# Selective Binding and Cleavage of DNA by Stereoisomers of *N*,*N*′-Bis(phenanthrolin-2-yl)-1,2-cyclohexanediamine Conjugates, and Their Copper Complexes

Keigo Hayashi, Ryouko Nakajima, Isao Kiyosawa, Hiroaki Ozaki, and Hiroaki Sawai*
*Department of Applied Chemistry, Faculty of Engineering, Gunma University, Kiryu, Gunma, 376-8515*

(Received March 15, 2004; CL-040289)

Stereoisomers, trans-*RR*, trans-*SS*, and cis forms, of *N*,*N*′-bis-(phenanthrolin-2-yl)-1,2-cyclohexanediamine conjugates were prepared, and their DNA binding activity was evaluated. The copper complexes of the conjugates (ligand:Cu(II) = 1:1 and 1:2) cleave DNA in the same order of the DNA binding activity of the conjugates, trans-*RR* > cis > trans-*SS*.

There has been considerable interest in the specific DNA binding and cleaving activity of phenanthroline derivatives and their metal complexes. Sigman et al. and others reported that the copper complex of 1,10-phenanthroline binds with DNA and induces cleavage of DNA in the presence of a reducing agent under an anerobic condition.[1–3] Barton and co-workers have shown that chiral ruthenium complexes of phenanthroline derivatives show differences in the binding with and cleaving DNA.[4,5] The structure of the metal complex likely determines the mode of interaction with DNA of a particular conformation.[1–8] Although various metal complexes of phenanthroline derivatives have been synthesized and their DNA binding and cleaving activity assessed, very few studies have been made on a chiral phenanthroline derivative as a ligand and its stereoselectivity in binding with DNA. Thus we undertook synthesis of a new chiral phenanthroline derivative that could bind to DNA with chiral selectivity. Here we report the synthesis of conjugates of phenanthroline with trans-*RR*, trans-*SS*, and cis forms of 1,2-diaminocylohexane, the differences in their binding with DNA and DNA cleaving activity by copper complexes of the stereosiomers of the conjugate.

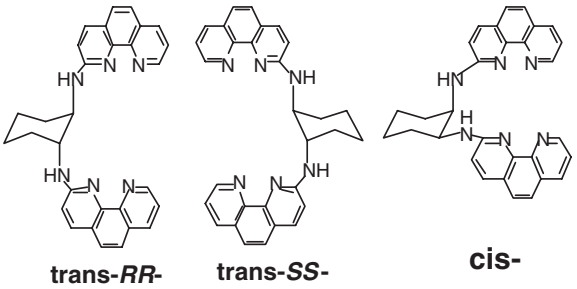


**Figure 1.** Stereoisomers of *N*,*N*′-bis(phenanthrolin-2-yl)-1,2-cyclohexanediamine conjugate.

The trans-*RR*-*N*,*N*′-bis(phenanthrolin-2-yl)-1,2-cyclohexanediamine conjugate was prepared from 2-chlorophenanthroline[9] and trans-*RR*-1,2-diaminocyclohexane using tetramethylguanidine as a coupling agent and a solvent at 100 °C for 50 h and was purified by silica gel chromatography with dichloromethane/methanol/aqueous ammonia (9:1:0.1) as an eluent. The conjugate was obtained in 18% isolated yield. The corresponding trans-*SS*- and cis-conjugates were prepared by the same method in 45 and 43% isolated yield, respectively. Their structures were confirmed by NMR and ESI-mass spectrum.[10] Each conjugate was converted to the hydrochloride salt by adding hydrochloric acid to the methanol solution of the conjugate and was re-crystallized from methanol–ethyl acetate. The elemental analysis of the hydrochloride of the conjugates indicates that they are tri-hydrochloride.[10] The CD spectra of the trans-*RR*- and trans-*SS* conjugates display the mirror-image CD, which demonstrates that they are enantiomers. The cis-conjugate showed no CD band indicating that it is meso form.

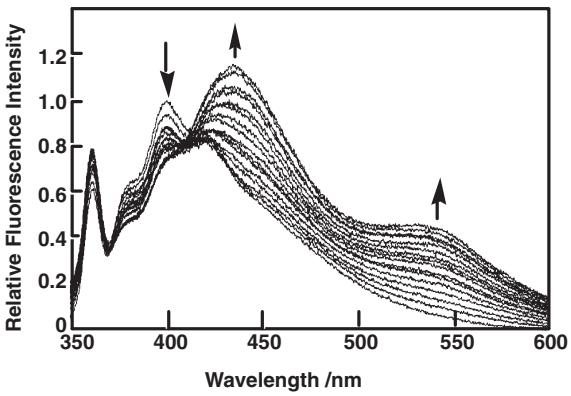


**Figure 2.** Fluorescence spectral titration of trans-*RR* conjugate with calf thymus DNA. Titration was carried out in a 1-mm-path length cell with 10 μM of the conjugate in 10 mM Tris-HCl buffer (pH 7.2) containing 20 mM NaCl at 25 °C by addition of DNA, 0, 38, 76, 114, 152, 190, 228, 266, 304, 342, 380, 418, 456, 494, 532, and 570 μM (based on nucleotide residue) in the direction of arrows.

The conjugates showed fluorescence at 400 nm by excitation at 320 nm. The addition of DNA to the solution of the conjugate caused a decrease in 400 nm fluorescence intensity and the emergence of a new band at 435 nm and a broad-band fluorescence at 500–600 nm, which indicates the interaction of the conjugate with DNA. The broad and long wavelength fluorescence at 500–600 nm is probably due to the excimer emission of the phenanthroline moieties of the conjugate. Similar broad and long wavelength emission is also observed in the case of *N*,*N*′-bis(phenanthrolin-2-yl)ethylenediamine upon binding with DNA.[11] The binding constant of the trans-*RR* conjugate with DNA was $1.2 \times 10^5\ M^{-1}$, estimated from McGhee and von Hippel equation analysis[12] of the decrease in fluorescence intensity at 400 nm by addition of DNA (0–570 μM). The binding constants of the trans-*SS* and cis form conjugate with DNA were $3.5 \times 10^4$ and $8.3 \times 10^4\ M^{-1}$, respectively, estimated by the same method as that for the trans-*RR* conjugate described above. The conjugates bind with DNA in the order of trans-*RR* > cis > trans-*SS* form. DNA is a chiral-macromolecule composed of a

Copyright © 2004 The Chemical Society of Japan

right-handed helix, and thus it discriminates the binding between the stereo-isomers of the DNA-binding agent.

We further carried out a viscometric study to explore the binding of the conjugate to DNA. 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 $t_0$ are flow times of CT-DNA solution in the presence and absence of the ligand, respectively. The relative viscosity increased slightly with increase in concentration of the trans-*RR* form conjugate. No viscosity change was observed when the trans-*SS* and cis form conjugate was added to the DNA solution. The typical DNA-intercalating agent, ethidium bromide, showed an increase in viscosity under the same conditions, as expected for a DNA intercalator.[13] The results indicate that the conjugate binds with DNA as a groove binder,[8] although a slight contribution of an intercalative mode was considered for the binding of the trans-*RR* form conjugate.

We conducted UV spectral titration of the conjugate against copper(II) chloride solution to investigate formation of a copper complex of the conjugate. The spectral titration indicates the formation of complexes of copper(II) and the conjugate in 2:1, 1:1, and 1:2 molar ratio.

The DNA cleaving activity of the copper complexes of the conjugates was assessed by conversion of a closed circular form of pBR322 DNA to a relaxed form or to a nicked form of DNA. The reaction mixtures (6 μL) containing the copper(II)-conjugate complex (10 μM based on the conjugate), mercaptopropionic acid (5 mM) and pBR322 DNA (0.1 μg) were mixed in 0.1 M Tris-HCl (pH 7.2) buffer containing 50 mM NaCl, and incubated for 30 min containing 50 mM NaCl, and incubated for 30 min at 37 °C. The closed circular DNA (form I) is cleaved to a nicked (form II) and then linear (form III) DNA by the copper complexes of the conjugates as shown in Figure 3. The DNA cleaving activities of the copper(II) complexes of the conjugates, estimated from the ratio of the cleaved DNA (lanes 3–11), were much stronger than that of the copper complex of phenanthroline (lane 2) under the same conditions. The DNA cleaving activity of the copper complexes of stereoisomers of the conjugate was in the following order; trans-*RR*- > cis- > trans-*SS*-conjugate, although 2:1 copper complex of the trans-*SS*-conjugate showed

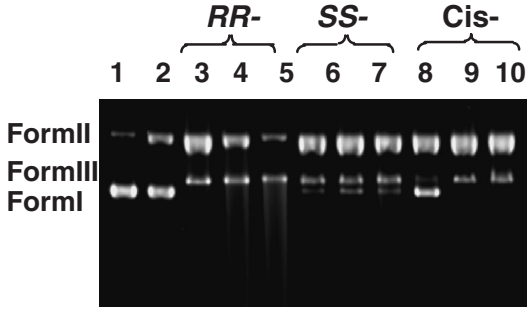


**Figure 3.** Strand scission of pBR 322 DNA by copper complexes of *N*,*N*′-bis(phenanthrolin-2-yl)-1,2-cyclohexanediamine conjugate. Lane 1, control DNA; lane 2, Cu(II)-phennathroline (1:2) complex; lanes 3–5, Cu(II)–trnas-*RR*-conjugate; lanes 6–8, Cu(II)–trans-*SS*-conjugate; lanes 9–11, Cu(II)–cis-conjugate. Lanes 3, 6, 9, Ligand:Cu(II) = 2:1; lanes 4, 7. 10, Ligand:Cu(II) = 1:1; Lanes 5, 8, 11, Ligand:Cu(II) = 1:2. The reactions were performed as described in the text and analyzed by 0.9% agarose gel electrophoresis after staining with ethidium bromide.

stronger DNA cleaving activity than the corresponding copper complex of the cis-conjugate (lanes 6, 9). The order of the DNA cleaving was the same as that of the DNA binding activity of the conjugates in cases of 1:1 and 1:2 copper complexes. The 1:2 and 1:1 copper complexes of trans-*RR* conjugate cleaved DNA to a shorter pieces (lanes 4, 5). Two phenanthroline moieties in the trans-*RR* form conjugate could be in the 1,2-diaxial position and could work cooperatively for the binding with right-handed double-stranded DNA and enhance cleavage of DNA.

In conclusion, we have prepared the enantiomeric and meso forms of conjugates composed from phenanthroline and stereoisomers of 1,2-diaminocyclohexane. The conjugates and their copper complexes show stereoselectivity in DNA binding and cleavage.

**References and Notes**

1 D. S. Sigman, A. Mazumder, and D. M. Perrin, *Chem. Rev.*, **93**, 2295 (1993).
2 O. Zalenko, J. Gallagher, and D. S. Sigman, *Angew. Chem., Int. Ed. Engl.*, **36**, 2776 (1997).
3 M. Pitié, C. Boldron, H. Gornitzka, C. Hemmert, B. Donnadieu, and B. Mounier, *Eur. J. Inorg. Chem.*, **2003**, 528.
4 K. E. Erkkila, D. T. Odon, and J. K. Barton, *Chem. Rev.*, **99**, 2777 (1999).
5 J. K. Barton, *Science*, **233**, 727 (1986).
6 R. Tamilarasan and D. R. MacMillin, *Inorg. Chem.*, **29**, 2798 (1990).
7 P. Lincoln, A. Broo, and B. Norden, *J. Am. Chem. Soc.*, **118**, 2664 (1996).
8 S. Satyanarayana, J. C. Dabrowiak, and J. B. Chaires, *Biochemistry*, **32**, 2573 (1993).
9 S. Ogawa, T. Yamaguchi, and N. Gotoh, *J. Chem. Soc., Perkin Trans. 1*, **1974**, 976.
10 trans-*RR* conjugate: ESIMS: $m/e = 471.1$ (Calcd. for M + H, 471.2); $^1$H NMR ($CDCl_3$) $\delta$ 1.62 (m, 4H, 3′-CH2-), 2.15 (m, 4H, 2′-CH2), 4.32 (m, 2H, 1′–CH–), 6.30 (m, 2H, –NH–), 6.50 (d, $J = 8.7$ Hz, 2H, 3-H), 7.39 (d, $J = 8.4$ Hz, 2H, 5-H), 7.50 (d, $J = 8.7$ Hz, 2H, 3-H), 6.50 (d, $J = 8.7$ Hz, 2H, 3-H); Anal. Found: C, 60.68; H, 5.68; N, 14.21%. Calcd for $C_{30}H_{26}N_6 \cdot 3HCl \cdot H_2O$: C, 60.26; H, 5.23; N, 14.05%. trans-*SS* conjugate: ESIMS: $m/e = 471.2$ (Calcd. for M + H, 471.2); $^1$H NMR ($CDCl_3$) $\delta$ 1.62 (m, 4H, 3′–CH2–), 2.15 (m, 4H, 2′–CH2), 4.32 (m, 2H, 1′–CH–), 6.30 (m, 2H, –NH–), 6.50 (d, $J = 8.7$ Hz, 2H, 3-H), 7.39 (d, $J = 8.4$ Hz, 2H, 5-H), 7.50 (d, $J = 8.7$ Hz, 2H, 3-H), 6.50 (d, $J = 8.7$ Hz, 2H, 3-H); Anal. Found: C, 59.60; H, 5.16; N, 13.39%. Calcd for $C_{30}H_{26}N_6 \cdot 3HCl \cdot 1.5H_2O$: C, 59.36; H, 5.31; N, 13.85%. cis conjugate: ESIMS: $m/e = 471.2$ (Calcd. for M + H, 471.2); $^1$H NMR ($CDCl_3$) $\delta$ 1.62 (m, 4H, 3′–CH2–), 2.15 (m, 4H, 2′–CH2), 4.32 (m, 2H, 1′–CH–), 6.30 (m, 2H, –NH–), 6.50 (d, $J = 8.7$ Hz, 2H, 3-H), 7.39 (d, $J = 8.4$ Hz, 2H, 5-H), 7.50 (d, $J = 8.7$ Hz, 2H, 3-H), 6.50 (d, $J = 8.7$ Hz, 2H, 3-H); Anal. Found: C, 59.75; H, 5.93; N, 13.98%. Calcd for $C_{30}H_{26}N_6 \cdot 3HCl \cdot 1.5H_2O$: C, 59.36; H, 5.31; N, 13.85%.
11 K. Hayashi, H. Akutsu, H. Ozaki, and H. Sawai, submitted for publication.
12 D. McGhee and P. H. von Hippel, *J. Mol. Biol.*, **86**, 469 (1974).
13 G. Cohen and H. Eisenberg, *Biopolymers*, **8**, 45 (1969).

Published on the web (Advance View) May 10, 2004; DOI 10.1246/cl.2004.684