Osmotically Induced Reversible Transitions in Lipid–DNA Mesophases

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Osmotically-induced reversible transitions in lipid-DNA mesophases

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Experimental procedures

Sample preparation

About 30 mg of lipid powder (Avanti Polar Lipids) was hydrated with purified water, and extruded at least 41 times using an Avanti mini-extruded with membrane hole size of 40 nm. Nucleosomal DNA of 146 base-pairs in length was extracted from chicken erythrocytes as described in (1, 2). Stock lipid and DNA solutions were mixed to form ca. 2 mg of lipoplex solution. After complex formation and initial overnight equilibration with several temperature cycles between 10 and 40 °C, different amounts of PEG with molecular weight 8000 were added, to apply osmotic pressures of 0 to 100 atm as measured using vapor pressure osmometery (Wescor 5560 vapor osmometer).

Small-angle x-ray scattering (SAXS)

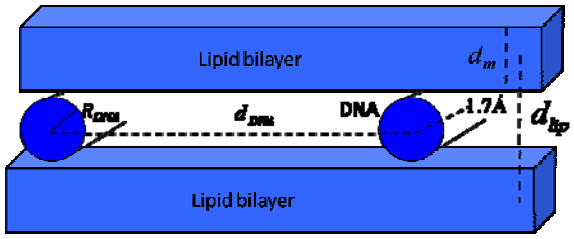
The procedure for SAXS measurements is detailed in ref (3). Briefly, samples were placed in sample-cells with a small amount of equilibrating solution, and then mounted into a temperature-controlled holder at 25 °C. Samples were then x-rayed for 1-3 hours with a fine-focus fixed copper anode x-ray source (Enraf-Nonius, Delft, The Netherlands). Diffraction patterns were recorded by direct exposure of Fujifilm BAS image plates and digitized with a Fujifilm BAS 2500 scanner. The images were analyzed using the FIT2D (copyright A. P. Hammersley, European Synchrotron Radiation Facility). Radially integrated plots were used to derive the intermolecular repeat spacings. Data was recorded for samples at different applied osmotic pressures.

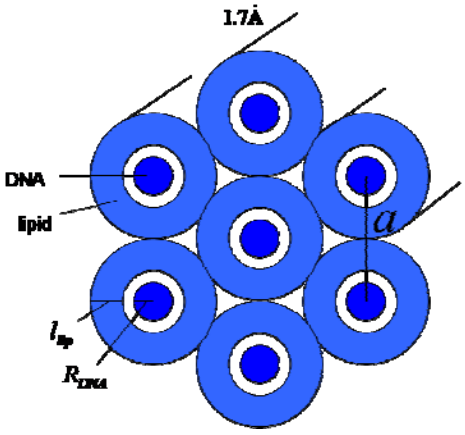
Freeze-fracture transmission electron microscopy (FF-TEM)

In the controlled-environment vitrification system (CEVS) which was saturated and equilibrated at 25 °C, a small drop of sample was placed between two copper grids, and the assembly was placed between two round gold planchettes. The formed "sandwich" was vitrified in liquid ethane, transferred into liquid nitrogen (LN_2), and inserted into a LN_2 pre-cooled sample table. The sample table was inserted into the vacuum chamber of the BAF-060 replication system (BAL-TEC AG, Liechtenstein) at a temperature between -160 °C to -170 °C, and was opened exposing two grids with sample fractures. The fractured surfaces were coated with a platinum-carbon electrode by sputtering a 2 nm layer at 45°, then with carbon at 90°, forming a 20 nm thick replica. The sample table with the replicas was taken out, and the planchettes were thawed in double-distilled water, permitting replicas' detachment from the dissolved sample. Replicas were placed on clean microscopy grids and dried, then examined in a Tecnai 12 G^2 TEM at room temperature. Images were recorded on an Ultrascan 1000 2k x 2k sensitive CCD camera at nominal magnifications of up to 50k (4).

Water volume calculation from a simple box-model

Lamellar phase. Using SAXS, we derive d_{DNA} and d_{lip}, the repeat spacing between adjacent DNA strands and the distance between adjacent bilayers in lamellar lipoplexes, respectively. We define the unit cell to contain one DNA and one lipid charge (see illustration). For B-DNA, the average distance between charges along the DNA strand is 1.7Å, so that the volume of the unit cell is $d_{DNA}' d_{lip}' 1.7Å$. We calculate the water volume contained in a unit cell by subtracting the DNA volume $p' R_{DNA}^{2}' 1.7Å$ and the volume of the lipid bilayer $d_m' D_{DNA}' 1.7Å$ from the total unit cell volume. Based on the work by Safinya (5), we use an estimate of R_{DNA} = 10Å for the B-DNA radius, and d_m= 40 Å for the bilayer thickness, though we note that because we are only considering volume changes due to osmotic stress, rather than actual volume estimates, our calculations are insensitive to the exact choice of d_m.





Hexagonal phase SAXS allows us to derive *a*, the unit cell spacing of the inverted hexagonal phase. As with the lamellar phase, we define the unit cell to contain one DNA charge (see illustration). The unit cell volume is, therefore, $\pi \times \left(\frac{a}{2}\right)^2 \times 1.7\,\text{Å}$. We calculate the water volume within the unit cell by subtracting from the total unit cell volume the volume of DNA and the volume of lipids: $\left[\left(\frac{a}{2} - l_{lip}\right)^2 - R_{DNA}^2\right] \times \pi \times 1.7\,\text{Å}$, where we have assumed that the radius of B-DNA is $R_{DNA} = 10\,\text{Å}$, and $l_{lip} = \frac{d_m}{2} = 20\,\text{Å}$ is the average thickness of a lipid monolayer surrounding the DNA.

Reversibility of lipoplex phase formation

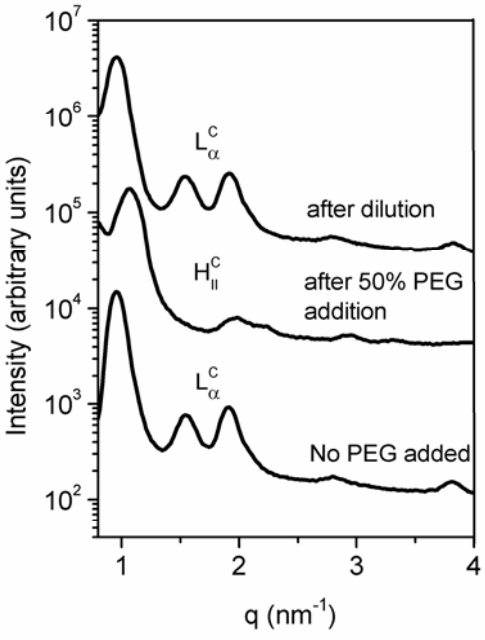


Figure S1: Small angle x-ray scans of lipid-DNA complexes at the isoelectric point showing reversibility in lipoplex phase formation. At no applied osmotic stress, lamellar complexes form. After addition of PEG at 50% by weight to solutions of lamellar complexes (bottom scan), hexagonal complexes are formed (center scan). After dilution, lamellar complexes are again found in solution (top curve).

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