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Columnar DNA Superlattices in Lamellar *o*-Ethylphosphatidylcholine Lipoplexes: Mechanism of the Gel-Liquid Crystalline Lipid Phase Transition

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ABSTRACT

DNA arranges into rectangular columnar superlattices between interdigitated lipid bilayers in the low-temperature gel phase of the lipoplexes of the cationic *o*-ethylphosphatidylcholines. The interlamellar correlation in the DNA ordering is not observed within the liquid crystalline lipid phase. The gel–liquid crystalline phase transition of the lipid induces a contraction of the DNA smectic array that matches the increased positive charge density of the membrane surface caused by the interdigitated–noninterdigitated transition. The transition proceeds via an intermediate state that has an expanded lamellar repeat period.

Complexes of DNA with lipids (cationic, zwitterionic) in water have attracted considerable attention recently, largely due to their use as nonviral DNA delivery vehicles in gene therapy.[1] However, there is still insufficient understanding at the molecular level of the nanoscale architecture and structural behavior of such lipid−DNA complexes (lipoplexes).

In the presence of DNA, aqueous lipid systems containing cationic lipid (properly, lipoid) have been shown to arrange into sandwich structures, with two-dimensional DNA smectic layers intercalated between lipid bilayers.[2−5] The major driving force for lipoplex formation is believed to be the entropy gain through counterion release.[4,6−10] The particular DNA arrangement that predominates depends on a number of parameters, including the intrinsic properties of the specific lipid system. The intercalated DNA strands have been found to exhibit in-plane positional correlation,[4,11] but in most cases, interbilayer coupling is not manifested. An example of strong interplane coupling has been reported for the system DMPC/DMTAP/DNA, in which DNA formed a rectangular columnar phase intercalated between tilted gel lamellar lipid bilayers $L^{c}_{\beta'}$ [12] (according to the accepted lipoplex designation, the superscript "c" is added to the common lipid nomenclature to indicate "complexed" [4]).

In the present letter, we report on the transmembrane coupling of the 2D DNA smectic structures in lipoplexes as a function of one of the major determinants of the molecular order in lipid systems − the gel-liquid crystalline ("main") phase transition. The lipid component of the lipoplexes was a cationic *o*-ethylphosphatidylcholine with saturated hydrocarbon chains of 14, 16, or 18 C atoms. Cationic lipids of this class are promising transfection agents since they are the only cationic membrane lipid derivatives shown to be metabolized by cells; moreover, they do not require a "helper" lipid for their activity and function in the presence of serum.[5] Here we demonstrate that, in the low-temperature gel lipid phase, DNA arranges into a centered rectangular columnar phase intercalated between interdigitated lipid bilayers. To the best of our knowledge, this is the first report of such an arrangement. Conversion of the DNA arrangement from the coupled 3D columnar phase to a decoupled 2D smectic manifold is triggered by the lipid phase transition from the interdigitated gel phase to the lamellar liquid crystalline phase.

Lipoplex components were dimyristoyl-, dipalmitoyl-, or distearoyl- *o*-ethylphosphatidylcholines (EDMPC, EDPPC, EDSPC, respectively), as triflate salts[5] (chloride salts from Avanti Polar Lipids, Inc.; Birmingham, AL, were also used in some experiments), and herring sperm DNA (Invitrogen, Carlsbad, CA). Samples were prepared by adding an aqueous DNA solution to the dry lipid film and vortexing at a temperature above the lipid phase transition, as previously described.[13] Small-angle X-ray diffraction (SAXD) measurements were performed at Argonne National Laboratory, Advanced Photon Source, BioCAT (beamline 18-ID) and DND-CAT (beamline 5-IDD), using 12 keV X-rays. A Linkam thermal stage (Linkam Sci Instruments, Surrey, England) provided temperature control. Linear heating and

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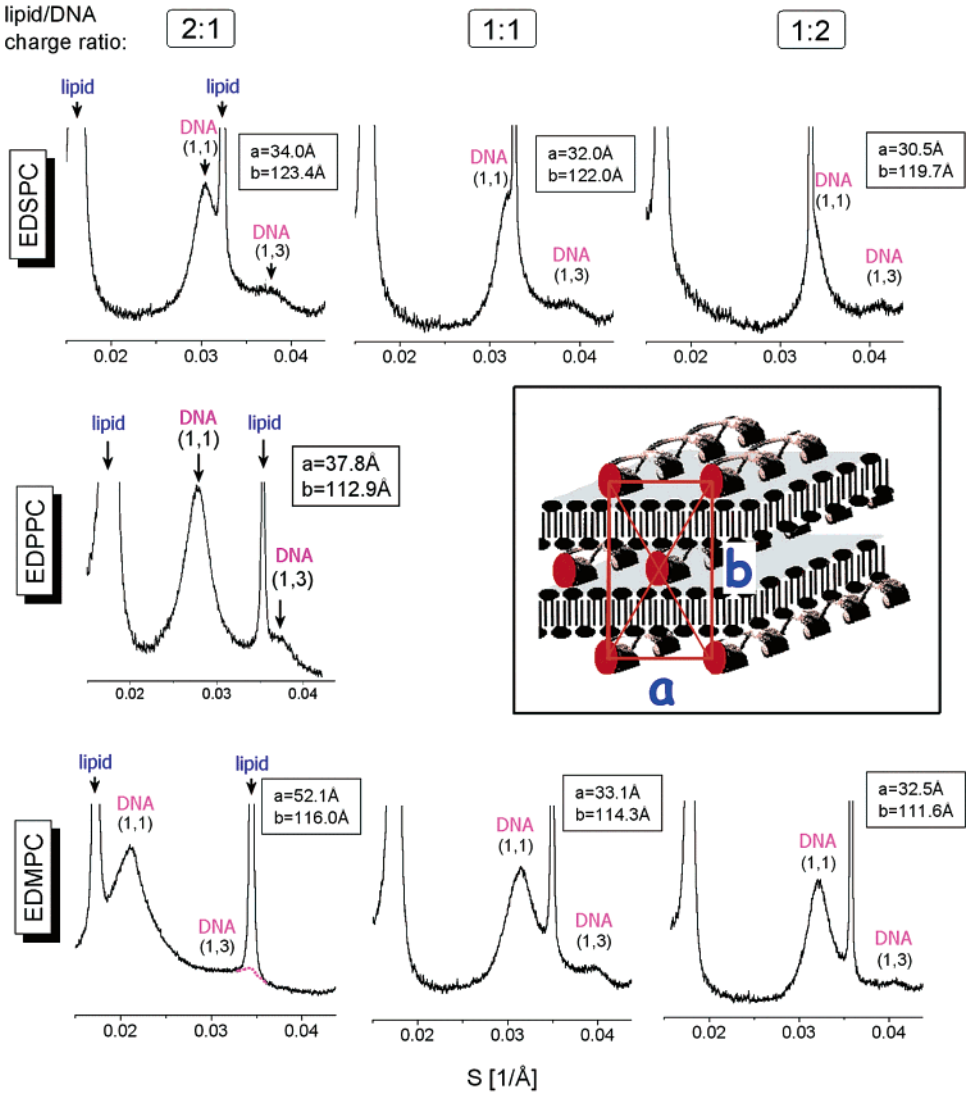

Figure 1. Small-angle X-ray diffraction profiles of the gel-phase ethyl-PC/DNA samples at different stoichiometries. Data for lipids with three different chain lengths are shown. In all samples, the diffuse DNA reflections index as a centered rectangular columnar lattice, depicted in the drawing in the inset. The X-ray patterns are registered at ~15 °C below the transition temperature of the lipids.

cooling scans were performed at rates of 0.8−2 °C/min. Exposure times were typically ~0.5 s.

Aqueous dispersions of the saturated-chain representatives of the *o*-ethylphosphatidylcholines have previously been shown to exhibit gel−liquid crystalline phase transitions at temperatures close to those of their parent phosphatidylcholines; addition of an isoelectric amount of DNA does not change significantly the lipid phase transition parameters.[5,14] Figure 1 displays the small-angle X-ray diffraction profiles of gel-phase ethyl-PC/DNA complexes in three different regimes: excess lipid (2:1 lipid/DNA charge ratio), isoelectric (1:1 lipid/DNA charge ratio), and excess DNA (1:2 lipid/DNA charge ratio). A set of sharp reflections typify the lamellar gel lipid phase $L^{c}_{\beta I}$ (d = 56.4 Å for the EDPPC/DNA system shown in the middle row). This lamellar repeat period is ~13.5 Å larger than that observed for the interdigitated gel phase of the pure aqueous EDPPC dispersions, and the increase is considered due to DNA strands intercalated between the interdigitated lipid layers.[5,14] In addition to the sharp lipid reflections, two or three additional diffuse reflections are seen; these are attributed to the DNA ordering both within the smectic layers between the lipid bilayers, as well as across bilayers, from one DNA layer to another. The positions of these reflections index to a centered rectangular columnar lattice, $S_{hk} = \sqrt{[(h/a)^2 + (k/b)^2]}$, with lattice constants a and b; the constant b is determined by the lamellar spacing d ($b = 2d$). The DNA scattering peaks index as (1,1) and (1,3), respectively (and in some cases (1,5); not illustrated). A schematic presentation of the centered rectangular columnar lattice of the DNA rods between the interdigitated lipid layers is shown in the inset of Figure 1, and the calculated lattice structural parameters are included in the corresponding panels of Figure 1. Reasoning similar to that suggested in ref 12 for verifying the centered symmetry of the lattice is appropriate here. Due to the interdigitated nature of the gel phase of the ethyl-PC lipoplexes, however, the parameter b of the DNA lattice is considerably smaller than in noninterdigitated bilayers.

Upon heating, the lipid layers of the lipoplexes undergo a phase transition from the interdigitated gel phase to a liquid

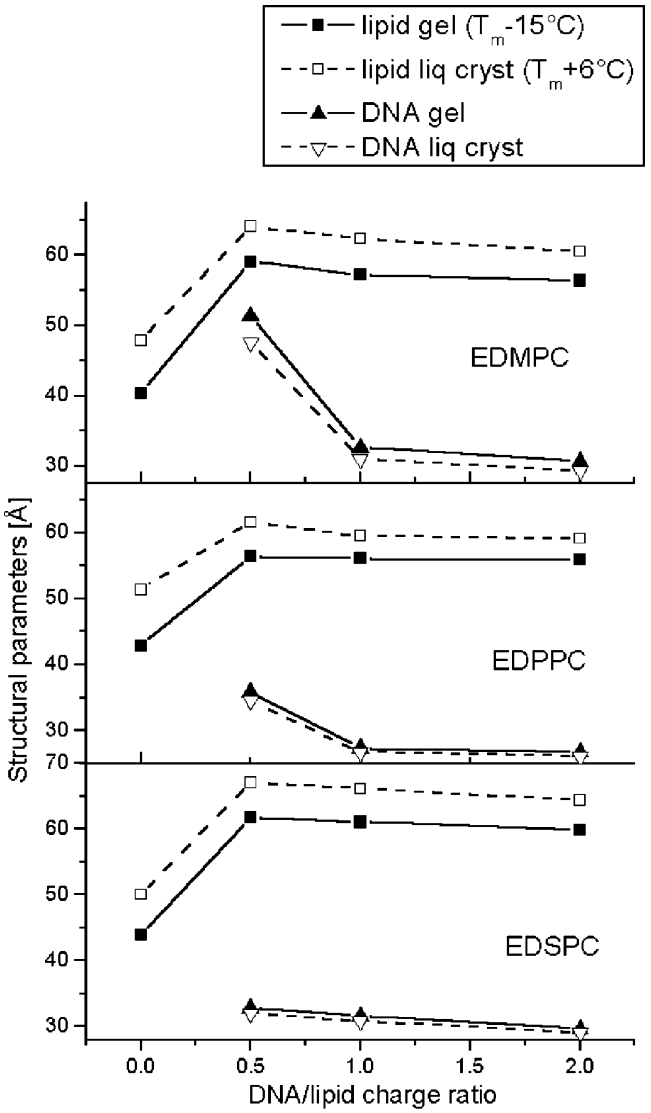

Figure 2. Variation of the lipoplex lamellar repeat distance d and the DNA interhelical distance d_{DNA} as a function of the lipid/DNA stoichiometry, in the low-temperature gel phase (taken at temperatures ~15 °C below the transition temperature), and in the high-temperature liquid crystalline phase (taken at ~6 °C above the transition temperature), as indicated. The solid lines and full symbols refer to the gel phase, and the dashed lines and open symbols refer to the liquid crystalline phase; squares refer to the lipid spacings, and triangles to the DNA spacings. At excess lipid, the higher lamellar repeat period is probably due to rippling or pinching, which disappears upon saturation of the lipoplexes with DNA.

crystalline lamellar L^c_α phase at temperatures close to those of the pure lipid dispersions: 23, 41, and 52 °C, respectively, for the EDMPC, EDPPC, and EDSPC complexes. The transition temperatures were independent of the amount of DNA, as previously reported.[14] This transition is associated with an expansion of the lamellar repeat distance d by 4−6 Å. The lamellar periods of the lipoplexes as a function of the DNA contents are shown in Figure 2.

The lipid phase transformation was immediately followed by a rearrangement of the DNA lattice, specifically, a decrease of the DNA in-plane distance by ~1 Å and a loss of the interlamellar correlation, the latter revealed by the disappearance of the multiple DNA reflections from the SAXD patterns. Thus, at temperatures above the lipid phase transition, a single broad DNA reflection, attributed to the in-plane, strand-to-strand positional correlation of the DNA strands, was seen. The change of the DNA interstrand distance for the three different lipid/DNA stoichiometries is shown in Figure 2 as a function of DNA/lipid charge ratio.

The reduction of the DNA spacing during the gel−liquid crystalline lipid transition of ethyl-PCs is opposite of that previously reported for the system DMPC/DMTAP (1:1)+DNA; in that case an expansion of the DNA interhelical distance was induced by the lipid phase transition.[15] This difference probably reflects the interdigitated nature of the ethyl-PC gel phase,[16,17] which is preserved in the presence of DNA.[14] Indeed, while noninterdigitated gel systems exhibit increasing area per headgroup at the melting transition, the change is in the opposite direction during interdigitated gel−liquid crystalline (noninterdigitated) phase transitions (see, e.g., refs 18−20). We can thus expect that the decrease in the DNA interstrand spacing is produced by the increased positive charge density of the membrane surface that occurs during the interdigitated−noninterdigitated transition. Quantitatively, if the charge densities of the lipid multilayers and the DNA smectic arrays are matched in an isoelectric system, then the DNA interstrand distance d_{DNA} can be estimated as[15]

$$d_{DNA} = (S_{DNA}\rho_{DNA})\cdot(L/D)/(d_{lip}\rho_{lip})$$

where $S_{DNA} = 186$ Å^2 and $\rho_{DNA} = 1.7$ g/mL are the cross-sectional area and the density of DNA,[21] respectively; d_{lip} is the lipid bilayer thickness, ρ_{lip} is the lipid density, and L/D is the lipid/DNA weight ratio. For the isoelectric EDSPC/DNA system ($L/D = 2.94$), lamellar spacings d of 61 and 66 Å for the gel and liquid crystalline phase, respectively, were measured in the present study; the water layer thickness of 27 Å was previously determined from electron density profiles[5]; thus, the lipid bilayer thicknesses are d_{lip} (gel) = 34 Å and d_{lip} (liquid crystalline) = 39 Å. A change in the DNA distance Δd_{DNA} ~1 Å was measured during the gel−liquid crystalline transition (Figure 3) for the EDSPC/DNA (1:1) system. If this change is supposed to be due solely to the change of the lipid surface charge density during the transition, the expected change in the lipid specific volume during the transition would be Δv ~0.1 mL/g (a value of 1.03 mL/g for the lipid specific volume in the liquid crystalline phase as measured for DSPC[22] was used for this estimation). Such a value for the specific volume change is within the limits of those typically reported for the melting transition of lipids, although close to the upper limit.[21,23] A similar value of $\Delta v = 0.098$ mL/g was measured, for example, with another charged lipid of 18 carbon atom chains − dioctadecyl-β-D-glucuronosyl-*sn*-glycerol.[24] A possible source for a large volume change of the cationic EDSPC upon the phase transition is also the interdigitated nature of its gel phase. Indeed, the volume change for the phase transition has been reported to be larger for an interdigitated gel than for a noninterdigitated gel.[19,25] Thus, the DNA rearrangement in the isoelectric lipoplexes during the transition could be roughly accounted for by the change in the

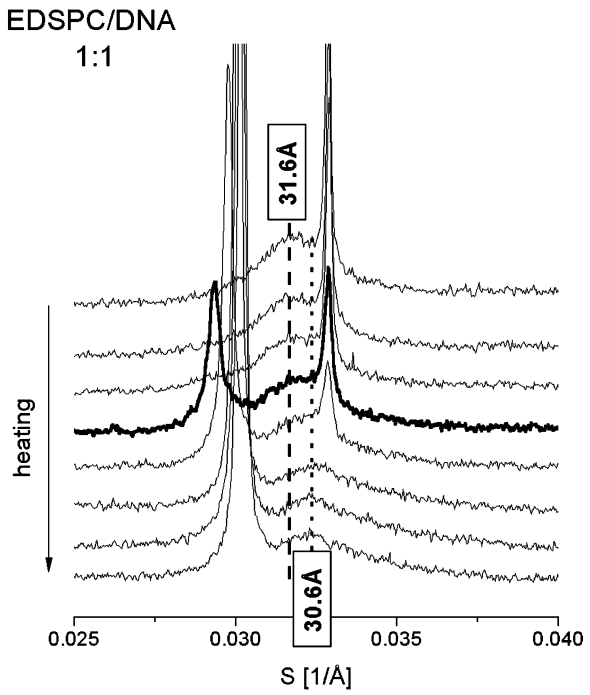

Figure 3. Small-angle X-ray diffraction patterns recorded with an EDSPC/DNA (1:1) sample upon heating from 30 °C to 58 °C at 1 °C/min. The evolution of the DNA interstrand peak could be followed during the lipid phase change; the DNA strand spacing becomes smaller to match the increased positive charge density of the membrane surface in the liquid crystalline phase.

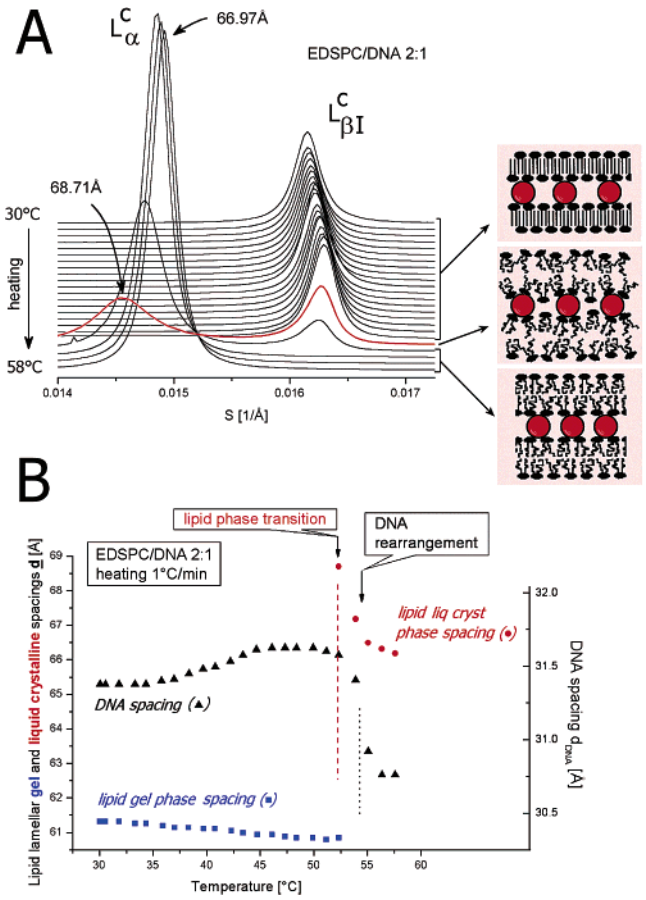

Figure 4. (A) Small-angle X-ray diffraction patterns recorded from EDSPC/DNA (2:1) upon heating from 30 °C to 58 °C at 1 °C/min. The phase transition from the interdigitated gel phase (with a short repeat distance) to the noninterdigitated liquid crystalline phase takes place at ~52−53 °C. An intermediate state with broader lamellar reflections at a larger repeat period can be seen. Suggested representations of the molecular arrangements are shown on the right side. (B) Spacings of the lipoplex lamellar repeat period d and the DNA arrangement d_{DNA} in the EDSPC/DNA (2:1) system upon heating from 30 °C to 58 °C at 1 °C/min. The lipid phase transition precedes the DNA rearrangement and includes an intermediate, disordered state of large lamellar repeat distance; the DNA interstrand distance remains typical for the gel phase in that intermediate state (due to the scaling, the DNA diffraction peaks cannot be seen in panel A).

lipid charge density during the interdigitated gel−noninterdigitated liquid crystalline phase transition (a conceivable reciprocal effect of the DNA on the lipid charge density would be much smaller and is not considered).

With the nonisoelectric samples (2:1 excess lipid, and 1:2 excess DNA), the lamellar repeat period is essentially the same as of the isoelectric lipoplexes (Figure 2); apparently DNA is tightly bound to the lipid layers in all cases. The minor decrease produced by increasing DNA content is likely a result of better ordering in the samples saturated with DNA. With respect to the DNA spacings in the different composition regimes, the results are qualitatively consistent with the theoretical analyses[6−8] predicting overcharging of the complex at compositions different from isoelectric (within certain composition ranges), driven by the entropy gain of a partial counterion release within the complex upon integration of the excess lipid or DNA into the complex; such overcharging has been previously reported to take place in DOTAP/DOPE lipoplexes.[4,26] Indeed, the DNA distance increases at DNA/lipid charge ratios <1, indicating that the excess lipid is incorporated into the lipoplexes (Figure 2). This increase is most pronounced with the shorter chain EDMPC, and is less so for the longer chain lipids (Figure 2). Such behavior possibly reflects the decreased area per molecule (increased charge density) of the longer chain lipids.[23]

The transmembrane coupling of the DNA ordering in the gel phase is a likely result of the membrane rigidity in that phase. Indeed, the solid lipid bilayer should favor long-range order. In contrast, the "fluid" bilayers in the liquid crystalline phase reduce that kind of order. The smaller thickness of the interdigitated gel bilayers might additionally facilitate, by increasing electrostatic interactions across the bilayer, the transmembrane coupling within the columnar DNA superlattice. The previous observation of such an arrangement referred to noninterdigitated gel bilayers, which are significantly thicker (by some 15 Å).[12] In that case, however, the membranes consisted of a mixture of cationic (DMTAP) and electrically neutral (DMPC) lipid. Thus, the centered rectangular stacking of DNA in that system might be facilitated by a local demixing of these lipids around the negatively charged DNA strands, along with local bending of the lipid bilayer. In fact, our ethyl-PC lipoplexes represent a sole example in which centered rectangular DNA ordering is attained within a single-lipid system.

Close inspection of the X-ray diffraction patterns within the transition region reveals a few intermediate profiles with

expanded, diffuse lamellar reflections preceding the equilibration of the liquid crystalline noninterdigitated arrangement (Figure 4A). This feature is observed with all three stoichiometries (lipid/DNA 1:2; 1:1; 2:1 charge ratios), and all three cationic ethyl-PC species studied. At that stage of the transition, the DNA reflection remains unchanged from that of the gel phase. It changes to a lower value immediately following the relaxation of the lamellar arrangement to its liquid crystalline state (Figure 3). The spacings of the lipid lamellar gel and liquid crystalline phases along with the DNA interstrand spacing for the EDSPC/DNA 2:1 sample that were recorded on heating are plotted in Figure 4B. A similar disordered state of diffuse and large lamellar repeat distances within the phase transition region could be identified in the cooling scans as well (not illustrated). It is conceivable that the lipid gel−liquid crystalline transition proceeds via an intermediate state having an expanded lipid lamellar distance (larger than that of the liquid crystalline phase) and an expanded DNA strand arrangement (typical for the gel lipid phase). This intermediate state precedes the shift of the DNA diffraction peak to the lower values that are characteristic of the liquid crystalline lipid phase (DNA condensation); it may thus reflect a short-lived rippling or disordering of the lamellar structure due to the transient mismatch between lipid and DNA charge densities during the transition.

In summary, in lipoplexes of the cationic *o*-ethylphosphatidylcholines with saturated hydrocarbon chains of 14, 16, and 18 C atoms, DNA arranges into rectangular columnar two-dimensional superlattices between interdigitated lipid bilayers in the gel phase. The DNA arrangements in the nonisoelectric samples (2:1 excess lipid and 1:2 excess DNA) are indicative of overcharging of the complex, as theoretically expected. The gel → liquid crystalline lipid phase transition, which gives rise to a noninterdigitated arrangement, proceeds with an increase of the lamellar lipid spacing. This transition induces a contraction of the DNA strand arrays until their mean charge density matches the increased positive charge density of the membrane surface produced by the interdigitated → noninterdigitated transition. The interlamellar correlation in the DNA ordering was not observed in the liquid crystalline lipid phase.

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