, H. Mio, P. Laggner, S. Bernstorff, J. Synchrotron Radiat. 5 (1998) 506.
[16] V. Luzzati, P. Mariani, H. Delacroix, Makromol. Chem., Macromol. Symp. 15 (1998) 1.
[17] I. Koltover, T. Salditt, C.R. Safinya, Biophys. J. 77 (1999) 915.

[18] G. Caracciolo, R. Caminiti, F. Natali, A. Congiu Castellano, Chem. Phys. Lett. 366 (2002) 200.
[19] G. Caracciolo, R. Caminiti, D. Pozzi, M. Friello, F. Boffi, A. Congiu Castellano, Chem. Phys. Lett. 351 (2002) 222.
[20] G. Caracciolo, D. Pozzi, R. Caminiti, A. Congiu Castellano, Eur. Phys. J. E 351 (2003) 222.
[21] J.J. McManus, J.O. Rädler, K.A. Dawson, J. Am. Chem. Soc. 126 (2004) 15966.
[22] M. Rappolt, P. Laggner, G. Pabst, Recent Res. Develop. Biophys. 3 (2004) 363.
[23] R. Zantl, L. Baicu, F. Artzner, I. Sprenger, G. Rapp, J.O. Rädler, J. Phys. Chem. B 103 (1999) 10300.
[24] M. Pasenkiewicz-Gierula, T. Rog, K. Kitamure, A. Kusumi, Biophys. J. 78 (2000) 1376.
[25] V. Luzzati, F.J. Husson, Cell Biol. 12 (1962) 207.
[26] V. Luzzati, Biological Membranes, Academic Press, New York, 1968.
[27] J.F. Nagle, S. Tristram-Nagle, Biochim. Biophys. Acta 1469 (2000) 159.
[28] G. Caracciolo, R. Caminiti, Chem. Phys. Lett. 400 (2004) 314.
[29] G. Caracciolo, D. Pozzi, H. Amenitsch, R. Caminiti, Langmuir (2006) (in press).
[30] A. Martin-Herranz, A. Ahmad, H.M. Evans, K. Ewert, U. Schulze, C.R. Safinya, Biophys. J. 86 (2004) 1160.
[31] M.E. Hayes, A.V. Gorelov, K.A. Dawson, Prog. Colloid Polym. Sci. 118 (2001) 243.
[32] M.T. Kennedy, E.V. Pozhaski, V.A. Rakhmanova, R.C. MacDonald, Biophys. J. 78 (2000) 1620.
[33] A.J. Lin, N.L. Slack, A. Ahmad, I. Koltover, C.X. George, C.E. Samuel, C.R. Safinya, J. Gene Med. 7 (2005) 739.
[34] T. Salditt, I. Koltover, J.O. Rädler, C.R. Safinya, Science 275 (1997) 810.
[35] T. Salditt, I. Koltover, J.O. Rädler, C.R. Safinya, Phys. Rev. E 58 (1998) 889.