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Reversible structural switching of a DNA-DDAB film

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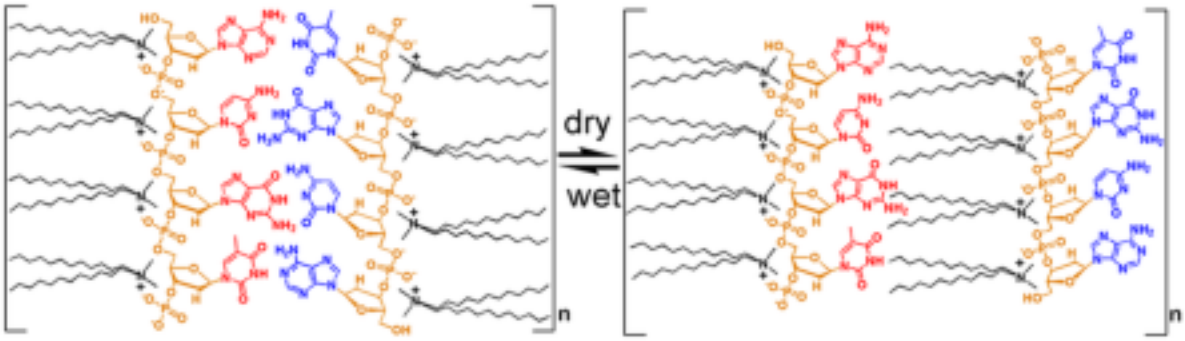
Abstract

We describe the novel structure and behavior of a DNA-DDAB complex film cast from an organic solvent which exhibits a structural switching transition as it is dried or wetted with water. The film can be easily prepared by forming a complex between the negatively charged phosphate groups of DNA and the positively charged headgroup of the surfactant DDAB. This complex is then purified, dried, dissolved in isopropanol and cast onto a glass slide to form a self-standing film by means of slow evaporation. While the structure of the dried film was found to be composed of single-stranded DNA and a monolayer of DDAB, upon hydration of the film the structure switched to double stranded DNA complexed to a bilayer of DDAB. We expect that this phenomenon would serve as a useful model for the design of new responsive materials and programmable self-assembly.



Nucleic acid self-assembling nanostructures interfaced with other biomolecules have great potential for materials science.[1] Naturally derived polyanions such as nucleic acids, can self-assemble with cationic lipids via electrostatic complexation, thermodynamically driven by the release of counterions.[2] These complexes dispersed in water have been extensively studied and recognized as useful for gene and siRNA delivery. Their structures in water, are dependent on factors such as temperature and co-lipid ratio, and often assume the forms preferred by the lipid. For example, DNA complexes with dimethyldidodecyl-ammonium bromide (DDAB) or dioleoylglycerophosphocholine (DOPC) tend to form lamellar structures whereas complexes with dilinoleoylglycerophospho-ethanolamine (DOPE) form inverted hexagonal structures, while the DNA itself remains double stranded (dsDNA).[3] Certain nucleic acid-cationic lipid mixtures are water-insoluble complexes able to form self-standing films when cast from an

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Supporting Information Available: Experimental procedures, further characterization of the structure and switching of the films. This material is available free of charge via the Internet at http://pubs.acs.org.

organic solvent such as chloroform or ethanol.[4] In a previous study, we found that the tensile and structural properties of these films can be tuned by blending RNA and DNA of different molecular weights.[5] However, the structure of nucleic acid-lipid films and the state of the DNA and lipid within the films in particular, has been a controversial subject. While it has been reported that the DNA within the film retains its double stranded helical form,[4] recent studies have suggested that the DNA in such films is single stranded[6] (ssDNA) but experimental evidence remains scarce as no structure is given to satisfactorily explain the state of the lipid. Herein, we report the structural characterization of the switching of a DNA-DDAB film summarized schematically in Figure 1A. Films are prepared by mixing aqueous solutions of DNA (~2000 bp) and DDAB to form a water insoluble complex that is purified, dried and dissolved in isopropanol, prior casting on a glass slide (Supporting Information (SI)). AFM analysis of the surface morphology shows planar surfaces separated by equal steps of 2.85 ± 0.3 nm in height (Figures 1C and S1). The phase image indicates that these flat surfaces are hydrophobic, resulting from an uppermost layer of lipid tails, and that the sides of the steps have some hydrophilic character, due to both the lipid head groups and phosphate backbone of DNA (SI). WAXS and SAXS studies confirm that the surface morphology represents the structure of the bulk film, yielding a repeat distance of 2.85 ± 0.2 nm from the main scattering peak at 2.2 nm^{-1}. X-ray reflectivity studies of a 49 nm thick dip-coated film also indicated that each layer has a repeat distance of 2.85 ± 0.2 nm (Figure S3). We conclude that the films in dry state have a lamellar structure, since two peaks were observed in a harmonic series (q_0=2.2 nm^{-1}, q_2=4.4 nm^{-1}) (Figure S2). However, a repeat distance of 2.85 nm does not agree with the dimensions of a model structure of dsDNA (about 2 nm in diameter) and a lipid bilayer of 2.4 nm (interdigitated, according to experimental results)[7] or 3.4 nm (theoretical calculations without interdigitation).[8]

Strikingly, WAXS studies indicate that the repeat distance of the film increases to 4.3 ± 0.2 nm (q=1.48 nm^{-1}) when immersed in water (Figure 1D). As we previously reported,[5] this corresponds to a layer of dsDNA and an interdigitated bilayer of DDAB (Figure 1A, left). This structural change can be repeatedly reversed when the film is dried. At a macroscopic level, a 155% increase in the cubic volume is observed when a thick film is immersed in water (data not shown). This swelling ratio is directly correlated to the percent difference of the repeat distance between the dry state and wet state at a nanoscopic level.

Our scattering data suggest that the film has a lamellar structure in the dry state formed by the repeat of a ssDNA layer of ~1.1 nm thickness[9] that interacts with a lipid monolayer of ~1.7 nm thickness corresponding to DDAB molecules with fully extended tails[8] (Figure 1A, right). As ssDNA is more hydrophobic than dsDNA,[10] its base moieties can interact hydrophobically with DDAB lipid tails and its phosphate groups can electrostatically complex with the cationic head groups of DDAB.

The DNA structures in the dry and wet films were further examined by FT-IR spectroscopy to analyze the interactions between the DNA bases, phosphate backbone and DDAB (Figure 2A). From the literature, it is known that both dsDNA and ssDNA have absorbance bands at 1652 cm^{-1} and 1691 cm^{-1} which correspond to the in-plane base vibrations, particularly the carbonyl stretching of thymine and guanosine. The band at 1691 cm^{-1} arises due to the C6 carbonyl stretch of base-paired guanine and the C2 carbonyl stretch of paired thymine, while the band at 1658 cm-1 arises due to the C6 carbonyl stretching vibrations of unpaired guanine, the C2 carbonyl stretches of unpaired cytosine, and the C4 carbonyl stretches of unpaired thymine. These peaks can then be used to determine whether hydrogen bonding is present. Our measurements of native DNA were comparable with surface-based measurements from the literature and show a higher absorption band at 1652 cm^{-1} relative to the band at 1691 cm^{-1}. Single-stranded DNA shows the opposite relation; a higher absorption band at 1693 cm^{-1} and a lower band at 1652 cm^{-1}.[11] In the dry state, the DNA-DDAB film exhibits a higher absorption

maximum at 1691 cm^{-1} with peaks similar to those of ssDNA (Figure 2C). By contrast, the film immersed in water has similar characteristics to B-form dsDNA in this region. The conversion of ssDNA into dsDNA can be monitored by FT-IR and occurs on the order of minutes (Figure S4). Circular dichroism (CD) spectra of the dry and wet films give further evidence that the nucleic acid is essentially single-stranded (non-helical) and double-stranded (helical) in the dry and wet films, respectively (Figure S5). In agreement with FT-IR and SAXS studies, intercalation studies with ethidium bromide treated-DNA-DDAB films corroborate the dsDNA to ssDNA transition when wet films in a wet state is dried (data not shown).

The lipid also undergoes a structural change from bilayer to monolayer as the film is dried. Evidence for this change can be seen in the IR absorbance band for the symmetric stretching of CH_2 groups at 2852 cm^{-1}(ν_s) and the asymmetric stretches at 2923 cm^{-1}(ν_{as}) (Figure 2B).[12] DDAB as well as DNA-DDAB films in water show a local maximum at 2923 cm^{-1} while the dry film shows a shifted band at 2927 cm^{-1}. The position of the absorption band at 2852 cm^{-1} is independent of the treatment of the DNA-DDAB film. The intensity ratio between the 2852 cm^{-1} and the 2923 cm^{-1} peak is 1:0.09 for the dry DNA-DDAB film, while for the wet film as well as DDAB alone it is in the range of 1:0.5 to 1:0.6. A higher intensity in ν_{as} indicates a lower amount of interdigitated lipids (i.e. a lipid monolayer).

The melting point of the DNA duplex decreases when organic solvents or long alkyl chains (e.g. reverse phase column) lower the base stacking energy due to hydrophobic/hydrophobic interactions.[13] Similarly, at low water content DDAB molecules interact with the bases and favor the dsDNA to ssDNA transition. By contrast, when the film is immersed in water, the hydrophilic interactions between the DNA and water molecules might favor the ssDNA to dsDNA transition.

In conclusion, DNA-DDAB films undergo a reversible structural transition, from double to single strands and from bilayer to monolayer, with reduced water content in the film. Our model for the film in both dry and wet states suggests that one of the two DNA strands and the DDAB molecules switch positions when the film dries (Figure 1A). This means that the lipid bilayer, which can be described as interdigitated alkyl chains, is disrupted and a monolayer of lipids is formed. The hydrophilic head group of the lipid monolayer interacts with the phosphate backbone of the DNA while the lipid alkyl chains interact with the bases of single stranded DNA. This local switching could also explain why both single strands of the DNA in the dry film are able to find their complementary partner quickly when immersed in water. The presence of water is thus essential to maintain the stability of the DNA double helix.

By taking advantage of this phenomenon and the interplay of nucleic acid self-assembly and lipid self-organization, DNA/lipid films are interesting models for the design of new responsive materials with applications in electronics, medicine and synthetic biology (for instance a new formulation for nucleic acid delivery).[4,5,6,14]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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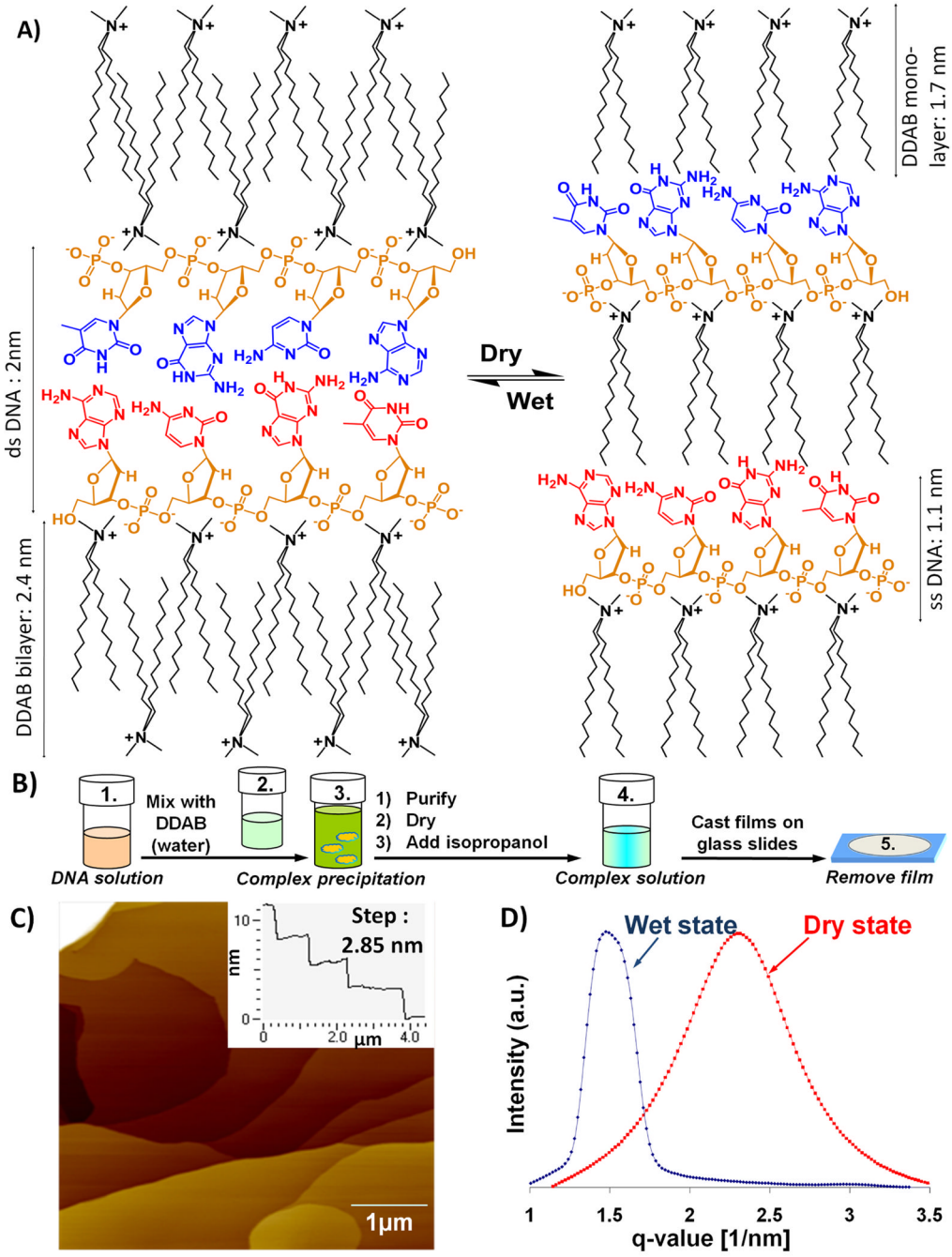

Figure 1.

(A) Model for the film structure in dry and wet states. (B) Preparation procedure of the DNA-DDAB films (see Supporting Information). [1] Native DNA (~2000 bp) dissolved in buffer (pH 8.5, TBE) and heated at 90°C prior to renaturation at 20°C, is mixed with DDAB in water [2] to form an insoluble complex [3]. After being purified, dried and dissolved in isopropanol [4], the complex is cast on a glass slide to form a dry self-standing film [5]. (C) AFM image of the surface of a DNA-DDAB film in dry state. (D) WAXS/SAXS data of films in dry and wet states.

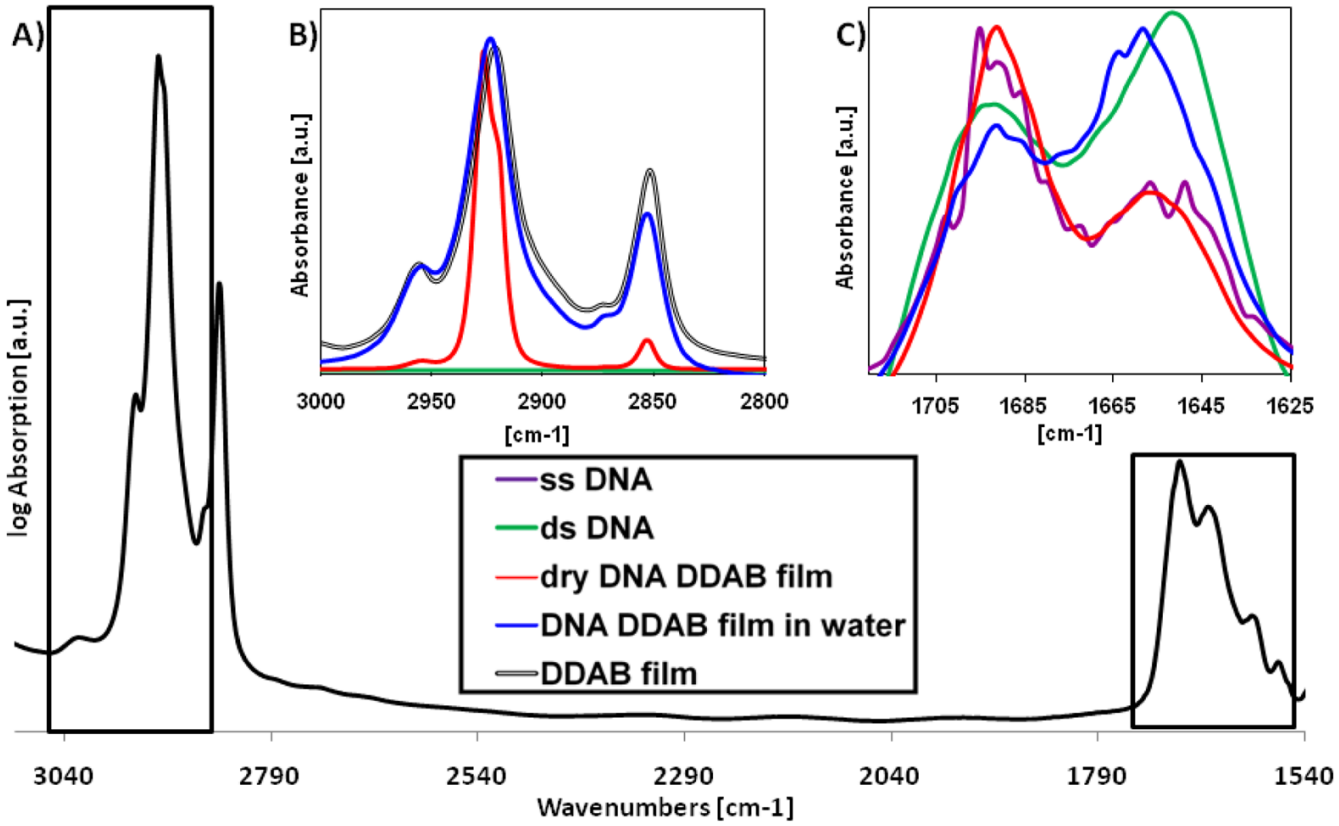

Figure 2.
(A) Full FT-IR spectrum of the DNA-DDAB film in the dry state and the specific absorbance bands for the lipid (B) the DNA bases (C) the DNA-DDAB film in dry and wet states and the controls of ssDNA, dsDNA and DDAB alone.