Observation of a Rectangular Columnar Phase in Condensed Lamellar Cationic Lipid-DNA Complexes

F. Artzner,[1,*] R. Zantl,[1] G. Rapp,[2] and J. O. Rädler[1,†]

[1]*Lehrstuhl für Biophysik, E22, Technische Universität München, James-Franck-Strasse 1, D-85748 Garching, Germany*

[2]*European Molecular Biology Laboratory, EMBL c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany*

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We report on a synchrotron x-ray diffraction study of saturated cationic lipid complexed with DNA. In the lipid gel phase $L^c_{\beta'}$ the complexes exhibit Bragg reflections due to lamellar lipid bilayer stacking and three nonintegral peaks in agreement with an intercalated centered rectangular columnar superlattice of DNA. The diffuse broadening of the DNA peaks is caused by in-plane translational disorder of the DNA strands and is approximated by Lorentzians with positional correlation lengths of $\xi_y \approx 250$ Å out of plane and $\xi_x > 2000$ Å in plane. We interpret our results in terms of a system of interacting two-dimensional smectic layers. [S0031-9007(98)07820-X]

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The thermotropic and lyotropic phase behavior of lipids as well as DNA has been investigated for many years [1]. More recently, it was discovered that DNA and oppositely charged cationic lipids (CL) form electrostatically stabilized composite liquid crystals. A structural characterization of CL-DNA complexes using synchrotron x-ray scattering revealed a lamellar phase of alternating lipid membranes and two-dimensional (2D) DNA smectic monolayers [2,3]. This structure found interest as the first experimental realization of a system of weakly interacting quasi-two-dimensional smectic manifolds [4,5]. A high resolution line shape analysis on CL-DNA aggregates revealed strong chain fluctuations within the layers and yielded the 2D compression modulus in the harmonic approximation [3]. Theoretically, three possible equilibrium phases of stacked 2D smectic layers can be distinguished depending on the transmembrane lattice interactions [4,5]: (i) a decoupled phase of strongly fluctuating 2D smectic; (ii) a weakly coupled stack of smectics with orientational long-range order, but short-range positional order; and, finally, (iii) a 3D columnar phase in the limit of strong interplane coupling. The second, new soft condensed matter phase was named "sliding phase" [4].

In this Letter we report on the existence of a columnar CL-DNA phase (Fig. 1) and analyze the strong, but finite, positional correlations over several layers. We investigated complexes made of cationic lipids with saturated alkyl chains, DMPC/DMTAP/DNA (dimyristoylphosphatidyl-choline/dimyristoytrimethyl-ammonium propane) using synchrotron x-ray scattering. The DMPC/DMTAP/DNA mixtures form an ordered $L^c_{\beta'}$ lipid-gel phase at low temperatures and undergo a first order transition to a L^c_α lipid-fluid phase [6]. The lamellar CL-DNA phase described here is one of the condensed lipid-polyelectrolyte phases, for which the lipid phase nomenclature plus an additional "*c*" for "condensed" or "complexed" is used [7].

Isoelectric CL-DNA complexes with a balancing number of cationic lipids, and DNA phosphate groups were investigated for DMPC/DMTAP mixtures with the molar ratio cationic lipid between 10% and 75%. The complexes were prepared by adding stoichiometrically isoelectric quantities of calf thymus DNA sodium salt to sonicated vesicle suspensions. Samples were temperature cycled several times between 10 and 60 °C and allowed to equilibrate for more than seven days. The x-ray experiments were carried out at the EMBL beam line X13 at DESY (Hamburg) using a monochromatic beam with a wavelength of 1.5 Å selected by a Ge(111) single crystal. The sample temperature was controlled within 0.1 °C by a water-bath regulating setup isolated with mica windows. The setup allowed us to record small- and wide-angle x-ray scattering simultaneously using two independent linear detectors [6]. In addition, 2D image plates were used to obtain a better signal-to-noise ratio.

In Fig. 2 the small- and wide-angle scattering of a DMPC/DMTAP/DNA sample is shown at low (15 °C)

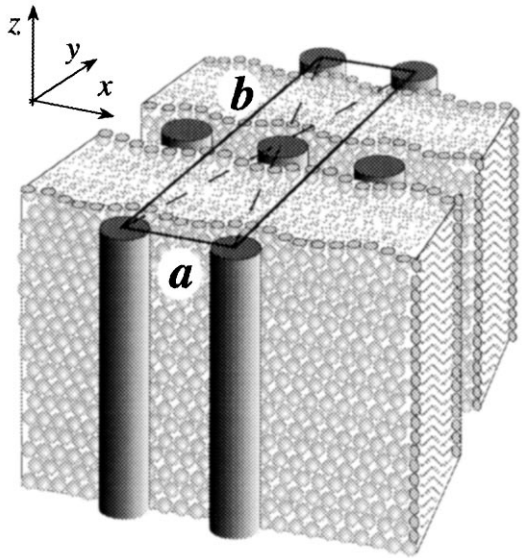

FIG. 1. Local structure of the centered rectangular columnar DNA lattice embedded in a cationic lipid lamellar phase.

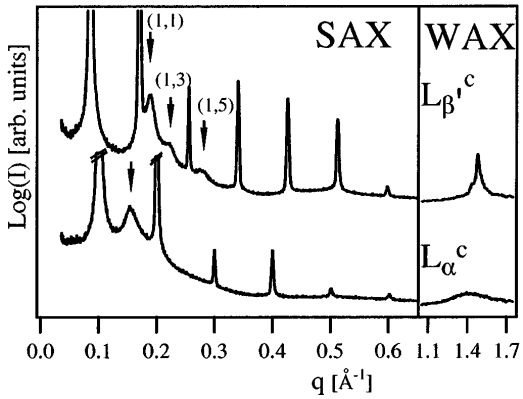

FIG. 2. Powder-averaged small- and wide-angle raw data of DMPC/DMTAP(1:1)-DNA complexes at 15 °C (L_{α}^{c}) and 55 °C ($L_{\beta'}^{c}$). The arrows indicate the position of diffuse scattering peaks arising from the intercalated DNA strands.

and high (55 °C) temperature. The crystalline order of the lipids in the $L_{\beta'}^{c}$ phase is revealed by the wide-angle reflection ($q = 1.51$ Å^{-1}). In either case, we observe a set of equally spaced small angle Bragg reflections at $q = 2\pi n/d_{lam}$ arising from the membrane repeat distance, d_{lam}. The lamellar Bragg reflections are resolution limited and can be called the fundamental reflections of the membrane-based composite structure. In the fluid phase, one additional broad asymmetric peak is seen, which is attributed to the in-plane positional correlation of an intercalated DNA rod lattice, as described in detail in Ref. [2,3]. At low temperatures three additional diffuse reflections are observed, which indicate a new superstructure of the intercalated DNA in the $L_{\beta'}^{c}$ phase. Their peak positions and peak widths will be analyzed below.

The peak positions of all $L_{\beta'}^{c}$ samples can be indexed to a centered rectangular columnar DNA lattice, $q_{hk} = 2\pi\sqrt{(h/a)^2 + (k/b)^2}$ with 2D lattice constants a and b. Thereby the lattice constant b is fixed by the fundamental lamellar spacing $b = 2d_{lam}$. A centered rectangular symmetry is built by oriented stacks of planar DNA rod lattices which are displaced in a centered *ABAB* configuration from layer-to-layer as shown in Fig. 1. We choose the DNA axis to be oriented along the z direction and the bilayer plane to be orthogonal to y. A simple rectangular phase corresponding to *AA* stacking cannot be brought into agreement with the measured sets of q values. The three DNA scattering peaks are indexed $(1, 1)$, $(1, 3)$, $(1, 5)$, respectively. The systematic missing of (h, k) peaks with $h + k = 2n + 1$ confirms the centered symmetry. Essential evidence for the proposed columnar superlattice is that all $L_{\beta'}^{c}$ samples independent of the DNA density, i.e., varying interaxial distance, can be mapped to it. The fit and the experimental data of the diffuse scattering of two slightly different lipid-to-DNA mass ratio samples are shown in Fig. 3.

An important distinction can be drawn between the scattering observed in decoupled 2D smectic manifolds and in the coupled rectangular columnar phase. The latter has locally the symmetry of a true 3D columnar phase with 2D orthorhombic density in the x-y plane, while on larges scales the positional correlation is lost, as we discuss in the following. The reflections $(1, 1)$, $(1, 3)$, and $(1, 5)$ are "off axis" in reciprocal space (see Fig. 3, top) as can be seen on the image plate x-ray data of oriented domains in powder samples [8]. In contrast, the high temperature L_{α}^{c} phase is some type of decoupled smectic missing cross-correlation peak. Only a "(10)" rodlike diffuse scattering (Fig. 3, top) with a typical asymmetric powder-averaged line shape is observed. An analogous behavior is found in alkylated smectic *B* liquid crystals, where the state of the alkyl chains determines different degrees of order [9].

The DNA peaks exhibit considerable line broadening, indicative of disorder in the columnar DNA lattice.

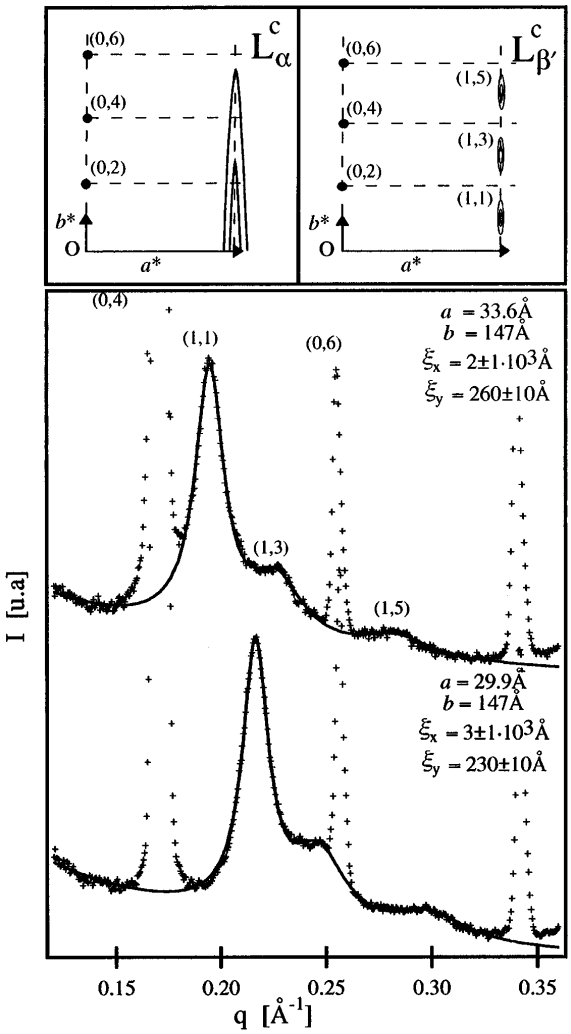

FIG. 3. Top: line broadening in a reciprocal lattice of the uncoupled and centered rectangular phases. Bottom: Lorentzian fit of the diffuse scattering to a centered rectangular superstructure. Two samples with different DNA-lipid ratio and hence DNA packing distance are shown.

We will introduce phenomenologically translational disorder which destroys the long-range correlation in the layers, as well as from layer-to-layer, and discuss the validity of this approach *a posteriori*. Knowing that the intercalated DNA is constrained by the closely spaced lipid membranes, we assume the displacement vector $\vec{u}$ to be restricted to lateral translations in plane: $\vec{u} = u(x, y)\vec{x}$. This assumption is justified by the fact the $(h, 0)$ peaks exhibit no measurable diffuse broadening. A corresponding 2D mass density correlation function is $g(x, y) = \langle \exp(iq_0[u(x, y) - u(0, 0)]) \rangle = e^{-h^2(x/\xi_x + y/\xi_y)}$ with in-plane positional correlation length ξ_x and out-of-plane correlation length ξ_y: In the reciprocal lattice, the reflections (h, k) appear broadened by a product of Lorenzians

$$I(ha^* + q_x, kb^* + q_y) = \frac{1}{q_x^2 + h^4/\xi_x^2} \frac{1}{q_y^2 + h^4/\xi_y^2} . \tag{1}$$

Note that the shapes of the (h, k) peaks are independent of k (Fig. 3, top). The powder averaging $\tilde{I}(q)$ of the scattering intensities can be carried out analytically:

$$\tilde{I}(q) = \frac{1}{4\pi} \int_{-\pi}^{\pi} d\theta \int_{-\pi/2}^{\pi/2} d\varphi I(q, \theta, \varphi) \cos\varphi . \tag{2}$$

Neglecting the curvature term of the sphere of radius q in the reciprocal space, we obtain in good approximation a sum of Lorentzian powder line shapes:

$$\tilde{I}(q) = \sum_{h,k} \frac{1}{(q - \tilde{q}_{hk})^2 + \tilde{\Delta}_{hk}^2} + O\left(\frac{\Delta_\perp^4}{q^4}\right), \tag{3}$$

with

$$\tilde{q}_{hk} = q_{hk} + \Delta_\perp^2/q_{hk} , \tag{4}$$

$$\tilde{\Delta}_{hk} = h^2(\xi_x^{-1} \cos\theta_{hk} + \xi_y^{-1} \sin\theta_{hk}) , \tag{5}$$

where q_{hk} and θ_{hk} are the positions of the peaks in cylindrical coordinates in the reciprocal space, and $\Delta_\perp^2 = h^4(1 + \tan\theta_{hk}^2)/(\xi_x^2 + \xi_y^2 \tan\theta_{hk}^2)$ is the square of the width of a (h, k) peak observed from the origin in the reciprocal space. As shown in Fig. 3 the three diffuse scattering peaks are well fitted simultaneously by using Eq. (3) and a simple base line $(c + d/q)$. The free parameters were the intensities $I_{1,k}$, the correlation lengths ξ_x, ξ_y, and the lattice constant a. Equations (4) and (5) determine the positions $\tilde{q}_{hk}$ as in Eq. (4) and the widths $\tilde{\Delta}_{hk}$ as in Eq. (5) of the Lorentzians. The fitted parameters are given in Fig. 3. The pertinence of the model is shown by the fact that the quality of the fits ($\chi_{\text{model}} = 1.97$) was not improved by allowing free positions and widths for each peak ($\chi_{\text{free}} = 1.93$). The shift of the peak positions is governed by the in-plane correlation length ξ_x [Eq. (4)] and improves χ by about 5% with respect to the naive positions q_{hk}. It should be furthermore noticed that the (20) and (22) reflections are not observed, in agreement with the h^2 dependence of the peak width.

We conclude from the above analysis that the observed line broadening in the $L_{\beta'}^c$ phase is consistent with translational disorder along $\vec{x}$. The determination of the in-plane correlation length is limited by the experimental resolution with lower limit 2000 Å and hence a low density of defects, and a large domain size has to be assumed. The out-of-plane disorder is well described by an exponential decay of the mass-density correlation function with a correlation length between three and four lamellar repeat distances, pointing out a translational disorder between neighboring 2D smectic layers.

In comparison, for the fluid L_α^c complexes, power-law-like diffuse scattering was reported which is caused dominantly by in-plane fluctuations of the 2D smectic manifolds [3]. The different positional coupling in the L_α^c and the $L_{\beta'}^c$ phase should originate from the fact that the $L_{\beta'}$ membrane is rigid, i.e., possesses a finite shear modulus. Hence we may consider the case that the DNA columnar lattice couples to the rigid membrane such that a nonzero macroscopic shear modulus stabilizes true long-range order. However, since we do not observe sharp DNA Bragg reflections this lattice must be perturbed by some kind of disorder. In principle, we have to distinguish topological disorder and thermal fluctuations, whereby the former kind of disorder might be intrinsic or quenched.

In an attempt to assess the degree of topological disorder we show a freeze fracture electron microscopy image of $L_{\beta'}^c$ complexes (Fig. 4). The picture shows large smooth terraces of layered lipid membranes, where membrane smectic-A edge dislocations are rarely seen. The electron microscopy (EM) image does not depict the DNA lattice, since freeze fracture tends to cleave the membrane in the midplane and is resolution limited by the grain size of the replica. The prevailing topological defects are most likely 3D stacking faults of the 2D DNA lattices caused by either translational or rotational mismatch. This type

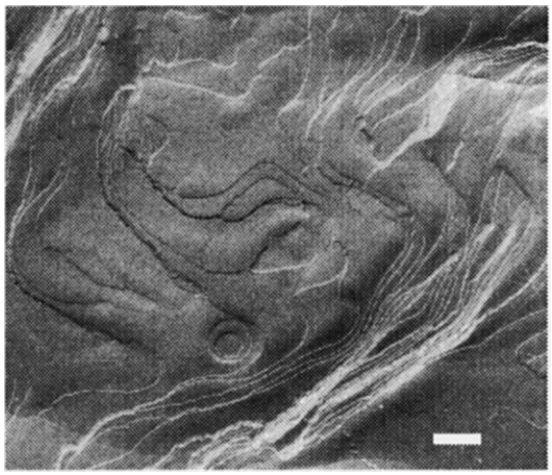

FIG. 4. Freeze fracture electron microscopy image of DMPC/DMTAP(1:1)-DNA complexes in the $L_{\beta'}^c$ phase showing a large scale defect-free lamellar organization of the lipid membranes. The bar equals 200 nm.

of disorder may result in an exponentially decaying correlation function [10].

On the other hand, thermal fluctuations become relevant if the DNA lattice is coupled to the rigid membrane in a way that can be overcome by thermal motion. One might picture this case as correlated units of DNA which are free to slide on the membrane but experience a weak periodic, washboardlike, potential transmitted from the next-layer 2D smectic lattice. The fluctuation behavior of this sliding phase was elucidated recently [4,5] and is expected to be dominated by a single exponential decay of the density correlation function out of plane.

Eventually, thermal fluctuations as well as topological disorder might contribute to the line broadening. We found that, during the first days of sample equilibration, temperature cycling improved on the peak intensities and line width, hence removed quenched disorder. In equilibrated samples, however, temperature jump experiments repeatedly and quickly yielded the same intrinsic peak width independent of sample history. The later experiments show that the kinetics of the L_α^c-L_β^c crystallization of the DNA lattice occurs on a time scale less than 10 s. The orientation of the DNA lattice in the fluid L_α^c phase seems to be conserved over a period of at least several hours.

Two mechanisms for the interlayer coupling can be proposed that will naturally lead to a centered *ABAB* stacking: (i) local elastic bending deformation of the lipid membrane and (ii) electrostatic repulsion across the membrane. Both contributions might go hand-in-hand by local demixing of the cationic and neutral lipid around the negatively charged DNA strand [11]. In the liquid crystalline L_α^c state a sinusoidal deformation on one side of the bilayer will be exponentially damped with a perpendicular penetration depth $l_p = \sqrt{B/K}\,(a/2\pi)^2$, where $B \approx 10^9$ dyn/cm^2 denotes the bulk compressiblity and $K \approx 10^{-5}$ dyn denotes the splay modulus [12]. For a periodicity $a = 30$ Å, the penetration length $l_p \approx 3$ Å is smaller than the bilayer thickness. In contrast, in the L_β, so-called lipid-gel state, the lipids exhibit short-range positional order with a finite in-plane and out-of-plane shear modulus. An increase in the compressibility of the bilayer in the crystalline state could possibly increase the penetration length of the deformation.

In this Letter we have provided evidence that CL-DNA complexes in the lipid-gel phase exhibit rectangular columnar order embedded in a lamellar lipid phase. The columnar phase established for the first time the centered symmetry of the interlayer correlations, which might prevail also in other cationic lipid systems [13]. The powder-averaged diffuse scattering is described by in-plane DNA positional disorder with the correlation length in plane being 1 order of magnitude larger than out of plane. Even though the phase has all of the characteristics of the predicted sliding phase, it is not possible at this stage to come to a conclusion as to the nature of the disorder. A higher resolution line shape analysis on aligned samples and a continuous control over the coupling strength by variation of the compounds in the layered complexes will be necessary to measure the crossover from 3D to 2D crystalline order.

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*Permanent address: Equipe Physico-chimie des systèmes Polyphasés, CNRS URA 1218, 5, rue J. B. Clément F-92296 Châtenay-Malabry, France.
†Email address: raedler@physik.tu-muenchen.de

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