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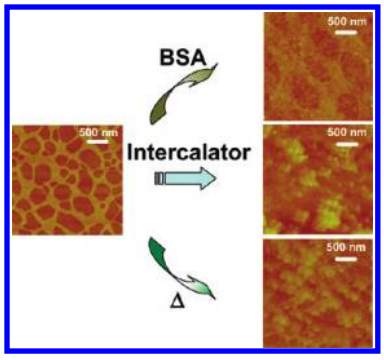
Direct Evidence on the External Stimuli Induced Dissembly of DNA through Microscopic Techniques

Mahesh Hariharan,[†,‡] Elizabeth Kuruvilla,[†] and Danaboyina Ramaiah*[,†]

[†]Photosciences and Photonics, Chemical Sciences and Technology Division, National Institute for Interdisciplinary Science and Technology (NIIST) CSIR, Trivandrum 695019, India, and [‡]School of Chemistry, Indian Institute of Science Education and Research (IISER), Trivandrum 695016, India

ABSTRACT Calf thymus DNA exhibited a regular network-like structure on mica and copper surfaces, respectively, under atomic force (AFM) and scanning electron (SEM) microscopic techniques while oily streak cholesteric birefringent texture was observed on the glass surface under optical polarizing microscopy (OPM). In the presence of an external stimuli such as temperature, intercalating compounds such as the viologen-linked pyrene **1** and *para*-tolylacridinium iodide (**2**) and the minor groove binding spermine (**4**) prevented the DNA−DNA interactions and thereby perturbed the self-assembly of DNA. In contrast, the major groove binding bovine serum albumin (BSA) and the noninteracting ligand *ortho*-tolylacridinium iodide (**3**) did not affect the overall morphology of DNA, as characterized through the AFM, SEM, OPM, and circular dichroism (CD) techniques. As far as we know, this is the first report that presents direct evidence for the perturbation of supramolecular assembly of DNA under various conditions and that can be visualized through different microscopic techniques.

SECTION Biophysical Chemistry



Supramolecular interactions, in all living cells, have remarkable influence on biochemical processes, leading to stabilization of compact morphologies, enhanced macromolecular associations, and altered reaction rates.[1−6] This is particularly manifested in the occurrence of cellular DNA structures as bundles that are formed from the mutual stabilization of neighboring duplexes.[7−11] In principle, such packaging of DNA can be precluded through high temperature and ultra high vacuum[12−15] leading to the dissembly of spatially ordered DNA. In addition, steric barrier arising from bound ligands can inhibit DNA−DNA interactions under ambient conditions. There has been a great deal of interest in understanding such DNA−DNA, DNA−protein, and DNA−ligand interactions through microscopic techniques. However, the majority of microscopic investigations are focused on ligand interactions with short stranded DNA oligonucleotides,[16,17] while interaction of ligands with more complex structures consisting of numerous duplexes organized into networks or mesostructures received less attention.

Unlike proteins,[14] which typically bind to DNA by making contacts within the major groove of the double helix, small molecules bind to DNA within the minor groove,[15] through intercalation[18−21] between adjacent base pairs or through electrostatic interactions with the phosphate backbone on the surface of DNA. Minor groove recognition relies on van der Waals' contacts, hydrogen bonds, Coulombic attraction, and intrinsic properties of the DNA such as flexibility, hydration, and electrostatic potential. On the other hand, the planar polycyclic aromatic moiety is essential for intercalation of ligands.[22−27] Such ligands $\pi-\pi$ stack between the base pairs and maintain contact with DNA over full length of its recognition site. Herein, we report the influence of temperature, protein (bovine serum albumin, BSA), intercalating (**1** and **2**) and minor groove binding (**4**) ligands (Figure 1) on the self-assembly of DNA through atomic force (AFM),[28−35] scanning electron (SEM) and optical polarizing (OPM) microscopic techniques.

To understand the morphology and arrangement of native calf thymus DNA (CT DNA), the AFM, SEM, and OPM images of the CT DNA were recorded on different surfaces. Figure 2A shows the orderly arrangement of highly interconnected network-like structure[32,33] obtained using AFM at 20 μM concentration of DNA on hydrophilic mica surface. Similar structure of DNA was observed on hydrophobic copper surface using SEM at 20 μM concentration (Figure 2B). Empty regions of the network-like structure of DNA were found to have a width of ca. 250 nm, while the OPM analysis indicates an oily streak cholestric texture for CT DNA on the glass surface under similar concentrations (Figure 2C). Interestingly, we observed concentration-dependent changes in the morphology of DNA on copper surface. For example, at 30 μM concentration, DNA exhibits a regular network-like structure, which changed to flaky structures embedded in network-like arrangement at 40 μM concentration. This morphology gets

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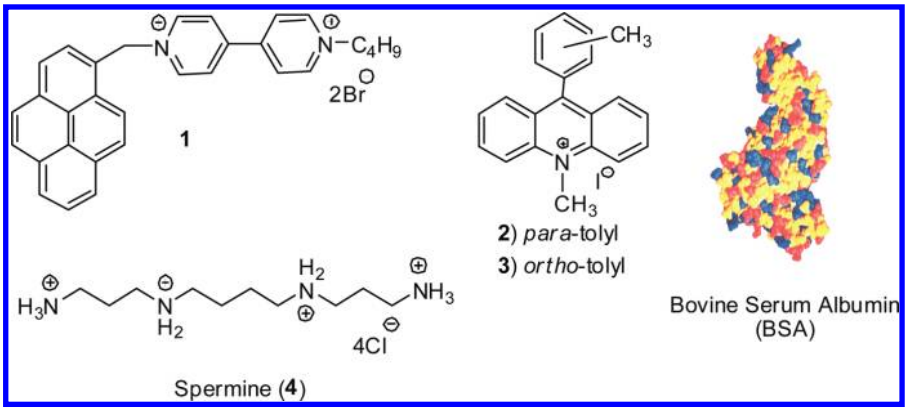

Figure 1. Structures of ligands used in the present study.

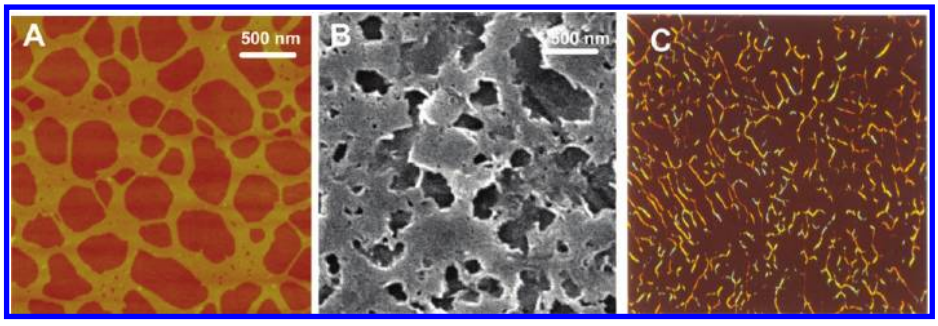

Figure 2. Images of DNA (20 μM): (A) AFM on mica (Scale x, y, 3.3 μm/div; z, 10 nm/div), (B) SEM on copper, and (C) OPM (40×) on glass surface.

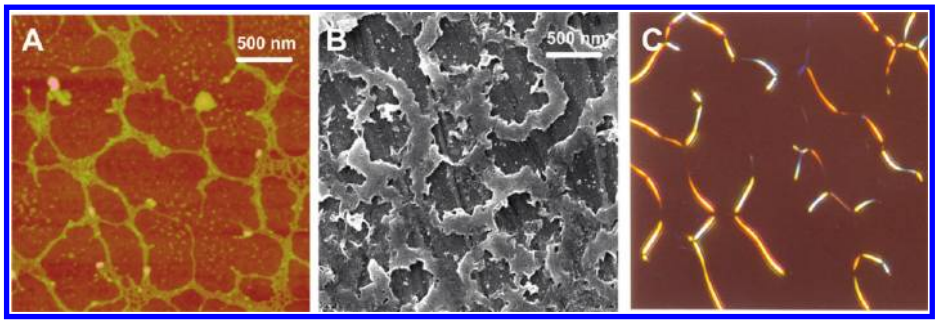

Figure 3. Images of (2:1) complex of DNA (20 μM) and the ligand **1** (10 μM): (A) AFM (Scale x, y, 3.3 μm/div; z, 10 nm/div), (B) SEM, and (C) OPM (40×) techniques.

transformed into a highly symmetric flower-like arrangement at 80 μM (Figure S1, Supporting Information).

Thermal denaturation and circular dichroism (CD) studies were carried out to understand the effect of temperature and ligands on the ordered structure of DNA in solution. A significant decrease in the CD signal corresponding to CT DNA was observed with increasing temperature. Thermal denaturation studies of CT DNA (T_m = 63 °C) in the presence of the ligand **1** exhibited significantly increased melting temperature of 78 °C (Figure S2, Supporting Information). Similarly, in the case of derivative **2**, we observed a T_m value of 70 °C. In contrast, the derivative **3** is found to have negligible effect on the melting temperature of DNA (63 °C). We observed no change in the absorbance at 260 nm corresponding to DNA bases in the presence of spermine (**4**), a minor groove binder.

To visualize the effect of temperature and ligands on DNA self-assembly, we have carried out detailed microscopic studies. Figure 3A shows the AFM image indicating partial disruption of the network-like structure of DNA at a ratio of 1:0.5 of DNA and the DNA intercalating viologen-linked pyrene derivative **1**. Further confirmation was obtained from SEM as shown in Figure 3B. As shown in Figure 3C, we observed cholesteric birefringent[36,37] texture of DNA in the presence of the ligand **1** with reduced abundance of streaks at these concentrations. At equimolar concentration of DNA and the ligand **1** (K_{ass} = 1.1 × 10^4 M^{-1}),[38] we observed a complete disruption of self-assembled spatial ordering of CT DNA

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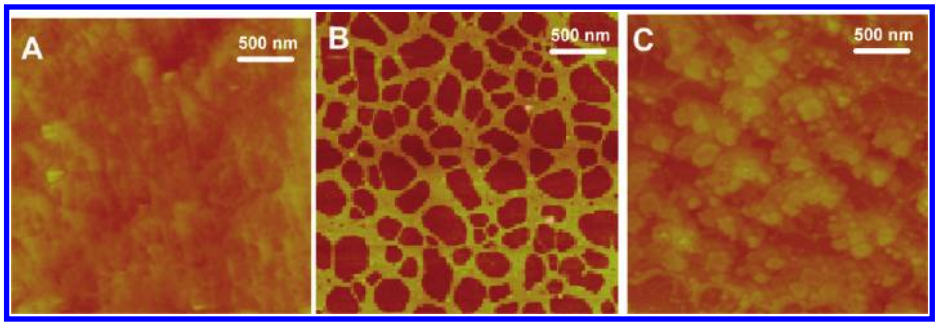

Figure 4. AFM images of DNA (20 μM) in the presence of (A) ligand **4** (20 μM), B) ligand **3** (20 μM), and (C) denatured DNA (Scale x, y, 3.3 μm/div; z, (A) 10, (B) 25, and (C) 10 nm/div).

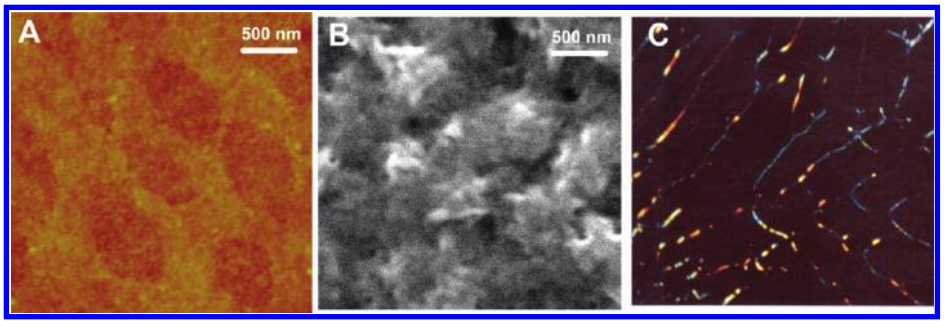

Figure 5. Images of DNA−BSA complex (20 μM each): (A) AFM (Scale x, y, 3.3 μm/div; z, 10 nm/div), (B) SEM, and (C) OPM (40×) techniques.

(Figure S3, Supporting Information). Similar results were obtained with *para*-tolylacridinium iodide **2** (Figure S4, Supporting Information), which exhibited significant affinity for DNA (K_{ass} = 71.1 × 10^4 M^{-1}).[22]

Spermine, a well-known groove binder of DNA, exhibited the complete destruction of the regular network-like structure of DNA (Figure 4A). In contrast, we observed negligible changes in the presence of *ortho*-tolylacridinium iodide **3**, which shows negligible binding to DNA, at equimolar concentrations (Figure 4B). Figure 4C shows the AFM image of the denatured DNA. In agreement with the AFM studies, we observed a complete destruction of the network-like structure of DNA using SEM (Figure S5, Supporting Information). Interestingly, the regular network-like structure of DNA was retained in the presence of BSA even at equimolar concentration (Figure 5). The AFM and SEM studies revealed that BSA alone showed a regular structure[39] with a mean roughness of 2 ± 0.1 nm (Figure S6, Supporting Information). Moreover, OPM studies showed no birefringent texture. BSA is found to adsorb in the network-like structures as well as empty regions of DNA without affecting the spatial ordering of DNA as shown in the Figures 5A and 5B. Figure 5C shows the characteristic oily streak cholestric structure of DNA in the presence of the equimolar concentration of BSA.

Regular network-like structure of DNA at 20 μM concentration on hydrophilic mica surface and hydrophobic copper surface could be attributed to the bundling of several DNA helices, which arises from the mutual stabilization of neighboring helices.[35] The observation of different morphologies of DNA on copper surface at various concentrations using SEM could be attributed to the greater extent of DNA−DNA interactions resulting from the negligible interactions between DNA and the hydrophobic copper surface. Such concentration dependent morphological changes of DNA was not observed using AFM due to the hydrophilic nature of the mica surface. DNA is well studied for its liquid crystalline behavior arising from the anisotropic long-range order.[36,37] In tune with the observations made with SEM and AFM techniques, we observed the characteristic oily streak cholesteric birefringent texture for the DNA on the glass surface at 20 μM concentration. All these microscopic observations point to the inherent property of the DNA to exist as an ordered assembly. The reduced CD signal and increase in the absorbance at 260 nm corresponding to DNA at elevated temperatures on the other hand can be attributed to the unwinding of the double helical structure of the duplex DNA, which in turn prevents the spatially ordered DNA−DNA interactions.

The observed increase in the melting temperature of DNA and concentration-dependent morphologically identical disruption of DNA−DNA self-assembly in the presence of the intercalating ligands **1** and **2** is due to their effective interaction with DNA.[22−25,38] This could be attributed to the intercalation of these derivatives with DNA, resulting in the prevention of DNA−DNA interactions. In contrast, negligible changes in T_m and unperturbed network-like structure of DNA in the presence of the noninteracting ligand **3**,[22,23] confirms the fact that effective interaction of the ligand is essential to prevent the DNA−DNA interactions either through intercalating or minor groove binding. The changes in the hydrophilic and hydrophobic balance of DNA in the presence of various interacting ligands could also be reason for the cause of the disruption of the DNA self-assembly. The observed disruption of the network-like structure of DNA in the presence of groove binding spermine further confirms the importance of

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DNA−ligand interaction in the dissembly of network-like DNA structure. While negligible changes in the morphology of DNA in the presence of BSA could be attributed to the interaction of BSA in the major groove of DNA and such interactions have insignificant influence on the DNA assembly.

In conclusion, we report the microscopic evidence on the effect of temperature, protein, and ligands that exhibit interaction on the self-assembly of DNA. The morphology of DNA was unaffected when bound to major groove binder BSA and the nonbinding compound **3**. Increase in temperature perturbed the self-assembly of DNA, which is not viable under biological conditions. Interestingly, the derivatives that stabilize duplex DNA through intercalation and minor groove binding interactions prevent the DNA−DNA interactions thereby perturb the self-assembly of DNA. The results presented here can provide important insights into the causes of rupture of the supramolecular assembly of DNA in cells and the consequences of such dissembly in various physiological functions.

SUPPORTING INFORMATION AVAILABLE

Experimental details, AFM images, CD spectra and thermal denaturation curves of CT DNA under different conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author:

*To whom correspondence should be addressed. E-mail: rama@niist.res.in or d_ramaiah@rediffmail.com. Tel: (+) 91 471 2515362. Fax: (+) 91 471 2491712.

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