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Observation of a Rectangular DNA Superlattice in the Liquid-Crystalline Phase of Cationic Lipid/DNA Complexes

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Over the last years complexes of DNA with cationic lipids (lipoplexes) have attracted much attention because of their potential use as nonviral gene delivery systems.[1] Among the possible arrangements, the lamellar phase is largely the most abundant nanoscale architecture:[2] this structure consists of smecticlike arrays of stacked bilayers with monolayers of DNA intercalated within the water gaps. The DNA strands usually exhibit 1D in-plane positional correlation,[2−4] while in some cases 2D interbilayer coupling is manifested when DNA is embedded within lipid bilayers in the gel phase.[5−7] Here we report, for the first time, on the observation of transbilayer correlation in the DNA ordering in the liquid-crystalline lipid phase. We also show that different DNA superstructures can be obtained by adjusting the membrane rigidity. Aside from clarifying the role of membrane rigidity in promoting formation of transbilayer DNA superstructures, our results may be potentially important for the fabrication of DNA sequencers, chips, and biosensors.

The lipid component of lipoplexes was a binary mixture of dimyristoyl phosphatidylcholine (DMPC) and the cationic Gemini surfactant (2*R*,3*R*)-2,3-dimethoxy-1,4-bis(*N*-hex-adecyl-*N*,*N*-dimethylammonium)butane dibromide, **1** (Figure 1).

DMPC was purchased from Avanti Polar Lipids and used without further purification. Extruded DMPC/**1** liposomes at 6/4 molar ratio were prepared as elsewhere described.[8] DMPC/**1**/DNA lipoplexes were prepared by addition, at room temperature, of known volumes of an aqueous 2 mM solution of calf thymus DNA (Sigma, St. Louis) in HEPES buffer (5 mM HEPES, 0.1 mM EDTA, at pH 7.4). The concentration of DNA was dictated by the concentration of **1** to obtain charge-neutral (ρ=[cationic headgroup]/[DNA single base] = 1) or cationic ($\rho = 2$) complexes. Synchrotron small and wide-angle X-ray diffraction (SWAXD) measurements were performed at the Austrian SAXS station of the synchrotron light source ELETTRA (Trieste, Italy). Detailed experimental conditions can be found elsewhere.[4] The unoriented samples were sealed in 1.5 mm diameter quartz X-ray capillaries. SAXD experiments 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.

In Figure 2 (top panel) the SWAXD patterns of isoelectric DMPC/**1**/DNA lipoplexes at low temperature (5 °C) are shown. The crystalline order of the lipids in the gel phase is revealed by the WAXD pattern showing a typical strong reflection. In the SAXD regime, we observe a set of equally spaced Bragg reflections arising from the membrane repeat distance $d = 2\pi/q_{001} = 52.4$ Å. In

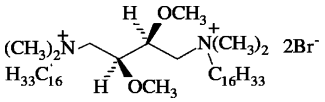

Figure 1. Chemical structure of the Gemini surfactant **1**.

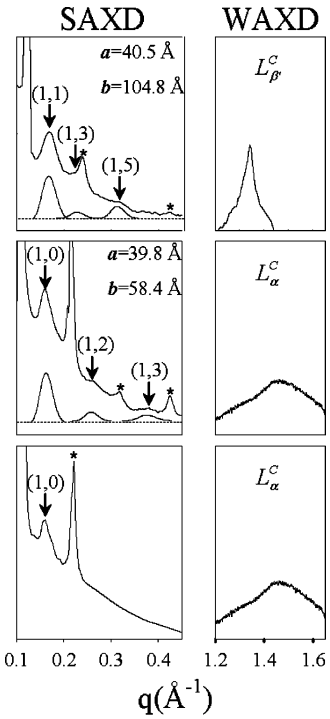

Figure 2. Small and wide-angle X-ray diffraction patterns of DMPC/**1**/DNA lipoplexes at different temperatures. At 5 °C (top panel), the diffuse DNA reflections in the SAXD regime (marked by arrows) index as a centered rectangular columnar lattice between gel bilayers. At 50 °C (middle panel), DNA arranged into a simple rectangular phase between fluid bilayers (as shown by the WAXD pattern). At 65 °C (bottom panel), transbilayer coupling disappeared and the single broad DNA reflection was attributed to the in-plane DNA−DNA positional correlational. Lipid reflections are marked with the symbol ∗. Solid lines are the best fit functions to the DNA reflections.

lamellar lipoplexes, the average thickness of the interlamellar water gaps corresponds to the diameter of DNA plus a hydration layer ($d_W \approx 20$ Å).[9,10] Because the complex consists of alternating layers of lipid and DNA, we could calculate the lipid bilayer thickness ($d_B = d - d_W = 32.4$ Å). This value is in very good agreement with the bilayer thickness of pure DMPC/**1** aggregates ($d_m = 30.7$ Å).[11] Such a thickness was much lower (by some 15 Å) than that of pure DMPC (∼47 Å)[12] and was ∼4.5 Å larger than that observed for the highly interdigitated phase of pure **1** bilayers.[13] Thus, our

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structural findings indicated that Gemini promoted the formation of interdigitated DMPC/**1** bilayers with a high degree of interpenetration of alkyl chains. Furthermore, the addition of DNA did not change significantly the membrane thickness of DMPC/**1** bilayers. In addition to the sharp lipid peaks (marked by stars), three diffuse reflections were seen and indexed as the (1,1), (1,3), and (1,5) reflections of a centered rectangular columnar DNA lattice, $q_{hk} = 2\pi[(h/a)^2 + (k/b)^2]^{1/2}$, with lattice constants $a = 40.5$ Å and $b = 104.8$ Å. The systematic missing of (h,k) peaks with $h + k = 2n + 1$ confirmed the centered symmetry.[5−7] As evident, the lattice parameter $b = 2d = 104.8$ Å was much smaller than that found in noninterdigitated bilayers.[5] This result was due to the interdigitated nature of the gel phase of DMPC/**1** bilayers and was in good agreement with the findings of Koynova and MacDonald.[7] Upon heating, the WAXD patterns of DMPC/**1**/DNA lipoplexes showed that the lipid bilayers underwent a gel−liquid-crystalline phase transition that was completed between 35 and 50°C. Thus, the transition temperature of DMPC/**1** bilayers was found to be definitely higher than that of pure DMPC ($T_m \approx 24$ °C). This indicated that Gemini surfactants raised significantly the transition temperature of the phospholipids. At 50 °C (Figure 2, middle panel), Bragg peaks in the SAXD regime show that the gel−liquid-crystalline transition is associated with an expansion of the lamellar repeat period, d, by 6 Å ($d = 58.4$ Å). According to ref 7, the enlargement in the lamellar period is ascribed to the temperature-induced bilayer expansion. Nevertheless, the estimated thickness of DMPC/**1** bilayers ($d_m \approx 38$ Å) remains much lower (by some 10 Å) than that of pure DMPC (∼47 Å).[12] This finding suggested that thermal fluctuations reduced the degree of interdigitation, but a partial interdigitation persisted in the liquid-crystalline phase.[14,15]

In the interdigitated liquid-crystalline phases, the acyl chains are more motionally restricted than in the noninterdigitated liquid-crystalline phase but much more disordered than in their gel counterpart.[14,15] Multiple DNA reflections are still observed but could not be indexed to a centered rectangular lattice (as in the low-temperature gel phase) nor to the simple DNA−DNA in-plane correlation usually observed in the liquid-crystalline phase of lipoplexes.[2−4] Conversely, a simple rectangular phase is indexed with the measured sets of q values. DNA peaks are indexed to the (1,0) and (1,2), (1,3) reflections of a simple rectangular lattice with $a = 39.8$ Å and $b = 58.4$ Å. Here we emphasize that our structural findings are the first report of a transbilayer DNA superlattice in the liquid-crystalline phase. The unexpected reduction of the DNA spacing (less than 1 Å) probably reflects the decreased area per molecule and the increase in membrane charge density.[7] At 65 °C, loss of interlamellar correlation occurred as revealed by the disappearance of the multiple DNA reflections from the SAXD pattern (Figure 2, bottom panel). In this temperature range ($50 < T < 65$ °C) the thermotropic behavior of DMPC/**1**/DNA lipoplexes resembled the typical behavior of lipoplexes.[2−4] Indeed, the increase in temperature induced a reduction of the bilayer thickness by ∼1.5 Å followed by a small expansion of the DNA spacing (∼1 Å).

At low temperatures, DNA arranged into a centered rectangular columnar phase intercalated between interdigitated bilayers in the gel phase (Figure 3, panel A) while, at higher temperatures (50 °C), DNA formed a simple rectangular DNA lattice between fluid but still partly interdigitated bilayers (Figure 3, panel B). At 65 °C, transbilayer coupling was lost and DNA superlattice was not observed (Figure 3, panel C). DNA lattice is coupled to the membrane rigidity by thermal motion.[16] When bilayers are stiff (as in the gel phase) Coulomb repulsions between DNA strands

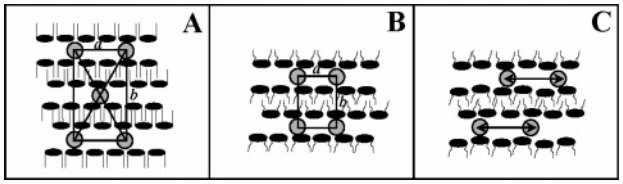

Figure 3. Schematic sketch of the local structure of the DNA ordering within DMPC/**1**/DNA lipoplexes as a function of temperature. At low temperatures, DNA arranges into a centered rectangular columnar phase between interdigitated gel bilayers (A). At intermediate temperatures (above the lipid main transition), DNA forms a simple rectangular DNA lattice between fluid bilayers (B). Increasing temperature transbilayer coupling is lost (C). The 2D lattice constants a and b are indicated.

favors a centered rectangular lattice.[5−7] Upon heating, thermal fluctuations continuously raise. In the first stage, thermal motion possibly becomes strong enough that correlations are not well developed in centered rectangular lattices but a partial interlayer coupling still persists. In this case, a tendency to form a simple rectangular rather than centered structure would appear.[16] When fluctuations become strong, interlayer coupling becomes weak and DNA strands in different layers are practically decoupled.[16] The explanation for the observation of a DNA superstructure in the liquid-crystalline phase of DMPC/**1** membranes is likely to be their interdigitation. Indeed, at higher DMPC/**1** molar ratios (8:2 and 7:3, not reported) the degree of interdigitation was found to decrease. Such a reduction of interdigitation resulted in a decrease of membrane rigidity with the consequence that the transbilayer coupling of the DNA ordering was not observed. The DMPC/**1** molar ratio was also found to alter the thermotropic of lipoplexes: the higher is the DMPC/**1** molar ratio, the lower is the lipid main transition temperature and the narrower is the temperature range where DNA superstructures exist.

In conclusion this study is the first report of a DNA transbilayer ordering in the liquid-crystalline phase of lipoplexes. Furthermore, we have also clarified that different DNA superstructures can be obtained by adjusting the membrane rigidity.

References

(1) Felgner, P. L.; Ringold, G. M. *Nature* **1989**, *331*, 461−462.
(2) Salditt, T.; Koltover, I.; Rädler, J. O.; Safinya C. R. *Science* **1997**, *275*, 810−814.
(3) Zantl, R.; Baicu, L.; Artzner, F.; Sprenger, I.; Rapp, G.; Rädler, J. O. *J. Phys. Chem. B* **1999**, *103*, 10300−10310.
(4) Caracciolo, G.; Pozzi, D.; Amenitsch, H.; Caminiti, R. *Langmuir* **2006**, *22*, 4267−4273.
(5) Artzner, F.; Zantl, R.; Rapp, G.; Rädler, *Phys. Rev. Lett.* **1998**, *81*, 5015−5018.
(6) McManus, J. J.; Rädler, J. O.; Dawson, K. A. *J. Am. Chem. Soc.* **2004**, *126*, 15966−15967.
(7) Koynova, R.; MacDonald, R. C. *Nano Lett.* **2004**, *4*, 1475−1479.
(8) Bombelli, C.; Caracciolo, G.; Di Profio, P.; Diociaiuti, M.; Luciani, P.; Mancini, G.; Mazzuca, C.; Marra, M.; Molinari, A.; Monti, D.; Toccaceli, L.; Venanzi, M. *J. Med. Chem.* **2005**, *48*, 4882−4891.
(9) Salditt, T.; Koltover, I.; Rädler, J. O.; Safinya, C. R. *Phys. Rev. E* **1998**, *58*, 889−904.
(10) Koltover, I.; Salditt, T.; Safinya C. R. *Biophys. J.* **1999**, *77*, 915−924.
(11) Caracciolo, G.; Piotto, S.; Bombelli, C.; Caminiti, R.; Mancini, G. *Langmuir* **2005**, *21*, 9137−9142.
(12) Pabst, G.; Katsaras, J.; Raghunathan, V. A.; Rappolt, M. *Langmuir* **2003**, *19*, 1716−1722.
(13) Caracciolo, G.; Mancini, G.; Bombelli, C.; Luciani, P.; Caminiti, R. *J. Phys. Chem. B* **2003**, *107*, 12268−12274.
(14) Schram, V.; Thompson, T. E. *Biophys. J.* **1995**, *69*, 2517−2520.
(15) Kranenburg, M.; Venturoli, M.; Smit, B. *J. Phys. Chem. B* **2003**, *107*, 11491−11501.
(16) Golubovic, L.; Lubensky, T. C.; O'Hern, C. S. *Phys. Rev. E* **2000**, *69*, 1069−1094.

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