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DNA–amine interactions: From monolayers to nanoparticles*

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Abstract

DNA is a polyanionic molecule that can electrostatically interact with positively charged ligands. Interaction with surfactants such as CTAB leads to cooperative binding of detergent molecules through synergistic effects of electrostatic and hydrophobic forces. DNA can also form a monolayer at air–water interface with positively charged octylamine and nanoparticles with cationic lysine capped gold nanoparticles. Formation of such electrostatic composites of DNA has practical implications for developing DNA diagnostic systems and for lipid-encapsulated DNA delivery systems.

Keywords: DNA–surfactant interactions. DNA–Langmuir Blodgett monolayers, DNA–gold nanoparticles.

1. Introduction

The understanding of the physical nature of DNA–cationic ligands has primary importance due to its significance in biomedical applications, particularly in therapeutics and diagnostics [1]. The interaction of cationic amines and polyamines with the anionic phosphate backbone of DNA leads to condensation of DNA by screening the phosphate–phosphate repulsion [2]. Thus cationic detergents condense DNA (plasmids) into discrete particles, which are released fast into cells with accompanying decondensation [3]. This feature of DNA–surfactant interaction has prompted biophysical characterization of complexes of large DNA molecule with different surfactants [4]. However, parallel studies on interaction of short oligonucleotides with lipids/detergents are lacking. As part of our study on DNA–amine interactions [5], we have investigated the ability of DNA to be an anionic template receptor for organizing different cationic ligands ranging from detergents to nanoparticles. This article summarizes recent results from our endeavour in this direction.

2. DNA–surfactant interaction in solution

The interaction of cationic lipids with DNA is well known to induce condensation and subsequent precipitation of the condensates [6]. Light scattering data on DNA–CTAB complexes corresponding to different relative stoichiometries indicated that at a CTAB to DNA phosphate ratio of 0.75–0.80, solution turns cloudy with the onset of precipitation of complex [7]. At submicellar concentrations of CTAB [(CTAB) 22 μM; cmc of CTAB 92 μM], the binding of CTAB to ODN (oligodeoxynucleotides) occurs predominantly in monomeric form and not in aggregated micellar form. The UV thermal melting of duplex ODN at varying concentrations of CTAB (0–22 μM)

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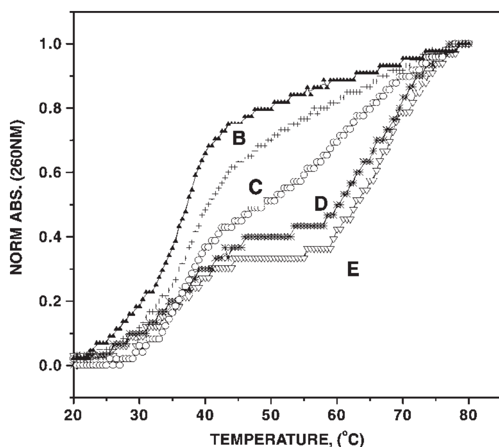

Fig. 1. UV–Temperature plot for ODN duplex **1:2** in the presence of increasing concentrations of CTAB (A) 0 μM, (B) 6.1 μM, (C) 12 μM, (D) 18 μM and (E) 22 μM.

exhibited an interesting split in the melting curve, leading to a double sigmoid pattern (Fig. 1). This suggests the existence of a second species of duplex ODN that is thermally more stable than the duplex ODN in the absence of CTAB. Such a split in the melting curve was not seen with ODN solution containing only either tetramethyl ammonium bromide (lack of hydrocarbon chain) or cetyl alcohol (lack of positively charged head group). Thus the formation of a higher stability DNA duplex in the presence of CTAB is the result of synergistic effect of simultaneous presence of cationic head group and the hydrophobic chain in the same molecule. When a double-chain cationic detergent such as DOTAP was used, the split in the melting curve was qualitatively similar, but the resolution was less pronounced. The CD spectra of complexes exhibited profiles different from the classical B-DNA pattern, but were not amenable for meaningful interpretation. The results can be fitted overall into a DNA–detergent binding model (Fig. 2). The surfactant binding is initiated by electrostatic interaction of cationic ligands with polyanionic DNA that templates the first surfactant. This is followed by electrostatic binding of next surfactant to the initial complex at the adjacent site, driven cooperatively by the hydrophobic interaction with the already bound surfactant chain. Continuation of such a cooperative binding phenomena leads to ODN duplex, which gets completely coated with CTAB, which is responsible for the higher melting species. Formation of such a complex is supported by CD spectra departing from classical B-DNA pattern and arising from a partial condensation of DNA induced by a combined effect of charge neutralization and hydrophobic compressibility. Since concentration of CTAB corresponds to less than 80% of that of ODN in terms of charge ratios, some bare duplex is left behind that is seen as the lower melting species in the thermal melting curve. Thus, when ODN is treated wih submicellar concentration of positive detergents such as CTAB, cooperativity in surfactant binding to DNA is coupled to melting transition and causes significant stabilization of DNA duplex.

3. DNA-surfactant interaction at monolayers

A novel way of realizing the template activity for cationic surfactants is through Langmuir–Blodgett (LB) films at air–water interface, as these are exce1lent media for 2-dimensional organization of heterophasic-binding components [8]. We have studied the electrostatic complexation of single-stranded ODN with octadecylamine (ODA) at air–water interface (Fig. 3) and subsequent hybridization with complementary ODN to generate duplex ODA complexes at interface

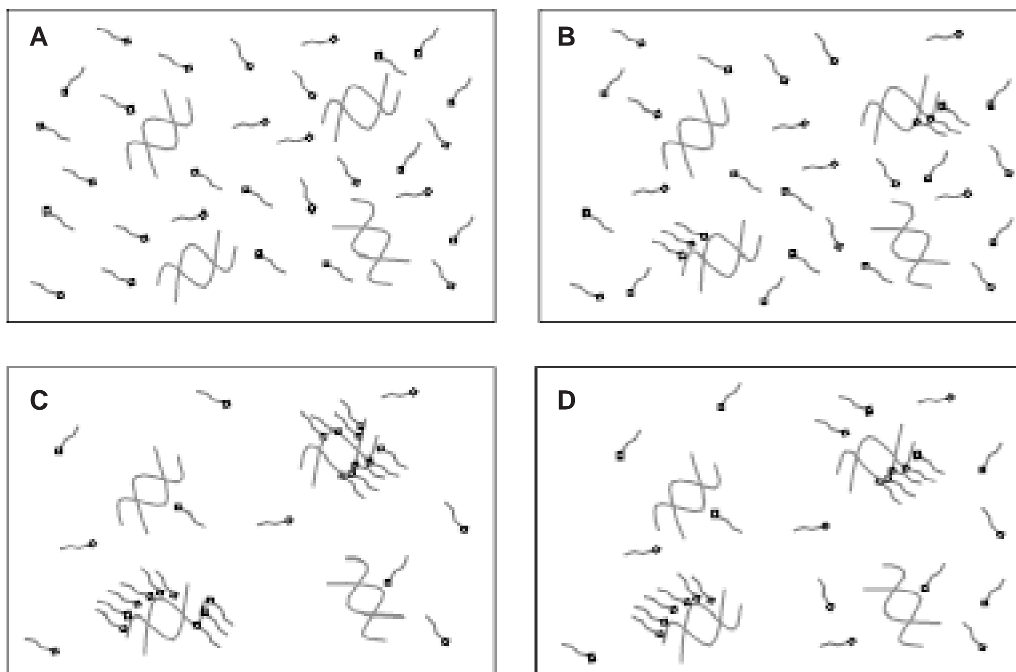

Fig. 2. Schematic representation of model for DNA–surfactant interactions. A. DNA and surfactant at sub-micellar concentrations, B. Initial binding of surfactant to some DNA molecules by electrostatic interactions, C. Hydrophobic interactions among surfactant chains, cooperatively drive new surfactant molecules to bind DNA molecules carrying prebound surfactants, and D. Phase separation 'in solution' of surfactant-bound and free duplexes, leading to two melting transitions, Tm_2 and Tm_1.

rather than in solution [9]. The complexation of ssODN molecules with ODA monolayers was followed in time by measuring the pressure (π)–area (A) isotherms. The LB films of ODA–DNA complex formed on different substrates were characterized using QCM, FTIR, UV-visible and fluorescence spectroscopic techniques [10].

A large (and slow) expansion of ODA/ODA–ODN monolayer was observed during each stage of complexation in the following sequence: ODA, ODA+ssODN, ODA+ODN duplex, ODA+duplex+ ethidium bromide. Curves 1 and 2 in Fig. 4 represent the π-A isotherm compression/

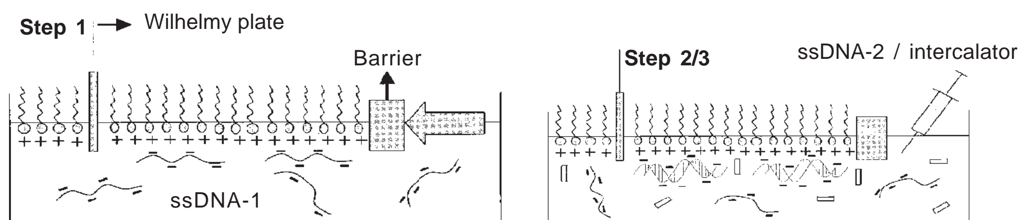

Fig. 3. Sequential immobilization–hybridization of ODA–DNA. Step 1: Spreading of ODA on the ssDNA-l subphase followed by immobilization of ssDNA-l at the air–water interface. Steps 2/3: Introduction into the trough and complexation of the complementary ssDNA-2–ethidium bromide intercalator molecules with ssDNA-l molecules immobilized at the air–water interface.

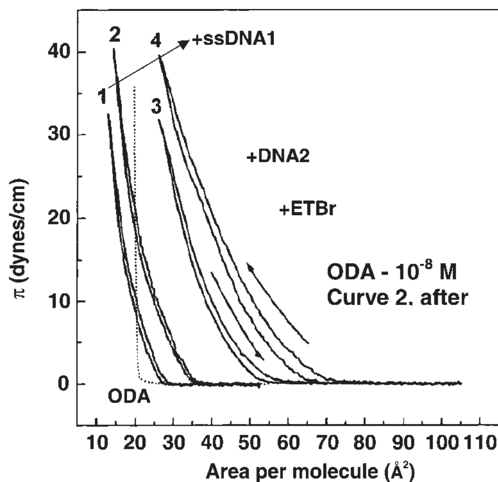

Fig. 4. π-A isotherms recorded from an ODA monolayer at various times after the introduction of DNA/ intercalator molecules into the subphase: curves 1 and 2, 1 and 12 h, respectively, after spreading ODA on the ssDNA-1 subphase, curve 3, 12 h after the introduction of ssDNA-2 into the subphase; curve 4, 12 h after the introduction of ethidium bromide intercalator into the subphase. The dotted line corresponds to the π-A isotherms recorded from an ODA monolayer on a 10^{-8}M solution of ethidium bromide in deionized water as the subphase.

expansion cycles of ODA Langmuir monolayer after 1 and 12 h, respectively, after spreading monolayer on ssODN 1 in subphase. As compared to the 21Å^2 per molecule takeoff area for ODA Langmuir monolayer, the ssODN–ODA complex monolayer gave 35Å^2 per molecule. The addition of complementary ssODN 2 into subphase resulted in a further expansion to 53Å^2 per molecule due to the formation of DNA 1:2 duplex at the interphase (curve 3). The formation of duplex by sequential immobilization was confirmed by the addition of a duplex intercalator such as ethidium bromide, which as expected further shifted the takeoff area to 70Å^2 per molecule. These results confirmed the hybridization of ssODN 2 to ODA-bound ssODN 1 to yield duplex by sequential electrostatic and H-bonding immobilization of complementary ODNs at air–water interphase via cationic Langmuir monolayer.

4. Electrostatic assembly of cationic gold nanoparticles on DNA template

The ordered arrangement of nanoparticles in two- and three-dimensional structures is an important research problem of practical goal [11]. While DNA molecules, or to be more precise, single-stranded oligonucleotide sequences, have been used for the organization of gold nanoparticles and supramolecular nanoparticle structure in solution [12], to the best of our knowledge, the use of DNA molecules as templates to realize linear nanoparticle assemblies has not been reported so far. Further, self-assembly of nanoparticles in hexagonal close packed thin film form is known; programmed organization of nanoparticles in superstructures of desired shape and morphology is relatively unexplored. Our experience on assembly of cationic lipids on DNA template led us to experiment on assembling cationic gold particle on anionic DNA, mediated by attractive coulombic interactions between the two components. Upon the addition of lysine-capped gold colloidal solution to 15/30-mer ODN duplexes, the solution colour changed rapidly from ruby red to blue indicating aggregation of gold particles [13]. After equilibration, ultracentrifugation and redispersion in H_2O (pH 7.0), the samples were drop-coated as film on carbon-coated grids for transmission electron microscopy (TEM) and on Si wafer for imaging by scanning tunneling microscopy (STM). In the TEM picture shown in Fig. 5, parallel dark bands were obtained with a high degree of order in stacking of duplex chains over the film surface. The dark bands arise from

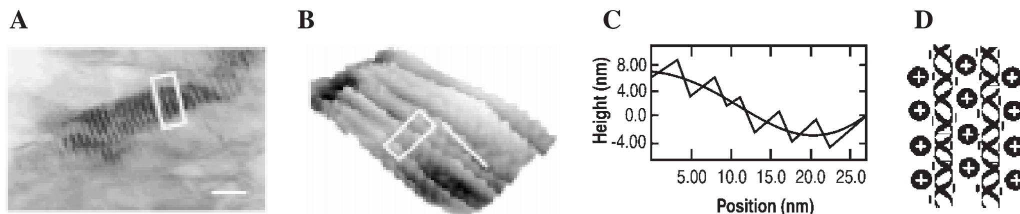

Fig. 5A. TEM picture of the synthetic DNA **1**–gold nanoparticle complex film showing the linear gold supercluster assemblies mediated by the DNA template. The scale bar corresponds to 100 nm. B. STM image of a synthetic DNA **2**–gold nanoparticle complex film dropcoated on a highly conducting Si substrate showing the linear gold nanoparticle assemblies. The length of the line in this image corresponds to 27.5 nm. C. Surface height variation with distance along the line shown for the STM image in B. D. Schematic showing the expected templating action of the DNA double helical molecules leading to the condensed, layered gold nanoparticle–DNA–gold nanoparticle–DNA structures observed in the TEM (A) and STM (B) images (magnified view of the regions circumscribed by the boxes in images A and B.

electron scattering from gold particles and the observed length of the bands 80Å^2 is in close agreement with the expected length of the DNA molecule (55Å^2). The average separation between the longer black bands was 90Å^2, corresponding to DNA double helix–gold nanoparticle sandwich assembly. The STM image shows individual gold nanoparticle assembly as a regular, linear pattern on DNA template in a lamellar fashion. The surface height variation–distance plot indicated a highly periodic variation in height and the observed periodicity of 4 nm is in excellent agreement with the size of gold nanoparticle (3.5± 0.7 nm) arranged in linear arrays. No such ordered structure was seen with uncapped gold particle–DNA films. Similar linear superstructures arising from gold nanoparticle on DNA template could be realized from a simpler protocol [14] in which drop-dried films of DNA duplexes were first deposited on a substrate followed by the addition of lysine-capped gold colloidal particles to the film (Fig. 6).

5. Conclusions

In summary, DNA duplex can be employed as a template receptor for electrostatic assembly of positively charged ligands such as detergents (CTAB), ODA and lysine-capped gold nanoparticles. The negative charges present on the phosphate backbone of DNA in a periodic spacing, spontaneously order the cationic ligands to form linear superstructures. Of particular interest is

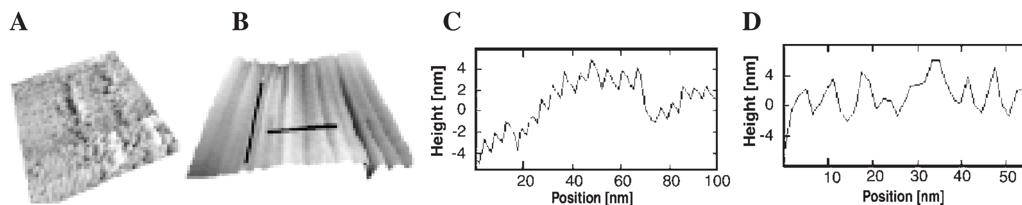

Fig. 6A. STM image recorded from a drop-dried DNA film deposited on a conducting Si substrate. The extent of this image is 100 x 100 nm. B. STM image recorded from a drop-dried DNA film deposited on a conducting Si substrate after the addition of lysine-capped gold nanoparticles. The extent of this image is 100 x 100 nm. C. Surface height variation with distance along the vertical line shown in the STM image of the DNA–gold nanoparticle film (B). D. Surface height variation with distance along the horizontal line shown in the STM image of the gold nanoparticle–DNA film (B).

the assembly of gold nanoparticles on unfunctionalized DNA duplex which holds great promise since templates with different geometries based on rational oligonucleotide design would result into topologically intricate structures, leading to generation of technologically important nanowires and semiconductor quantum dots.

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