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Cu^{2+}-Mediated Assembly of the Minor Groove Binders on the DNA Template with Sequence Selectivity

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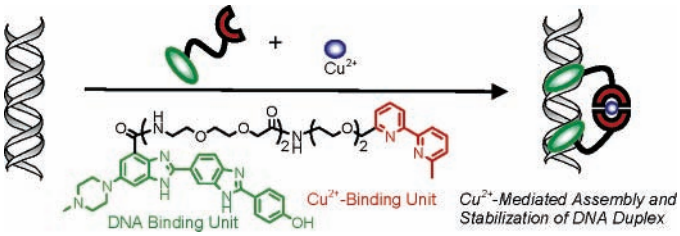
ABSTRACT



The ligands (Bpy-H, 5) have been designed to connect the Hoechst33258 skeleton for DNA binding and 2,2′-bipyridine for Cu^{2+} complexation. It has been revealed that the new Hoechst ligand long Bpy-H (5L) having a long linker exhibits Cu^{2+}-mediated assembly on the DNA template having two A_3T_3 sites in a selective manner depending on the length of the linker of the ligand as well as on the distance between the two A_3T_3 sites of DNA.

Oligonucleotides and their analogues have provided potential tools for artificial regulation of gene expression in a sequence-specific manner, and some of them are currently undergoing clinical evaluation. Recently, small molecular DNA binders have attracted much attention because of their unique binding properties,[1] although they normally recognize a limited area of DNA compared to oligonucleotides. For example, because of their small size, they are easily accessible to DNA not only with normal structures but also with noncanonical ones. Thus, they target a large repertoire of nucleic acid structures such as nucleosomal DNA,[2] quadruplex,[3] triplet repeats,[4] branched DNA,[5] and so on. In our approach to recognition of a noncanonical DNA structure with small molecules, we have recently reported new bidentate minor-groove binders (bis-Hoechst, bis-H) for the DNA sequence with two remote A_3T_3 binding sites as well as for the three-way junction DNA (Figure 1A).[6] The structure of the bidentate ligands is characterized by the polyether linker that is introduced at the convex side of the

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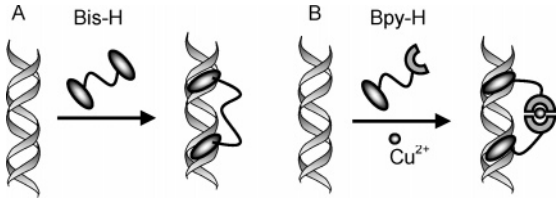
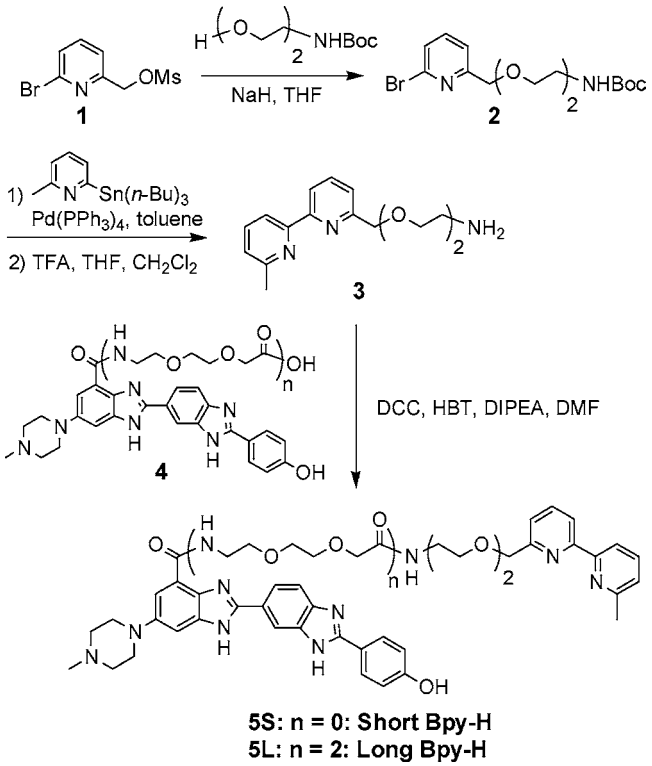


Figure 1. General concept of a self-assembling system on a DNA template by multiple coordination with a metal cation. (A) Covalently connected bis-Hoechst binds nearby binding sites. (B) The noncovalent assembly of Bpy-H is mediated by Cu^{2+} coordination on a DNA template.

Hoechst molecule. A multiple-binding mode as shown in Figure 1A might be expanded to multidentate binding toward long DNA sequences. In this study, we have attempted to establish a new binding mode in which small molecular ligands may assemble on a DNA template by multidentate coordination with the metal cation (Figure 1B). As a starting point for such an assembling system, we designed a new Hoechst ligand having a 2,2′-bipyridine as a metal-chelating unit (long Bpy-H (**5L**) and short Bpy-H (**5S**) in Scheme 1).

Scheme 1. Synthesis of Bpy-H Ligands



It is expected that two bipyridine units form a tetracoordinated complex with a divalent cation to effect assembly of the Bpy-H ligand on the duplex DNA template.

The Hoechst compounds bearing a 2,2′-bipyridine unit were synthesized starting with a 2-bromopyridine derivative (**1**)[7] (Scheme 1). The pyridine derivative (**2**) was obtained from **1** with *N*-Boc protected 2-(2-aminoethoxy)ethanol, which was coupled with 2-methyl-6-(tributylstannanyl)-pyridine,[8] followed by deprotection of the *N*-Boc group to yield the bipyridylamine (**3**). The Hoechst derivative (**4**)[6] containing a different length of linker was condensed with the bipyridyl unit (**3**) to produce the corresponding bipyridyl-connected Hoechst with a short linker ($n = 0$), short Bpy-H (**5S**), and with a long linker ($n = 2$), long Bpy-H (**5L**).

The binding constants of Bpy-H (**5L** and **5S**) were determined by fluorescence titration with DNA**1** having a single A_3T_3 site (Figure 2). It was shown from a titration

DNA**1**	5′-AGTGCGCCG**AAATTT**GACACGCTGCACGACG-3′ 3′-TCACGCGGC**TTTAAA**CTGTGCGACGTGCTGC-5
DNA**2**(1)	5′-AGTGCGCCG**AAATTT**G**AAATTT**CGCACGACG-3′ 3′-TCACGCGGC**TTTAAA**C**TTTAAA**GCGTGCTGC-5
DNA**2**(3)	5′-GTGCGCCG**AAATTT**GCC**AAATTT**CGCACGAC-3′ 3′-CACGCGGC**TTTAAA**CGG**TTTAAA**GCGTGCTG-5′
DNA**2**(6)	5′-GCGCCG**AAATTT**GGACCC**AAATTT**CGCACGA-3′ 3′-CGCGGC**TTTAAA**CCTGGG**TTTAAA**GCGTGCT-5′
DNA**2**(9)	5′-CGCCG**AAATTT**GGCCGACCC**AAATTT**CGCAC-3′ 3′-GCGGC**TTTAAA**CCGGCTGGG**TTTAAA**GCGTG-5′

Figure 2. Sequences of the duplexes used in this study. DNA**1** contains a single A_3T_3 site, and DNA**2**(*x*) has two A_3T_3 sites separated by (*x*) nonbinding base pairs.

curve that complexation between Bpy-H (**5L** or **5S**) and DNA**1** took place in a 1:1 ratio with $K_s = 9.8 \times 10^8$ M^{-1} for **5L** and $K_s = 5.2 \times 10^8$ M^{-1} for **5S**.[9] Unlike complexation with DNA**1**, fluorescence intensity of Bpy-H complexed with DNA**2** decreased time dependently.[10] Titration experiments by CD spectroscopy showed that Bpy-H (**5S** and **5L**) bound to two A_3T_3 sites in DNA**2** with a similar affinity regardless of the distance between these two sites.[11] These results show that minor groove bindings of the Hoechst unit of Bpy-H were not altered by each other when they bound to two A_3T_3 sites of DNA**2**.

The binding affinity of the Bpy-H ligand (**5S** and **5L**) to the metal cation was evaluated by UV titration experiments (Figure 3). Long Bpy-H (**5L**) has absorption bands at 235 and 270 nm corresponding to the bipyridine unit and also at 370 nm of the Hoechst unit, and its spectrum was changed by the addition of $CuCl_2$ with five isosbestic points at 238,

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(9) Figure S1 in the Supporting Information.

(10) Measurement of fluorescence spectroscopy is also hampered by quenching with $CuCl_2$ as shown in Figure S2 in the Supporting Information.

(11) CD titration data are shown in Figure S6 in the Supporting Information.

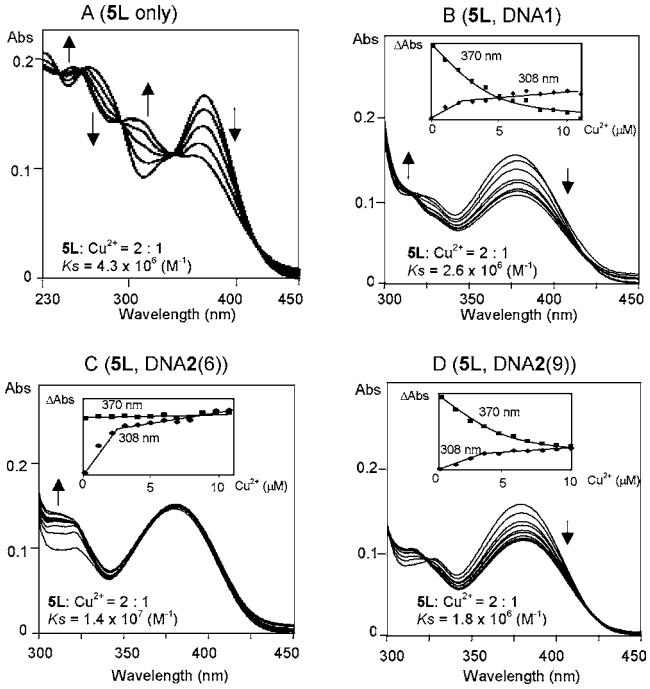

Figure 3. UV−titration by the addition of $CuCl_2$ to long Bpy-H (**5L**) in the absence or the presence of DNA. (A) **5L** in the absence of DNA, (B) **5L** in the presence of DNA**1**, (C) **5L** in the presence of DNA**2**(6), and (D) **5L** in the presence of DNA**2**(9). $CuCl_2$ was added into a solution of the ligand (5 μM) and DNA (2.5 μM) buffered with 0.01 M HEPES and 0.01 M NaCl at pH 7.4. Arrows indicate hyper- or hypochromic effects observed by the addition of $CuCl_2$.

255, 289, 345, and 405 nm (Figure 3A). The hyperchromic effect observed at ca. 310 nm indicates complex formation between the bipyridine units and Cu^{2+}. The hypochromism at 370 nm may reflect aggregation of the Hoechst units induced by complexation between the bipyridine units with Cu^{2+} as schematically shown in Figure 4A.

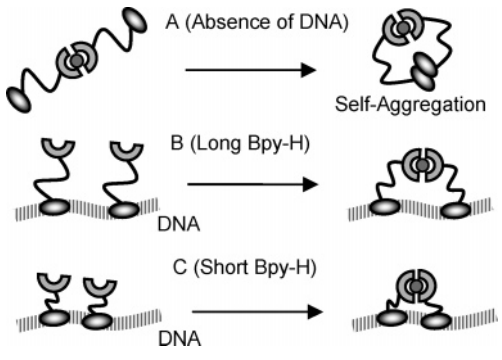

Figure 4. Schematic illustration of complexes with Cu^{2+} and long Bpy-H (**5L**) or short Bpy-H (**5S**). (A) Complex in the absence of DNA, (B) Cu^{2+}-mediated assembly of **5L** on the DNA template, and (C) 2:1 **5S**−Cu^{2+} complex on the DNA template having two close binding sites.

The titration curves were obtained by plotting the absorbance change at 308 nm with the use of short and long Bpy-H ligands (**5S** and **5L**) to reveal a complexation property of a 1:2 Cu^{2+}−ligand ratio with K_s (M^{-1}) = 4.0×10^6 (**5S**) and 4.3×10^6 (**5L**). It was also found that Co^{2+} formed 1:2 Co^{2+}−ligand complexes with K_s (M^{-1}) = 3.4×10^4 (**5S**) and 1.6×10^4 (**5L**). Complexation was not observed with Mg^{2+}, Ca^{2+}, Mn^{2+}, Ni^{2+}, or Zn^{2+}.

To check the template effect of the DNA substrate on complexation with Cu^{2+}, similar titration experiments were performed in the presence of DNA**1** and **2**. Figure 3B−D illustrates the results obtained with **5L**. Addition of $CuCl_2$ induced different effects on the hyperchromism at ca. 310 nm (bipyridine region) and on the hypochromism at 370 nm (Hoechst region) depending on the bound DNA sequence. The titration curve obtained by plotting the absorbance at 308 nm indicated that complexation between Cu^{2+} and **5L** took place in a 1:2 ratio as shown in the insetted graphs in Figure 3B−D. In the complex between **5L** and DNA**2**(6), the largest hyperchromism at 308 nm and the smallest hypochromism at 370 nm were observed. The former suggests that the bipyridine unit of **5L** forms complexes with Cu^{2+} most effectively, which was proven by the highest affinity of $K_s = 1.4 \times 10^7$ (M^{-1}). The latter implies that the binding of bipyridine units with Cu^{2+} may not alter the minor groove binding of the Hoechst unit of **5L** with DNA**2**(6). Preorganization of **5L** in the complex with DNA**2**(6) may be beneficial for 1:2 Cu^{2+}−ligand complexation as schematically shown in Figure 4B.

On the contrary, although a similar spectral change was observed with the short Bpy-H ligand (**5S**), complexation between **5S** and Cu^{2+} was found to take place in a 1:1 ratio in the presence of the DNA template. Exceptionally, a 2:1 ligand−Cu^{2+} ratio was observed in the titration experiment with DNA**2**(3).[12] These results suggest that the linker of **5S** is suitable for 2:1 ligand−Cu^{2+} complexation in DNA**2**(3). Thus, the UV titration experiments in the presence of the DNA substrate have clearly proven that the template effects of DNA on Cu^{2+} binding depend on the linker length of the ligands.

We next attempted to prove the template effects of DNA on the ligand binding with DNA. CD titration experiments showed that the binding affinity of **5L** to DNA**2**(6) was enhanced in the presence of Cu^{2+}.[11] As changes of CD spectroscopy reflect the conformational change of DNA, we next measured the effects of metal−ligand complexation on the thermal stability of the duplex. Consequently, effects of Cu^{2+} on DNA binding of the Bpy-H ligands were investigated by thermal denaturation experiments.

The melting temperature of DNA**2**(6) (T_m = 59.5 °C) was increased by 6.7 °C in the presence of **5L**, and importantly, further stabilization was exhibited by the addition of Cu^{2+} (Figure 5A). Table 1 summarizes effects of Cu^{2+} and Mg^{2+} on the melting temperature (ΔT_m) of DNA**2** with **5L**. The fact that the magnesium ion did not show such stabilization effects has supported that Cu^{2+}−bipyridine complexation

(12) Figure S4 and S5 in the Supporting Information.

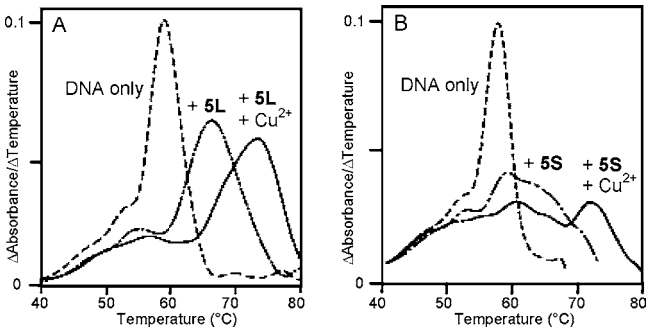

Figure 5. Effects of the ligands on the thermal melting behavior of DNA**2**(6): (A) long Bpy-H (**5L**) and (B) short Bpy-H (**5S**). Derivatives of the melting curve were shown.

enhances binding of the Hoechst units with the DNA substrate. By contrast, the melting curve of DNA**2**(6) became so broad in the presence of **5S** that the T_m value was not calculated (Figure 5B), suggesting that **5S** distorted the DNA structure. Additional stabilization effects of Cu^{2+} were not clearly observed in the other complexes of **5S** with DNA**2**. It should be noted that a selective increase of the T_m value by **5L** was observed for DNA**2**(3) (ΔT_m = +6.3 °C) and DNA**2**(6) (ΔT_m = +6.5 °C). These results, together with the UV−titration experiments, show that **5L** exhibits binding properties similar to those for Cu^{2+} in the complex with DNA**2**(3) and DNA**2**(6). The flexible nature of the polyether linker of **5L** may be responsible for its broad selectivity.

Table 1. ΔT_m Values for the Duplexes Containing Two A_3T_3 Sites in the Presence of Cu^{2+} or Mg^{2+} [a]

DNA substrate (T_m, °C)	ΔT_m/°C[c]	
	$+Cu^{2+}$	$+Mg^{2+}$
DNA**2**(1) (T_m[b] = 66.9)	+2.7	+0.7
DNA**2**(3) (T_m[b] = 66.6)	+6.3	+1.5
DNA**2**(6) (T_m[b] = 66.2)	+6.5	+1.1
DNA**2**(9) (T_m[b] = 69.3)	+0.5	+0.1

[a] 1 μM of each DNA duplex was used in 10 mM HEPES containing 10 mM NaCl, pH 7.4. [b] Containing 2 μM of long Bpy-H (**5L**). [c] 2 μM each of $CuCl_2$ or $MgCl_2$ was used, which did not affect T_m values in the absence of **5L**.

A plausible structure of the assembly of $Cu(\mathbf{5L})_2^{2+}$−DNA**2**(6) was calculated by MD to suggest that the length of the long linker of **5L** is suitable to reach each other across the phosphate backbone and that a complex of $Cu(bpy)_2^{2+}$ may fit within the major groove of the DNA duplex (Figure 6). An additional interaction of the $Cu(bpy)_2^{2+}$ complex with DNA such as an electrostatic interaction[13] may become advantageous for stabilization of the DNA duplex.

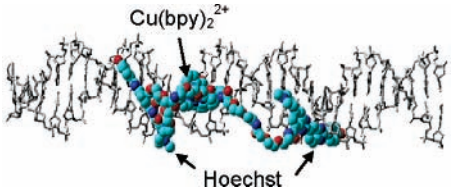

Figure 6. Illustrations of the simulated complex of the $Cu(\mathbf{5L})_2^{2+}$ with the duplex DNA**2**(6). MD and MM calculations were performed with the AMBER 94 force field.

In summary, this study has revealed that the new Hoechst ligand long Bpy-H (**5L**) with 2,2′-bipyridine exhibits Cu^{2+}-mediated assembly on the DNA template having two A_3T_3 sites in a selective manner depending on the length of the linker of **5L** as well as on the distance between the two A_3T_3 sites. The new binding mode established in this study will be useful for the application of small molecular ligands to higher-ordered assembly on the long DNA template. Further study is now ongoing along this line.

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Supporting Information Available: Experimental procedures, fluorescence and CD spectra, and ^{1}H NMR and MS spectra of compounds **3**, **5L**, and **5S**. This material is available free of charge via the Internet at http://pubs.acs.org.

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