Double Strand DNA Cleavage by a Dinuclear Cu(II) Complex

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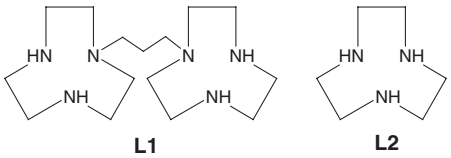
A dinuclear Cu(II) complex with 1,3-bis(1,4,7-triaza-1-cyclononyl)propane cleaved plasmid DNA to relaxed circular and linear forms at pH 7.3 and 30 °C. Under the same conditions, double strand cleavage by the corresponding mononuclear Cu(II)([9]aneN$_3$) complex was negligible.

Natural nucleases such as alkaline phosphatase, phospholipase C, and P1 nuclease contain more than two metal ions in the active site,[1] and there have been reported quite a few dinuclear Co(III), Cu(II), La(III), and Fe(III) complexes that hydrolyze phosphate esters including DNA.[2]

Unlike simple models such as bis-(4-nitrophenyl) phosphate(BNPP), DNA cleavage mechanisms by the same type of metal complexes are not clearly established. The active species leading DNA cleavage are yet to be determined.

In this study, we report that the dinuclear Cu(II) complex with 1,3-bis(1,4,7-triaza-1-cyclononyl)propane(L1), cleaves DNA over 10 times faster than the corresponding mononuclear Cu([9]aneN$_3$: L2) complex does under mild conditions. Since the CuL2 complex is rather more efficient in hydrolyzing BNPP than the Cu$_2$L1 complex,[3] there must be other factors or mechanisms operating in the complex mediated DNA cleavage.



The cleavage of DNA was followed by monitoring conversion of supercoiled plasmid DNA (form I), 1.02×10^{-4} M in base pairs, to relaxed circular (form II) and linear (form III) forms (Figure 1).[4] Under the experimental conditions used, form III formation by the CuL2 complex was negligible. The extents of DNA cleavage at various concentrations of the Cu complexes were listed in Table 1. The extent of DNA cleavage was proportional to the concentration of the Cu$_2$L1 complex up to 20 μM and decreased thereafter. The reaction was pH dependent, where maximum cleavage was obtained around pH 8.4.[5]

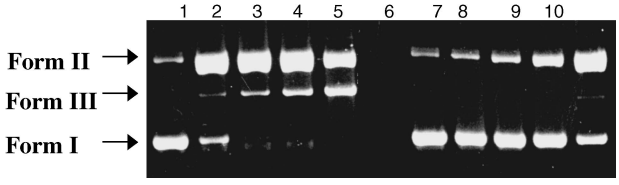


Figure 1. DNA(pCMV-Myc: 102 μM bp) cleavage reaction by the Cu$_2$L1 and the CuL2 complexes at pH 7.3(10 mM HEPES) and 30 ± 0.5 °C. lane 1–5: DNA + Cu$_2$L1(4 μM): lane 6–10: DNA + CuL2(8 μM): t = 0.1, 3, 6, 12, 24 h, respectively.

Table 1. The extent of DNA cleavage(%) at the various concentrations of the Cu$_2$L1 and the CuL2 complexes at pH 7.3 and 30 ± 0.5 °C[a]

Catalysts/μM		Form I	Form II	Form III
Cu$_2$L1	4	3.2	90.2	6.6
	12	2.0	73.2	24.8
	32	1.5	76.4	22.2
	48	1.6	79.0	19.4
CuL2	8	84.0	16.0	0
	40	38.8	61.3	0
	96	30.5	69.5	0

a. after 6 h incubation:[DNA]=102 μM bp and 10 mM HEPES buffer used.

The pseudo first order rate constants of degradation of form I by the Cu$_2$L1 and CuL2 complexes (4 μM) were 1.0×10^{-2}/min and 5.2×10^{-3}/min, respectively at pH 7.3 and 30 °C.[6] Plasmid DNA was cleaved at least 10 times faster by the Cu$_2$L1 complex than the CuL2 complex.[7]

Recently, we have shown that the Cu$_2$L1 complex hydrolyzed BNPP in almost the same rate, while 4-nitrophenylphosphate(NPP) with 300 fold rate enhancement over the CuL2 complex.[3] We proposed a double Lewis activation by two Cu(II) ions which was more effective for hydrolysis of phosphate diesters of RNA type or phosphate monoesters than phosphate diesters.[2a]. In hydrolyzing BNPP, the Cu$_2$L1 and CuL2 complexes shared the mechanism involving an intramolecular metal hydroxide attack on the bound substrate in monodentate fashion. As active species, CuL2(OH$_2$)(OH) was proposed in hydrolyzing BNPP.[8] The maximum rate obtained near pH 8.4 in DNA cleavage reaction by the Cu$_2$L1 complex indicated that monomeric unit of the dinuclear complex might participate in the cleavage.[9] Unlike the CuL2 complex, the Cu$_2$L1 complex has additional positive charges by the neighboring Cu(II) ion and might facilitate DNA binding. Polyamines are known to bind DNA with their protonated forms and sometimes to lead DNA cleavage upon addition of metal ions.[10] Ionic strength dependencies for nicking and linearization of plasmid DNA supported the strong electrostatic interaction by the Cu$_2$L1 complex (Figure 2a). Form III production by the Cu$_2$L1 complex was inhibited at lower NaNO$_3$ concentration than form II production. Binding associated with linearization, which may occur near a negatively charged nicked gap, appears to have higher sensitivity to ionic strength than that associated with nicking.[11]

To verify active species in DNA cleavage reaction, we added radical scavengers to the reaction mixtures of the Cu$_2$L1 complex (Figure 3). Catalase inhibited the reaction and superoxide dismutase(SOD) had little effect in the reaction. Other additives such as DMSO and NaN$_3$ did not affect the reaction. Catalase inhibition indicates peroxide species might be involved

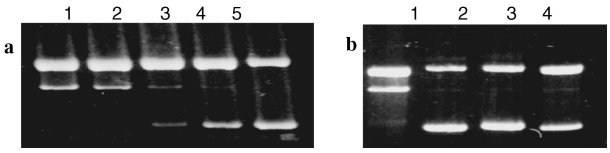


Figure 2. a) Ionic strength dependence of the Cu_2L1 complex(10 μM) mediated DNA(pCMV-Myc: 102 μM bp) cleavage at pH 7.3(10 mM HEPES), 25 °C, t = 3 h. lane 1–5: I = 0, 10, 30, 50, 100 mM $NaNO_3$. b) DNA cleavage mediated by the various Cu complexes(10 μM) at pH 7.3 and 37 ± 0.5 °C, t = 3 h.: lane 1: DNA + Cu_2L1, lane 2: + CuL2, lane 3: + Cu_2L3, lane 4: + Cu_2L4 complexes.

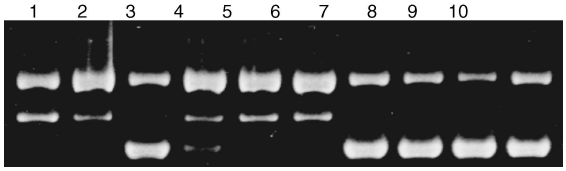


Figure 3. DNA(pCMV-Myc) cleavage in the presence of various conditions at pH 7.3(10 mM HEPES) and 29 ± 0.5 °C: lane 1: DNA(102 μM bp) + Cu_2L1(10 μM), lane 2: + DMSO(0.1 M), lane3: + catalase (1 unit/10 μL), lane 4: + SOD(1 unit/10 μL), lane 5: + NaN_3(30 mM), lane 6: + NaCl(30 mM), lane 7: DNA + $Cu(NO_3)_2$(28 μM), lane 8: + L1(10 μM), lane 9: DNA only (control), lane 10: DNA + CuL2(24 μM).

in the reaction.[12] In our experimental conditions, O_2 was not excluded so that there might be participation of O_2 from the air or from endogenous reductants(amine buffer).[13] We tested the efficiency of other dinuclear Cu complexes, Cu_2L3 (L3=1,5-bis(1,5,9-triaza-1-cyclododecyl)propane and Cu_2L4 (L4=1,5-bis(1,4,7-triaza-1-cyclononyl)-*m*-xylene) complexes in cleaving DNA.[14] The participation of O_2 should be the same in all reactions carried out in our experiments. As shown in Figure 2b, the Cu_2L1 complex cleaved plasmid DNA more efficiently than other Cu complexes including the Cu_2L4 complex, which had been previously shown comparable reactivity in hydrolyzing GpppG.[15] Although an involvement of metal-peroxide species is still yet to be verified, hydrolytic mechanism is likely operating in the DNA cleavage reaction.[16]

In the Cu_2L1 complex mediated DNA cleavage, the binding and catalytic ability of the Cu_2L1 complex to DNA should be investigated further. However, it is remarkable to observe large rate enhancement in cleaving DNA with a flexible dinuclear Cu_2L1 complex.

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References and Notes

1 a) N. S. Strater, W. N. Lipscomb, T. Klabunde, and B. Krebs, *Angew. Chem., Int., Ed. Engl.*, **35**, 2024 (1996). b) D. E. Wilcox, *Chem. Rev.*, **96**, 2435 (1996).
2 a) J. Chin, *Curr. Opin. Chem. Biol.*, **1**, 514 (1997). b) N. H. Williams, B. Takasaki, M. Wall, and J. Chin, *Acc. Chem. Res.*, **32**, 485 (1999). c) R. Ott and R. Kramer, *Appl. Microbiol Biotechnol.*, **52**, 761 (1999). d) C. Li, S. Yu, D. Li, Z. Liao, X. Sun, and H. Xu, *Inorg. Chem.*, **41**, 913 (2002).
3 J. Kim, *Chem. Lett.*, **2000**, 156.
4 *Cleavage of DNA*: solutions (40 μL of total volume) containing 102 μM base pair DNA (supecoiled plasmid pCMV-Myc, from Clonetech. Labs.) were prepared with varying concentrations of the Cu complexes (1–100 μM) at ice bath. The pH was maintained with 10 mM HEPES. About 4–5 μL of an aliquot was placed in eppendorf tubes and incubated at 30 ± 0.5 °C. At certain time intervals, the reaction was quenched by adding 1 μL loading buffer (bromophenol blue, xylene cyanol, 50% glycerol) containing 4 mM EDTA, and stored at −20 °C until analysis by gel electrophoresis. DNA cleavage reactions in the presence of scavengers (SOD, catalase, DMSO, NaN_3) were performed by the same method as described above. The same experiments on DNA cleavage by the CuL2 complex could not be done since the reactions were not fast enough to use enzymes. *Product analysis and Quantitation*: cleavage products were analyzed in 1% agarose gels. The gels were stained in buffer containing 1 μg/mL ethidium bromide and the extent of DNA degradation was determined by using volume quantitation method with Bio-Rad Molecular Analyst/PC software version 1.4. The relative amounts of the different forms of DNA were determined by dividing absorbance intensity for a particular band by the total intensities of the each band in the same lane. The correction factor of 1.22 for form I DNA obtained from the calibration experiments was utilized.
5 DNA cleavage was performed at pH 6.7 (MES), 7.3 and 8.0 (HEPES), 8.9 and 9.5 (CHES).
6 The decreases in form I were plotted against time and they were fitted well with first order exponential decay curve (pseudo-first order kinetics), in the case where enough amounts of supercoiled DNA were remained during the reaction([Cu_2L1] < 4 μM, [CuL2] < 40 μM). All experiments were performed at least in triplicate. The kinetic run by electrophoresis was reproducible within 20% error.
7 Two moles of Cu(II) ions in the Cu_2L1 complex was considered.
8 a) K. A. Deal and J. N. Burstyn, *Inorg. Chem.*, **35**, 2792 (1996). b) E. L. Hegg and J. N. Burstyn, *Inorg. Chem.*, **35**, 7474 (1996). c) K. M. Deck, T. A. Tseng, and J. N. Burstyn, *Inorg. Chem.*, **41**, 669 (2002).
9 The kinetic pKa and the pKa of the bound water to the CuL2 complex were 7.3 and the maximum rate was observed near pH 8.5 in hydrolyzing BNPP. The Cu_2L1 complex showed the similar pH rate profile in DNA cleavage reaction.
10 T. Ihara, S. Sueda, A. Inenaga, R. Fukuda, and M. Takagi, *Supramol. Chem.*, **8**, 93 (1997).
11 F. V. Pamatong, C. A. Detmer, III, and J. R. Bocarsly, *J. Am. Chem. Soc.*, **118**, 5339 (1996).
12 a) R. Hertzberg and P. B. Dervan, *J. Am. Chem. Soc.*, **104**, 313 (1982). b) M. S. Melvin, J. T. Tomlinson, G. R. Saluta, G. R. Kucera, N. Lindquist, and R. A. Mandervile, *J. Am. Chem. Soc.*, **122**, 6333 (2000).
13 S. T. Frey, H. H. J. Sun, N. N. Murthy, and K. D. Karlin, *Inorg. Chim. Acta*, **242**, 329 (1996).
14 Ligands L1-L4 and their Cu(II) complexes were synthesized by literature methods. a) X. Zhang, W. Hsieh, T. N. Margulis, and L. J. Zompa, *Inorg. Chem.*, **34**, 2883 (1995). b) B. DasGupta, C. Katz, T. Israel, M. Watson, and L. J. Zompa, *Inorg. Chim. Acta*, **292**, 172 (1999).
15 K. P. McCue and J. R. Morrow, *Inorg. Chem.*, **38**, 6136 (1999).
16 According to ref. 8, the reactivity under anaerobic condition retained 70% of that under aerobic condition in hydrolyzing BNPP and DNA by the Cu complexes of L2 and its synthetic analogue, *i*-Pr_3[9]aneN_3. And there proposed O_2 independent, hydrolytic mechanism.

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