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Role of Surfactants in the Interaction of Dye Molecules in Natural DNA Polymers

H. You, H. Spaeth, V. N. L. Linhard, and A. J. Steckl*

Nanoelectronics Laboratory, University of Cincinnati, Cincinnati, Ohio 45221-0030

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Solutions and powders formed from salmon sperm deoxyribonucleic acid (DNA) reacted with the cationic surfactant cetyltrimethylammonium chloride (CTMA-Cl) incorporated fluorescent rhodamine molecules: anionic sulforhodamine 640 (SRh) or cationic/zwitterionic rhodamine 640 perchlorate (RhP). The role of the cationic surfactant in the interaction between rhodamine dye and DNA−surfactant molecules has been investigated in both solution and solid state using optical spectroscopy and electrophoresis. Unexpectedly, the dye molecules did not interact directly with DNA, rather the DNA double helix acted as a template for the interaction between dye molecules and CTMA in the DNA/CTMA complex. The SRh and RhP molecules yield different fluorescence characteristics with increasing DNA/CTMA amount, indicating different configurations between the CTMA ligands.

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Introduction

Deoxyribonucleic acid (DNA) polymers, the source of genetic information, are soluble only in aqueous solutions. However, after bonding with cationic surfactants, such as cetyltrimethylammonium chloride (CTMA-Cl), the DNA−surfactant complex is soluble in organic solvents. Formed into thin films, the DNA-based biopolymer material displays many useful properties,[1] such as low optical loss, high temperature stability, tunable refractive index, and low microwave insertion loss. Currently, natural DNA processed from salmon roe and milt sacs, waste products of the fishing industry, is quite abundant and relatively inexpensive. To date, the DNA-based biopolymer has been reported to be a promising new material for photonics,[2] holographic information inscription,[3,4] nonlinear optics,[5,6] electronics,[7] and chemical sensors[8] applications. Significant enhancement in the efficiency and brightness of organic light-emitting diodes that incorporate DNA nanometer thin films has been previously reported.[9,10]

It is well known that many small molecules can readily interact with DNA double helix structures through three different modes: ionic, groove, and intercalation binding.[11] Many fluorescent dye molecules[12,13] intercalate into the double helix of DNA, whereby the intensity of fluorescence is greatly enhanced.[14,15] Amplified spontaneous[16] and stimulated emission[17] from structures containing a DNA−surfactant complex gain medium doped with the fluorescent dye molecules have been reported with quite a low emission threshold. One possible mechanism for the highly efficient light amplification was ascribed[16,18] to the interaction between dye molecules and DNA double helix by either intercalation or groove binding. However, a recent study[19] on circular dichroism (CD) in DNA/CTMA thin films and solutions that incorporated sulforhodamine (SRh) molecules clearly indicated the key role of the surfactant in imparting chirality to the SRh molecules. Actually, considering the structure of DNA−surfactant complexes in the precipitation[20] and solution form,[21] the fluorescent dye molecules may be incorporated between surfactant ligands. Dye−surfactant interactions are of great importance in the dyeing and photographic industries, biological and medicinal photosensitization, and analytical and environmental sciences.[22] More specific to this work, 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 biopolymers with various light-emitting molecules in optoelectronic devices. Therefore, the relationship between the structure of selected dye molecules and their location and orientation relative to the DNA−surfactant complex needs to be better understood.

*Corresponding author. E-mail: a.steckl@uc.edu.

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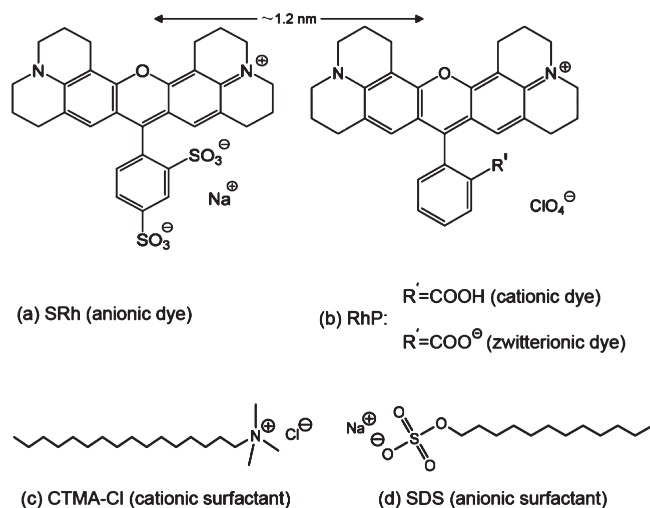

Figure 1. Chemical structure of molecules employed in this study: (a) SRh, anionic dye; (b)RhP, cationic/zwitterionic dye; (c) CTMA-Cl, cationic surfactant; (d) SDS, anionic surfactant.

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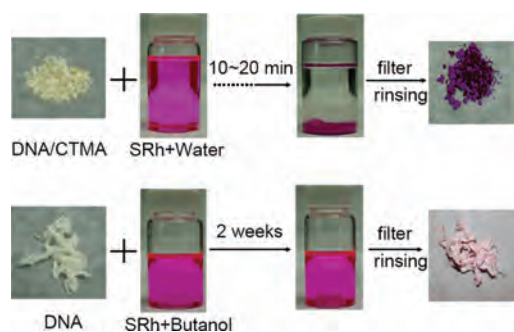

Figure 2. Photographs illustrating the interaction between DNA/CTMA powders and DNA fibers and SRh aqueous and butanol solution.

In this paper we report on the nature of the interaction between the DNA-Na polymer complexed with a cationic surfactant (CTMA-Cl) and sulforhodamine sodium (SRh) or rhodamine perchlorate (RhP) dye molecules (see molecule structures in Figure 1). The triggering event for this investigation was the dramatic effect observed when DNA/CTMA whitish fibrous powder was added to SRh aqueous solution, which was normally pink in color. As seen in Figure 2, the solution became clear, and the DNA/CTMA powder (which does not dissolve in water) became pink. This change occurred in 20−30 min for a still solution and in 10−15 min for an agitated solution. The DNA/CTMA powder retained its pink color after repeated rinsing in clean water. A similar phenomenon was observed when RhP was substituted for SRh.

Since SRh is soluble in organic solvents as well as water, a complementary experiment was carried out. DNA-Na fibers were introduced into a SRh butanol solution. After duration as long as 2 weeks, the solution did not change color and the DNA-Na fibers regained their whitish color after rinsing in clear butanol solvent (see Figure 2). Since the DNA−surfactant complex is tested in SRh aqueous solution, whereas the DNA-Na is tested in SRh butanol solution; additional DNA-Na experiments were performed with longer chain alcohols to simulate the surfactant environment. The results for DNA-Na in SRh 1-hexadecanol ($C_{16}H_{33}OH$) solution were similar to those in the case of DNA-Na into SRh butanol solution.

Therefore, we conclude that the dye molecules did not interact directly with the DNA double helix, which just acted as a template for the interaction between dye molecules and CTMA in the DNA/CTMA complex. The SRh and RhP molecules yield different fluorescence characteristics with increasing DNA/CTMA amount, indicating different configurations inside the CTMA ligands.

Experimental Methods

Materials and Sample Preparation. Salmon sperm DNA is extracted by an enzyme process at the Chitose Institute of Science and Technology, Japan.[23] To improve film forming characteristics,[24] the as-received high molecular weight (MW) DNA (25−10 MDa) was sonicated, reducing the average MW to approximately 200 KDa. Next, the DNA-Na is reacted with the cationic surfactant CTMA-Cl (0.01 M) in water. This forms a DNA/lipid (DNA/CTMA) complex, which precipitates out of the aqueous solution but is soluble in simple organic solvents. For the DNA/dye/CTMA solution, fluorescent dye (5 wt % to DNA/CTMA) is added into the DNA-Na aqueous solution, and then reacted with the surfactant solution, resulting in the precipitation of a pink DNA/dye/CTMA complex, which dissolves into butanol to obtain the DNA/dye/CTMA solution. For the DNA/CTMA/dye solution, the DNA/CTMA complex is formed first. Then it is reacted with the dye molecules in an organic solvent to obtain the DNA/CTMA/dye solution. Butanol was utilized as the organic solvent because of its relatively slow evaporation (due to a low vapor pressure) and moderate viscosity. All solution samples were dissolved on a rotary mixer overnight at room temperature. The DNA/CTMA/dye solutions were made with different weight ratios of DNA/CTMA to dye molecules. Optical absorption of DNA/CTMA/dye and DNA/dye/CTMA solution was performed over the near-ultraviolet and visible (UV−vis) spectrum.

For dye absorption experiments, dye molecules in 0.0025 wt % aqueous and organic solutions incorporating insoluble DNA/CTMA powders and DNA-Na fibers, respectively, were used. The amount of the dye introduced into the solution was chosen such that there was 1 dye molecule for 10 DNA base pairs. UV−vis absorption spectrum measurements were performed at 15 min intervals, utilizing 0.8 mL of the pure solution without undissolved DNA-Na fibers or DNA/CTMA powders, which was then returned into the main mixture container after the test.

For the decomposition experiments, equal amounts (0.84 mmol) of DNA/SRh (5 wt %) /CTMA and DNA/CTMA were treated with 10 mL of sodium dodecylsulphate (SDS) (Aldrich) aqueous solution (0.168 M). This is equivalent to 1 SRh molecule for every 10 DNA base pairs and 20 CTMA molecules and 20 SDS molecules. The mixtures were placed in a rotary mixer for 48 h at room temperature and then filtered with 0.45 μm PVDF syringe filter to separate the solution from any remaining solid. The solutions were first used for UV absorption measurements. This was followed by treatment with 1 mL of diluted DNA-specific PicoGreen (Invitrogen, Inc.) dye solution, and then used for the photoluminescence (PL) measurements. Solutions with the same amount of DNA/CTMA and DNA/SRh/CTMA in water without SDS were also tested for comparison. The solutions without PicoGreen dye were also concentrated for the electrophoresis experiment.

Measurement. A Perkin-Elmer spectrometer (Lambda 900) was used to measure the UV absorption spectrum of different solutions. The PL measurements were performed with He−Cd laser excitation (Omnichrome Series 74) at 325 nm or Argon laser excitation (Omnichrome 532−100MBS) at 488 nm. The emission spectra were analyzed by an Acton Research spectrometer

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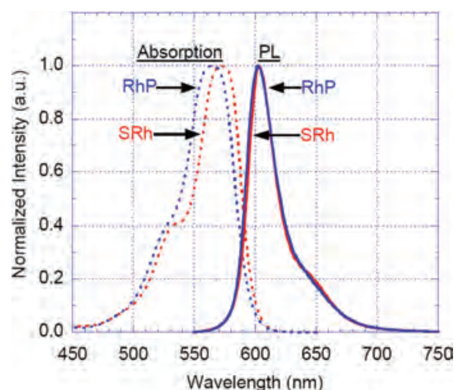

Figure 3. Optical absorption and PL spectra (488 nm excitation) of SRh and RhP in butanol solution.

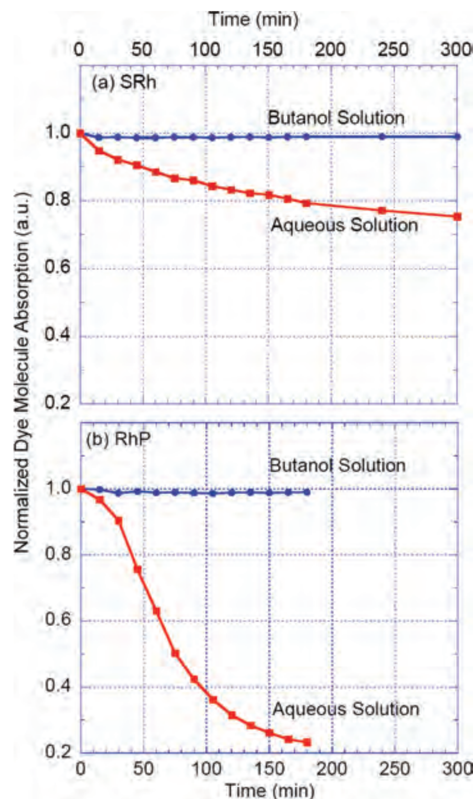

Figure 4. Time dependence of the normalized dye molecule absorption (~560−570 nm) in butanol (with DNA-Na fiber) and in aqueous solution (with DNA/CTMA powder): (a) SRh; (b) RhP.

(SP2550) equipped with a photomultiplier sensitive in the UV−vis spectrum. High-pass filters and dichroic mirrors were utilized to block the laser pump light. The spectrometer resolution was 0.16 nm. The PicoGreen PL measurements were performed with a NanoDrop 3300 Fluorospectrometer. The electrophoresis was performed with 0.5 wt % agarose gel in TAE buffer. Ethidium bromide was used to label the DNA. All measurements were performed at 300 K.

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Results and Discussion

The rhodamine dye family is a derivative of xanthene, incorporating a central group of heterocyclic fused rings. Rhodamine dyes are widely used as the gain medium in dye lasers and in fluorescence analysis. SRh is an anionic molecule in both aqueous and organic solutions, whereas the RhP molecule can take both the cationic and zwitterionic form in solution. The relative abundance of the two forms depends on the dye concentration, solvent viscosity, and dielectric constant.[25−27] The UV−vis absorption and PL spectrum of the dye molecules in butanol solution are shown in Figure 3.

The relationship between the state of the DNA (Na salt vs surfactant complex), the SRh molecules, and the solvent was first quantified by measuring the optical absorption of the dye solutions. Figure 4 shows the time dependence of the normalized SRh and RhP absorption in butanol, into which DNA-Na fibers were added, and in aqueous solution, into which DNA-CTMA powder particles were added. The SRh and RhP absorption in butanol did not change with time upon the introduction of DNA-Na fibers, indicating that, to a first approximation, there is no direct dye−DNA reaction. On the other hand, the dye absorption in aqueous solution dramatically decreased with time with addition of DNA/CTMA powder particles. This demonstrates that the DNA/CTMA can react with dye molecules and remove them from their aqueous solution, whereas the DNA-Na is unable to perform the counterpart reaction in butanol solution. This clearly indicates there is no direct interaction between the DNA and dye molecules. Also, no evidence was observed for ion exchange between the CTMA ligands in the DNA/CTMA complex and dye molecules when the dye was mixed with DNA/CTMA in butanol solution. CD spectroscopy also supports these conclusions, as there was a strong induced CD signal for SRh in DNA/CTMA butanol solution, whereas SRh in DNA aqueous solution did not display a CD signal.[19]

It is well known that DNA−cationic surfactant complexes can be decomposed by the addition of anionic surfactants[28] or monovalent salts.[29] The DNA is released from the DNA−cationic surfactant complex and surfactant aggregate structures are formed because the interaction between oppositely charged surfactants is stronger than the one between DNA and cationic amphiphiles.[30] This presents an opportunity to further understand the interaction between DNA, surfactant, and dye molecules. The decomposition of DNA/CTMA complexes was investigated with the addition of the anionic surfactant SDS in aqueous solution (see structure in Figure 1d). As the DNA/CTMA powder is not soluble in water, the decomposition is not complete even after 48 h. UV−vis absorption and PL of the DNA/CTMA−SDS solution were used to characterize the degree of decomposition. The influence of SRh dye molecules on the decomposition of DNA/CTMA complexes was investigated. PicoGreen was used to identify the presence of DNA double helix molecules. In Figure 5, spectra from DNA/SRh/CTMA and DNA/CTMA aqueous and SDS solutions are compared. Since DNA/CTMA complexes do not readily dissolve in water, all solutions were filtered prior to optical measurements to remove particulates. Comparing the SDS-based solutions in Figure 5a, it is clear that the presence of the SRh molecules greatly amplified the decomposition process, leading to very strong UV (265 nm) absorption from DNA and red (590 nm) absorption from SRh. Similarly, in Figure 5b, the decomposed DNA/SRh/CTMA+SDS solution exhibited strong PL signal from intercalated PicoGreen

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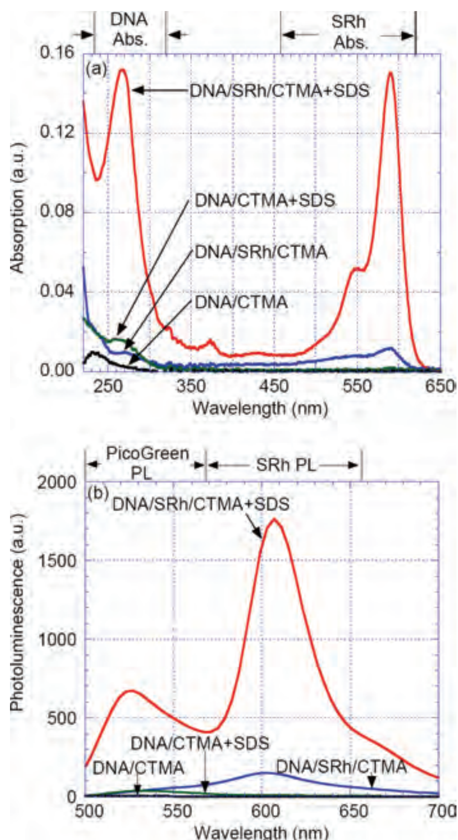

Figure 5. Decomposition of DNA/SRh/CTMA and DNA/CTMA in SDS solution: (a) UV−vis absorption spectra; (b) PL spectra. Solutions were filtered to remove solids remaining after 48 h. For comparison, spectra of DNA complexes in plain aqueous solutions are also shown.

molecules (with a peak at 520 nm) and from SRh molecules (at 605 nm). On the other hand, the DNA/CTMA+SDS solution had very weak UV absorption and PicoGreen fluorescence, indicating a much less effective decomposition. As expected, the DNA/SRh/CTMA and DNA/CTMA plain aqueous solutions showed very weak absorption or PL signals. When the filtered DNA/SRh/CTMA+SDS solution was concentrated and investigated by gel electrophoresis, the DNA and SRh molecules separated under the electric field, with DNA moving toward the anode and SRh moving toward the cathode. All of these observations demonstrate that the dye molecules react with CTMA and not directly with DNA in the DNA/CTMA complex.

The interaction between the two oppositely charged surfactant and dye molecules, SRh and CTMA-Cl (without DNA), in aqueous solution ([SRh] = 40 μM, [CTMA] = 0.8 mM) was investigated by optical measurements. The results are shown in Figure 6. For comparison, the results for DNA/SRh/CTMA butanol solution with the same SRh concentration are also presented. Interaction between SRh and CTMA-Cl molecules in aqueous solution was indicated by the UV−vis absorption and PL intensity decrease and slight blue shift in the peak position. The significant differences in absorption and PL between the plain dye solution and the dye−surfactant aqueous solution clearly indicate the strong interaction between the oppositely charged molecules. A similar effect has been reported[22] for rhodamine 6G and SDS solutions and attributed to an ion pair aggregation process. The fact that the SRh and SRh/CTMA butanol solutions exhibited nearly the same absorption and PL is attributed to the absence of micelle formation in organic solution (unlike the case

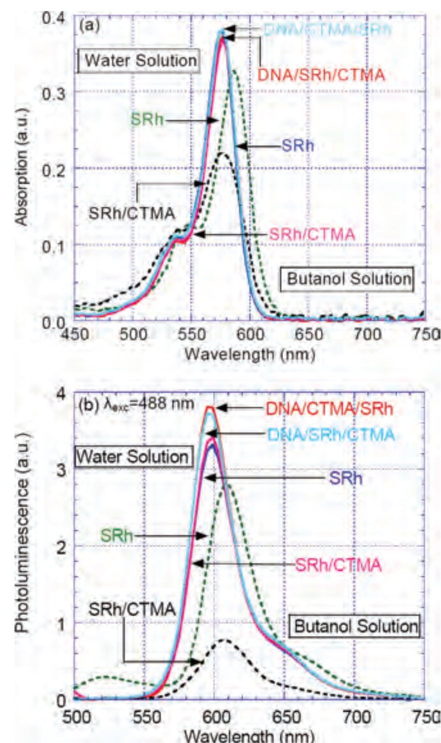

Figure 6. UV−vis absorption spectrum (a) and PL spectrum (488 nm excitation) (b) of SRh molecules with the addition of CTMA in butanol (solid line) and in aqueous solution (dash line), respectively. Data for DNA/SRh/CTMA and DNA/CTMA/SRh in butanol solutions is also shown for comparison.

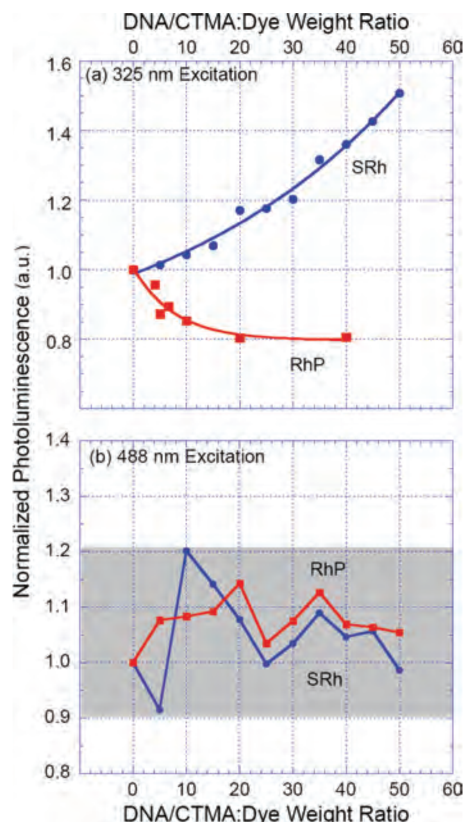

Figure 7. Normalized PL intensity of SRh (left) and RhP (right) in butanol solution with the addition of different weights of DNA/CTMA with excitation at 325 nm (up) and 488 nm (down), respectively.

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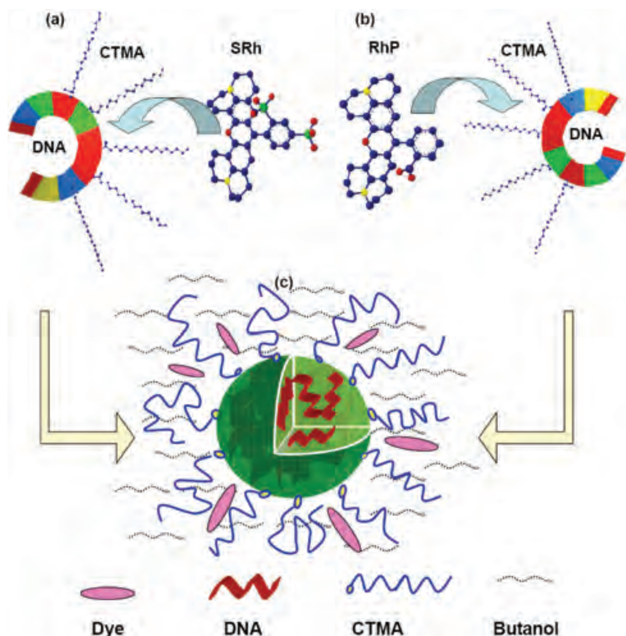

Figure 8. Expected SRh (a) and RhP (b) molecule incorporation dynamics inside the CTMA molecules in the DNA/CTMA and the final structure of the solvable DNA/dye/CTMA complex in solution (c). For simplicity, only CTMA ligands located on the surface of the globule are shown.

in aqueous solution). This indicates that the interaction between the CTMA and dye molecules in DNA/CTMA complex can not be attributed to the formation of ion-pair aggregates, which would result in significant changes in the intensity and wavelength of the absorption and PL from the dye molecules.

To more fully understand the dye molecule configuration in DNA/CTMA, the PL intensity of SRh and RhP with different weight ratios between DNA/CTMA and dye in butanol solutions was measured. The results are shown in Figure 7. It is very interesting to note that, when the 325 nm laser excitation was used, the SRh PL intensity increased with increasing DNA/CTMA in the solution, whereas the RhP PL intensity decreased with DNA/CTMA concentration. When the excitation source was changed to 488 nm argon laser, the PL intensity of both SRh and RhP stayed within a common band with different DNA/CTMA concentrations in the butanol solution. On the other hand, as shown in Figure 4, compared to SRh, RhP molecules are easier to remove from their aqueous solutions by DNA/CTMA powders. This indicates that the SRh and RhP molecules interact with the CTMA ligands in the DNA/CTMA complex with different mechanisms. Considering the structures of these two dye molecules[25] (Figure 1) and of the DNA−surfactant complex in alcohol solution,[21] it is reasonable to ascribe this difference to the dye molecular structures. For SRh, the steric effect due to two sulfates in the phenyl group is important as the SRh molecules incorporate into CTMA ligands in the DNA/CTMA complex. It is likely that the xanthene group incorporates first and then the phenyl group, as shown schematically in Figure 8. By comparison, the phthalide group in RhP molecules is more easily incorporated into CTMA ligands without hindrance, resulting in the fast removal of RhP dye from aqueous solution by DNA/CTMA powders. After incorporation, the xanthene group, which is the fluorescing group in rhodamine dyes, is located closer to the DNA base pairs for SRh compared to RhP molecules. This would lead to more efficient energy transfer between SRh molecules and DNA base pairs. Using 325 nm laser excitation, which is in the range of DNA absorption, energy is mostly absorbed by the DNA base pairs and then transferred to SRh molecules, followed by emission from SRh excited states. As the DNA/CTMA concentration increases, more energy is absorbed by base pairs and transferred to SRh, leading to increasing PL signal from SRh molecules. For RhP solutions, the greater distance between the xanthene group and the DNA base pairs prevents efficient energy transfer. Therefore, increasing DNA/CTMA concentration leads to more laser energy being absorbed by base pairs and less energy available for RhP excitation, resulting in decreasing RhP PL. When 488 nm excitation is used, which is in the range of rhodamine absorption (Figure 1c), the energy is mainly directly absorbed by the dye molecules, and therefore the PL signal is independent of the DNA/CTMA concentration in the solution.

In summary, the interaction between the rhodamine dye molecules and DNA−surfactant complexes has been investigated. In contrast to the common assumption that dye molecules will interact directly with DNA double helices through either groove or intercalation binding, it appears that the DNA double helix structure simply acts as a template for the interaction between the CTMA ligands and xanthene dye molecules. Anionic SRh and cationic/zwitterionic RhP dyes incorporate into the CTMA with different directions, which yields different fluorescence characteristics in DNA/CTMA alcohol solution. Understanding and controlling the complex interactions between DNA polymers, surfactants, and dye molecules can lead to novel optoelectronic devices.

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