Structural States of Self-Assembled Lamellar DNA-Membrane Templates During Artificial Bionanomineralization of CdS Nanorods

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INTRODUCTION

Self-assembled complexes comprised of anionic polyelectrolytes and cationic lipids have recently received intense experimental and theoretical attention. In this preprint, we examine CdS growth within self-assembled lamellar DNA-membrane templates, a simple prototypical bionanomineralization system in which the structures, charge distributions, and phase behavior of the components can be controlled precisely. DNA-membrane complexes were originally conceived as non-viral gene delivery systems. The addition of DNA to cationic liposomes induces a topological transition from liposomes to rafts. Condensed in the presence of divalent counterions into close-packed cationic and neutral lipids. DNA within these lamellar complexes can condense in the presence of divergent counterions into close-packed rafts. In this work, we use CdS, the cationic component of CdS, to drive this 2-D DNA condensation within the membrane lipid galleries. Since the Cd2+ ions are confined and organized by the inter-DNA nanotubes, we can use the DNA-membrane complex as a nanoreactor to template the growth of CdS nanorods via reaction with H2S. We also examine how the structure of the templated CdS nanorods are affected when the charge of the membrane is tuned relative to the charge of the DNA, as well as when the degree of overcharging is varied for both positively and negatively charged templates. We show that crystallographic control of inorganic nanostructures is possible using these simple DNA-membrane complexes as templates.

EXPERIMENTAL

λ-phage DNA (New England Biolabs, Inc., Beverly, MA.) were precipitated out using standard procedure. The positively charged lipid dioleoyl phosphatidyl choline/phosphatidyl ethanolamine (DOTAP) and the negatively charged dioleoyl phosphatidyl choline (DOPC) with the same alkyl chain length were used (both from Avanti Polar Lipids, Alabaster, AL) at different ratio to control membrane charge density. DNA, lipid and CdCl2 stock solutions were added together at different charge stoichiometries and different global Cd2+ concentrations. The DNA to lipid charge stoichiometry (D/L ratio) is defined as the total negative charge of the phosphate groups on DNA divided by the total positive charge of the cationic DOTAP head groups in the membrane. The DNA-membrane complexes are defined as isoelectric when D/L=1, and as negatively overcharged when D/L>1 and positively overcharged when D/L<1. All DNA-membrane complexes were sealed in 1.5 mm quartz capillary for XRD.

DNA-membrane complexes with different [Cd2+] were reacted with H2S gas to form CdS. The DNA-membrane complexes with CdS grown inside were dissolved in a 1% sodium dodecyl sulfate (SDS) solution and centrifuged to collect the CdS nanocrystals. The CdS-dispersed solution was put on 200 mesh size holy carbon grid (from SPI, West Chester, PA) and investigated using a JEOL 2010F energy filtering, field-emission analytic TEM/STEM operating at 200 kV.

RESULTS AND DISCUSSION

Isoelectric Complexes With and Without Cd2+ Ions. The structures of isoelectric λ-phage DNA-membrane complexes at different global [Cd2+] were studied by SAXS. We observe the 2-D collapse of DNA into close-packed condensed rafts within isoelectric complexes at high global [Cd2+] for all the membrane charge densities studied. Two typical sets of SAXS data at different membrane charge densities and global [Cd2+] are shown in Figure 1.

![Figure 1. SAXS data for isoelectric λ-phage DNA-membrane complexes at different membrane charge densities and global [Cd2+]: a: 70/30 DOTAP/DOPC; b: 30/70 DOTAP/DOPC. Curves 1, 2, 3 and 4 on each of the data sets correspond to global [Cd2+] at 0, 10, 30 and 40 mM respectively. The sharp, regularly spaced peaks correspond to the lamellar organization. The arrows indicate the positions of the in-plane inter-DNA correlation peaks. Note the large change in the DNA peak positions for the lower membrane charge density complex (b), due to the condensation of DNA into close-packed 2-D rafts. The two regularly-spaced sharp peaks correspond to the 1st and 2nd order of the lamellar correlation. For DNA-membrane complexes comprised of 70/30 DOTAP/DOPC membranes without added Cd2+ (see curve 1 in Figure 1 a), the peaks correspond to a lamellar periodicity of 62.2 Å, which corresponds to the membrane bilayer thickness (37.2 Å for 70/30 DOTAP/DOPC) plus the diameter of hydrated DNA (25 Å). For complexes with 30/70 DOTAP/DOPC membranes without added Cd2+ (see curve 1 Figure 1 b), the peaks correspond to a lamellar periodicity of 67.8 Å, which is again the membrane bilayer thickness (42.8 Å for 30/70 DOTAP/DOPC) plus the diameter of hydrated DNA (25 Å). The peaks under the arrow correspond to the in-plane DNA correlation. For both membrane charge densities, DNA is observed to condense at elevated global [Cd2+], evidenced by a large change in the inter-DNA distance in the 1-D lattice. For the high membrane charge density sample (DOTAP/DOPC=70/30) with no added Cd2+, the inter-DNA spacing is initially 29.8 Å, which is close to 25 Å, the diameter for hydrated DNA (see curve 1 in Figure 1 a). When global [Cd2+] was increased, this peak shifted to higher q. The final condensed d_{HSA} is 28.4 Å at global [Cd2+]=40 mM (see curve 4 in Figure 1 a), which is just enough to fit one diameter of hydrated DNA rod plus one diameter of hydrated Cd2+ ions (~4 Å). For the low membrane charge density complexes (DOTAP/DOPC=30/70), the initial DNA separation is significantly larger than the DNA diameter (d_{HSA}=58.1 Å, see curve 1 in Figure 1 b). When the global [Cd2+] is increased beyond a threshold value, this DNA spacing decreased sharply. The most drastic decrease occurs near [Cd2+]=30 mM, where d_{HSA} drops to 30.5 Å from an initial value of 58.1 Å. Further increases of the global [Cd2+] to 40 mM only causes a small decrease of the DNA spacing, resulting in a final condensed DNA spacing of 28.8 Å, which is similar to that for complexes with 70/30 DOTAP/DOPC membrane. The lamellar spacing is slightly expanded as global [Cd2+] is increased. The observed expansion of the lamellar
spacing after DNA condensation is 2.6 Å and 4.9 Å for the 
DOTAP/DOPC=70/30 and DOTAP/DOPC=30/70 complexes 
respectively. This is likely due to the additional Cd\textsuperscript{2+} ions that were 
organized into the complex, along with their associated hydration 
layers. Since typical hydrated diameters of divalent ions (~4 Å) are 
significantly smaller than the surface to surface distance between 
membranes in the complex (~25 Å), this observed expansion of the 
lamellar spacing suggests that the ions are organized not just in the 2-
D plane, but along the layering direction as well. The degree of 
expansion along the layering direction after DNA condensation is 
different for complexes with different membrane charge density. This 
suggests that different densities of Cd\textsuperscript{2+} ions can be accommodated in 
complexes with the same inter-DNA distances.

Figure 2. SAXS for overcharged λ-phage DNA-membrane complexes 
at different membrane charge density and global [Cd\textsuperscript{2+}]. a: Positively 
overcharged 70/30 DOTAP/DOPC complexes, D/L=0.6; b: Negatively 
overcharged 30/70 DOTAP/DOPC complexes, D/L=1.4. Curves 1, 2, 3 
and 4 for both data sets correspond to global [Cd\textsuperscript{2+}] at 0, 10, 30 and 40 
mM respectively. The arrows indicate the positions of the in-plane 
inter-DNA correlation peaks. Note the large change in the DNA peak 
positions in the negatively overcharged complexes (b), due to the 
condensation of DNA into close-packed 2-D rafts within the lipid 
galleries. DNA does not condense for the positively overcharged 
complex (a).

Non-Isoelectric Complexes With and Without Cd\textsuperscript{2+} Ions. By 
changing the ratio of DNA charge to lipid charge (by making the 
complexes positively overcharged or negatively overcharged), the 
initial DNA separation d\textsubscript{DNA} can be tuned, while maintaining the basic 
lamellar structure. Overcharged (non-isoelectric) λ-phage DNA-
membrane complexes at different global [Cd\textsuperscript{2+}] have been studied 
using SAXS. The lamellar phase is preserved, and the characteristic 
diffraction signature of a regularly spaced series of diffraction peaks 
can be clearly observed. For positively overcharged complexes (70/30 
DOTAP/DOPC membrane charge density) without added Cd\textsuperscript{2+} (see 
curve 1 in Figure 2 a), the peaks correspond to a lamellar periodicity of 
62.8 Å; For negatively overcharged complexes (30/70 DOTAP/DOPC 
membrane charge density) without added Cd\textsuperscript{2+} (see curve 1 in Figure 2 
b), the peaks correspond to a lamellar periodicity of 70.0 Å. Those 
lamellar periodicities are slightly larger than that of the corresponding 
isolectric complexes. It is interesting to note that no DNA 
condensation is observed for positively overcharged complexes 
(D/L<1), in contrast, for the negatively overcharged complexes 
(D/L>1), DNA exhibits behavior analogous to that of isoelectric 
complexes, and condenses at sufficiently high global [Cd\textsuperscript{2+}]. These 
trends can be seen in the two typical sets of SAXS data in Figure 2 for 
overcharged λ-phage DNA-membrane complexes. For positively 
overcharged complexes (D/L=0.6, 70/30 DOTAP/DOPC membrane, 
Figure 2 a), the initial d\textsubscript{DNA} is 37.8 Å (compare with 29.8 Å for the 
corresponding isoelectric complex of the same membrane charge 
density, curve 1 in Figure 1 a). As [Cd\textsuperscript{2+}] is increased, d\textsubscript{DNA} remained 
unchanged. For negatively overcharged complexes (D/L=1.4, 30/70 
DOTAP/DOPC membrane, Figure 2 b), the initial d\textsubscript{DNA} is 45.8 Å 
(compare with 58.1 Å for isoelectric complex of the same membrane 
charge density, curve 1 in Figure 2 b). As [Cd\textsuperscript{2+}] is increased, the inter-
DNA spacing decreases in the same manner as the isoelectric 
complexes, and reaches a 2-D condensed DNA state.

Structural Characterization of the CdS Grown within DNA-
Membrane Templates. Using a combination of membrane charge 
density, DNA to lipid charge ratio, and global [Cd\textsuperscript{2+}], structural 
parameters of the DNA-membrane template such as the condensed 
ion density and the nanopore size can be controlled. DNA-membrane 
complexes with condensed Cd\textsuperscript{2+} ions are reacted with H\textsubscript{2}S in the gas 
phase to form CdS nanocrystals at room temperature. The existence of 
CdS with wurtzite structure is confirmed using Wide Angle X-ray 
Scattering (WAXS). CdS grown in DNA-membrane complexes can be 
isolated by dissolving the organic matrix. CdS crystals grown in 
complexes with different initial condensed Cd\textsuperscript{2+} ions density have been 
studied by Transmission Electron Microscope (TEM) and compared 
with the CdS grown in free solution. Instead of micron-sized single 
crystals with a hexagonal shape, the templated CdS are of one-
dimensional rod-like nanocrystals. The condensed Cd\textsuperscript{2+} ions 
are confined in arrays of nanopores in DNA-membrane complexes, which 
are aligned along the DNA axis. This implies that during the H\textsubscript{2}S 
reaction, the CdS growth is confined in directions perpendicular to the 
DNA axis, and is unconfined along the DNA, hence resulting in rod-like 
nanocrystals.

Figure 3. Molecular casting of CdS nanorods. a, b: HRTEM of typical 
individual CdS nanorods templated by isoelectric DNA-membrane 
complexes comprised of 30/70 DOTAP/DOPC membrane with λ-phage 
DNA (a) or calf thymus DNA (b). (scale bar is 5 nm): Note 60° tilt of 
(002) planes relative to rod axis. (c) Schematic representation of B-
form DNA, showing the negatively charged phosphate groups (white) 
on the backbone, which organize the Cd\textsuperscript{2+} ions and guide the 
nucleation of CdS; d: Single nanorod diffraction reveal the 
crystallographic structure of the templated CdS nanorods. A single 
CdS nanorod (different from that shown in a and b) is illuminated by 
an electron beam probe, with a probe size of ~43 nm. Note the tilt of (002) 
lattice fringes. e: Resultant nano-beam diffraction pattern of the CdS 
nanorod in d. The single rod diffraction pattern unambiguously 
indicates the tilt of the (002) planes.

The widths can be effectively controlled by the initial condensed 
Cd\textsuperscript{2+} ion densities, which in turn can be controlled by membrane
charge density and/or DNA to lipid charge stoichiometry. It must be emphasized that it is the concentration of condensed ions rather than the nanopore size that controls the templated CdS nanorod width. Moreover, it is interesting to note that it is the width rather than the length that is controlled.

Anionic and cationic components in the DNA-membrane template work synergistically together in the templating process: DNA is highly anionic. The mean distance between negative charges on DNA (0.17 nm) is less than the Bjerrum length (0.78 nm), defined as \( \frac{e^2}{\epsilon k T} \), where \( e \) is an elementary charge, \( \epsilon \) is the static dielectric constant, \( k \) is the Boltzmann constant, and \( T \) is the temperature. This implies that the linear charge density of DNA is beyond the Manning limit, and a layer of condensed ions, in the present case Cd\(^{2+} \), is expected to condense on its surface. The concentration of Cd\(^{2+} \) ions condensed on the DNA within a composite DNA-membrane template, which determines the final CdS morphology, is modulated by the charge density of the cationic membrane. A similar interplay between anionic and cationic components in biomolecular templates may be operative in more complex biomineralization systems.

Current approaches for making wurtzite II-VI semiconductor nanorods exploit the anisotropic growth rates for different lattice planes. For this reason, the growth direction is usually along the c-axis, e.g. the (002) planes are usually perpendicular to the rod direction. Surprisingly, nanorods grown from the DNA-membrane complexes are not oriented along the c-axis. This is clearly seen in the two different high-resolution TEM (HRTEM) images of representative nanorods (Figure 3 a,b). templated from isoelectric complexes of calf thymus DNA with 30/70 DOTAP/DOPC membrane with global [Cd\(^{2+} \)] at 40 mM. The lattice fringes correspond to (002) planes of CdS (d = 0.336 nm), which are tilted by 60° from the rod axis, in contrast to wurtzite nanorods prepared from other approaches. This observed tilt of the (002) lattice planes can be related to the orientation of the DNA sugar-phosphate backbone, which is tilted by ~60° with respect to the helix axis in B-form DNA when projected onto a 2D plane (Figure 3 c). The spatial distribution of the positively charged Cd\(^{2+} \) ions, and therefore the nucleation of the CdS polar (002) planes, is organized by this negatively charged “ridge” on the DNA surface. In fact, recent experiments have shown that it is possible for biopolymers to spatially organize ions on the nanometer scale via electrostatic interactions.

Because the templated CdS nanorods are confined to grow along the nanopores defined by adjacent DNA strands, the (002) planes are tilted by ~60° with respect to the nanorod major axis. The periodicity of each helical turn of B-form DNA is ~5.4 nm, which is just enough to fit 10 (002) planes with nearly no mismatch. This unusual crystallographic orientation was further confirmed by nano-beam diffraction (NBD). In these NBD measurements, a 43 nm diameter electron beam probe is used to illuminate a single nanorod with (002) planes tilted ~60° away from the rod direction (see Figure 3 d). The diameter of the probe beam can be seen relative to the width of the horizontally-oriented CdS nanorod, on which lattice fringes can be clearly observed. The resultant diffraction pattern of the single nanorod is shown in Figure 3 e, and confirms the crystallographic orientation of the (002) planes with respect to the long axis of the nanorod.

**CONCLUSIONS**

In summary, we have investigated how the anionic and cationic components of DNA-membrane templates affect the CdS templating process. Depending the charge of the membrane, different concentrations of Cd\(^{2+} \) ions are condensed into the template, and different morphologies of CdS are templated as a result. Crystallographic control of the inorganic nanostructures is possible using DNA-cationic membrane complexes. The strong electrostatic interactions within such complexes align the CdS (002) polar planes parallel to the negatively charged sugar-phosphate DNA backbone, which suggests that molecular details of the DNA molecule have been replicated onto the inorganic crystal structure.

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