Microdiffraction of DNA-Membrane Self-Assemblies for Gene Delivery Applications

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Introduction

It has been recently reported that the addition of DNA to cationic lipid mixtures can induce a topological transition from liposomes into condensed multi-lamelllar self-assembly, where a periodic one dimensional (1D) lattice of parallel DNA chains is confined between stacked two dimensional (2D) lipid sheets (Fig. 1 middle) [1]. These DNA-membrane complexes are currently being developed as gene carriers for gene therapy as an alternative to virus based delivery vectors. In addition, these DNA-membrane complexes constitute a new class of tunable nanostructured materials with intriguing technological possibilities, which generally require control of pore sizes as well as orientation. Small Angle X-ray Scattering (SAXS) of the DNA-membrane complexes indicates isotropic orientational distribution of the smetic domains. However, optical microscopy has revealed that DNA-membrane complexes form birefringent globules, which aggregate into randomly oriented fibers at mesoscopic (~1 μm) length-scales [1]. In general, however, the organization of local domains and any resultant modulations of the molecular structure within such DNA-membrane fibers are unknown. We report here experiments using a micro-focussed hard x-ray beam at the Advanced Photon Source (APS) to probe the mesoscopic structure of the DNA-membrane complexes.

Methods and Materials

We examined lamellar complexes formed from λ-phage DNA (48,502 base pairs) and liposomes made from a 50%-50% binary mixture of the neutral and cationic lipids, DOPC (dioleoyl-phosphatidylcholine) and DOTAP (dioleoyl-trimethylammonium propane) respectively. The mass ratio of DOTAP to DNA is 2.5, close to the isoelectric point of the system. The sample is thoroughly mixed and then sealed between two 170 μm cover slips using a 13 μm Kapton spacer ring and vacuum epoxy.

The microdiffraction x-ray experiments were conducted at the beamline 2-ID-D at the APS. Monochromatized x-rays at 11.2 KeV were focussed to a beam size of 1 μm x 4 μm using a blazed Au/Si zone plate[3]. The thin transmissive sample cell was positioned at the focus of the zone plate, behind an order sorting aperture of 10 μm diameter. Intensities of scattered x-rays were measured using a LN<sub>c</sub> cooled Charge-Coupled Device (CCD) area detector, and the resultant 2D image of the diffraction pattern covered the Q-range from 0.07 Å<sup>–1</sup> – 0.53 Å<sup>–1</sup>

Results and Discussions

Diffraction patterns taken from two different local regions of the same DNA-membrane complex sample. The strong modulation in intensity along the inner ring of intensity (~0.1Å<sup>–1</sup>) can be seen in the adjoining χ-scans in (b), which indicates partial alignment of the DNA-membrane fibers.

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References