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Thermotropic structural changes of saturated-cationic-lipid–DNA complexes

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Abstract. – We investigated the thermotropic behavior of fully hydrated saturated cationic lipid mixture of DMPC/DMTAP (dimyristoylphosphatidyl-choline/dimyristoyl-trimethyl-ammonium propane) complexed with DNA. Using simultaneous small- and wide-angle X-ray scattering, we found two condensed lamellar phases, a fluid L_α^c- and a gel-state $L_{\beta'}^c$-phase. The chain-melting transition was accompanied by a decrease in the bilayer thickness and an expansion of the intercalated one-dimensional DNA rod lattice. The interaxial DNA-DNA spacings are quantitatively described by conservation of local volume fractions for both phases in excess water. The line broadening of the wide-angle scattering exhibited a systematic variation in the hydrocarbon chain tilt as a function of the cationic lipid molar fraction.

Polyelectrolytes and oppositely charged phospholipid vesicles condense into mixed aggregates [1]. Recently it was discovered that in particular DNA and cationic lipids form liquid crystalline complexes. The structure of such *lipid*-DNA *composite phases* is of current interest, because cationic liposomes complexed with recombinant plasmids are promising candidates for synthetic gene delivery systems in future human gene therapy [2]. Cationic DOPC/DOTAP (dioleoylphosphatidyl-choline / dioleoyl-trimethyl-ammonium propane) liposomes rupture in the presence of λ-phage DNA and reorganize into *cationic-lipid*–DNA *complexes* (CL-DNA), which were well characterized using synchrotron X-ray scattering [3, 4]. A lipid lamellar phase with intercalated planar DNA rod lattices was discovered as revealed by a pronounced DNA-DNA correlation peak. The interhelical spacing was set by the lipid-to-DNA ratio, in the case of isoelectric complexes, where the number of cationic lipids equals the number of phosphate groups on the DNA. Furthermore, an analysis of the thermal diffuse scattering of

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the latter DNA peak was in quantitative agreement with an elastic 2D smectic continuum model [4]. The phase behavior of CL-DNA as a function of the lipid-to-DNA ratio can be understood from Poisson-Boltzmann theory as shown by recent theoretical studies [5]. Upon CL-DNA complex formation the cationic lipids replace the DNA counterions and act like two-dimensionally confined counter-lipids in the intercalated complexed smectic phase.

In this letter we report on structural investigations of dimyristoylphosphatidyl-choline/dimyristoyl-trimethyl-ammonium propane (DMPC/DMTAP) mixed with DNA. In contrast to the previously studied dioleoyl lipids, these mixtures exhibit saturated (diC14) hydrocarbon chains and hence undergo a chain order-disorder transition. Using small- and wide-angle X-ray diffraction, we found a lamellar gel phase and a lamellar liquid-crystalline phase in the low- and high-temperature regime of the CL-DNA aggregates in excess water. The phases are denoted $L^c_{\beta'}$ and L^c_α in analogy to the corresponding pure-lipid phases, whereby "c" stands for *condensed* or *complexed*. The gel–to–liquid-crystalline transition is first order and characterized by an anisotropic structural transition. We will show that the increase in the DNA spacing is directly related to the area dilation of the lipid bilayer. The wide-angle diffraction of the $L^c_{\beta'}$-phase, which is investigated here for the first time, reveals that the hydrocarbon chain tilt gradually changes with the content of cationic lipid.

The sodium salt of DNA calf thymus was obtained from Sigma (Deisenhofen, Germany). The dried DNA (MW 660) was dissolved in Millipore water ($R = 18.2$ MΩ/cm) and the concentration determined by 260/280 nm UV absorption. Varying amounts of the DNA solution (33 mg/ml) were filled into 1 mm X-ray quartz capillaries by weight and lyophilized in the capillaries. DMPC (dimyristoyl-phosphatidyl-choline, MW 677.9) and DMTAP (dimyristoyl-trimethyl-ammonium-propane, MW 590.4) were purchased from Avanti Lipids (Birmingham, AL, USA) in chloroform (purity > 99%) and mixed by volume in defined molar ratios. SUVs (small unilamellar vesicles) were prepared by solvent evaporation under nitrogen flow, vacuum drying, resuspension in Millipore water at 70 °C for 4 hr and by 10 minutes sonication. The DNA-lipid complexes were prepared by adding 50 mg/ml vesicle suspension onto the freeze-dried DNA inside the quartz capillaries. The samples were temperature cycled several times between 10 °C and 60 °C and allowed to equilibrate for more than seven days, at which time the samples exhibit a stable diffraction signal of only one phase. The X-ray experiments were carried out at the EMBL beam line X13 at DESY (Hamburg) designed for simultaneous small- and wide-angle scattering [6]. A monochromatic X-ray beam with a wavelength of 1.5 Å was selected by use of a Ge(111) crystal. The sample environment allowed temperature control within 0.1 °C and is described in ref. [6]. Typically the samples were equilibrated for 10 min at each temperature. During these periods a solenoid-driven shutter controlled by the data acquisition system was used to protect the samples from irradiation. Image-plate recordings were used for electron density calculations. In this case the Scherrer rings were radially integrated and corrected for the Lorentz-polarization factor [7].

A temperature series of simultaneous small- and wide-angle X-ray scattering (SAXS and WAXS) measurements of CL-DNA complexes is shown in fig. 1. The complexes consist of DMPC/DMTAP (1:1) mixed with a stoichiometrically isoelectric amount of calf thymus DNA, *i.e.* the number of cationic head groups was chosen to be equal to the number of anionic phosphate groups along the DNA. The Bragg reflections in the SAXS regime are attributed to the lamellar membrane stacking. The diffuse scattering peak (see arrow in fig. 1) arises from in-plane packing of the intercalated DNA strands [4]. A schematic sketch is shown in fig. 2b. At low temperatures a wide-angle peak appears that indicates the existence of an ordered hydrocarbon chain conformation. With increasing temperature a gel–to–liquid-crystalline phase transition is manifested by the disappearance of the chain correlation as well as a discontinuous jump of the SAXS Bragg reflections. Figure 2a shows the temperature

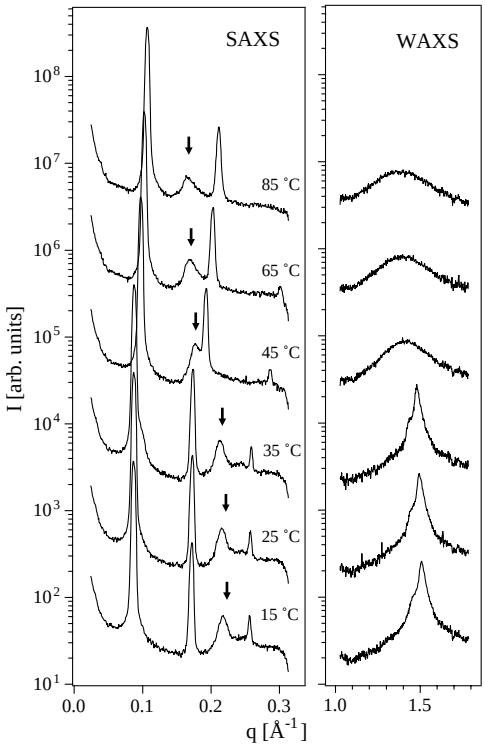

Fig. 1. – Small- and wide-angle X-ray scattering of isoelectric DMPC/DMTAP(1:1)-DNA complexes as a function of temperature (data log-stacked for clarity). The WAXS reflection indicates an order-to-disorder transition of the hydrocarbon chains between 35 °C and 45 °C. The lamellar repeat membrane distance decreases at the gel–to–liquid-crystalline transition, while the DNA in-plane correlation (see arrow) exhibits an expansion of the DNA in-plane rod lattice.

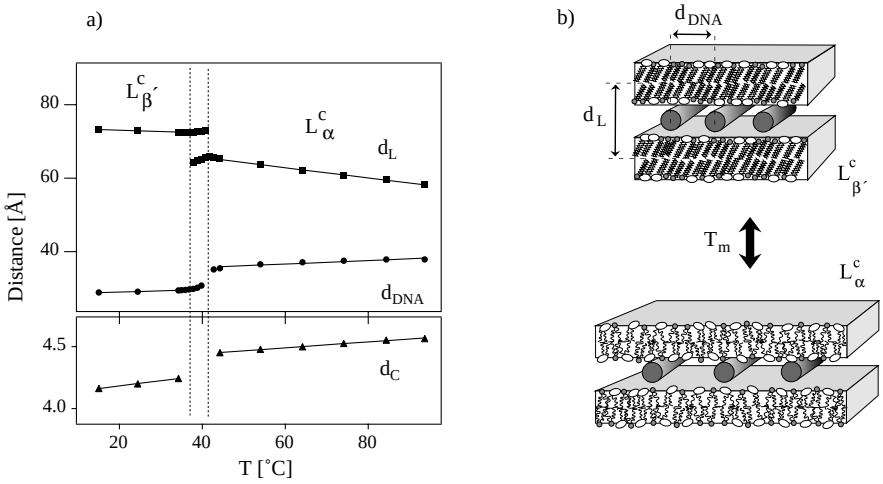

Fig. 2. – (a) Temperature dependence of the lamellar repeat distance, d_{L}, the interhelical distance, d_{DNA}, and the distance, d_{c}, corresponding to the WAXS reflection. (b) Schematic drawing of the thermotropic transition of the smectic lipid- columnar DNA lattice. The membrane thickness decreases due to chain melting, while the DNA lattice constant follows the area dilation of the lipid head groups.

TABLE I. – *Summary of the structural data of three isoelectric samples with different cationic molar fraction ϕ.* L/D*: lipid-to-*DNA *mass ratio,* d_{lam}*: lamellar repeat distance,* d_{DNA}*:* DNA *lattice constant,* δ_{mem}*: membrane thickness;* d_{c}*: distance corresponding to the* WAXS *reflection* (*in angstrom*) *and* ϑ_{T}*: chain tilt in the* $L_{\beta'}$*-phase.*

ϕ (%)	L/D	d_{lam}		d_{DNA}		d_{c}	δ_{mem}		ϑ_{T}
		$T = 15°\mathrm{C}$	$T = 45°\mathrm{C}$	$T = 15°\mathrm{C}$	$T = 45°\mathrm{C}$	$T = 15°\mathrm{C}$	$T = 15°\mathrm{C}$	$T = 45°\mathrm{C}$	$T = 15°\mathrm{C}$
25	9.5	75.6	67.0	–	–	4.16	39.6	35.0	0
50	4.5	73.2	65.3	28.9	35.3	4.17	37.2	33.3	20°
75	2.7	66.8	60.4	26.2	31.2	4.19	30.8	28.4	40°

dependence of the lamellar repeat spacing d_{L}, the DNA-DNA spacing d_{DNA} and the chain-chain correlation distance $d_{\mathrm{c}} = 2\pi/q_{\mathrm{c}}$, where q_{c} is the peak position observed in WAXS. A coexistence region characterized by two lamellar repeat spacings was found in the temperature interval T = 38–41 °C. The transition was completely reversible: no hysteresis effects in the X-ray diffraction pattern were observed after equilibration periods of about 15 min. The lamellar repeat distance decreased at the transition from 73 Å to 65 Å, while the lattice constant of the DNA rod lattice jumps up from 29 Å to 35 Å.

In fig. 3 the electron density profile calculated from the intensities of the seven lamellar reflections is shown for three different temperatures. The phase choice was found in order that the profiles exhibit the usual bilayer density plus an additional maximum in the middle of the water region due to the DNA strand. In this paper we will define the membrane thickness δ_{mem} to be the distance between head group peaks. The water layer thickness d_{w} is the difference between the lamellar repeat distance d_{lam} and membrane thickness, $d_{\mathrm{w}} = d_{\mathrm{lam}} - \delta_{\mathrm{mem}}$, and was found to be 35 Å in the ordered gel-phase, 32 Å in the fluid phase at 45 °C and finally 26 Å at 90 °C. The membrane thickness decreases furthermore with increasing molar fraction, Φ, of cationic lipid both in the fluid L_{α}^{c}- and the gel-like $L_{\beta'}^{c}$ phase (table I). In the L_{α}^{c}-phase this effect is partly due to the smaller head group size of the cationic lipid compared with the zwitterionic DMPC. In the $L_{\beta'}^{c}$-phase, moreover, the membrane thickness is affected by tilt of the alkyl chains. It is well known that in the fully hydrated $L_{\beta'}$ gel-phase of pure DMPC the lipid chains form a distorted hexagonal lattice with chain tilt towards next neighbours [7-10]. The signature in the WAXS scattering is a sharp reflection at 4.24 Å surrounded by a broader peak centered at 4.1 Å (fig. 4). In contrast the DMPC/DMTAP CL-DNA complexes with 25% molar fraction cationic lipid display a narrow symmetric peak at 4.16 Å indicative of an untilted L_{β}-phase. With increasing amount of cationic lipid the peak broadens and a shoulder at the low-q side of the chain packing peak appears (fig. 4). It is important to note that the width of the wide-angle reflection broadens more than the tilted $L_{\beta'}$-phase of pure DMPC. This behavior is explained by a tilt of the hydrocarbon chains between next neighbours rather than towards next neighbours. In the reciprocal space this unusual $L_{\beta\mathrm{F}}$ phase [10] gives 6 finite rod-lines perpendicular to the layers which are not centered in the equatorial plane [9]. The evolution of the tilt with the addition of cationic lipid is plausible, since the small TAP head group decreases the effective head group area [11]. At high TAP concentration, however, the electrostatic repulsion between the TAP head groups increases the effective head group area and favors tilt. Ultimately the question concerning hydrocarbon tilt can only be answered by X-ray analysis of oriented membranes [10]. However, we estimated the tilt angle from the width of the WAXS peaks, as well as from the change in membrane thickness [9]. In this context it

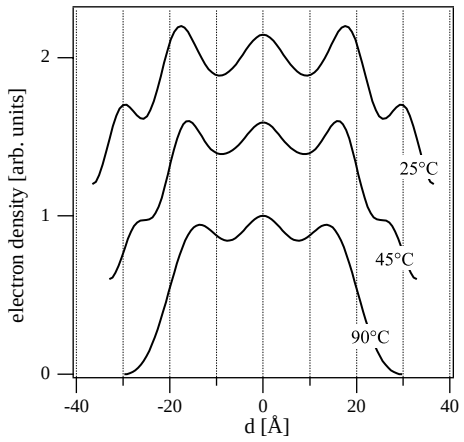

Fig. 3

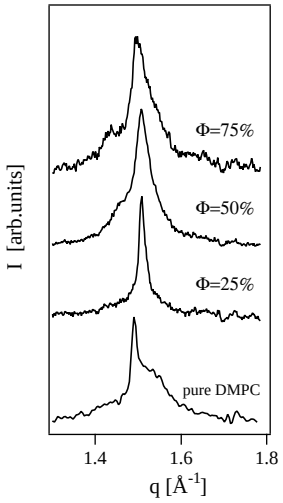

Fig. 4

Fig. 3. – Electron density profile of the cationic bilayer-DNA cross-section calculated from small-angle scattering of DMPC/DMTAP(1:1)-DNA at three temperatures (8 Å resolution).

Fig. 4. – Wide-angle X-ray scattering of the hydrocarbon chain order of pure DMPC in the tilted $L_{\beta'}$-phase, and DMPC/DMTAP-DNA complexes with three different cationic molar fractions ϕ. All samples at $T = 15$ °C. Data stacked.

is also relevant to note that the $P_{\beta'}$ ripple phase does not exist in CL-DNA complexes with molar contents of DMTAP larger than 20% [12].

The peculiar anisotropic structural changes of the CL-DNA complexes at the chain melting transition are typical for lipid membranes. The transition from an ordered chain configuration to a liquid-like organization is associated with an aliphatic chain area dilatation and a thinning of the bilayer thickness [8]. Figure 2b presents a schematic sketch of the CL-DNA chain-melting transition. We will show that the increase in the DNA spacing is a direct measure of the area dilatation of the lipid bilayer. Firstly, it must be noticed that the thermotropic changes in the DNA lattice (listed in table II) agree with the known area changes of pure lipid membranes. In micromechanical experiments on DMPC giant liposomes an area fractional change $(A_\alpha - A_\beta)/A_0 = 0.13$ at the main transition was reported and area thermal expansivity coefficients $\alpha(L_\alpha) = (4\text{–}6) \cdot 10^{-3}/°C$ and $\alpha(L_{\beta'}) = (5\text{–}8) \cdot 10^{-4}/°C$, respectively [13]. Secondly

TABLE II. – *The thermal expansion coefficients, α, and the fractional changes, $\Delta_{\alpha\beta}$, at the chain-melting transition of a* DMPC/DMTAP/DNA *complex with cationic volume fraction $\Phi = 0.5$. The coefficients are expressed relative to the spacing, d_0, defined as the corresponding spacing in the gel-phase at the transition temperature, T_m. δ_{mem}: membrane thickness, d_{DNA}:* DNA *lattice constant.*

	$\alpha(L_\alpha) = \frac{1}{d_0}\frac{\partial d_\alpha}{\partial T}[K^{-1}]$	$\alpha(L_\beta) = \frac{1}{d_0}\frac{\partial d_\beta}{\partial T}[K^{-1}]$	$\Delta_{\alpha\beta} = \frac{d_\alpha - d_\beta}{d_0}[K^{-1}]$
δ_{mem}	$-7.1 \cdot 10^{-4}$	$-9.5 \cdot 10^{-4}$	-0.11
d_{DNA}	$1.6 \cdot 10^{-3}$	$1 \cdot 10^{-3}$	0.19

the absolute DNA-DNA spacing can be calculated from the lipid-to-DNA mass ratio L/D. Assuming that the aggregates form one homogeneous phase the conservation of the local volume fractions predicts [3]

$$d_{\mathrm{DNA}} = \frac{A_{\mathrm{DNA}}\rho_{\mathrm{DNA}}}{\delta_{\mathrm{mem}}\rho_{\mathrm{mem}}} \cdot \frac{\mathrm{L}}{\mathrm{D}}, \tag{1}$$

where A_{DNA} and ρ_{DNA} denote the area cross-section and volume density of DNA and δ_{mem} and ρ_{mem} the thickness and density of the lipid bilayer. This simple formula was found to describe the DNA spacing in the regime between 30 Å and 70 Å (or $\Phi = 0.25$–0.6) of DOPC/DOTAP/DNA complexes using $A_{\mathrm{DNA}} = 186$ Å^2, $\rho_{\mathrm{DNA}} = 1.5$ g/ccm and $\rho_{\mathrm{mem}} = 1.07$ g/ccm [3]. Our data on DMPC/DMTAP/DNA follow the same equation within 1% accuracy for $\Phi = 0.5$. Most importantly, we find that the equation also holds in the L'_β gel-phase, if the L'_β membrane thickness and the L'_β lipid mass density are used. The latter increases by 5% from the fluid to gel-like state. The same relative change is found in the measured volume change of the membrane $\Delta_{\mathrm{V}} = \Delta_{\mathrm{DNA}} + \Delta_{\mathrm{mem}} \approx 5\%$ (table II). Equation (1) and the agreement with known lipid behavior indicates that the structural changes at the $\mathrm{L}^{\mathrm{c}}_\alpha$-$\mathrm{L}^{\mathrm{c}}_\beta$ transition are purely governed by the volumetric changes of the lipid bilayer. In the case of large cationic lipid content ($\Phi = 0.75$), however, the theoretical DNA spacing exceeds the measured values, most likely due to the short-range steric interaction between the DNA strands.

In conclusion we found that binary mixtures of saturated cationic lipids and saturated neutral lipids complexed with DNA form two intercalated lamellar CL-DNA phases, the fluid $\mathrm{L}^{\mathrm{c}}_\alpha$ and the $\mathrm{L}^{\mathrm{c}}_{\beta'}$ gel phase [12]. The chain-melting transition in the CL-DNA complexes is entirely dominated by the lipid phase behavior and exhibits the well-known anisotropic structural expansion of lipid membranes. The interaxial spacing is quantitatively described, both in the $\mathrm{L}^{\mathrm{c}}_\alpha$- and $\mathrm{L}^{\mathrm{c}}_{\beta'}$-phase, based on a model, which conserves the local volume fractions of lipid and DNA. The line shape of the $\mathrm{L}^{\mathrm{c}}_{\beta'}$-wide-angle reflection is consistent with the assumption that the smaller head group area of the DMTAP with respect to DMPC leads to an untilted L_β phase at a cationic molar fraction $\Phi = 0.25$ and possibly to a $\mathrm{L}_{\beta\mathrm{F}}$ phase with tilt between next neighbours at molar fractions $\Phi = 0.5$ and larger. We hope that in the future the preparation of aligned films of CL-DNA will clarify the nature of the $\mathrm{L}^{\mathrm{c}}_{\beta'}$-phase in more detail.

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