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Acridine−Viologen Dyads: Selective Recognition of Single-Strand DNA through Fluorescence Enhancement

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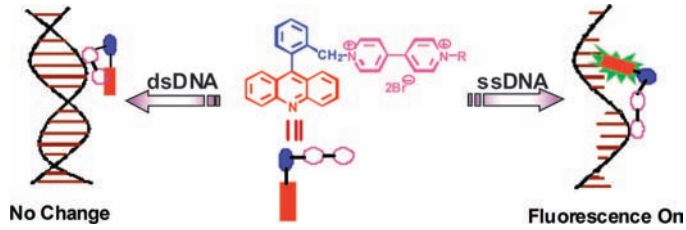
ABSTRACT



Tolylacridine−viologen dyads show distinct fluorescence emission changes in the presence of double-strand DNA (dsDNA) and single-strand DNA (ssDNA) depending on the position of the linkage. The para isomer shows fluorescence quenching in the presence of both dsDNA and ssDNA, while the ortho isomer interacts selectively with ssDNA with enhancement in fluorescence intensity.

Study of interactions of small molecules with nucleic acids is important from the viewpoint of developing new probes for quantification of nucleic acids, in establishing carcinogenic potential of a chemical and in developing drugs targeted to DNA.[1] A better understanding of the ligand−DNA interactions can lead to the development of probes that can discriminate between various nucleic acid structures.[2] An example is distinguishing between single (ssDNA) and double strand (dsDNA) to quantify single-strand or double-strand breaks in DNA.[3] In this context, the direct measurement of ssDNA and dsDNA through optical methods have several advantages. Of the optical methods, the fluorescence-based techniques offer high sensitivity.[4] These methods rely on measuring either changes in fluorescence intensity or lifetimes of the probe when bound to DNA.[3b,5] However, many of the available probes cannot efficiently differentiate between dsDNA and ssDNA.[5−7] As a result, the development

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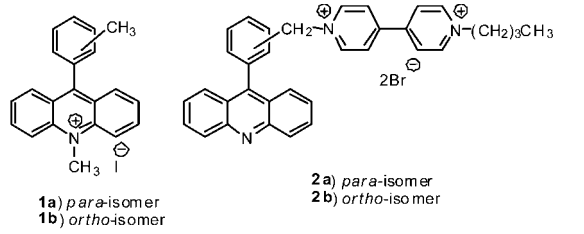
of fluorescent probes as well as methods to distinguish between various forms of DNA is gaining importance.

Recently, we reported that substitution at the ninth position of the acridinium ring plays a major role on the intercalating ability of the acridinium chromophore with dsDNA.[8] For example, the acridinium derivative **1a** with a *p*-methylphenyl group exhibited efficient intercalating interactions with dsDNA, while negligible changes were observed with **1b** having an *o*-methylphenyl substituent (Figure 1). In contrast,



dA40: 5'-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3'
dG40: 5'-GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG-3'
dC40: 5'-CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'
dT40: 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3'
dN40: 5'- GCATATCGTAGCATCGATCGATCGATGCATCGATCGATCG -3'
ssDNA: Calf Thymus ssDNA (denatured as well as commercial)

Figure 1. Structures of the acridine derivatives **1a**,**b** and **2a**,**b** and the single-strand sequences used in the present study.

1b showed efficient binding with ssDNA having nucleobases more exposed to the external medium as compared to dsDNA resulting in quenching of its fluorescence intensity. In this context, functional molecules that differentiate between dsDNA and ssDNA and show fluorescence enhancement are expected to be advantageous.[9] Herein, we report the interactions of the acridine−viologen dyads **2a** and **2b** with ssDNA and dsDNA under different conditions. These dyads showed high solubility and quenched fluorescence yields in buffer (Φ_F = 0.006 and 0.0008, respectively, for **2a** and **2b**) due to the existence of an efficient photoinduced electron transfer between the excited acridine chromophore and viologen moiety.[10] Of these dyads, **2b** undergoes selective interactions with ssDNA as compared to **2a** and uniquely signals the event through a "turn on" fluorescence mechanism.

Figure 2shows the change in the absorption spectra of **2a** with increasing addition of ssDNA in phosphate buffer (pH 7.4), while the inset shows the corresponding changes in the fluorescence spectra. Addition of ssDNA showed a gradual decrease in absorbance of **2a** with a bathochromic shift of 5 nm at the absorption maximum (357 nm). Correspondingly, we observed a regular quenching in fluorescence intensity of **2a** at 450 nm with the addition of ssDNA with a net quenching of ca. 30% at 1 mM of DNA. In the presence of dsDNA, **2a** exhibited hypochromicity as well as more efficient fluorescence quenching (80%) than observed with ssDNA (Figure S1, Supporting Information). In contrast, negligible changes were observed with the *o*-dyad **2b** in the presence of dsDNA, indicating thereby the importance of steric factors (Figure S2, Supporting Information). For an

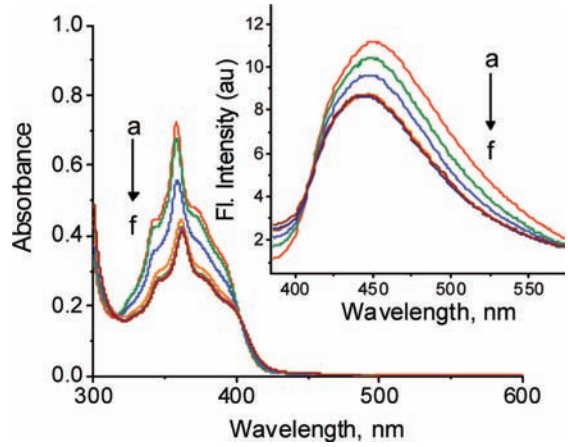


Figure 2. Changes in absorption and fluorescence (inset) spectra of **2a** (0.06 mM) with addition of ssDNA in phosphate buffer (pH 7.4). [ssDNA]: (a) 0, (c) 0.545, (f) 1 mM. λ_{ex}, 330 nm.

efficient DNA intercalation,[11] the aryl moiety present at the ninth position of the acridine chromophore has to undergo rotation before intercalation. Such a rotation is inhibited by the presence of the sterically bulky viologen substituent in the case of **2b**, and hence, negligible interactions were observed with dsDNA.

To examine the potential of the *o*-dyad **2b** as a selective probe for ssDNA, we evaluted its interactions with ssDNA and also in the presence of dsDNA. Addition of ssDNA to a solution of **2b** (experimental details for ssDNA, see the Supporting Information), we observed a gradual decrease in absorbance with 20% hypochromicity at 1 mM of ssDNA (Figure 3). Interestingly, the increasing in addition of ssDNA

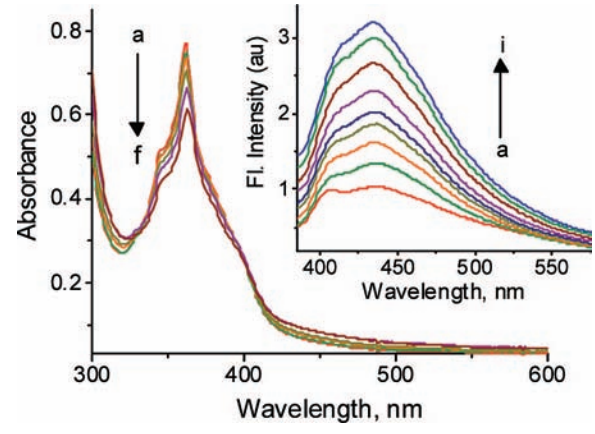


Figure 3. Changes in absorption and fluorescence (inset) spectra of **2b** (0.08 mM) with addition of ssDNA. [ssDNA]: (a) 0, (c) 0.55, (f) 1 mM. Inset shows the changes in the fluorescence spectra. [ssDNA]: (a) 0, (c) 0.1, (f) 0.33, (i) 0.92 mM. λ_{ex}, 330 nm.

led to a significant enhancement in the emission intensity (Inset of Figure 3). At 1 mM of ssDNA, we observed ca. 3-fold enhancement in fluorescence intensity of **2b**. Based

on the fluorescence changes, we obtained an association constant of 1.1×10^4 M^{-1} for **2b** according to the method of McGhee and von Hippel by using the data points of the Scatchard plot.[12] Figure 4 shows the relative changes in

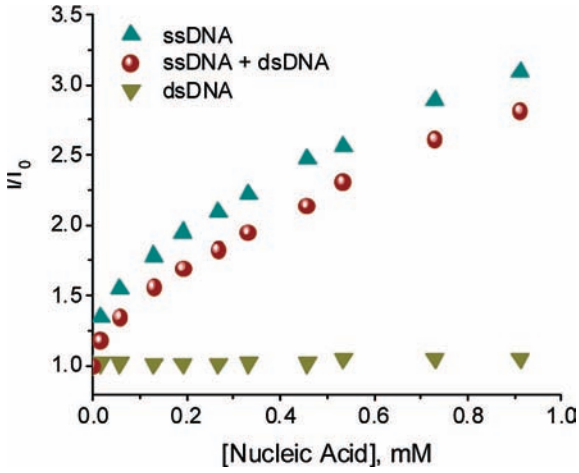


Figure 4. Relative changes in fluorescence intensity of the dyad **2b** (0.08 mM) with addition of ssDNA, dsDNA, and an equimolar mixture of ssDNA and dsDNA.

fluorescence intensity of **2b** with the addition of ssDNA, dsDNA, and a mixture of ssDNA and dsDNA. As shown in this figure, the presence of a nonbinder such as dsDNA has little effect on selective recognition of ssDNA by the dyad **2b**.

Picosecond time-resolved fluorescence analysis of **2a** in buffer showed a monoexponential decay with a lifetime of 5.4 ns (Figure S3, Supporting Information). However, in presence of ssDNA, it showed a biexponential decay having lifetimes 4.2 (75%) and 9.4 ns (25%). On the other hand, **2a** showed a biexponential decay with significantly reduced lifetimes of 0.92 and 4.5 ns in the presence of dsDNA. The *o*-dyad **2b**, which showed a biexponential decay with lifetimes of 10.1 (52%) and 0.4 ns (48%), exhibited increased lifetimes of 11 (45%) and 0.63 ns (55%) in the presence of ssDNA. As expected, **2b**showed negligible changes in lifetimes in the presence of dsDNA, indicating its selective interactions only with ssDNA.

Evidence for the binding mode of **2b** with ssDNA was obtained through the study of effect of ionic strength on DNA association constants and CD measurements.The enhancement in the fluorescence intensity of **2b** with ssDNA was found to show a regular decrease with increasing sodium ion concentration of 10, 20, 50, and 100 mM (Figure S4, Supporting Information). At 100 mM NaCl, the fluorescence intensity changes were not significant indicating the importance of electrostatic interactions of the dicationic viologen moiety in the binding of **2b** with ssDNA. Further, the number of sodium ions released per ligand bound to DNA were calculated from the plot of $\log[K_{asc}]$ against $-\log[NaCl]$. The slope gave a value of 1.8, which corresponds to the presence of two positive charges in **2b**. The dyad **2b**, which is inherently CD inactive, showed an induced CD (ICD) signal corresponding to the acridine chromophore at around 360 nm in the presence of ssDNA, whereas no such signals were obtained with the additon of dsDNA (Figure S5, Supporting Information). Interestingly, the intensity of the ICD signal was found to be significantly reduced at higher ionic strengths but the shape of the spectrum remained the same. This indicates the fact that the acridine moiety of **2b** can only undergo partial intercalative interactions with ssDNA due to presence of sterically bulky viologen unit.

To evaluate the sequence selectivity, we investigated the interactions of the dyad **2b** with ssDNA having 40 bases (Figure 1).[13] As shown in Figure 5, the enhancement in the

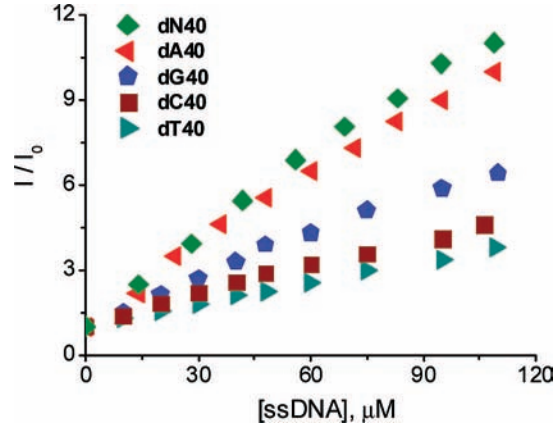


Figure 5. Relative changes in fluorescence intensity of **2b** (0.08 mM) with the addition of different ssDNA sequences such as **dN40**, **dA40**, **dG40**, **dC40**, and **dT40** in phosphate buffer (pH 7.4) containing 2 mM NaCl.

fluorescence intensity was found to be significantly higher with the oligonucleotides as compared to the CT ssDNA. The fluorescence intensity change was highest for the random

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sequence oligonucleotide **dN40** (ca. 11-fold) at 110 μM followed by **dA40**, **dG40**, **dC40**, and **dT40**. The DNA association constants calculated using the fluorescence titration data gave values in the range of $(2.1-3.8) \times 10^5\ M^{-1}$, which is one order higher than that observed for the CT ssDNA. The differences in binding of CT ssDNA as compared to various oligonucleotides could be attributed to the presence of double-stranded regions and various secondary structures formed during renaturation of the genomic ssDNA.

The acridine−viologen dyads **2a** and **2b** showed contrasting behavior upon interaction with ssDNA and dsDNA. In the case of the dyad **2a**, which exists only in the extended conformation, the viologen moiety does not interfere with intercalative interactions with either dsDNA or ssDNA resulting in its fluorescence quenching. The fluorescence quenching of **2a** by ssDNA and dsDNA can be attributed to an energetically favorable electron-transfer reaction from the nucleobases to the acridine moiety as reported previously.[10] However, the extent of quenching observed with ssDNA is less as compared to dsDNA. This is because apart from the intercalative interactions of **2a**, its viologen moiety can undergo electrostatic and groove binding interactions with dsDNA. Such interactions are not favored with ssDNA, because its nucleobases are more exposed and form supramolecular assemblies as compared to the dsDNA nucleobases. Therefore, the observed differences can be due to the different binding geometry of **2a** with ssDNA as compared to dsDNA leading to differential quenching of its fluorescence.

The *o*-dyad **2b**, on the other hand, exists in both extended and folded type conformers as evidenced from the picosecond lifetime studies. In the folded conformer, the viologen moiety lies above the acridine plane, where considerable spatial interactions exist between the donor and the acceptor and hence it exhibits a shorter lifetime of 0.4 ns. The extended conformer, on the other hand, has a long lifetime of 10.1 ns because the viologen uint is away from the acridine moiety. The observation of increased lifetimes of both the conformers of **2b** in the presence of ssDNA suggests a greater stabilization of the extended conformer, while in the folded conformer, the viologen moiety tends to move away from acridine moiety due to binding with ssDNA.

The strong dependence of the association constants on ionic strength of the buffer indicates that the electrostatic interactions of the viologen moiety play an important role in the binding of **2b** with ssDNA. Further, its acridine moiety undergoes partial intercalative interactions as evidenced from the observation of weak ICD signals even at 100 mM NaCl. Both the partial intercalative and electrostatic interactions of **2b** with ssDNA alter the interactions between the acridine and viologen moieties and thereby resulting in fluorescence enhancement. Such interactions are not possible with dsDNA, and hence, we observed negligible changes in the fluorescence intensity of the dyad **2b**.

In conclusion, we demonstrated the contrasting behavior of the dyads **2a** and **2b** with DNA. Interestingly, **2a** shows quenching in fluorescence intensity in presence of both dsDNA and ssDNA, while **2b** shows sequence selective interactions with ssDNA exhibiting enhanced fluorescence intensity (ca. 3−11-fold). The DNA binding mode of **2b** is proposed to be predominantly involving electrostatic interactions through the viologen moiety and partial intercalative binding of the acridine chromophore. These results indicate the potential use of the dyad **2b** as a probe for the selective recognition of ssDNA through "turn on" fluorescence intensity. Further studies are in progress to understand the factors and to develop fluorescence probes for the selective detection of ssDNA.

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Supporting Information Available: Experimental techniques, Figures S1−S9 and Table S1 showing absorption, fluorescence, CD, and NMR spectra of **1a**,**b** and **2a**,**b**, and DNA association constants of **2b** with oligonucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

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