Two-Dimensional Smectic Ordering of Linear DNA Chains in Self-Assembled DNA-Cationic Liposome Mixtures

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We report a synchrotron x-ray scattering study of linear DNA chains and cationic liposome mixtures which spontaneously self-assemble into a coupled two-dimensional (2D) smectic phase of DNA chains imbedded between lipid bilayers of a 3D smectic phase. The DNA peak is quantitatively described by *anisotropic exponentially decaying* chain-chain correlations. The measured interchain compressibility modulus $B(d)$ as a function of the interhelical spacing d of the 2D smectic, with $25 < d < 60$ Å, is not described by hard core repulsions but, rather, is dominated at larger spacings $d > 35$ Å by long-range electrostatic repulsions. [S0031-9007(97)04054-4]

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Over the last two decades the elastic and charge properties of the structurally well defined DNA macromolecule has lead to many important experimental studies [1]. Structural studies have centered around probing the various dense conformations of giant DNA molecules, with similarity to their biologically active native state, either induced by multivalent cations [2] or in high density liquid crystalline phases, both in vitro and in vivo in packing of DNA in cells [3].

More recently, there has been a tremendous surge in interest in elucidating the structures in complexes consisting of DNA mixed with oppositely charged (cationic) liposomes (closed bilayer shells of lipid molecules). This is because they have been shown to be able to mimic certain characteristics of natural viruses in their ability to act as efficient chemical carriers of extracellular DNA across outer cell membranes and nuclear membranes for gene therapy applications [4]. The complexes are novel statistical mechanical systems forming interacting tensionless one-dimensional (1D) polymeric chains physisorbed onto and confined between 2D fluid membranes with curvature elasticity [5]. More generally, the nature of interactions between polymers and membranes is actively studied and remains far from being understood [6].

In this Letter we report a synchrotron x-ray study with quantitative line shape analysis which demonstrates that in this recently discovered multilayered DNA-cationic liposome complex shown schematically in Fig. 1 [5], the 1D array of DNA chains sandwiched between cationic membranes exhibits a novel anisotropic chain-chain correlation function $g(\mathbf{r})$ which decays faster than algebraic and falls into the experimentally rare 2D smectic class [7]. The system constitutes the lower dimensional analog of three-dimensional smectic-*A* phases studied in numerous condensed matter materials in thermotropics [8], in lyotropic multilayer membranes [9], and in polymeric smectics [10].

The free energy density of a 2D smectic is given by

$$H/A = \frac{1}{2} B\left(\frac{\partial u(x,z)}{\partial z}\right)^2 + \frac{1}{2} K\left(\frac{\partial^2 u(x,z)}{\partial x^2}\right)^2, \quad (1)$$

where $u(x,z)$ is a continuum displacement field of the DNA chains with respect to a perfect lattice in the local coordinate system defined in Fig. 1. The 2D smectic lattice compressibility modulus is denoted by B (erg/cm^2), while the splay modulus $K = \kappa/d$ (erg) is related to the bending modulus of a single chain κ (erg cm). Equation (1) differs from the Hamiltonian of previously studied 2D systems such as stripe-phase domain walls in that there is no line tension term [11], so that DNA chain fluctuations are governed by their bending rigidity alone.

The correlation function $g(x,z) = \langle \exp(iq_0[u(x,z) - u(0,0)]\rangle$ can be calculated from Eq. (1) analogous to the derivation of the three-dimensional counterpart [8,12],

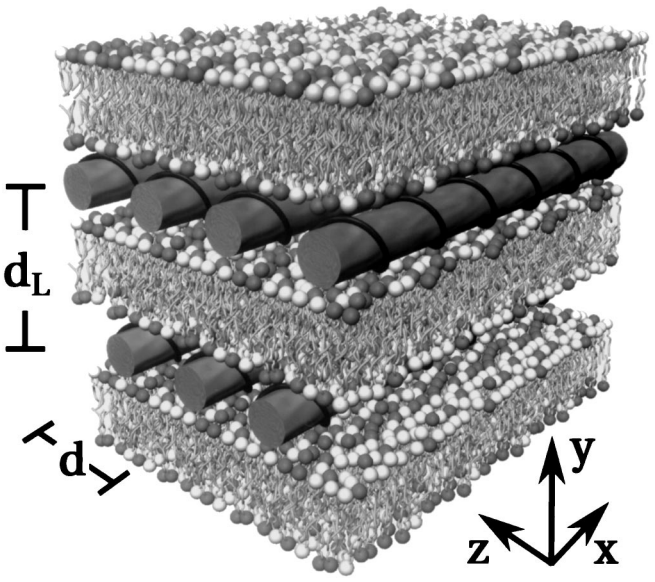

FIG. 1. Sketch of the self-assembled DNA/lipid complex with the DNA double helices represented by rods in between the lipid membrane comprising the neutral and cationic lipids. The corresponding lipid headgroups are shown in light and dark shades, respectively. d_L denotes the multilamellar periodicity and d the DNA spacing.

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yielding

$$g(x,z) = \exp\Big[-\eta\frac{2\pi}{\lambda}\sqrt{\pi\lambda|z|}\,e^{-x^2/(4\lambda|z|)} - \eta\frac{\pi^2}{\lambda}|x|\mathrm{erf}\Big(\frac{|x|}{2\sqrt{\lambda|z|}}\Big)\Big], \quad (2)$$

with $q_0 = 2\pi/d$, d = DNA interhelical spacing (Fig. 1), $\lambda = (K/B)^{0.5}$, and $\eta = k_B T q_0^2/(2\pi)^2 B$ and erf(z) denoting the error function. Parallel to the chains the correlation function decays exponentially $g(x, z \to 0) = \exp[-|x|/\xi_x]$ with a corresponding correlation length $\xi_x = \lambda/\eta\pi^2$. Normal to the chains the correlations decay as $g(x \to 0, z) = \exp[(z/\xi_z)^{0.5}]$, with $\xi_z = \lambda/(2\pi\eta)^2\pi$.

The samples were prepared by mixing lambda-phage DNA (48502 bp, contour length of 16.5 μm) with liposomes in ultrapure water as described previously [5]. When mixing aqueous solutions of DNA with a suspension of cationic lipid vesicles (cationic liposomes), a highly condensed system (complex) is formed in a self-assembled manner, where the cationic lipids neutralize the negative phosphate groups on the DNA (Fig. 1). The liposomes consisted of cationic DOTAP (dioleoyl-trimethyl-ammonium-propane) and neutral DOPC (dioleoyl-phophatidylcholine) at various weight ratios ν = mass[DOPC]/mass[DOTAP] with DOTAP/DNA = 2.2 (wt./wt.) kept constant at the isoelectric point of the complexes where the cationic DOTAP lipids exactly balance the negative phosphate groups of DNA. The unoriented samples were sealed in 1.5 mm quartz x-ray capillaries. The x-ray experiments (8 keV) were carried out at the Stanford Synchrotron Radiation Laboratory. The measured line shape did not depend on the instrument resolution function [5,9], which had a width typically 10 times smaller than the peak, and much faster decaying tails.

Representative small-angle scans are displayed in Fig. 2(a), shifted by multiplicative factors for samples of increasing mass ratio ν of neutral to cationic lipid (from top to bottom ν = 0, 0.67, 1.5, 2.33, and 5.25). While the lamellar (001) peaks only move slightly with ν towards smaller q, the much broader and weaker peak arising from the DNA-DNA correlations (vertical arrows) shifts over a wide range corresponding to a change in the DNA interhelical spacing d from essentially closed packed at 26 Å to significantly dilute at 54 Å. The measured curve $d(\nu)$ is displayed in Fig. 2(b) in membranes comprised of either DOPC/DOTAP or DLPC/DDAB (which results in thinner membrane) mixtures. This increase agrees well with the geometric packing relationship $d = (A_D/\rho_D)/(\delta_m/\rho_l)\,(L/D)$ [solid, dashed lines in Fig. 2(b)] derived previously [5]. Here, A_D is the DNA cross sectional area, ρ_D and ρ_l the densities of DNA and lipid, respectively, δ_m the membrane thickness, and L/D the total lipid to DNA mass ratio. The average thickness of the water gap $\delta_w = d_L - \delta_m$ remains nearly constant corresponding to the diameter of DNA = $2r_D$ = 20 Å plus a hydration layer. At higher dilution the system eventually phase separates to a phase of complexes in coexistence with a pure lipid lamellar phase [Fig. 2(a) bottom at ν = 5.25] [13].

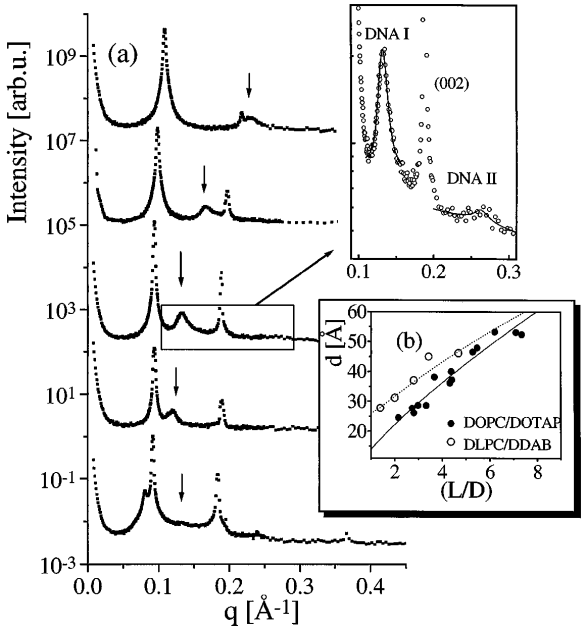

FIG. 2. (a) Small-angle scattering of charge-neutral DNA/complexes in excess water. Curves for samples of increasing ratios between neutral and cationic lipid ν are shifted by multiplicative factors: from top to bottom ν = 0, 0.67, 1.5, 2.33, and 5.25. Apart from very sharp Bragg reflections with the typical power-law tails of multilamellar phases, a much broader and weaker peak arising from DNA-DNA correlations is observed (vertical arrows). In some samples a weak peak is observed at the position of the second DNA harmonic (see inset). (b) From the peak positions as a function of ν the average interhelical spacing d between the DNA chains is obtained, and can be compared to the simple relationship expected for simple 1D packing (see text).

The tails in the lamellar (001) peaks decay algebraically, but simulations showed significant deviations from the standard Caille line shape [12]. This is not surprising since it may be expected that the bending rigidity κ_m of the membrane will be locally anisotropic, with one direction stiffened by the presence of the DNA chain [13]. Already on the large scale of Fig. 2(a), one recognizes the peculiar asymmetric line shape that directly relates to the confinement of DNA chains in a 2D layered structure. In some samples a very weak and broad second harmonic of the DNA was observed [see inset in Fig. 2(a)].

To model the data, we numerically calculate the structure factor of the DNA correlation peaks from $g(x,z)$. Since the data were taken from perfectly isotropic suspensions the "single crystal" result then has to be "powder averaged" in 3D. First, the structure factor is averaged in the two-dimensional $q_x - q_z$ space over the angle ϕ between the average helical axis of the DNA and the q_x axis, resulting in an expression that is applicable when the DNA is powderlike with a finite domain size L [9], but the

membrane stacks are perfectly oriented,

$$S_{2D}(q) = \frac{1}{2\pi}\int_{-\pi}^{\pi} d\phi \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} dz \times g(x,z)e^{-r^2\pi/L^2}e^{i(\mathbf{q}-\mathbf{G})\cdot\mathbf{r}}. \quad (3)$$

Here $\mathbf{q} = (q_x, q_z)$, $\mathbf{r} = (x, z)$, $\mathbf{G} = q_0\hat{\mathbf{z}}$. Investigating the resulting line shapes one finds that curves of fixed ξ_z and L become independent of ξ_x (apart from an absolute scaling factor), if ξ_x becomes larger than d [13]. In other words in this limit, the line shape is the same as that of an equivalent ensemble of rigid rods fluctuating only in their positions along z.

In the next step, the membrane normal is summed over all configurations with respect to $\mathbf{q}$ [14],

$$S_{3D}(q) \propto \int_{-\pi/2}^{\pi/2} d\theta\, \sin(\theta) S_{2D}(q\,\sin(\theta)) \times \left[\frac{J_1(r_D q\,\cos(\theta))}{r_0 q\,\cos(\theta)}\right]^2 f_y(q\,\cos(\theta)). \quad (4)$$

The second factor in the integrand is the cylindrical form factor of DNA with radius $r_D = 11$ Å, and $f_y(q_y)$ is the structure factor of the truncation rod which measures DNA-lattice correlations between layers and is a constant for a true 2D system. However, $f_y = 1$ does not fit the data with the right tail of the simulated peak being systematically too high [dotted line of Fig. 3(c)]. In other words, the model of perfectly flat and uncorrelated planes of DNA cannot explain all the data. After adding an effective Debye-Waller factor for the DNA rod which takes into account the thermal fluctuations in the y component (normal to the membrane) in the DNA-chain displacement function $u_y(x, z)$, the data can be fitted well for samples with small $d \leq 30$ Å with an rms roughness $(\langle u_y^2\rangle)^{0.5} \approx 10$ Å (with the lateral length scales of the DNA domains $L \approx 500$ Å). However, for larger d the required fluctuations increase tremendously with $(\langle u_y^2\rangle)^{0.5}$ between $0.4d_L$ and $0.6d_L$. Such strong fluctuations would affect the (001) peak significantly and completely wipe out the second harmonic lamellar peak, in contradiction to the experimental observation. We therefore conclude that at larger d a correlation between DNA of neighboring layers (correlation in the y direction) becomes important. To account for this we take $f_y(q_y) = 1/(1/\xi_y^2 + q_y^2)$ in Eq. (4) which describes exponentially decaying positional and orientational correlations along y.

Figure 3 shows 4 representative fits of the data to Eq. (4) along the lipid dilution line, for (*a*) $\nu = 0$, (*b*) 1, (*c*) 1.5, and (*d*) 3 (d spacings of 27.6, 38.1, 47.9, and 54.7 Å), with respective least-squares deviations of $\chi^2 =$ 1.24, 1.66, 1.65, and 0.83. The curves are plotted as a function of the normalized wave vector $(q - q_0)/q_0$, and have been shifted by additive constants along the abscissa for better comparison. In curve (*a*) the lamellar (002) peak falls on the left slope of the DNA correlation peak so that the affected data points had to be removed resulting in larger fitting uncertainties. The fitting parameters of the model were ξ_z, ξ_y, L, and d. ξ_x was kept constant in the range where it does not affect the line shape $\xi_x > 2d$ (see above).

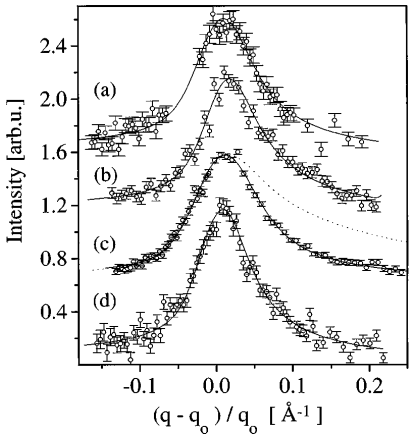

FIG. 3. The DNA correlation peaks of four representative samples: (*a*) $\nu = 0$, (*b*) $\nu = 1$, (*c*) $\nu = 1.5$, and (*d*) $\nu = 3$, corresponding to $d = 27.6$, 38.1, 47.9, and 54.7 Å, respectively. The normalized peaks are displayed as a function of the normalized wave vector $(q - q_0)/q_0$ and after background subtraction. The straight lines represent least-squares fits to the 2D smectic structure factor with exponential correlation across different layers. The dotted line in curve (*c*) is a simulation without such a correlation, i.e., with $f_y = 1$ in Eq. (4).

In the simulations, L determined by the width of the Gaussian-like center of the curve is found to vary nonsystematically with d, in a range between 500 and 1200 Å, corresponding to 2 to 4 times the correlation length ξ_z. The fitting results for ξ_z shown in Fig. 4(a) exhibit a moderate, roughly linear increase of ξ_z with d. Using the above definitions of ξ_z and ξ_x, one can extract the 2D chain-chain compressional modulus $B = (2\pi)^2 2^{-2/3}\{[(k_BT)^{4/3}/K^{1/3}](\xi_z/d)^{2/3}(1/d^2)\}$. The splay

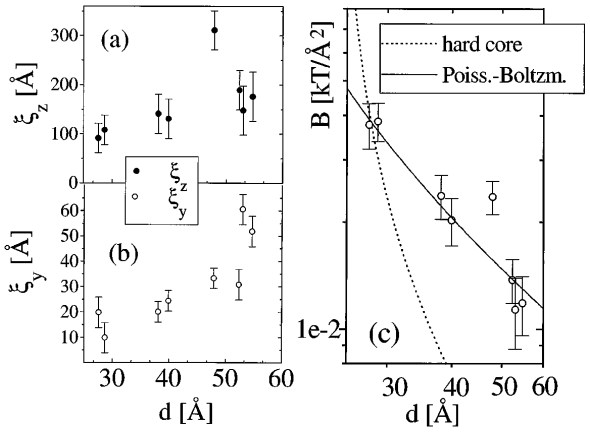

FIG. 4. The parameters obtained from the line shape analysis. (a) Correlation lengths ξ_z along the packing axis. (b) The correlation length ξ_y across the layers. (c) The values for the compressional modulus B in double-logarithmic scale. The decrease with d is much more moderate than the theoretic prediction (dotted line) for 1D packing of rigid rods with hard core repulsion (radius $r_D = 11$ Å). The solid line corresponds to the Poisson-Boltzmann model of electrostatically induced lipid counterion pressure (see text).

modulus K can be estimated from the DNA chain persistence length in two dimensions $\xi_p = 2\kappa/k_BT = 2Kd/k_BT$, which has been experimentally measured $\approx$500 Å [15]. The corresponding values of $B(d)$ are plotted in (c). Different values of ξ_p merely change the curve by a multiplicative factor, but not the d dependence.

First, $B(d)$ can be compared to the prediction for a one dimensionally packed hard-core system (e.g., hard cylinders) with radius $r_D = 11.5$ Å, which is shown as a dotted line in Fig. 4(c), using the analytical expression derived in [16]. The measured values are found to be significantly higher at large $d > 35$ Å. Thus, the ordering cannot just be an effect of close packing although at short distances $d < 35$ Å we expect hydration repulsion forces to contribute to B [17]. Exponentially decaying electrostatic interactions similar to those observed in hexagonal DNA liquid crystalline phases which result in $B \propto \exp[-d/\lambda_D]$ [17] can be ruled out for $d > 35$ Å, since a fit to this form is only consistent for a Debye length $\lambda_D = 25$ Å which corresponds to salt concentrations more than an order of magnitude larger than in the mixtures prepared in millipore water with free ion concentration <1 mM.

At larger $d > 35$ Å, outside of the hydration regime, our data are consistent with DNA chain-chain interactions being dominated by longer-ranged electrostatics and the entropic pressure of the lipid counterions (with the latter vanishing as $\nu \to 0$ for pure DOTAP membranes with $d \approx 27$ Å). At low salt concentrations the cationic lipids represent the dominant counterions to the DNA. As the lower dimensional analog to the solved case of charged membranes in pure water with no added salt [18], we expect the 2D counterion pressure $P(d)$ [force/length] between DNA chains to fall off to lowest order as $P \propto \pi k_BT/[2l_B(d - \rho)]$, where $l_B = e^2/(\varepsilon k_BT) = 7.1$ Å is the Bjerrum length in water (e = unit charge, ε = dielectric constant = 80), and $\rho < r_D$ is an effective excluded radius which is expected to be determined by a combination of the finite size of the counterion lipid (DOTAP) head group and r_D. The compressional modulus $B(d) = -d\,\partial P/\partial d$ is plotted as the solid line in Fig. 4(c) for the reasonable value of $\rho = 4.4$ Å. An overall prefactor to the computed B was also a fitting parameter. Thus, the simple dimensionality argument is in qualitative agreement with the experimental results. A fit to a recent calculation of the d dependence of B [19], including the long-ranged electrostatics and entropic effects contributing to B, is forthcoming [13]. In particular, we expect lipid chain stretch/compression, which changes the membrane cationic charge density in response to local changes in the DNA density, to contribute to B. We find that the correlation (ξ_y) between adjacent DNA layers increases for larger d [Fig. 4(b)] consistent with a simple electrostatic calculation where the electric field decays exponentially along y with a decay length proportional to d [19].

We have demonstrated that DNA orders as a finite sized 2D smectic phase in the 3D smectic DNA-lipid complex with weak correlations between layers [20]. The ordering is found to be consistent with the presence of long-ranged repulsive electrostatic forces. While we have measured the interhelical distance dependence of $B(d)$ of the 2D smectic phase, B and K could not be determined independently in unoriented samples. Studies in aligned samples measuring K will open up a new way to determine the persistence length of surface adsorbed polyelectrolytes.

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