Communication

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Observation of a Rectangular Columnar Phase in a DNA−Calcium−Zwitterionic Lipid Complex

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Complexes composed of DNA and cationic lipids have been studied in detail in recent years, mainly due to their potential to deliver DNA to cells in gene therapy.[1] It has been recognized that different structures can be prepared, based on these compositions and lipid chemistry. In this communication, we give the first example of a higher-order arrangement of DNA within an entirely noncytotoxic complex.

This opens the pathway to explore the consequences of such ordering on the issue of delivery and other arenas where DNA arrays may have biological significance. We believe such nontoxic ordered arrays of DNA may in the future represent novel components of structural and functional significance at the interface of the "synthetic biology" endeavor.[2]

Until now most studies on these synthetic systems have been done with the focus of creating artificial devices for gene delivery. In these cases, some researchers have screened large numbers of different lipid−DNA formulations to identify successful candidates in vitro.[3] Others, however, have tried to understand the complexes on a more fundamental level, studying structure, phase behavior, and other physical characteristics, in the hope that this would ultimately enable a "design"-based approach to delivery,[4−8] but no systematic understanding has emerged.

However, we consider that there emerges a general role for the engineering of ordered arrays of DNA that can protect the DNA in a biological environment but which is easily manipulated to provide compact readable and addressable devices. Such complexes typically cannot be based on direct electrostatic interaction between cationic lipids and DNA, as these are quite cytotoxic and the degree of binding is ill-controlled after initial preparation. However, complexes composed entirely of natural, zwitterionic lipid can complex with DNA when the interaction is mediated by a divalent cation.[9−14] Complexes formed by neutral lipids in the absence of the divalent cation have also been described.[15,16] The divalent cation−lipid complexes are entirely noncytotoxic and thereby have a remarkable status among the candidate systems in that the binding is highly tunable. They have been studied less, possibly due to the erroneous assumption that the interaction between divalent cations and zwitterionic lipids is weak. We have shown previously that the complex forms over a number of calcium concentrations. Structural studies of both the cationic and zwitterionic DNA lipoplexes have been reported over the past number of years.[4−6,13−21] However, here we show for the first time that, in addition to the enhanced divalent cation binding,[17] this complex is capable of arranging DNA into higher orders of local organization.

The complex is prepared by mixing DNA (calf thymus, purified by a method described before[18]), unilamellar liposomes of DPPC, prepared by bath sonication at 50 °C, and calcium from concentrated stocks in the wide part of a 1 mm X-ray capillary. The complex was allowed to form for 1 h before being centrifuged to the end of the capillary and equilibrated by a cycle of heating and cooling, above and below the melt transition temperature of the lipid over a number of days. The complex was prepared at a mole ratio of DNA:lipid of 1:4 in 10 mM $CaCl_2$. Small-angle X-ray experiments were then carried out at either the A2 beamline at the HASYLAB, Hamburg, Germany, or the ID2 beamline at the ESRF, Grenoble, France. The details of these experiments have been described elsewhere.[18]

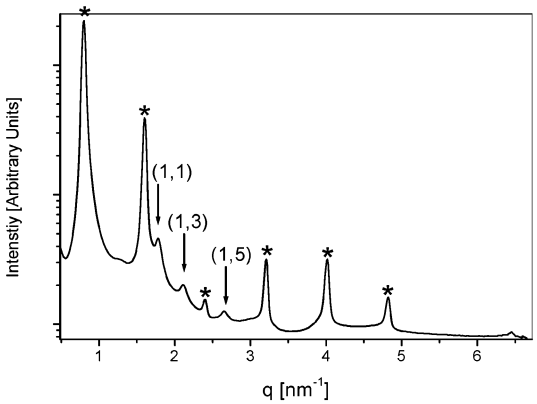

Figure 1. SAXS pattern observed for a complex prepared at a DNA:lipid ratio of 1:4 in 10 mM $CaCl_2$ from calf thymus DNA, 4 months after preparation. The SAXS pattern shown here is taken from the same sample scanned after 1 week. Intensity vs q [nm^{-1}] for the complex is shown. The equally spaced Bragg peaks of the DPPC lamellar phase are present (marked with *), however, three additional peaks corresponding to the (1,1), (1,3) and (1,5) indices of a DNA rectangular columnar phase are indicated with arrows.

We have already shown that DNA, DPPC, and calcium form a multilamellar complex, in which DNA is embedded between the DPPC layers.[17,18] Depending on the preparation conditions, in some of these complexes a DNA−DNA in plane correlation is also observed. This complex exhibits a rich phase behavior, with a two-phase DPPC region observed for complexes prepared from multilamellar liposomes and also interesting thermotropic phase behavior.[18] In this article, however, the complex described is prepared from unilamellar vesicles and therefore exhibits only one lipid phase in the SAXS patterns, one in which the DNA is embedded between the lipid lamellar layers.

A DNA multilamellar structure with equally spaced Bragg reflections at $q = 2\pi n/d_{lam}$ with $d_{lam} = 7.85$ nm and a DNA−DNA in plane repeat distance of 5.35 nm was observed in the complex, 1 week after preparation. However, after long-term incubation (4 months at 4 °C) of the complex, the true nature of the DNA phase became apparent. Three new DNA peaks were observed in addition to the lamellar peaks of the complexed lipid (Figure 1). These we have indexed as the (1,1), (1,3), and (1,5) indices of a rectangular

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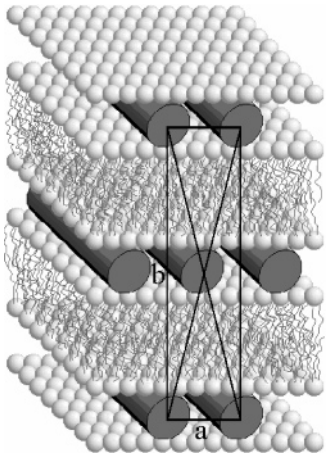

Figure 2. Arrangement of DNA in a complex composed of DNA, DPPC, and calcium (calcium atoms not shown) in which a rectangular columnar phase of DNA is present. The 2D lattice constants a and b are indicated; where $b = 2d_{lam}$, and d_{lam}, the interlamellar distance is 7.83 nm.

columnar phase of DNA, since the peak positions occur at $q_{hk} = 2\pi\sqrt{(h/a)^2+(k/b)^2}$ with two-dimensional (2D) lattice constants a and b. From Figure 2 we see that b, the lattice constant, is determined from $b = 2d_{lam}$ and $a = 3.53$ nm. This SAXS pattern is taken from exactly the same sample as in the 1-week-old case. While the factors controlling the DNA−DNA interaxial spacing (a) are understood in cationic lipid−DNA complexes,[4] it is not yet fully understood for the neutral lipid system.

The first rectangular columnar phase of DNA was identified by Artzner et al. in cationic DNA/DMPC/DMTAP complexes.[21] In our complex the calculated lamellar repeat distance for the bound DPPC in the sample measured after 4 months is 7.83 nm and is consistent with the value obtained for the same sample after 1 week. As described by Artzner et al., the observed DNA superstructure has a centered rectangular symmetry and is built by oriented stacks of planar DNA rod lattices which are displaced in a centered *ABAB* configuration from layer to layer. This structure is shown in Figure 2. The absence of (h,k) peaks with $h + k = 2n + 1$ confirms the centered symmetry. For symmetry reasons no (2,0) or (2,2) peaks were observed. This is discussed in detail by Artzner et al.[21]

It is unclear if this is the only DNA:lipid composition and calcium concentration at which this structure is observed. Complexes prepared at other compositions and calcium concentrations did not appear to have this higher level of DNA organization, even extended times after preparation. Previous work has shown a compact arrangement of the complex at 5mM calcium is reached,[17] but calcium when present in higher concentration may also help to mediate DNA ordering within the complex. Divalent cations have been shown to condense DNA in cationic lipid−DNA complexes.[19]

Until now it has been understood that complexes form very rapidly after the components are mixed,[22−24] but very little is known about the short or long time scale kinetics of these complexes. It will be important in the future in developing the science of dense complex ordered structures of DNA to be aware of these very long times and to explore them. In pursuing the engineering of ordered arrays of DNA overcoming and shortening these long times will be necessary (e.g. by cycling temperature for longer times).

In summary, we report for the first time a noncytotoxic highly ordered structure of DNA formed from a three-component system where calcium acts as the "cement" for complexation. The lipid bilayers in such complexes are analogous to the cell membrane, and it would also be possible to distribute transmembrane proteins into them, possibly providing a route to the extracellular and in situ reading of the DNA. In addition, the development of such arrays is potentially a very rich strand of future research, in that it opens the pathway to modulating the degree of binding of DNA in ordered arrays, merely by modulating the local calcium concentration.

Our research has also highlighted the importance of a very long time scale relaxational process in ordering complex soft and biopolymer systems. These will be interesting to explore from the viewpoint of fundamental science[25] but will also represent a challenge as one seeks to create novel synthetic systems at the interface of biology.

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