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ORIGINAL ARTICLE

DNA cleavage promoted by Cu^{2+} complex of cyclen containing pyridine subunit

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Abstract The tetraazamacrocycle crown ether (cyclen) containing two pyridine subunits was prepared by a modified procedure and the interaction of its metal complexes with DNA was studied by agarose gel electrophoresis analysis. The results indicate that the Cu^{2+} complex as nuclease model can promote the hydrolysis of phosphodiester bond of supercoiled DNA. The rate of degradation of the supercoiled DNA (form I) to nicked DNA (form II) obtained at physiological condition in the presence of 2.14 mM Cu^{2+} complex is 2.31×10^{-3} min^{-1}. The dependence of the rate of supercoiled DNA cleavage from the complex concentration shows an unusual profile and a hydrolytic cleaving mechanism of two monometallic complexes through cooperation from two-point binding to DNA is proposed.

Keywords Cyclen · DNA cleavage · Phosphodiester · Enzyme mimic · Electrophoresis

Introduction

The artificial nucleic acid cleaving agents have attracted extensive attention due to their potential applications in the fields of molecular biological technology and drug development [1]. Some metal complexes as cleaving agents for phosphodiester bond of nucleic acids have widely been investigated and found to be quite efficient [2]. Especially, the complexes of macrocyclic polyamines, such as 1,4,7,10-tetraazacyclododecane (cyclen) and 1,4,7-triazacyclononane (tacn), with transition metals actually exhibit excellent ability of hydrolytically cleaving nucleic acids [3] and anti-tumor as well as anti-HIV in cells [4]. 2,11-Diaza[3.3](2,6)pyridinophane (DAP) employing two pyridine moieties as parts of the cyclic backbone is an important member of tetraazamacrocycle (cyclen) compounds and its unique complexing behavior for metal ions has been reported [5]. However, the activity of cleaving nucleic acid like nuclease has not been reported to date. Recently, DAP was synthesized by previous method [6] with some modifications (Scheme 1) in our group. The interaction of metal complexes of DAP with DNA was studied and we found that Cu^{2+} complex as nuclease model can cleave double-stranded DNA under physiological condition. The dependence of the rate of supercoiled DNA cleavage on the complex concentration shows an unusual profile, on which the interaction pattern of the metal complex with DNA and the cleaving mechanism are proposed.

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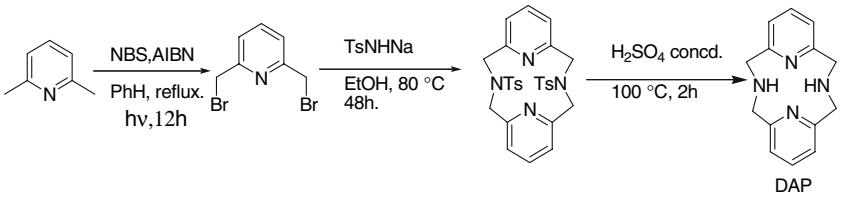
Results and discussion

Cleavage of plasmid DNA

Plasmid DNA cleavage experiments were performed using pUC19 DNA(25 μM bp) in 15 μL of Tris



Scheme 1 Synthesis of tetraazamacrocycle crown ether containing two pyridine subunits

buffer(0.1 M, pH 8.5) at 37 °C in the dark and quenched by loading buffer(3~3.5 μL). A preliminary study showed that Cu^{2+} complex exhibit more effective cleaving activity than other transition metal complexes due to Cu^{2+} ion's superior Lewis acidity [2p, 2r], moreover Cu^{2+} ion or DAP alone could not cleave plasmid DNA under the same conditions. This is kind of normal as mentioned in lots of work [7]. Therefore, Cu^{2+} complex was picked as cleavage agent and the dependence of the rate of plasmid DNA cleavage on pH and Cu^{2+} complex concentration were examined.

Dependence of the plasmid DNA cleavage on pH

pH is always one of the important factors in cleaving reaction. Form II (nicked DNA) content produced by DAP-Cu^{2+} complex cleaving DNA in pH range 6.5~8.5 at 37 °C (Fig. 1a and b) was studied. When pH value is higher than 7.5, Form II increases rapidly. Such behavior is similar to the work reported by Palumbo et al. [2f], which is diagnostic of the deprotonation of a metal-coordinated water molecule to form the active nucleophile. Hence, Tris–HCl buffer with a pH of 8.5 was used for all other experiments.

Dependence of the plasmid DNA cleavage on time

Figure 2 shows time course of pUC19 DNA (25 μM bp) cleavage by DAP-Cu^{2+} complex (2.14 mM) in Tris–HCl buffers at pH 8.5 and 37 °C. It reveals that 50% pUC19 DNA (Form I) was degraded to relaxed circular DNA (Form II) about 120 min. However, the linear DNA was not observed under present experimental conditions. This case is very similar to the reported works [8] using triazacyclononane derivative Cu^{2+} complex as DNA cleaving agent.

Dependence of the plasmid DNA cleavage on the concentrations of DAP-Cu^{2+} complex

Figure 3 shows that the amount of the cleaved DNA under the same conditions remarkably increases with the concentrations of DAP-Cu^{2+} complex up to a maximum at approximately 2.2 mM , but the amount of the cleaved DNA decreases when the complex

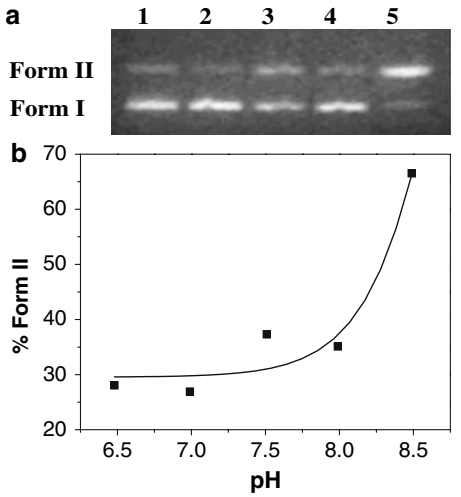


Fig. 1 (**a**) Agarose gel (1%) pUC19 DNA (25 μM bp) incubated for 214 min at 37 °C with 2.14 mM DAP-Cu^{2+} complex in a total volume of 15 μL of Tris–HCl buffers in different pH: (1).6.5; (2). 7.0; (3). 7.5; (4). 8.0; (5). 8.5. (**b**) pH dependent Form II content profile for DNA cleavage

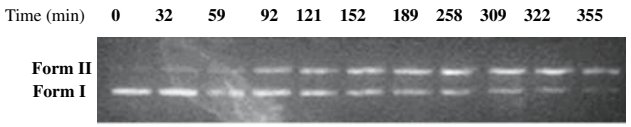


Fig. 2 Agarose gel (1%) pUC19 DNA (25 μM bp) incubated at 37 °C and pH 8.5 with DAP-Cu^{2+} complex (2.14 mM) in a total volume of 15 μL of Tris–HCl buffers for 0~355 min

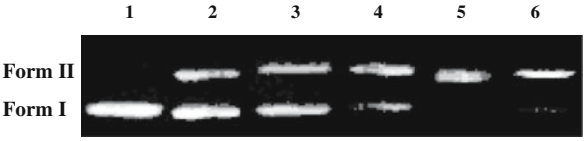


Fig. 3 Agarose gel (1%) pUC19 DNA (25 μM bp) incubated for 350 min at 37 °C and pH 8.5 with increasing concentrations of DAP-Cu^{2+} complex: Lane 1, DNA control; Lane 2, 0.54 mM; Lane 3, 1.07 mM; Lane 4, 1.61 mM; Lane 5, 2.14 mM; Lane 6, 2.68 mM

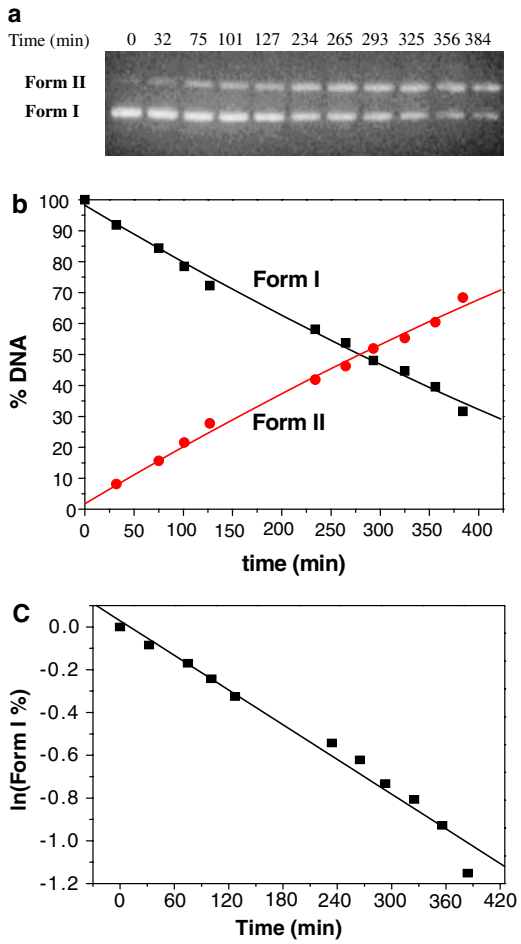


Fig. 4 (**a**) Agarose gel (1%) pUC19 DNA (25 μM bp) incubated at 37 °C and pH 8.5 with DAP-Cu^{2+} complex (1.61 mM) in a total volume of 15 μL of Tris–HCl buffers for 0~385 min. (**b**) Quantification of pUC19 DNA forms. (**c**) Data fit for pseudo first order reaction constant for the DNA cleavage: $k_1 = 2.15 \times 10^{-3}$ min^{-1}

concentration further increases. To better understand the unusual behavior of this system, we investigated the kinetics of DNA cleavage by the DAP-Cu^{2+} complex (0.54 mM~2.68 mM). Figure 4a shows a typical agarose gel generated by incubating the pUC19 (25 μM bp) plasmid DNA in the presence of 1.61 mM DAP-Cu^{2+}. Figure 4b shows the dependence of the rate of disappearance of supercoiled pUC19 DNA (Form I) and appearance of nicked DNA (Form II) on the DAP-Cu^{2+} complex concentration. Data fit for the pseudo first order reaction constant for the DNA cleavage to obtain $k_1 = 2.15 \times 10^{-3}$ min^{-1} (Fig. 4c). The values for the kinetic rate constant at different complex concentrations are reported in Table 1. The profile describes an unusual bell-shape changing tendency (Fig. 5) with the increase of complex concentrations

Table 1 Pseudo first order kinetic constants (k_1, min^{-1}) for the cleavage pUC19 DNA (25 μM bp) incubated at 37 °C and pH 8.5 in the presence of DAP-Cu^{2+} complexes

[DAP-Cu^{2+}]/ mM	k_1 ($\times 10^{-3}$ min^{-1})	[DAP-Cu^{2+}]/ mM	k_1 ($\times 10^{-3}$ min^{-1})
0.54	1.01 (0.18[a])	2.14	2.31 (0.32[a])
1.07	0.68 (0.17[a])	2.68	1.72 (0.24[a])
1.61	2.15 (0.12[a])		

[a] Standard deviation value

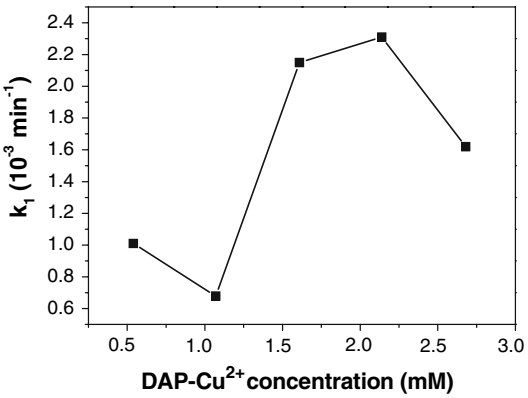


Fig. 5 Pseudo first order rate constants (k_1, min^{-1}) versus DAP-Cu^{2+} complex concentration for the nicking process of supercoiled pUC19 DNA (25 μM bp) at 37 °C and pH 8.5 in Tris–HCl buffer

and the maximum is 2.31×10^{-3} min^{-1} (k_1) at complex concentration of 2.14 mM. Although a rigorous comparison is made difficult by the different experimental conditions employed, this value is comparable to that reported for metal complexes of cyclen or tacn ligand in hydrolytic condition [2b, 3p, 8a, 9], and it is 3.85×10^6 times higher than DNA natural degradation rate constant 6×10^{-10} min^{-1} [10, 1h].

Cleavage mechanism

To investigate the mechanism of the plasmid DNA cleavage promoted by DAP-Cu^{2+} complex, the hydroxyl radical scavengers DMSO and t-BuOH were introduced to the system [3h, 8, 11]. No evident inhibition of DNA cleavage was observed in the presence of scavengers (Fig. 6, Table 2), which suggested that hydroxyl radical oxidative cleavage might not occur in the reaction. Therefore, DNA cleavage promoted by DAP-Cu^{2+} complex takes place probably via a hydrolytic pathway.

As shown in Fig. 4, the dependence of the rate of plasmid DNA cleavage on the DAP-Cu^{2+} complex concentration follows an unusually profile. The steep

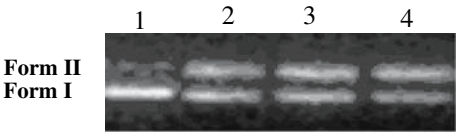


Fig. 6 Agarose gel (1%) pUC19 DNA (25 μM bp) incubated for 120 min at 37 °C in Tris–HCl buffer (pH 8.5) with DAP-Cu^{2+} complex (2.14 mM) and different scavengers: Lane 1, DNA control; Lane 2, DNA + DAP-Cu^{2+}; Lane 3, DNA + DAP-Cu^{2+} + t-BuOH (17.5 mM); Lane 4,. DNA + DAP-Cu^{2+} + DMSO (17.5 mM)

Table 2 Data of pUC19 plasmid DNA (25 μM bp) cleaved by 2.14 mM of DAP-Cu^{2+} incubated for 120 min at 37 °C in Tris–HCl buffer (pH 8.5) in the presence of radical scavengers

	% Form I	% Form II	*Scavengers*	% Form I	% Form II
DNA control	95.64	4.36	t-BuOH	47.05	52.95
DAP-Cu^{2+}	50.85	49.15	DMSO	49.72	50.28

increase of the reactivity in the first part of the curve could indicate a very high affinity of the Cu^{2+} complex for the DNA backbone, and then the following decrease suggests the concentration-dependent formation of an unreactive species. Burstyn [8a] and Palumbo [2f] reported the plasmid DNA cleavage promoted by 1,4,7-triazacyclononane (tacn)-Cu^{2+} complex and 2,4,6-triamino1,3,5-trihydrooxycyclohexane (taci), respectively. In their works, the bell-like shape of profile for the first-order rate constant versus the Cu^{2+} complex is similar to the present work. Palumbo and co-workers [2f] suggested that the rate decrease can be attributed to the formation of unreactive μ-hydroxo dimmers. According with such hypothesis, an unreactive μ-hydroxo dimmer could also be formed in the present work.

The dependence of the reaction rates on pH leads us to postulate that the active species in the deprotonated form of complex DAP-$Cu^{2+}(OH^{-1})$ formed when a proton is removed from the metal-coordinated water molecule in the complex DAP-$Cu^{2+}(OH_2)$. To confirm the presence of a hydroxide anion coordinated to Cu^{2+}, ESI-MS analysis (Fig. 7) was carried out at pH 8.5 aqueous solution. The peaks at *m*/*z* 319.86 and 321.97 show the DAP-$Cu^{2+}(OH^{-1})$ signals (calcd. 320.07 and 322.07) and *m*/*z* 337.78 and 339.85 show the DAP-$Cu^{2+}(Cl^{-1})$ signals (calcd. 338.04 and 340.03), which assesses without doubt the presence of an hydroxide anion coordinated to Cu^{2+}, as a result of a water molecule deprotonation. Such metal-coordinated hydroxide is an effective nucleophile. The evidence for copper hydroxides as the active catalyst [12] in the hydrolysis of phosphodiesters and the hydrolytic cleavage of DNA were reported [2f, 2o, 2p, 8a, 13]. Based on these proposals, a cleaving mechanism promoted by DAP-Cu^{2+} complex is proposed as in Scheme 2. Cu^{2+} ions usually form five-coordinated complexes, which is satisfied by four nitrogen atoms of cyclen and OH^{-1} or water molecule in complex DAP-$Cu^{2+}(OH^{-1})$ or DAP-$Cu^{2+}(OH_2)$. Therefore, it is considered that two DAP-Cu^{2+} molecules cooperate in the hydrolysis for phosphodiester bond of DNA, which is similar to phosphodiester hydrolysis promoted by binuclear copper^{2+} complex. One of DAP-Cu^{2+} complexes serves to bind the phosphodiester of DNA, whereas another one works to activate the hydroxide [2f, 2p, 8a, 13a–c].

Conclusion

In summary, the copper^{2+} complex of tetraazamacrocycle crown ether (cyclen) containing two pyridine subunits as nuclease model can promote the hydrolysis of phosphodiester bond of supercoiled DNA. The rate of degradation of the supercoiled DNA (form I) to nicked DNA (form II) obtained at physiological condition in the presence of 2.14 mM copper^{2+} complex is 2.31×10^{-3} min^{-1} and it is 3.85×10^{6} times higher than DNA natural degradation rate. The dependence of the

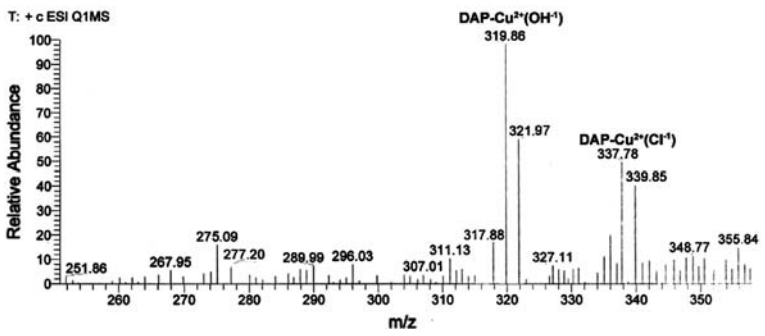
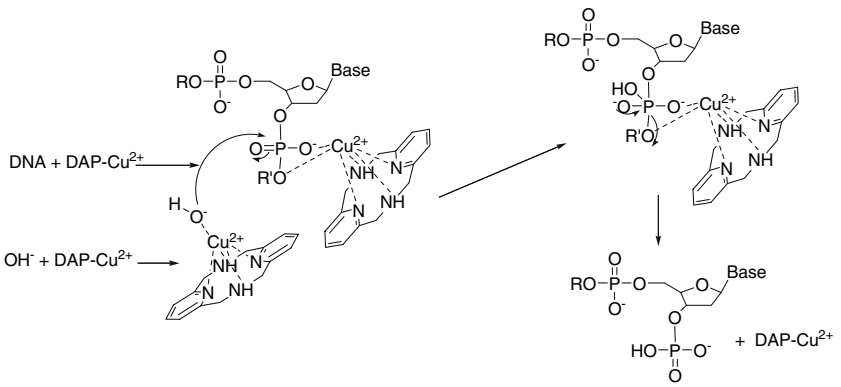


Fig. 7 ESI-MS spectra of DAP-Cu^{2+} complex in aqueous solution at pH 8.5



Scheme 2 Proposed hydrolytic mechanism of supercoiled DNA in the presence of DAP-Cu^{2+} complexes

rate of supercoiled DNA cleavage from the complex concentration shows an unusual profile and a hydrolytic mechanism of two monometallic complexes through cooperation from two-point binding to DNA is proposed.

Experimental

Apparatus

Melting points were tested on a Yanaco micro melting point apparatus. ^{1}H NMR data were recorded on a Brucker AM 300 (Germany). Mass spectra were obtained on an electrospray mass spectrometer (LCQ, Finnigan) in negative mode. The gel electrophoresis was conducted by DYY-5 electrophoresis. The gel imaging and documentation DigiDoc-It^{TM} system (version 1.1.23, UVP, Inc. Unpland, CA) was assessed using Labworks Imaging and TotalLab 2.01analysis software

Materials

pUC19 plasmid DNA was purchased from TaKaRa Biotechnology (Dalian) Co., Ltd., and the purity was checked by agarose gel electrophoresis and their concentration was determined by UV spectroscopy using the extinction coefficient appropriate for double-stranded DNA (1.0 OD_{260} = 50 μg/mL^{-1}). Agarose was from Oxoid Limited of Basingstoke, UK, Ethidium Bromide (EB) was from Amresco. Inc., tris(hydroxymethyl)aminomethane (Tris-Base) was from Robiot Co. Ltd., and Bromophenol Blue, Saccharose and ethyl diamine tetraacetic acid (EDTA) were commercially available. Deionized water was obtained by ionized column from distilled water. Stock solution of EB (2 g/L) was prepared by dissolving 100 mg of EB in 50 mL of deionized water. Kinds of buffer solutions were prepared by Tris-Base and hydrochloric acid, and the pH was confirmed by ORION868 with an Ag/AgCl electrode as the reference electrode in saturated KCl solution at room temperature. Loading buffer *IV* was prepared according to literature [14] as follows: 0.125 g of bromophenol blue was added into the solution containing 20 g of saccharose, 50 mL of deionized water and 8 mg of EDTA (the final solution was containing 0.25% bromophenol blue, 40% saccharose and 500 μM of EDTA and was stored under 4 °C). 2 × TAE buffer [11a, 14] was prepared by dissolving 48.5 g of Tris-Base, 11.5 mL of acetic acid and 2.9 g of EDTA in 500 mL of deionized water. And 1% (w/v) agarose gel was prepared with 0.5 × TAE buffer. All solvents were purified by standard procedure.

Synthesis of ligand DAP and preparation of DAP-Cu^{2+}complex solution

The ligand DAP was synthesized by a modification of published procedure [6] with (Scheme 1). Pre-manufactured sodium *p*-tolunesulfonamide (0.965 g, 5 mmol) was dissolved in 100 mL of ethanol. To this mixture was added dropwisely 50 mL of alcoholic solution containing 2,6-bis(bromomethyl)pyridine (1.32 g, 5 mmol) during half an hour. Then, the mixture was stirred under refluxing for two days. The mixture gradually turned muddy. After cooled to room temperature, the solution was filtrated and evaporated to nearly dryness. A glass of ice water (50 mL) was injected into the residue and normally white solid was obtained. Filtrated, the solid was recrystallized with chloroform/methanol to give *N′,N*-ditosyl-DAP (0.47 g, 48.1%), white powder, m.p. 260–264 °C. ^{1}H NMR ($CDCl_3$) δ(ppm): 2.48(s, 6H, 2 × CH_3), 4.48(s, 8H, 4 × CH_2), 7.17(d, J = 7.1Hz, 4H, 4 × Ar–H),

7.28(t, J = 8.0 Hz, 2H, Ar–H), 7.39(d, J = 7.1Hz, 4H, 4 × Ar–H), 7.80(d, J = 8.0, 4H, 4 × Ar–H); MS(E-SIMS) m/z: 549.2 ($[M+H]^+$, calc.for $C_{28}H_{28}N_4O_4S_2$: 549.2. *N′,N*-ditosyl-DAP (0.2 g, 0.36 mmol) was added into concentrated sulfuric acid(0.7 mL, 1.3 g) and heated at 100 °C for 2 h. Cooled, the mixture was carefully neutralized with 20% sodium hydroxide aqueous solution(1.4 mL), followed by extraction with chloroform. The organic layer was separated and dried with anhydrous sodium sulfate for half an hour. Then chloroform was evaporated under vacuum and 0.5 g of a white solid was obtained with a melting point >250 °C, yield: 57.8%. ^{1}H NMR(D_2O) δ(ppm): 3.97(s, 8H, 4 × CH_2), 7.31–7.58(m, 6H, 6 × Ar–H). MS(ESIMS)m/z: 241.3 ($[M+H]^+$, calc. for $C_{14}H_{16}N_4$: 241.1. The stock DAP-Cu^{2+}complex solutions were prepared by mixing equimolar amounts of ligand DAP and copper chloride in deionized water. MS(ESIMS)m/z: 338.1, 340.1 ($[M+Cl^-]^+$, calc.for $C_{14}H_{16}N_4Cu$ + Cl: 338.0, 340.0; 362.0 ($[M+CH_3COO^-]^+$, calc.for $C_{16}H_{19}N_4O_2Cu$: 362.1.

Cleavage of plasmid DNA

The plasmid DNA cleavage experiments were performed using pUC19 DNA in Tris buffer (pH 8.5). Reactions were performed by incubating DNA (25 μM bp) at 37 °C in the dark in the presence/absence of increasing amount of DAP-Cu^{2+}complex for the indicated time. When required, the incubation was carried out in the same buffer but in the presence of radical scavengers DMSO and t-BuOH [2f, 8a, 11]. All reactions were quenched by loading buffer(3~3.5 μL). Agarose gel electrophoresis was carried out on a 1% agarose gel in 0.5× Tris-acetate-EDTA buffer containing 0.5 μg mL ethidium bromide at 80 V for 1.5 h. The resolved bands were visualized with a UV transilluminator and quantified using TotalLab 2.01 software. The intensity of the band relative to the supercoiled form has been multiplied for 1.43 to take account of its reduced affinity to ethidium bromide [2f].

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References

1. (a) Liu, C., Wang, M., Zhang, T., Sun, H.: DNA hydrolysis promoted by di- and multi-nuclear metal complexes. Coord. Chem. Rev. **248**, 147–168 (2004), (b) Burer, R.M.: Cleavage of nucleic acids by bleomycin. Chem. Rev. **98**, 1153–1169 (1998), (c) Pogozelski, W.K., Tullius, T.D.: Oxidative Strand Scission of Nucleic Acids: Routes Initiated by Hydrogen Abstraction from the Sugar Moiety. Chem. Rev. **98**, 1089–1108 (1998), (d) Armitage, B.: Photocleavage of nucleic acids. Chem. Rev. **98**, 1171–1200 (1998), (e) McMillin, D., McNett, K.M.: Photoprocesses of copper complexes that bind to DNA. Chem. Rev. **98**, 1201–1220 (1998), (f) David, S.S., Williams, S.D.: Chemistry of glycosylases and endonucleases involved in base-excision repair. Chem. Rev. **98**, 1221–1262 (1998), (g) Rajski, S.R., Williams, R.M.: DNA crosslinking agents as antitumor drugs. Chem. Rev. **98**, 2723–2796 (1998), (h) Hegg, E.L., Burstyn, J.N.: Toward the development of metal-based synthetic nucleases and peptidases: a rationale and progress report in applying the principles of coordination chemistry. Coord. Chem. Rev. **173**, 133–165 (1998), (i) Cowan, J.A.: Chemical nucleases. Curr. Opin. Chem. Biol. **5**, 634–642 (2001), (j) Morrow, J.R., Iranzo, O.: Synthetic metallonucleases for RNA cleavage. Curr. Opin. Chem. Biol. **8**, 192–200 (2004), (k) Chen, C-A., Cowan, J.A.: In vivo cleavage of a target RNA by copper kanamycin A. Direct observation by a fluorescence assay. Chem. Commun. 196–197 (2002), (l) Baker, B.F., Lot, S.S., Kringel, J., Cheng-Flournoy, S., Villiet, P., Sasmor, H.M., Siwkowski, A.M., Chappell, L.L., Morrow, J.R.: Oligonucleotide-europium complex conjugate designed to cleave the 5′ cap structure of the ICAM-1 transcript potentiates antisense activity in cells. Nucleic Acids Res. **27**, 1547–1551 (1999), (m) Perreault, D.M., Anslyn, E.V.: Unifying the current data on the mechanism of cleavage-transesterification of RNA. Angew. Chem. Int. Ed. **36**, 432–450 (1997)
2. (a) Feng, G., Mareque-Rivas, J.C., Torres Martin de Rosales, R., Williams, N.H.: A highly reactive mononuclear Zn(II) complex for phosphodiester cleavage. J. Am. Chem. Soc. **127**, 13470–13471 (2005), (b) Branum, M.E., Tipton, A.K., Zhu, S., Que L. Jr.: Double-strand hydrolysis of plasmid DNA by dicerium complexes at 37 degrees C. J. Am. Chem. Soc. **123**, 1898–1904 (2001), (c) Komiyama, M., Kina, S., Matsumura, K., Sumaoka, J., Tobey, S., Lynch, V.M., Anslyn, E.: Trinuclear copper(II) complex showing high selectivity for the hydrolysis of 2′-5′ over 3′-5′ for UpU and 3′-5′ over 2′-5′ for ApA ribonucleotides. J. Am. Chem. Soc. **124**, 13731–13736 (2002), (d) Kuzuya, A., Mizoguchi, R., Morisawa, F., Machida, K., Komiyama, M.: Metal ion-induced site-selective RNA hydrolysis by use of acridine-bearing oligonucleotide as cofactor. J. Am. Chem. Soc. **124**, 6887–6894 (2002), (e) Scheffer, U., Strick, A., Ludwig, V., Peter, S., Kalden, E., Göbel, M. W.: Metal-free catalysts for the hydrolysis of RNA derived from guanidines, 2-aminopyridines, and 2-aminobenzimidazoles. J. Am. Chem. Soc. **127**, 2211–2217 (2005), (f) Sissi, C., Mancin, F., Gatos, M., Palumbo, M., Tecilla, P., Tonellato, U.: Efficient plasmid DNA cleavage by a mononuclear copper(II) complex. Inorg. Chem. **44**, 2310–2317 (2005), (g) Iranzo, O., Elmer, T., Richard, J.P., Morrow, J.R.: Cooperativity between metal ions in the cleavage of phosphate diesters and RNA by dinuclear Zn(II) catalysts. Inorg. Chem. **42**, 7737–7746 (2003), (h) Rossi, L.M., Neves, A., Bortoluzzi, A.J., Hörner, R., Szpoganicz, B., Terenzi, H., Mangrich, A.S., Pereira-Maia, E., Castellano, E.E., Haase, W.: Synthesis, structure and properties of unsymmetrical-alkoxo-dicopper(II) complexes: biological relevance to phosphodiester and DNA cleavage and cytotoxic activity. Inorg. Chim. Acta. **358**, 1807–1822 (2005), (i) Yang, M-Y., Richard, J.P., Morrow, J.R.: Substrate specificity for catalysis of phosphodiester cleavage by a dinuclear Zn(II) complex. Chem. Commun. 2832–2833 (2003), (j) Feng, G., Mareque-Rivas, J.C., Williams, N.H.: Comparing a mononuclear Zn(II) complex with hydrogen

bond donors with a dinuclear Zn(II) complex for catalyzing phosphate ester cleavage. Chem. Commun. 1845–1847 (2006), (k) Kondo, S., Shinbo, K., Yamaguchi, T., Yoshida, K., Yano, Y.: Cooperativity of binuclear Zn(II) complexes of bisimidazolyl ligands in the hydrolysis of bis(2,4-dinitrophenyl) phosphate in aqueous solution. J. Chem. Soc., Perkin Trans. **2**, 128–131 (2001), (l) Baldini M., Belicchi-Ferrari, M., Bisceglie, F., Capacchi, S., Pelosi, G., Tarasconi, P.: Zinc complexes with cyclic derivatives of ketoglutaric acid thiosemicarbazone: Synthesis, X-ray structures and DNA interactions. J. Inorg. Biochem. **99**, 1504–1513 (2005), (m) Erkkila, K.E., Odom, D.T., Barton, J.K.: Recognition and reaction of metallointercalators with DNA. Chem. Rev. **99**, 2777–2796 (1999), (n) Shao, Y., Zhang, J., Tu, C., Dai, C., Xu, Q., Guo, Z.: Steric effect on the nuclease activity of Cu(II) complexes with aminoquinoline derivatives. J. Inorg. Biochem. **99**, 1490–1496 (2005), (o) Ren, R., Yang, P., Zheng, W., Hua, Z.: A simple copper(II)-L-histidine system for efficient hydrolytic cleavage of DNA. Inorg. Chem. **39**, 5454–5463 (2000), (p) Zhu, L., dos Santos, O., Koo, C.W., Rybstein, M., Pape, L., Canary, J.W.: Geometry-dependent phosphodiester hydrolysis catalyzed by binuclear copper complexes. Inorg. Chem. **42**, 7912–7920 (2003), (q) Feng, G., Mareque-Rivas, J.C., Williams, N.H.: Bioinorganic models for the catalytic cooperation of metal ions and functional groups in nuclease and peptidase enzymes. Chem. Commun. 1845–1847 (2006), (r) Metzler-Nolte, N., Klein, C., Weyhermüller, T., Hrubanova, S., Happel, C.M., Kirin, S.I.: Synthesis, structure and comparison of the DNA cleavage ability of metal complexes M(II)L with the N-(2-hydroxyethoxyethyl)-bis(2-picolyl)amine ligand L (M = Co, Ni, Cu and Zn). Dalton Trans. 1201–1207 (2004), (s) Krämer, R.: Bioinorganic models for the catalytic cooperation of metal ions and functional groups in nuclease and peptidase enzymes. Coord. Chem. Rev. **182**, 243–261 (1999)

3. (a) Boseggia, E., Gatos, M., Lucatello, L., Mancin, F., Moro, S., Palumbo, M., Sissi, C., Tecilla, P., Tonellato, U., Zagotto, G.: Toward efficient Zn(II)-based artificial nucleases. J. Am. Chem. Soc. **126**, 4543–4549 (2004), (b) Iranzo, O., Kovalevsky, A.Y., Morrow, J.R., Richard, J.P.: Physical and kinetic analysis of the cooperative role of metal ions in catalysis of phosphodiester cleavage by a dinuclear Zn(II) complex. J. Am. Chem. Soc. **125**(7), 1988–1993 (2003), (c) Williams, N.H., Lebuis, A.M., Chin, J.: A structural and functional model of dinuclear metallophosphatases. J. Am. Chem. Soc. **121**, 3341–3348 (1999), (d) Kikuta, E., Murata, M., Katsube, N., Koike, T; Kimura, E.: Novel recognition of thymine base in double-stranded DNA by zinc(II)-macrocyclic tetraamine complexes appended with aromatic groups. J. Am. Chem. Soc. 5426–5436 (1999), (e) Sissi, C., Rossi, P., Felluga, F., Formaggio, F; Palumbo, M; Tecilla, P., Toniolo, C., Scrimin, P.: Dinuclear Zn(2+) complexes of synthetic heptapeptides as artificial nucleases. J. Am. Chem. Soc. **123**, 3169–3170 (2001), (f) Kimura, E., Kodama, Y., Koike, T., Shiro, M.: Phosphodiester hydrolysis by a new zinc(II) macrocyclic tetraamine complex with an alcohol pendant: Elucidation of the roles of Ser-102 and zinc(II) in alkaline phosphatase. J. Am. Chem. Soc. **117**, 8304–8311 (1995), (g) Young, M.J., Chin, J.: Dinuclear copper(II) complex that hydrolyzes RNA. J. Am. Chem. Soc. **117**, 10577–10578 (1995), (h) Bencini, A., Berni, E., Bianchi, A., Giorgi, C., Valtancoli, B., Chand, D.K., Schneider, H.-J.: Proton and Cu(II) binding to tren-based tris-macrocycles. Affinity towards nucleic acids and nuclease activity. Dalton Trans. **(5)**, 793–800 (2003), (i) Kim, J.H.: Selective hydrolysis of 4-nitrophenyl phosphate by a dinuclear Cu(II) complex. Chem. Lett. 156–157 (2000) (j) Chand, D.K., Schneider, H.-J., Bencini, A., Bianchi, A., Giorgi, C., Ciattini, S., Valtancoli, B.: Supramolecular chemistry, part 99. Affinity and nuclease activity of macrocyclic polyamines and their Cu^{II} complexes. Chem. Eur. J. **6**(21), 4001–4008 (2000), (k) Williams, N.H., Wyman, P.: Phosphate diester hydrolysis within a highly reactive dinuclear cobalt(III) complex. Ligand effect on reactivity, transition state and dissociation. J Chem. Soc. Perk Trans **2**(11), 2068–2073 (2001), (l) McCue, K.P., Morrow, J.R.: Hydrolysis of a model for the 5′-cap of mRNA by dinuclear copper(II) and zinc(II) complexes. Rapid hydrolysis by four copper(II) ions. Inorg. Chem. **38**, 6136–6142 (1999), (m) Jeung ,C.S., Kim, C.H., Min, K., Suh, S.W., Suh, J.: Hydrolysis of plasmid DNA catalyzed by Co(III) complex of cyclen attached to polystyrene. Bioorg. Med. Chem. Lett. **11**, 2401–2404 (2001), (n) Kikuta, E., Matsubara, R., Katsube, N., Koike, T., Kimura, E.: Selective recognition of consecutive G sequence in double-stranded DNA by a zinc(II)-macrocyclic tetraamine complex appended with an anthraquinone.J. Inorg. Biochem. **82**, 239–249 (2000), (o) Ragunathan K.G., Schneider, H.J.: Supramolecular chemistry. 51. Complexes from polyazacyclophanes, fluorescence indicators, and metal cations - an example of allosterism through ring contraction. Angew. Chem. Int. Ed. Engl. **35**, 1219–1221 (1995), (p) Rammo, J., Hettich, R., Roigk, A., Schneider, H.J.: Catalysis of DNA cleavage by lanthanide complexes with nucleophilic or intercalating ligands and their kinetic characterization. J. Chem. Soc. Chem. Commun. 105–107 (1996), (q) Fry, F.H., Fischmann, A.J., Belousoff, M.J., Spiccia, L., Brügger, J.: Kinetics and mechanism of hydrolysis of a model phosphate diester by $[Cu(Me_3tacn)(OH_2)_2]^{2+}$ (Me3tacn = 1,4,7-Trimethyl-1,4,7-triazacyclononane). Inorg. Chem. **44**, 941–950 (2005), (r) Peng, W., Liu, P.-Y., Jiang, N., Lin, H.-H., Zhang, G.L., Liu, Y., Yu, X.-Q.: Dinuclear macrocyclic polyamine zinc(II) complexes linked with flexible spacers: Synthesis, characterization, and DNA cleavage. Bioorg. Chem. **33**, 374–385 (2005)
4. (a) Michaelis, K., Kalesse, M.: Selective cleavage of the HIV-1 TAR-RNA with a peptide-cyclen conjugate. Angew. Chem. Int. Ed. **38**, 2243–2245 (1999), (b) Parker, L.L., Lacy, S.M., Farrugia, L.J., Evans, C., Robins, D.J., O'Hare, C.C., Hartley, J.A., Jaffar, M., Stratford, I.J.: A novel design strategy for stable metal complexes of nitrogen mustards as bioreductive prodrugs. J. Med. Chem. **47**, 5683–5689 (2004), (c) Parker, L.L., Anderson, F.M., O'Hare, C.C., Lacy, S.M., Bingham, J.P., Robins, D.J., Hartley, J.A.: Synthesis of novel DNA cross-linking antitumour agents based on polyazamacrocycles. Bioorg. Med. Chem. **13**, 2389–2395 (2005), (d) Liang, F., Wang, P., Zhou, X., Li, T., Li, Z., Lin, H., Gao, D., Zheng, C., Wu, C.: Nickel(II) and cobalt(II) complexes of hydroxyl-substituted triazamacrocyclic ligand as potential antitumor agents. Bioorg. Med. Chem. Lett. **14**, 1901–1904 (2004), (e) Liang, X.-Y., Sadler, P.J.: Cyclam complexes and their applications in medicine. Chem. Soc. Rev. **33**, 246–266 (2004), (f) De Clercq, E.: Timeline: The bicyclam AMD3100 story. Nat. Res. Drug. Disc. **2**, 581–587 (2003), (g) Jang, Y.H., Blanco, M., Dasgupta, S., Keire, D.A., Shively, J.E., Goddard, W.A.: Mechanism and energetics for complexation of 90Y with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic Acid (DOTA), a model for cancer radioimmunotherapy. J. Am. Chem. Soc. **121**, 6142–6151 (1999), (h) Fichna, J., Janecka, A.: Synthesis of target-specific radiolabeled peptides for diagnostic imaging. Bioconjugate Chem. **14**, 3–17 (2003)
5. (a) Kim, W.D., Hrncir, D.C., Kiefer, G.E., Sherry, A.D.: Synthesis, crystal structure, and potentiometry of

pyridine-containing tetraaza macrocyclic ligands with acetate pendant arms. Inorg. Chem. **34**, 2225–2232 (1995), (b) Marchand, A.P., Chong, H.-S., Alihodžić, S., Watson, W.H., Bodige, S.G.: Synthesis and alkali metal picrate extraction capabilities of novel, cage-functionalized, pyridine containing crown ethers and cryptands. Tetrahedron, **55**, 9687–9696 (1999), (c) Koch, W.O., Kaiser, J.T., Krüger, H.-J.: First structural characterization of an eight-coordinate cobalt(II) complex containing five-membered chelate rings: Evidence for d-orbital-stabilization energy favoring eight-over six-coordination at cobalt(II) ions. Chem. Commun. **22**, 2237–2238 (1997), (d) Koch, W.O., Barbieri, A., Grodzicki, M., Schünemann, V., Trautwein, A.X., Krüger, H.-J.: Eight-coordinate iron(II) and iron(III) ions in complexes with distorted dodecahedral FeN_8 environments: synthesis and structures of bis(2,11-diaza[3.3](2,6)pyridinophane)iron complexes. Angew. Chem. Int. Ed. **35**, 422–424 (1996)

6. Bottino , F., Di Grazia, M., Finocchiaro, P., Fronczek, F.R., Mamo A., Pappalardo, S.: Reaction of tosylamide monosodium salt with bis(halomethyl) compounds: an easy entry to symmetrical N-tosyl aza macrocycles. J. Org. Chem. **53**, 3521–3529 (1988)
7. (a) Dai, W.-M., Fong, K.C., Lau, C.W., Zhou, L., Hamaguchi, W., Nishimoto, S.: Synthesis and DNA cleavage study of a 10-membered ring enediyne formed via allylic rearrangement. J. Org. Chem. **64**, 682–683 (1999), (b) Basile, L.A., Raphael, A.L., Barton, J.K.: Metal-activated hydrolytic cleavage of DNA. J. Am. Chem. Soc. **109**, 7550–7551 (1987), (c) Branum, M.E., Tipton, A.K., Zhu, S., Que, L. Jr.: Double-strand hydrolysis of plasmid DNA by dicerium complexes at 37 degrees C. J. Am. Chem. Soc. **123**, 1898–1904 (2001), (d) Takeda, N., Shibata, M., Tajima, N., Hirao, K., Komiyama, M.: Kinetic and theoretical studies on the mechanism of alkaline hydrolysis of DNA. J. Org. Chem. **65**, 4391–4396 (2000)
8. (a) Deck, K.M., Tseng, T.A., Burstyn, J.N.: Triisopropyltriazacyclononane copper(II): An efficient phosphodiester hydrolysis catalyst and DNA cleavage agent. Inorg. Chem. **41**, 669–677 (2002), (b) Shi, S., Liu, J., Li, J., Zheng, K.C., Tan, C.P., Chen, L.M., Ji, L.N.: Electronic effect of different positions of the $-NO_2$ group on the DNA-intercalator of chiral complexes $[Ru(bpy)2L]^{2+}$ (L = o-npip, m-npip and p-npip). Dalton Trans. 2038–2046 (2005)
9. (a) Hegg, E.L., Burtyn, J.N.: Copper(II) macrocycles cleave single-stranded and double-stranded DNA under both aerobic and anaerobic conditions. Inorg. Chem. **35**, 7474–7481 (1996), (b) Itoh, T., Hisada, H., Sumiya, T., Hosono, M., Usui, Y., Fujin, Y.: Hydrolytic cleavage of DNA by a novel copper(II) complex with cis,cis-1,3,5-triaminocyclohexane. Chem. Commun. 677–678 (1997), (c) Sreedhara, A., Cowan, J.A.: Efficient catalytic cleavage of DNA mediated by metalloaminoglycosides. Chem. Commun. 1737–1738 (1997)
10. (a) Sigman, D.S., Mazumder, A., Perrin, D.M.: Chemical nucleases. Chem. Rev. **93**, 2295–2316v (1993), (b) Westheimer, F.H.: Why nature chose phosphates. Science **235**, 1173–1178 (1987)
11. (a) Subramanian, M., Shadakshari, U., Chattopadhyay, S.: A mechanistic study on the nuclease activities of some hydroxystilbenes. Bioorg. Med. Chem. **12**, 1231–1237 (2004), (b) Song, Y.-F., Yang, P.: Synthesis, DNA scission chemistry, and an investigation of the reactive oxygen species of two 2,6-dimethoxyhydroquinone-3-mercaptoacetic acid-peptide conjugates. Aus. J. Chem. **54**, 253–259 (2001), (c) Scarpellini, M., Neves, A., Horner, R., Bortoluzzi, A.J., Szpoganics, B., Zucco, C., Nome Silva, R.A., Drago, V., Mangrich, A.S., Ortiz, W.A., Passos, W.A., de Oliveira, M.C.B., Terenzi, H.: Phosphate diester hydrolysis and DNA damage promoted by new *cis*-aqua/hydroxy copper(II) complexes containing tridentate imidazole-rich ligands. Inorg. Chem. **42**, 8353–8365 (2003)
12. Molenveld , P., Engbersen, J.F.J., Reinhoudt, D.N.: Dinuclear metallo-phosphodiesterase models: application of calix[4]arenes as molecular scaffolds. Chem. Soc. Rev. **29**, 75–86 (2000)
13. (a) Selmeczi, K., Giorgi, M., Speier, G., Farkas, E., Reglier, M.: Mono- versus binuclear copper(II) complexes in phosphodiester hydrolysis. Eur. J. Inorg. Chem. 1022–1031 (2006), (b) Rossi, L.M., Neves, A., Hörner, R., Terenzi, H., Szpoganicz, B., Sugai, J.: Hydrolytic activity of a dinuclear copper(II,II) complex in phosphate diester and DNA cleavage. Inorgan. Chim. Acta **337**, 366–370 (2002), (c) Rossi, L.M., Neves, A., Bortoluzzi, A.J., Hörner, R., Szpoganicz, B., Terenzi, H., Mangrich, A.S., Pereira-Maia, E., Castellano, E.E., Haase, W.: Synthesis, structure and properties of unsymmetrical-alkoxo-dicopper(II) complexes: biological relevance to phosphodiester and DNA cleavage and cytotoxic activity. Inorganica Chimica Acta **358**, 1807–1822 (2005), (d) Reddy, P.A., Nethaji, M., Chakravarty, A.R.: Hydrolytic cleavage of DNA by ternary amino acid Schiff base copper(II) complexes having planar heterocyclic ligands. Eur. J. Inorg. Chem. 1440–1446 (2004)
14. Reed , R., Holmes, D., Weyers, J., Jones, A.: Practical skills in biomolecular sciences, Addison-Wesley, Boston, (2001) 8–33