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DNA Intercalators Bearing Metal Chelating Moiety. Ternary Complexes of Polyamine Containing Anthraquinone Derivative-DNA-Cu(II) and Its DNA Cleavage Activity

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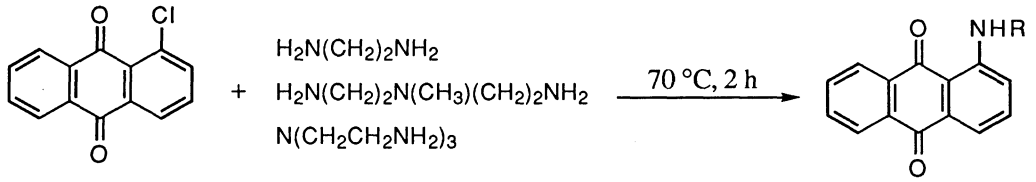
Polyamine-linked DNA-intercalators formed stable ternary complexes with Cu(II) and ds-DNA, some of which induced the chain cleavage of ds-DNA. The reactivity was related to the stereochemical features of the complexes.

In the last decade, several articles treated the effect of metal ions on the interaction of native DNA ligands, e.g., zinc finger protein,[1] anthracycline antibiotics[2] etc., with DNA. An extension of this *Ligand-DNA-Metal ternary interaction* to non-natural system should contribute to the elucidation of the mechanisms of the function of antibiotics and to the design of effective antitumor drugs. With this view, man-designed such ternary interactions or complexes and their intended regulation have been of our great concern.

The authors have already described some DNA intercalators carrying an oligo(oxyethylene) chain as a metal binding group.[3] A cooperativity between the metal ion and the DNA ligand for the binding to DNA and the cleavage (possibly hydrolytic) of DNA was confirmed. The metal binding capability of these DNA intercalators so far reported were, however, rather low. This prompted us to design new series of DNA ligands, in which a polyamine (which is a typical "border line" metal chelator) is attached to anthraquinone nucleus.

Treatment of 1-chloroanthraquinone in ethylenediamine (en), *N, N*-bis(2-aminoethyl)methylamine (dien) and tris(2-aminoethyl)amine (tren) at 70 °C for 2 h afforded 1-[(2-aminoethyl)amino]anthraquinone (**1**) (75 %), 1-[2-[*N*-(2-aminoethyl)-*N*-methylamino]ethylamino]anthraquinone (**2**) (50 %) and 1-[2-[*N*, *N*-bis(2-aminoethyl)amino] ethylamino]anthraquinone (**3**) (19 %), respectively (Scheme 1).[4]



1: R = -$(CH_2)_2NH_2$
2: R = -$(CH_2)_2N(CH_3)(CH_2)_2NH_2$
3: R = -$(CH_2)_2N(CH_2CH_2NH_2)_2$

Scheme 1.

All these compounds exhibited, upon binding to double stranded DNA (ds-DNA, from calf thymus), peculiar hypochromic and bathochromic shifts in the absorption spectra which are characteristic to DNA

intercalation.[5] By analyzing the spectra, the binding constants (K) at 298 K were obtained (Table 1) in a standard manner according to the equation of McGhee and von Hippel.[6]

Table 1. Binding constants[a] of anthraquinone derivatives with calf thymus DNA at 298 K

Ligand	Binding constant, K x 10^{-4} / dm^3mol^{-1}	
	without Cu(II)	with Cu(II)
1	4.6	6.4
2	5.3	2.8
3	14	29

a) In a 0.3 mmol dm^{-3} HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) buffer with 0.1 mol dm^{-3}NaCl (pH 7.0).

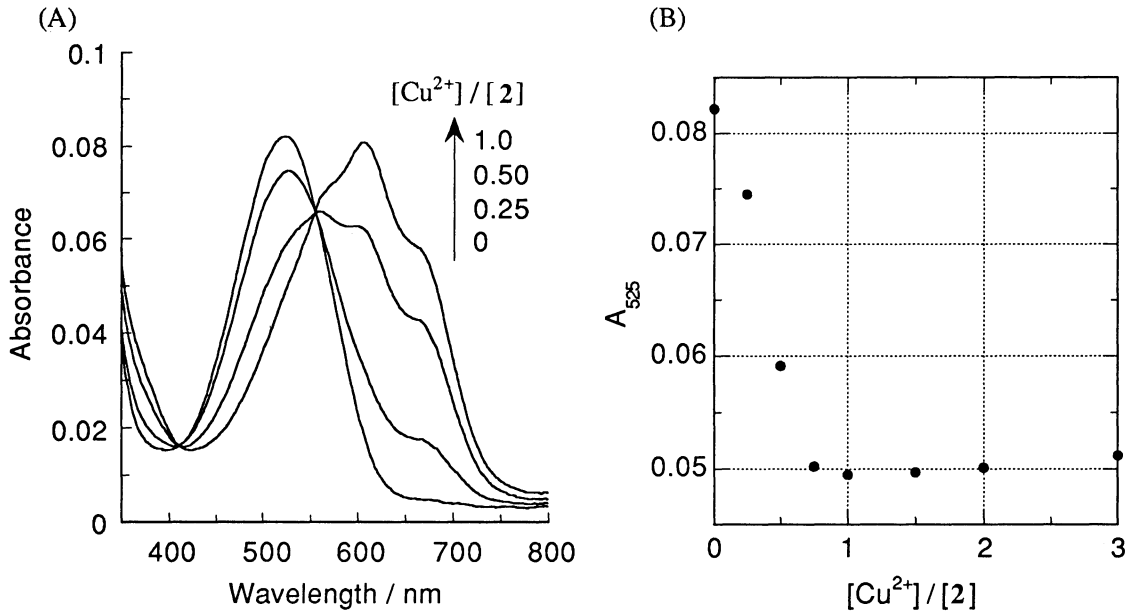


Fig. 1. A; Spectral change of **2**-DNA conjugate on addition of Cu(II). The titration was carried out in the presence of ten-molar excess of DNA-phosphate to **2** (30 μmol dm^{-3} **2**) in 0.1 mol dm^{-3} NaCl and 3.0 mmol dm^{-3} HEPES (pH 7.0). B; The corresponding Job's plot. The absorbance of the solution at 525 nm was measured while Cu(II) was added to **2**-DNA conjugate.

The addition of Cu(II) to ds-DNA-ligand solution produced a further change in the spectra of ligands. Figure 1 shows the spectra (A) of ds-DNA-**2** conjugate on addition of Cu(II) and the corresponding Job's plot (B). The Cu(II)-**2** complexation stoichiometry is clearly unity. Similar results were obtained for **1** and **3**. Although it is known that Cu(II) binds to the phosphate and bases of ds-DNA,[7] Cu(II), in the present system, binds strongly to the polyamine moieties of intercalating ligands even in the presence of large excess of DNA phosphate and bases. This is a good indication of an intimate ternary interaction among DNA strand,

heterocycle, and Cu(II). The binding constants in the presence of Cu(II) (equimolar to ligands) are summarized in Table 1. It is notable that the complexation of intercalating ligands with Cu(II) stabilizes the ternary complex with DNA for **1** and **3**, but destabilizes for **2**.

To take a closer view to the nature of these ternary complexes, DNA cleavage assay was adopted. The binding of polyvalent metal ions to the phosphate site in ds-DNA can cause a cleavage by hydrolytic or radical mechanisms.[8] Then if the metal in the present ternary complex is favorably situated in the vicinity of DNA backbone, an enhancement of DNA cleavage should be expected. Super-coiled plasmid DNA (pBR322 DNA), which is relaxed by a single nicking, was used as a substrate. The observed scission efficiency at pH 7.0 is summarized in Fig. 2. It should be emphasized that a cooperation of **3** with Cu(II) was observed, but not in the case of **1** or **2**. In the reference assay, ethylenediamine, *N*, *N*-bis(2-aminoethyl)methylamine or tris(2-aminoethyl)amine did not cause any cleavage enhancement. What is notable is the difference in DNA cleaving capability between **1**, **2** and **3**, in spite of their comparable DNA binding rate in the DNA relaxation study.

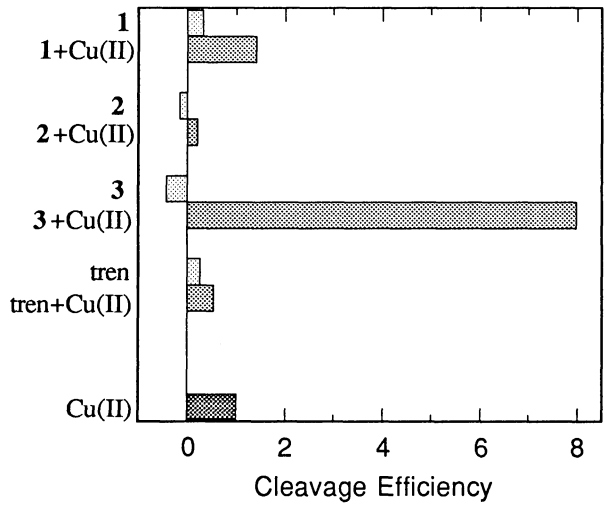
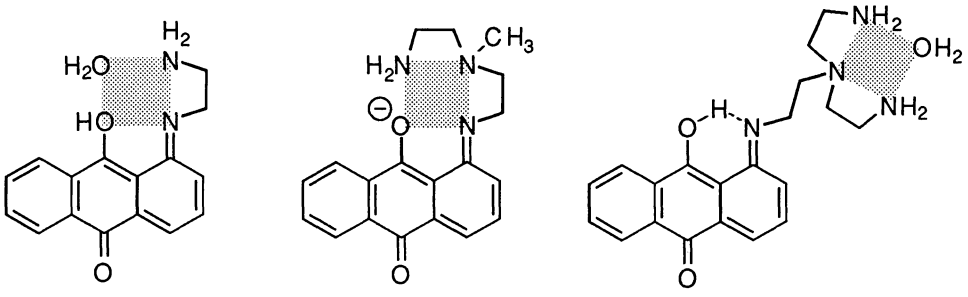


Fig. 2. Cleaving efficiencies by super-coiled DNA relaxation assay. After incubation of 50 μmol dm^{-3} DNA-p, 50 μmol dm^{-3}Cu(II) and 50 μmol dm^{-3} ligands in 0.5 mmol dm^{-3}phosphate buffer (pH 7.0) at 37 °C for 12 h, DNA fragments were separated by agarose gel (1 %) electrophoresis. Super coiled and relaxed DNAs are determined by conventional densitometry. Cleavage efficiency refers to the relaxed DNA produced, Cu(II) reaction (bottom row) being taken as reference (cleavage efficiency 1).

The Cu(II) complex formation of **1**, **2** and **3** was studied by pH titration photometry. The data suggested that (i) the structures in Fig. 3 dominate at pH 7, (ii) the Cu(II)-**2** is formed at pH 6 and stays unchanged at higher pH, and (iii) the complexes of **1** and **3** undergo further deprotonation at pH above 7 (data not shown). It is emphasized that the deprotonation of aromatic hydroxyl group takes place only for **2**-Cu(II) at moderately low pH (pH 7).[9] This is in agreement with the observed decrease of DNA-binding constant of **2** in the presence of Cu(II). These informations imply that the pictures in Fig. 3 represent the complex structures that are bound to ds-DNA under the conditions of super-coil relaxation assay (pH 7). The oxygen and nitrogen atoms on the anthraquinone nucleus are always involved in complexation with Cu(II) for **1** and **2**, while it is not the case for **3**.

The Cu(II)-**3** complex (Fig. 3, **3**) can afford the anthraquinone nucleus for full intercalation with ds-DNA, while its Cu(II) site can be placed at the same time at a favorable position for a direct interaction with the phospho-diester backbone of DNA. Thus, the phosphate chain at the complexation site is endowed with both bond strain caused by intercalation and Lewis acid activation through coordination to Cu(II). This is likely a mechanism of cooperativity of Cu(II) and **3** for cleaving DNA. For **1** and **2**, meanwhile, anthraquinone and

Cu(II) moieties are held together into an intimate compact entity, which obviously can not extend an intercalation strain and Lewis acid activation in a cooperative manner. In this meaning, Cu(II)-**3** complex, which can cause both of the effects simultaneously, is hopefully deemed as an enzyme model for cleaving DNA. A detailed complex-chemical study is in progress in this laboratory.



1 **2** **3**

Fig. 3. Possible structures of ligand-Cu(II) 1 : 1 complexes at pH 7.0. Square-planer coordination around Cu(II) is indicated by shade.

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References

1) J. Kuwahara and J. E. Coleman, *Biochemistry*, **29**, 8627 (1990); L. Regan and N. D. Clarke, *ibid.*, **29**, 10878 (1990); J. W. R. Schwabe, D. Neuhaus, and D. Rhodes, *Nature*, **348**, 458 (1990); J. Nardelli, T. J. Gibson, C. Vesque, and P. Charnay, *ibid.*, **349**, 175 (1991).

2) C. E. Myers, L. Gianni, C. B. Simone, R. Klecker, and R. Greene, *Biochemistry*, **21**, 1707 (1982); M. Spinelli and J. C. Dabrowiak, *ibid.*, **21**, 5862 (1982); H. Beraldo, A. G. -Suillerot, and L. Tosi, *Inorg. Chem.*, **22**, 4117 (1983); V. Malatesta, F. Morazzoni, A. Gervasini, and F. Arcamone, *Anti-Cancer Drug Design*, **1**, 53 (1985).

3) S. Takenaka, T. Ihara, and M. Takagi, *J. Mol. Recog.*, **3**, 156 (1990); R. Fukuda, S. Takenaka, and M. Takagi, *J. Chem. Soc., Chem. Commun.*, **1990**, 1029.

4) **1**: red solid, ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.08 (2.1H, t, J=6.1Hz), 3.44 (2.1H, t, J=6.1Hz), 7.10 (2.0H, dd, J=1.2, 8.2), 7.55 (2.0H, t, J=7.3), 7.61 (2.0H, dd, J=1.2, 7.3), 7.71 (2.0H, dt, J=1.5, 7.3), 7.77 (2.0H, dt, J=1.2, 7.3), 8.24 (2.0H, dd, J=1.5, 7.6), 8.30 (2.0H, dd, J=1.2, 7.6); Anal. Found: C, 67.33; H, 5.97; N, 9.36%. Calcd for $C_{16}H_{14}N_2O_2$+1H_2O; C, 67.60; H, 5.67; N, 9.39%; **2**, **3**: red solid, NMR data and elemental analysis of **2** and **3** also supported their structures (data not shown).

5) W. D. Wilson and I. G. Lopp, *Biopolymers*, **18**, 3025 (1979).

6) J. D. McGhee and P. H. von Hippel, *J. Mol. Biol.*, **86**, 469 (1974); S. Takenaka, T. Ihara, M. Hamano, and M. Takagi, *J. Chem. Soc., Chem. Commun.*, **1990**, 1271.

7) D. C. Liebe and J. E. Stuehr, *Biopolymers*, **11**, 167 (1972); H. Sigel, *Chem. Soc. Rev.*, **1993**, 255.

8) L. A. Basile, A. L. Raphael, and J. K. Barton, *J. Am. Chem. Soc.*, **109**, 7550 (1987).

9) J. Hine, "Structural Effects on Equilibria in Organic Chemistry", John Wiley & Sons, Inc. (1975).

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