Chirality of sulforhodamine dye molecules incorporated in DNA thin films

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Thin films formed from salmon sperm DNA reacted with a cationic surfactant (CTMA-Cl) included up to 25 wt % fluorescent molecule sulforhodamine (SRh). SRh effect on DNA chirality and vice versa was investigated by circular dichroism (CD) spectroscopy. The CD signals at 250–265 nm indicate that DNA chirality was maintained or enhanced. Induced CD (iCD) signal at 580–610 nm indicates that SRh is chiral in DNA/CTMA. iCD signal from both solutions and thin films generally increases with SRh concentration. The chirality induced in SRh molecules and the absence of significant DNA reduction in chirality are clear indicators of strong binding to DNA/CTMA. © *2008 American Institute of Physics*. [DOI: 10.1063/1.3027070]

The double helix structure of DNA has prompted research in novel and improved devices in several fields, including photonics,[1] electronics,[2] and spintronics.[3] Enhancement in the efficiency and brightness of organic light emitting diodes, which incorporate DNA nanometer thin films, has been previously reported.[4,5] Stimulated emission from thin film lasing structures containing a DNA gain medium doped with the fluorescent dye molecule sulforhodamine 640 (Exciton Inc., Dayton OH) (SRh) has been reported to have quite a low threshold.[6] A much more complete understanding of the biophysical mechanisms involved in the interaction between DNA, surfactants, and fluorophores is required in order to fully exploit the potential of combining the unique structure and properties of DNA (and other biopolymers) with various light emitting molecules in optoelectronic devices. Since most of the device applications utilize thin film structures, an understanding of processes and mechanisms is particularly needed as these material undergo a liquid ("wet") to solid ("dry") state transition.

In this paper we report on the nature of the interaction between DNA-Na polymer complexed with a cationic surfactant cetyltrimethylammonium chloride (CTMA-Cl) and SRh molecules. Circular dichroism (CD) spectroscopy[7] has been employed to investigate chirality in DNA/CTMA thin films and solutions containing various amounts of SRh molecules. CD is the result of differential absorption of left-and right-circularly polarized beams in molecules that have a chiral structure. A chiral structure is frequently found in biopolymers, such as nucleic acids and proteins.

The backbone of the DNA double helix consists of alternating ribose sugar and phosphate groups forming two spirals running in opposite directions. Nitrogenous bases attached at approximately right angles to the sugar/phosphate groups join the two spirals through hydrogen bonds. The four bases found in DNA, adenine (A), cytosine (C), guanine (G), and thymine (T), are uniquely paired (A-T, G-C) such that they allow a constant DNA helix diameter (~2.37 nm). In the common DNA polymorph (B-DNA), the helix has a right-handed (or clockwise) rotation. The chirality in the DNA molecule is introduced by the sugar units, with the other units (phosphate groups, bases) being intrinsically achiral. However, in the overall structure chirality is usually observed as changes in the ultraviolet (UV) absorbance of the bases in the 200–300 nm region induced by coupling to the chiral backbone. The chromophore component of the SRh molecule is a planar sequence of seven fused rings. SRh molecules incorporated in DNA/CTMA thin films have[6] main absorption and luminescence peaks at ~575 and 605 nm, respectively. Differences in the CD spectral characteristics between DNA/CTMA and DNA/CTMA:SRh samples result from an induced CD (iCD) effect,[8] caused by the interaction between the chiral DNA polymer and the achiral SRh molecules.

CD experiments were performed both on liquid and thin film samples. The samples consisted of DNA/CTMA dissolved in butanol and combined with SRh dye. The thin films were prepared from the same solutions used for the liquid samples in order to make comparisons between the two states more meaningful.

Salmon sperm DNA is extracted by an enzyme process[9] at the Chitose Institute of Science and Technology, Japan. To improve its film forming characteristics, the as-received high molecular weight (MW) DNA was sonicated[10] to reduce the average MW to approximately 200 kDa. Next, the DNA-Na is reacted with the cationic surfactant CTMA-Cl in water. This forms a DNA/lipid (DNA/CTMA) complex, which precipitates out of the aqueous solution, but is soluble in simple organic solvents. Thin film formation by spin coating is markedly easier with organic solvents versus aqueous solutions. We utilized butanol as the solvent because of its relatively slow evaporation (due to a low vapor pressure) and moderate viscosity. The butanol solutions were all 10 wt % DNA/CTMA. This was dissolved on a rotary mixer overnight at room temperature. Sulforhodamine was added to each solution as a percentage of the weight of DNA/CTMA. The solutions were each filtered through a 0.45 μm syringe-tip polytetrafluoroethylene filter. Solutions of 2.5, 5, 10, 15, 20, and 25 wt % SRh to DNA/CTMA were prepared.

Thin films produced by spin coating were typically ~1.5 μm thick. The spin-coating process started by completely flooding the substrate, followed by spreading the solution for 8 s at 300 rpm, a spin of 1 min at 400 rpm, and finally a drying spin of 10 min at 200 rpm. The film thick-

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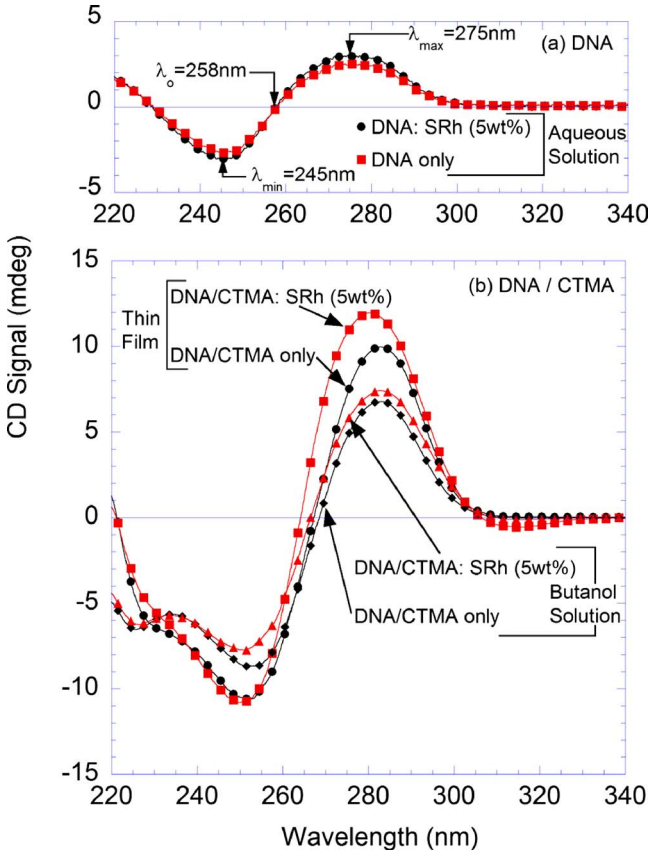

FIG. 1. (Color online) CD signal spectral response for the DNA absorption region (220–340 nm): (a) DNA and DNA/SRh (5 wt %) aqueous solutions and (b) DNA/CTMA and DNA/CTMA:SRh (5 wt %) butanol solutions and thin films.

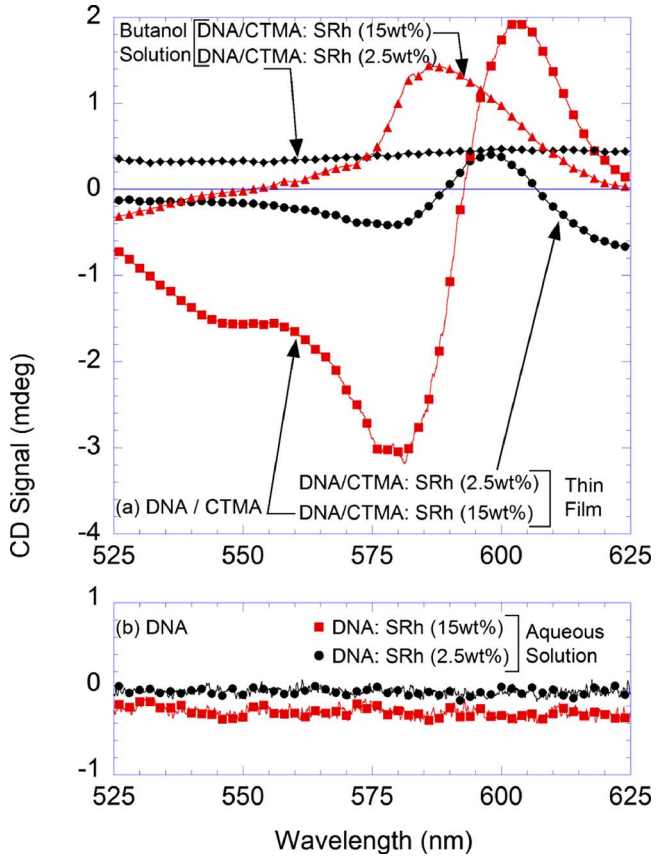

FIG. 2. (Color online) iCD signal spectral response for the SRh absorption region (525–626 nm): (a) DNA/CTMA and DNA/CTMA:SRh (2.5 and 15 wt %) butanol solutions and thin films and (b) DNA and DNA/SRh (2.5 and 15 wt %) aqueous solutions.

ness was verified with a Dektak contact profilometer.

The actual concentrations of SRh in both solution and thin film samples probably differed somewhat from the nominal values given above due to possible losses because of incomplete dissolution or the filtering process. Furthermore, in the case of the thin films it is possible that some of the SRh molecules were aggregated and not uniformly distributed. However, we have no evidence that any of these possibilities resulted in significant reductions in concentration.

CD measurements were performed with a Jasco CD spectrometer. Solution samples used a two part cylindrical cuvette (bottom with cavity and flat top) with a liquid path length of 0.01 mm (10 μm). Background correction was performed using a cuvette filled with butanol. For measurements of solid samples, thin films were spun on the top part of the cuvette. In this case, background correction was performed using a clean cuvette half. The spectrometer settings were a scan speed of 5 nm/min, response time of 1 s, and bandwidth of 15 nm. Three scans were performed and averaged for each sample. The output signal was filtered by the spectrometer software to improve the signal-to-noise ratio. We concentrated our measurements in two spectral regions: 220–340 nm for observing the DNA effects and 525–625 nm for SRh.

As a baseline reference, we measured the CD spectra of aqueous solutions of pure DNA (1 wt %) and of DNA plus SRh (5 wt % to DNA). As shown in Fig. 1(a), both aqueous DNA solutions exhibit the characteristic CD spectral features[7] of B-DNA in the near UV (220–340 nm) region: (a) a negative band centered at 245 nm, (b) a positive band centered at 275 nm, and (c) a zero crossing at 258 nm. It is clear that the presence of a significant amount of SRh has not affected the CD signal and, hence, the conformation and structure of the DNA molecule.

Near UV CD spectra from DNA/CTMA butanol solutions and thin films were obtained as a function of SRh concentration. Shown in Fig. 1(b) are the results for liquid and solid samples containing DNA/CTMA only and those also containing 5 wt % SRh. Interestingly, the CD results for the different samples in Fig. 1(b) retain most of the spectral features of the DNA aqueous solutions, with a ~7–8 nm shift to longer wavelengths. This indicates that the helical DNA structure is not materially affected by the reaction of DNA with CTMA, by the use of organic solvents for the liquid samples, or by the absence of a liquid environment for the solid samples.

iCD spectra were obtained in the SRh absorption-luminescence wavelength region (525–625 nm). Shown in Fig. 2(a) are spectra for DNA/CTMA thin film and solution samples containing 2.5 and 15 wt % SRh. While the 2.5 wt % SRh solution sample does not show an iCD signal in this spectral window, the 15 wt % SRh solution sample displays a clear positive lobe with a peak at 585 nm. All thin film samples displayed an SRh iCD signal, always with a higher intensity than their solution counterparts due to their much higher effective volume SRh concentration. The 15 wt % SRh thin film exhibits significant negative and positive iCD lobes with maxima at 580 and 598 nm, respec-

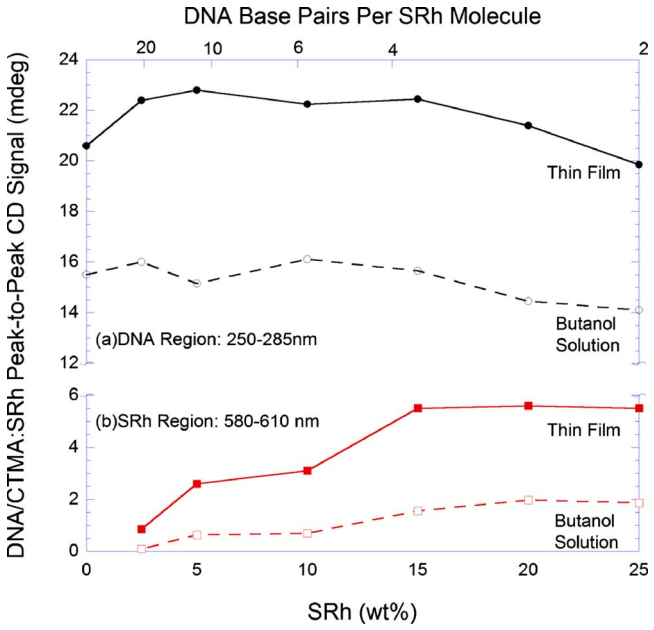

FIG. 3. (Color online) Peak-to-peak CD signal vs SRh concentration: (a) DNA absorption region and (b) SRh absorption region.

tively. Interestingly, aqueous DNA:SRh solutions did not exhibit an SRh iCD signal, as shown in Fig. 2(b). These results lead us to the conclusion that the presence of the CTMA surfactant is critical (in either a direct or indirect way) in enabling SRh to interact with the DNA double helix in order to induce SRh chirality.

The peak-to-peak (negative-to-positive lobe excursion) iCD amplitude is shown in Fig. 3 as a function of SRh concentration in both DNA/CTMA solutions and thin films. In the DNA absorption region (250–285 nm), the CD signal in thin films at first increases with SRh concentration up to ~5 wt %, after which it is roughly constant up to a concentration of ~15 wt %, and is followed by a reduction in signal, as shown in Fig. 3(a). It is important to point out that at 25 wt % SRh concentration, which corresponds to one SRh molecule for every two DNA base pairs, the CD signal is only slightly lower than for the film with no SRh. This is a surprisingly large amount of dye molecules being accommodated by the DNA-CTMA polymer. A similar behavior is observed for the solution samples, but at lower signal levels than for the thin film samples.

The iCD signal in the SRh absorption region is shown in Fig. 3(b) as a function of SRh concentration. A monotonic increase in iCD signal strength is observed for both thin film and solution samples with increasing SRh concentration up to ~15 wt %. The SRh iCD signal becomes essentially saturated at greater SRh concentrations. It is interesting to point out that the SRh iCD signal saturation onset occurs at the same concentration at which the DNA CD signal begins to decrease.

A reasonable explanation of the observed behavior is that the SRh molecules are strongly bound to the DNA/CTMA polymer, but probably not intercalated in the DNA double helix since the DNA/SRh aqueous solutions do not exhibit any iCD in either the DNA or SRh wavelength regions. Strong bonding would explain the presence of significantly enhanced CD for the DNA/CTMA polymer and iCD for the otherwise achiral SRh molecules. The strong, but nonintercalating, bonding would also explain the ability of DNA/CTMA to absorb a very large SRh concentration without losing its chirality.

In summary, CD spectroscopy was utilized to investigate the incorporation of SRh fluorescent dye molecules in DNA/CTMA polymers and to compare their behavior in the liquid state (solution) and the solid state (thin film). A strong iCD signal for SRh in DNA/CTMA indicates that the achiral dye molecule acquired chirality by binding to the DNA/CTMA. In contrast, SRh in DNA aqueous solution does not display an iCD signal, nor is there an enhanced CD signal on the part of the DNA. This clearly indicates the critical role of the CTMA-Cl surfactant in enabling SRh binding. Interestingly, the CD characteristics of thin films and solution samples, while different in signal intensity, followed similar overall trends. Further investigation is required to identify the nature of the SRh-DNA/CTMA binding and the mechanisms that allow such large loading without denaturing of the DNA secondary structure.

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