Biomolecular Recognition

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DNA-Assisted Long-Lived Excimer Formation in a Cyclophane**

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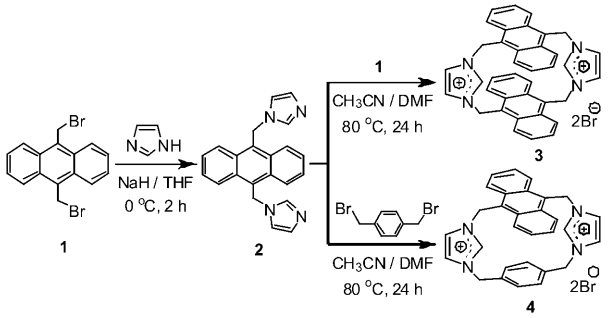
The chemistry of cyclophanes has been an area of interest because of the potential applications of functionalized cyclophanes in supramolecular chemistry,[1] in molecular recognition,[2] in molecular electronics and machines,[3,4] as drug carriers,[5] and as catalysts in organic synthesis.[6] These systems are associated with a high degree of structural rigidity and are known to form unusually strained structures.[7] By virtue of having a rigid structure with a defined cavity, these systems encapsulate and stabilize a large number of guest molecules through noncovalent interactions.[8] Even though several cyclophane derivatives have been effectively utilized for host–guest complexation,[9] the design of functional cyclophanes that are soluble in aqueous media and undergo specific interactions with biomacromolecules such as oligonucleotides, DNA, and proteins has been challenging in recent years.[10]

We have been interested in developing functionalized water-soluble cyclophanes and in the factors governing their biomolecular recognition.[11] In this context, we have designed two novel cyclophane derivatives (**3** and **4** in Scheme 1) containing anthracene and imidazolium moieties and we have investigated their photophysical and optoelectronic properties in the presence and absence of DNA, micelles, and proteins. In the presence of DNA, the symmetrical cyclophane **3** exhibited a novel sandwich-type excimer with bathochromic-shifted emission and significantly increased lifetimes. This is the first report of DNA assisting the formation of an anthracene excimer that exhibits an unusually long fluorescence lifetime of 143.1 ns and an emission maximum at 570 nm. The uniqueness of cyclophane **3** is that it exhibits high solubility and stability in the aqueous medium, undergoes selective interactions with DNA in buffer and under agarose gel electrophoresis conditions, and signals the event through a "turn on" excimer emission mechanism.

The synthesis of cyclophanes **3** and **4** has been achieved in moderate yields (Scheme 1; see the Supporting Information for details). In methanol and acetonitrile, both **3** and **4** exhibited characteristic anthracene absorption and fluorescence maxima at 375 and 430 nm, respectively (Figure S1 in the Supporting Information). However, in the aqueous medium, cyclophane **3** showed similar absorption spectra to those observed in methanol and acetonitrile but exhibited a



Scheme 1. Synthesis of the cyclophane derivatives **3** and **4**. DMF: *N*,*N*-dimethylformamide.

dual emission consisting of a structured band with a λ_{max} value of 430 nm and a broad band centered at 550 nm, with an I_{550}/I_{430} ratio of 0.8 (Figure 1 A). In contrast, model compound **4** in the aqueous medium showed similarly structured absorption and emission spectra to those obtained in methanol and acetonitrile. To ascertain the origin of the dual emission, excitation spectra of cyclophane **3** were recorded at 430 and 550 nm (Inset of Figure 1 A). These spectra were found to be identical to the absorption spectrum of **3**, thereby indicating that the absorbing species is the same for both of these emission bands.

Picosecond time-resolved fluorescence measurements of **3** in methanol and acetonitrile showed monoexponential decay with lifetimes of (15.0 ± 0.5) and (18.7 ± 1) ns, respectively. In contrast, **3** exhibited biexponential decay with lifetimes of (13.4 ± 0.5) and (52.6 ± 2) ns in the aqueous medium (Figure S2 in the Supporting Information). However, when we monitored the lifetimes at 550 nm, the species with the longer lifetime was observed to be the major component (96 %), whereas we observed both of these species in equal amplitudes at 430 nm. As expected, model compound **4** exhibited only monoexponential decay, with lifetimes of (8.9 ± 0.2), (9.7 ± 0.2), and (13.5 ± 0.3) ns in acetonitrile, methanol, and the aqueous medium, respectively. When the excited-state behavior of cyclophane **3** was analyzed immediately after excitation (60 ps) through TRES, we obtained a spectrum that only had the emission band with a λ_{max} value of 420 nm (Figure 1 B). However, gradually with time, we observed the formation of a new broad band at around 550 nm. This band grew in intensity and, after 26 ns, an

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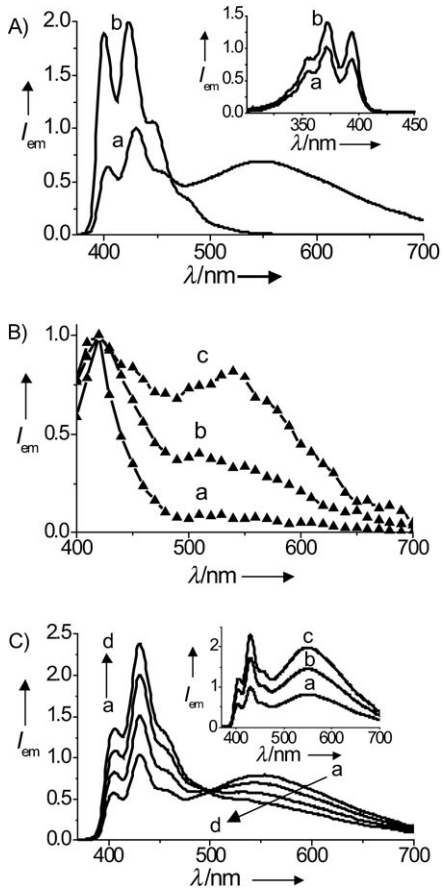

Figure 1. A) Emission spectra of the cyclophanes a) **3** (10 μM) and b) **4** (10 μM) in aqueous medium for an excitation wavelength, λ_{ex}, of 355 nm. The inset shows the excitation spectra of **3** monitored at a) 550 and b) 430 nm. B) Time-resolved emission spectroscopy (TRES) of **3** in aqueous medium recorded at different time points: a) 60 ps, b) 2.2 ns, and c) 26 ns. $\lambda_{ex} = 375$ nm. C) Effect of temperature on the emission spectra of **3** in aqueous medium: a) 298 and d) 353 K. The inset shows the emission spectra of **3** in aqueous medium at different concentrations: a) 15, b) 29, and c) 44 μM. $\lambda_{ex} = 355$ nm.

emission spectrum that was similar to that obtained in the steady-state was observed.

We assign the dual emission observed in the case of cyclophane **3** in the aqueous medium to the locally excited singlet state of the anthracene chromophore (monomer) at 430 nm and the intramolecular excimer at 550 nm, on the basis of the experimental evidence and literature reports.[12] Of these species, the locally excited state exhibited a short lifetime of 13.4 ns, whereas the intramolecular excimer showed a lifetime of 52.6 ns, as confirmed by the monitoring of lifetimes at different wavelengths and by TRES. The observation of a bathochromic-shifted emission maximum at 570 nm and a long lifetime of 52.6 ns for the excimer clearly supports the conclusion that the anthracene chromophores of **3** are in an extensively stacked conformation. The intramolecular nature of the excimer was further evidenced by the negative results obtained with model compound **4** and by monitoring of the emission spectrum of **3** as a function of temperature and concentration (Figure 1C). When the temperature was gradually increased from 25 to 80 °C, we observed a decrease in the excimer emission intensity at 550 nm and a concomitant increase in the monomer emission at 430 nm, with an isoemissive point at 500 nm. When the emission spectrum of cyclophane **3** was recorded at different concentrations (15–44 μM), we observed concentration-independent spectral features with a constant excimer-to-monomer (I_{550}/I_{430}) ratio of 0.8 ± 0.01 (Inset of Figure 1C). These observations establish the fact that the locally excited singlet state of cyclophane **3** undergoes geometric relaxation and forms a stable intramolecular excimer due to the existence of strong hydrophobic interactions in the aqueous medium.

Since cyclophane **3** exhibited favorable optoelectronic properties and high solubility under physiological pH conditions, it was of interest to us to evaluate its potential application for biomolecular recognition.[13] In this context, we investigated the interactions of cyclophane **3** with DNA, polyoligonucleotides, micelles, and proteins. Gradual addition of DNA to a solution of **3** in phosphate buffer resulted in a steady decrease in the absorbance, with isosbestic points at 332, 382, and 402 nm (Figure 2A). At 40 μM DNA, we observed significant hypochromicity (45%), along with a bathochromic shift of approximately 5 nm. In the emission spectrum, with the gradual addition of DNA, we observed a steady and nonnegligible decrease in the fluorescence intensity of the monomer at 430 nm, whereas the excimer emission intensity showed significant enhancement (Figure 2B). After addition of 40 μM DNA, we observed an approximately ninefold increase in the I_{570}/I_{430} ratio and a bathochromic shift of 20 nm. As a result, the excimer of **3** exhibited enhanced emission and a maximum at 570 nm in the presence of DNA,

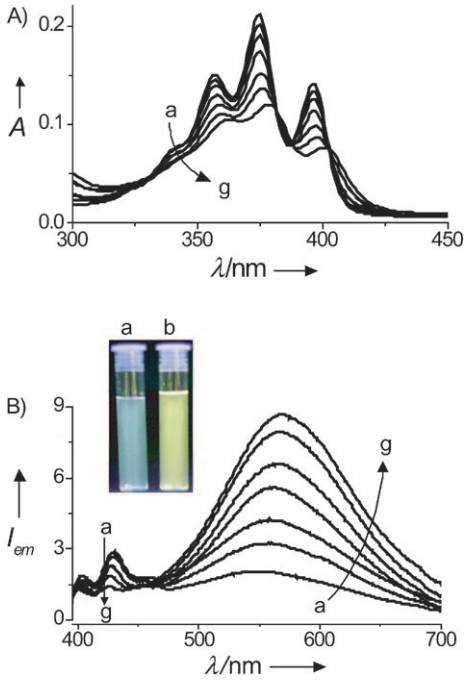

Figure 2. Changes in the A) absorption and B) emission spectra of **3** (14 μM) upon addition of DNA in phosphate buffer (pH 7.4). [DNA]: a) 0; b) 40 μM. The inset of (B) shows the color difference for visual observation of the different emission intensities of **3** a) in buffer and b) in the presence of DNA (40 μM).

thereby allowing the visual detection of DNA in buffer (inset of Figure 2B). By contrast, we observed negligible changes in the absorption and emission spectra of model compound **4** upon addition of DNA (Figure S3 in the Supporting Information). The association constant (K_{DNA}) between the cyclophane **3** and DNA was determined by using the absorption and emission spectral data for the half-reciprocal and Scatchard methods, respectively.[14,15] Both these methods gave a value of $K_{DNA} = (7.6 \pm 1) \times 10^4\,M^{-1}$, which indicates the strong binding affinity of cyclophane **3** towards DNA (Figure S4 in the Supporting Information).

To evaluate whether **3** exhibits sequence selectivity, we studied its interactions with polynucleotides such as poly-(dA):poly(dT), poly(dG):poly(dC), and poly(dG–dC):poly-(dG–dC) (Figure S5 and S6 in the Supporting Information). Addition of poly(dA):poly(dT) to a solution of cyclophane **3** in buffer initially gave an enhancement in the excimer emission at 565 nm. However, subsequent additions from 32.8 to 68.6 μM yielded a significant enhancement in the monomer emission at 438 nm, along with a concomitant decrease in the excimer emission intensity at 565 nm. In contrast, the addition of poly(dG):poly(dC) and poly(dG–dC):poly(dG–dC) under similar conditions resulted in approximately 3- and 2.5-fold enhancements in the I_{570}/I_{430} ratio, respectively; this indicates the strong sequence dependence of the excimer formation.

Picosecond time-resolved fluorescence measurements for cyclophane **3** in the presence of DNA showed a significant enhancement in the lifetime of the excimer at 570 nm, with a concomitant decrease in the monomer lifetime (Figure 3A). At 40 μM DNA, we observed an excimer lifetime of 143.1 ns, as compared with the lifetime of 52.6 ns in buffer. In contrast, the monomer at 430 nm showed a decrease in lifetime ($\tau_F =$ 11.5 ns), as compared with the lifetime of 13.4 ns in the absence of DNA. In the TRES analysis[16] measured immediately after excitation (60 ps), we observed higher excimer intensity in the emission spectrum than that in the absence of DNA. Subsequently, the emission spectrum evolved with time and, after 96 ns, the spectrum was dominated exclusively by the excimer emission with the λ_{max} value at 570 nm (Figure 3B).

To have a better understanding of the binding of cyclophane **3** with DNA, we investigated the effect of the ionic strength of the buffer and carried out circular dichroism (CD) studies (Figure S7 and S8 in the Supporting Information). When DNA was successively added to a solution of **3** in buffer containing 50 mM NaCl, we observed an I_{550}/I_{430} ratio of 0.42, as compared with that of 7.1 observed in the absence of NaCl. However, with 500 mM NaCl, we observed a negligible influence of DNA in the formation of the excimer. Cyclophane **3**, which is inherently CD inactive, showed an induced CD (ICD) signal corresponding to the anthracene chromophore at 375 nm in the presence of DNA. Successive additions of a solution of cyclophane **3** to DNA resulted in the steady development of a bisignated ICD signal with a negative band at 405 nm and a positive peak at around 375 nm. The corresponding absorption spectra, on the other hand, showed linearity with concentration, thereby ruling out the formation of aggregates under these conditions. These results

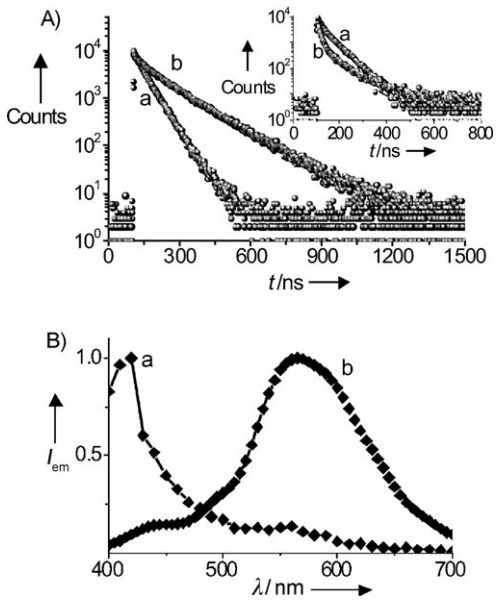

Figure 3. A) Fluorescence decay profiles of **3** (14 μM) a) alone and b) in the presence of DNA (40 μM) monitored at 550 (main) and 430 nm (inset). B) The time-resolved emission spectra of **3** in the presence of DNA monitored a) 60 ps and b) 96 ns after excitation. $\lambda_{ex} = 375$ nm.

demonstrate that cyclophane **3** undergoes both electrostatic and nonclassical intercalative interactions in the minor groove of DNA.[17]

To understand the uniqueness of the DNA template in inducing the excimer formation with enhanced emission intensity, we investigated the interactions of cyclophane **3** with a negatively charged micellar medium and with proteins (Figure S9 and S10 in the Supporting Information). Successive additions of sodium dodecylsulphate (SDS), at and above critical micelle concentration (8.3–32.3 mM), or proteins such as bovine serum albumin (BSA, 21.6 μM) and fibrinogen (7 mM) led to negligible changes in the excimer emission of cyclophane **3**, while a gradual nonnegligible increase in the monomer emission was observed upon addition of SDS. As only the DNA template induced excimer formation in cyclophane **3**, we further examined its potential use for the detection of DNA under gel electrophoresis conditions.[18] To demonstrate this, we prepared different concentrations of plasmid DNA and then used cyclophane **3** for staining after gel electrophoresis. Interestingly, we observed DNA-concentration-dependent emission intensity, which can be effectively used for the quantification of DNA in gels through "turn on" excimer emission (Figure S11 in the Supporting Information).

The optimized geometry obtained through B3LYP-level theoretical calculations[19] by using a 6-31G basis set showed a rigid conformation for cyclophane **3** with an interplanar distance of 5.26 Å, while a distance of 5.24 Å was observed for model cyclophane **4** (Figure S12 in the Supporting Information). Based on the literature evidence,[20] the formation of the excimer in cyclophane **3** is unexpected because such a process is reported to be feasible only in systems having an interplanar distance of less than 4 Å. This unusual behavior of cyclophane **3** in the aqueous medium could be attributed to

the existence of a highly ordered conformation due to hydrophobic interactions, which favor the excimer formation, as evidenced by the excitation and TRES spectral analysis as well as by concentration- and temperature-dependent experiments. The negligible formation of the excimer emission at and above 353 K confirms the fact that the ordered conformation of cyclophane **3** is essential and such a conformation no longer exists at these temperatures.

The interaction of cyclophane **3** with DNA is novel because it resulted in the exclusive formation of the excimer with a bathochromic-shifted emission maximum at 570 nm and with a significantly enhanced intensity (approximately ninefold) and lifetime (143.1 ns). These observations indicate that DNA acts as a unique template in stabilizing the highly organized sandwich-type conformer of cyclophane **3**. Based on the negative results obtained with proteins and micellar media, the driving force for the formation of such an excimer in the presence of DNA could be attributed as the synergistic effects of both hydrophobic interactions in the minor groove and electrostatic interactions between the cationic cyclophane and the phosphate backbone. The involvement of electrostatic interactions was established through observation of the negligible influence of DNA on the excimer formation at the higher ionic strengths of buffer. Based on steric considerations, the classical intercalative binding of cyclophane **3** with DNA could be ruled out, but **3** can undergo nonclassical intercalative interactions in the minor groove of DNA, as evidenced from the observation of a bisignated ICD signal and the sequence-dependent excimer formation.[21]

In conclusion, we have developed novel water-soluble symmetrical cyclophane **3**, which, interestingly, exhibited emission from the locally excited state (monomer) at 430 nm in methanol and acetonitrile. In the aqueous medium, in addition to the monomer emission, it showed intramolecular excimer emission at 550 nm. In contrast to model compound **4**, cyclophane **3** undergoes sequence-selective interactions with DNA that result in the exclusive formation of a highly organized sandwich-type excimer with a bathochromic-shifted emission at 570 nm and significant enhancement in the intensity and the lifetime of 143.1 ns. As far as we know, this is the first report of DNA assisting in the formation of an excimer with a longest lifetime at 25 °C. The uniqueness of cyclophane **3** is that it selectively recognizes DNA rather than micelles and proteins in buffer and under agarose gel electrophoresis conditions and it signals the event through a "turn on" excimer emission mechanism.

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