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# Bis(phenanthroline)-ethylenediamine conjugate displays excimer fluorescence upon binding with DNA†

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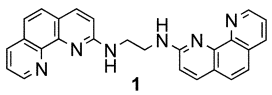
**1,2-Bis(1,10-phenanthrolin-2-yl)ethylenediamine (1) displays a unique long-life time excimer fluorescence at 528 nm by binding with calf thymus DNA, while its monomer fluorescence at 400 nm shows a decrease upon binding with DNA.**

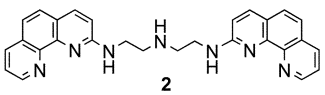
A number of polycyclic aromatic compounds form excimer or exciplex by inter- or intra-molecular interaction, and display a unique emission spectrum.[1] Thus, these compounds such as pyrene are widely used as fluorescent molecular probes for bio-macromolecules such as nucleic acids and proteins.[2] However, phenanthroline and its derivatives are usually non-luminescent, although some Cu(I) and Ru(II) complexes of phenanthroline derivatives show emission spectrum due to their metal–ligand charge-transfer transitions.[3–5] Metal complexes containing phenanthroline derivatives have been shown to be useful as probes for DNA. Several chemical and physical methods have been used to characterize the binding and cleaving of DNA with the complexes.[4–6] Some ruthenium and copper complexes of phenanthroline or its derivatives display characteristic luminescence signals when they bind to DNA.[4,5,7]

We have prepared phenanthroline–polyamine conjugates and their metal complexes to explore strong DNA-binding and cleaving agents. During our studies on phenanthroline–polyamine conjugates, we found that *N*,*N*′-bis(1,10-phenanthrolin-2-yl)ethylenediamine shows a fluorescence at 400 nm and a unique broad-band emission around 528 nm due to excimer formation upon binding with DNA accompanying decrease in monomer emission at 400 nm. We will report here first example of excimer formation of a phenanthroline derivative by binding with DNA.

1,2-Bis(1,10-phenanthrolin-2-yl)ethylenediamine (**1**) was prepared from ethylenediamine and 2-chloro-1,10-phenanthroline.[8] $N^1$,$N^5$-bis(1,10-phenanthrolin-2-yl)diethylenetriamine (**2**) was prepared in the similar method from $N^3$-BOC-diethylentrimaine[9] and 2-chloro-1,10-phenanthroline, followed by de-protection of $N^3$-Boc group with 5 M HCl. The conjugates, **1** and **2**, were obtained as a hydrochloride and crystallized from ethanol.



**1**



**2**

Both **1** and **2** display fluorescence at 400 nm by excitation at 320 nm. The quantum yields of **1** and **2** in aqueous buffer solution (pH 7.2) were 0.011 and 0.017, respectively. 1,2-Bis(1,10-phenanthrolin-2-yl)ethane was reported to show a similar fluorescence in the 320–470 nm region in acetonitrile when excited in their π–π* absorption band.[10] The addition of DNA to the solution of **1** and **2** caused a decrease in fluorescence intensity, which indicates the binding of **1** and **2** with DNA. Fig. 1 shows the fluorescence spectra of **1** and **2** in a neutral aqueous solution with or without the addition of calf thymus DNA. The binding constants of **1** and **2** with DNA, were $2.4 \times 10^4$ (number of DNA binding sites, $n$ = 16 nucleotides) and $3.7 \times 10^4$ $M^{-1}$ ($n$ = 17 nucleotides), respectively, estimated from the Mcghee and von Hippel equation analysis[11] of the decrease in fluorescence intensity at 400 nm by the addition of DNA. Surprisingly, the addition of DNA to the solution of **1** displayed a new broad-band fluorescence at 528 nm which was probably due to the excimer emission.[1] This emission band was not observed in the complexes with metal (Cu(II), Zn(II), or Fe(II)), indicating that the emission band does not arise from the complex of the phenanthroline derivatives. Corresponding to the decrease in emission at 400 nm, the emission at 528 nm increased as DNA concentration increased until the DNA nucleotide residue to **1** ratio reached to 70 : 1. In contrast, no fluorescence band was observed at 500–550 nm for **2** in the presence of DNA.

We carried out the fluorescence spectral titration by addition of **1** to DNA solution (data in the supporting information†). From the data, the excimer emission at 528 nm appeared even at low concentration of **1**. This result indicates the eximer produced by intramolecular interaction.

We further examined fluorescence of **1** in the presence of poly(dAdT)/poly(dAdT) and poly(dGdC)/poly(dGdC) (Fig. 2). The addition of poly(dAdT)/poly(dAdT) caused a shift of the 400 nm emission to a longer wavelength, 440 nm, and an increase in the fluorescence intensity. The shift of the wavelength and the increase

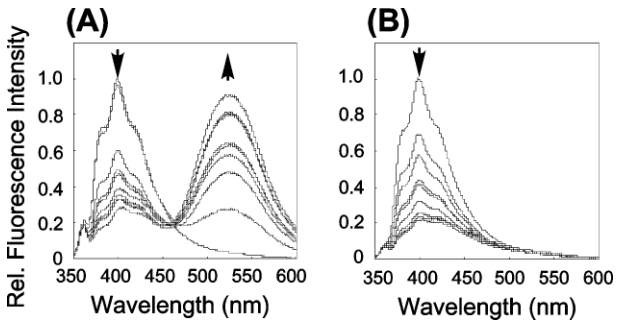



**Fig. 1** Fluorescence spectral titration of **1** (**A**) and **2** (**B**) with calf thymus DNA. Titration was carried out in a 1 mm cell with 20 μM of **1** or **2** in 10 mM Tris-HCl buffer (pH 7.6) containing 20 mM NaCl at 25 °C by addition of DNA, 0, 0.14, 0.28, 0.43, 0.57, 0.71, 0.85, 0.99, 1.14 and 1.42 mM (based on nucleotide residue) in the direction of arrows.

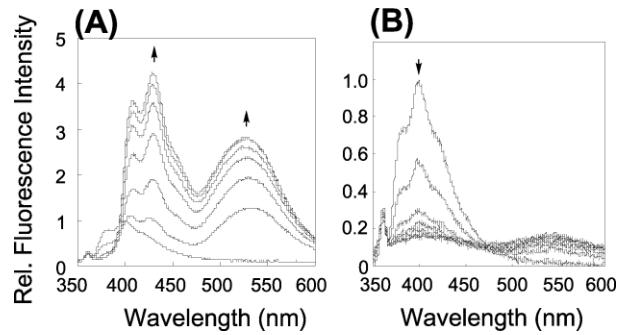



**Fig. 2** Fluorescence spectral titration of **1** with poly(dAdT)/poly(dAdT) (**A**) and poly(dGdC)/poly(dGdC) (**B**). Titration was carried out in the same way as that for the calf thymus DNA except for the DNA concentration, (**A**): 0, 0.13, 0.27, 0.54, 0.80, 1.07 and 1.68 mM; (**B**): 0, 0.09, 0.18, 0.27, 0.36, 0.54 and 0.72 mM.

† Electronic supplementary information (ESI) available: synthetic procedures and analytical data, binding isotherms, and fluorescence titration by addition of **1** to calf thymus DNA. See http://www.rsc.org/suppdata/cc/b3/b316126a/

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in the emission suggest that **1** is located in a hydrophobic region because of its binding to poly(dAdT)/poly(dAdT) and suppression of the fluorescence-quenching of **1** by water molecules.[13] Moreover, the 528 nm emission due to excimer formation was also enhanced in the presence of poly(dAdT)/poly(dAdT). On the contrary, the addition of poly(dGdC)/poly(dGdC) caused a sharp decrease in the 400 nm emission and the emergence of the 528 nm excimer emission was very small. The guanine base in the DNA could work as a fluorescence quencher for **1** because of energy or electron transfer.[13–15] The decrease in the fluorescence of **1** upon binding with DNA could be caused by quenching with guanine residues. The variation of fluorescence intensity depending on the GC content of DNA was reported in the case of a Ru(II)-complex of phenanthroline derivative that interacts in a groove of the double helix DNA.[13]

The lifetime measurement of 400 nm emission of **1** without the addition of DNA showed a short-life time, $\tau$ = 3.1 ns, of the single-phased emission decay (Table 1). When DNA was present, a biphasic decay of the 528 nm fluorescence of **1** was observed with a long-lived component, $\tau$ = 17 ns, and short-lived component, $\tau$ = 2.5 ns, in a 70 : 30 ratio, respectively. The short-lived emission is consistent with monomer emission. This monomer emission may result from the long-wavelength tail of the emission curve in the presence of DNA, especially from binding to AT rich region because it is estimated that the monomer emission of **1** was present even at 528 nm with binding to poly(dAdT)/poly(dAdT) from Fig. 2A. The long lifetime and long wavelength of the 528 nm fluorescence indicate that an excimer is likely formed between the phenanthroline moiety of **1** upon binding with DNA. The broad band of the 528 nm emission is also consistent with excimer formation.[1,12]

**Table 1** Fluorescence lifetime of **1** (20 μM) in the absence (A) and in the presence of 1.42 mM DNA (B)

| | $\tau_1$/ns | $\alpha_1$ | $\tau_2$/ns | $\alpha_2$ | $\chi^2$ |
|---|---|---|---|---|---|
| A | 3.11 | 1.00 | — | — | 0.99 |
| B | 2.46 | 0.27 | 17.2 | 0.73 | 1.18 |

Fluorescence decay was monitored at 400 nm for (A) and at 528 nm for (B) on excitation.

Fig. 3 shows the results of a comparative viscosity study designed to explore the mode of binding of **1** and **2** to DNA. Hydrodynamic data may provide the most critical test for intercalating binding except for X-ray crystallographic or NMR data.[4,16] The relative viscosity, $\eta/\eta_0$, was estimated from the ratio of flow time of the solution with a viscometer, $t/t_0$, where $t$ and $t_0$

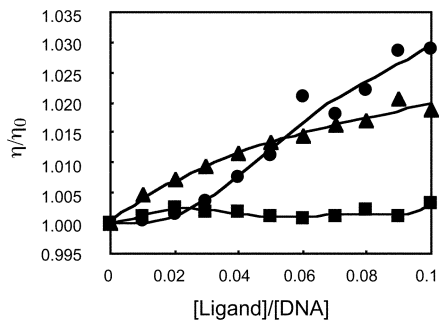


**Fig. 3** Viscometric titration of calf thymus DNA with ethidium (circles), **1** (squares), **2** (triangles). Titration was carried out in 10 mM Tris-HCl buffer (pH 7.6) with calf thymus DNA (200 μM bp) at 25 °C. The reduced specific viscosity ratio was plotted *versus* the molar ratio of compound to DNA base pairs.

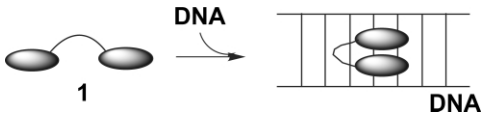


**Fig. 4** Simplified mechanistic scheme for the excimer formation of **1** upon binding with double stranded DNA.

are flow times of CT-DNA solution in the presence and absence of the ligand, respectively. Fig. 3 shows the effect of the concentration of **1** or **2** on the relative viscosity of the CT-DNA solution. The relative viscosity increased with the increase in concentration of **2** in a way similar to the typical DNA-intercalating agent, ethidium bromide.[4,16] On the contrary, no viscosity change was observed when **1** was added to the DNA solution. The results indicate that **2** binds with DNA by an intercalative mode,[4,16] while **1** works as a groove binder. Phenanthroline moieties of **1**, which are bound to DNA from the groove, could interact with each other to form an excimer as shown in Fig. 4. In the case of **2**, which binds with DNA by intercalation, the phenanthroline units could not interact with each other, thereby inhibiting the excimer formation.

In conclusion, we have prepared a phenanthroline–ethylendiamine conjugate which binds with DNA exhibiting a unique fluorescence due to the excimer formation of its phenanthroline moiety. The fluorescence was quenched by guanine residue of the DNA. This is a first example of excimer formation from a phenanthroline moiety upon binding with DNA, and may be applicable as a new DNA probe.

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