

## 双核铜(II)配合物在 $\text{H}_2\text{O}_2$ 存在下对 DNA 的氧化切割活性

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### Oxidative Cleavage of DNA by a Novel Dinuclear Copper(II) Complex in the Presence of $\text{H}_2\text{O}_2$

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**Abstract:** A novel dinuclear copper(II) complex,  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$  (phen = 1,10-phenanthroline, dipicH<sub>2</sub> = 2,6-pyridinedicarboxylic acid), has been prepared and structurally characterized. The complex crystallizes in the triclinic system, space group  $P\bar{1}$  with cell parameters  $a=0.846\ 0(17)$  nm,  $b=1.289\ 5(3)$  nm,  $c=1.452\ 7(3)$  nm,  $\alpha=77.42(3)^\circ$ ,  $\beta=79.11(3)^\circ$ ,  $\gamma=87.08(3)^\circ$ , and  $V=1.518\ 8(6)$  nm<sup>3</sup>. The dinuclear complex shows potential DNA cleavage activity at micromolar concentration in the presence of  $\text{H}_2\text{O}_2$  and exhibits higher nuclease efficiency than mononuclear complex  $[\text{Cu}(\text{dipic})(\text{H}_2\text{dipic})] \cdot \text{H}_2\text{O}$ . Without external reductants, the added  $\text{H}_2\text{O}_2$  may contribute to the generation of hydroxyl radicals that result in DNA strand scission. CCDC:291786.

**Key words:** dinuclear copper(II) complex; DNA; oxidative cleavage; crystal structure

## 0 Introduction

Transition metal complexes capable of cleaving DNA have been the focus of numerous investigations in the past two decades due to their potential use as structural probes in nucleic acids chemistry and as therapeutic agents<sup>[1~7]</sup>. Among them, the most famous and extensively studied is bis (1,10-phenanthroline) copper complex, which was first reported by Sigman et

al. to be able to efficiently mediate DNA strand scission in the presence of thiol and hydrogen peroxide<sup>[8~10]</sup>. This complex has also been widely used as a footprinting reagent and as a probe of DNA or RNA secondary structures<sup>[11~13]</sup>. Lately, various other copper complexes with different structural ligands exhibiting nucleolytic activities have also been studied, such as copper complexes with tamblamine<sup>[14]</sup>, Clip-phen<sup>[15]</sup>, o-quinacridines<sup>[16]</sup>, the tripeptide Gly-Gly-His<sup>[17]</sup>, and

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some ternary copper complexes of 1,10-phenanthroline with another ancillary ligand<sup>[18–20]</sup>.

Recently, multinuclear copper complexes showing potential DNA cleavage activity have attracted increasing interest<sup>[21–25]</sup>. More than one metal centers in multinuclear complexes may enhance the interaction between the complexes and DNA, which may facilitate the binding of complexes to DNA. Moreover, the synergy between two and more copper ions in multinuclear copper complex may contribute to higher DNA cleavage activity than mononuclear copper complex.

In the present paper, we describe the structure of a new dinuclear copper complex,  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ , where phen=1,10-phenanthroline and  $\text{dipicH}_2$ =2,6-pyridyldicarboxylic acid. The nuclease activity of the dinuclear copper complex has been studied and the preliminary cleavage mechanism of the complex is discussed.

## 1 Experimental

### 1.1 Materials and General Methods

Common reagents such as ethanol, anhydrous ether,  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ , NaOH,  $\text{NaClO}_4 \cdot \text{H}_2\text{O}$  and 1,10-phenanthroline monohydrate are all analytical grade and used as received. 2,6-pyridinedicarboxylic acid was purchased from Sigman. Plasmid pBR322 was purchased from MBI Fermentas.

The infrared spectra were recorded on a Bruker VECTOR22 spectrometer as KBr pellets (4 000~500  $\text{cm}^{-1}$ ), and elemental analysis was performed on a Perkin-Elmer 240C analytical instrument. The contrast mononuclear compound  $[\text{Cu}(\text{dipic})(\text{H}_2\text{dipic})](\text{H}_2\text{O})$  was prepared by literature method<sup>[26]</sup>.

### 1.2 Synthesis of $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$

To the mixture of 2,6-pyridinedicarboxylic acid (167 mg, 1 mmol) and NaOH (40 mg, 1 mmol) in 8 mL water, an aqueous solution (5 mL) of  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  (241.5 mg, 1 mmol) was added with stirring. 10 minutes later, a solution of 1,10-phenanthroline monohydrate (198 mg, 1 mmol) in methanol (5 mL) was added, and then the blue solution continued to stir for about 2 h at 60 °C. On cooling the solution to ambient temperature, an aqueous solution of  $\text{NaClO}_4 \cdot \text{H}_2\text{O}$

(140.5 mg, 1 mmol) was added and then stirred for about 30 min. The resulting light-blue precipitate was filtered and washed with ethanol and ether. Blue single crystals suitable for X-ray diffraction were obtained on slow evaporation of an aqueous methanol solution of the complex (286 mg, 80.6%). Anal. Calcd. for  $\text{C}_{26}\text{H}_{22}\text{Cu}_2\text{N}_4\text{O}_{12}$  (%): C, 44.0; H, 3.10; N, 7.89. Found (%): C, 44.5; H, 3.08; N, 7.86. IR (KBr pellet,  $\text{cm}^{-1}$ ): 3 422, 1 633, 1 589, 1 517, 1 424, 1 374, 854, 723.

**CAUTION!** Perchlorate salts of metal complexes containing organic ligands are potentially explosive. Only small quantity of material should be prepared and handled with suitable safety measures.

### 1.3 Determination of crystal structure

The crystal of  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$  was determined on a Siemens SMART CCD diffractometer at 293 K. The program SAINT<sup>[27]</sup> was used for data reduction and an empirical absorption correction was carried out using the SADABS program<sup>[28]</sup>. The structure was solved by Patterson methods that revealed the position of all non-hydrogen atoms and refined using the full-matrix least-squares method on  $F_{\text{obs}}^2$  with the SHELXTL software package<sup>[29]</sup>. Hydrogen atoms were placed in calculated positions. Atomic scattering factors and anomalous dispersion corrections were taken from ref<sup>[30]</sup>.

CCDC: 291786.

### 1.4 DNA cleavage

A typical reaction was carried out by mixing 1  $\mu\text{L}$  of pBR322 DNA (0.335  $\mu\text{g} \cdot \mu\text{L}^{-1}$ , 0.1185  $\mu\text{mol} \cdot \text{L}^{-1}$ ), 4.5  $\mu\text{L}$  of 3-morpholinopropane sulfonic acid (MOPS) buffer (pH=7.4) and 8.5  $\mu\text{L}$   $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$  solution (20  $\mu\text{mol} \cdot \text{L}^{-1}$ ) in 40  $\text{mmol} \cdot \text{L}^{-1}$  MOPS buffer (pH=7.4, 4% DMF) with 3  $\mu\text{L}$  of  $\text{H}_2\text{O}_2$  at a 50 fold molar excess relative to the complex to yield a total volume of 17  $\mu\text{L}$ . After mixing, the sample was incubated at 37 °C. The reactions were quenched at appropriate time by the addition of sodium diethyldithiocarbamate trihydrate (DDTC) and loading buffer (0.25% brom-phenol blue, 50% glycerol). Then, the solution was subjected to electrophoresis on 0.7% agarose gel in TAE buffer (40  $\text{mmol} \cdot \text{L}^{-1}$  Tris acetate/1

mmol $\cdot\text{L}^{-1}$  EDTA) at 100 V and visualized by ethidium bromide staining. The Gel Imaging and Documentation DigiDoc-It<sup>TM</sup> System (Version 1.1.23, UVP, Inc. Unpland, CA) was assessed using labworks Imaging and Analysis Software (UVP, Inc. Unpland, CA).

Scavengers of reactive oxygen intermediates,  $\text{NaN}_3$ ,  $\text{D}_2\text{O}$ , dimethyl sulfoxide (DMSO) and tert-butyl alcohol (6 mmol $\cdot\text{L}^{-1}$ ) were added alternatively to the reaction mixtures. Cleavage was initiated by the addition of the complexes and quenched with 3 L loading buffer (0.25% bromphenol blue, 25% glycerol, 1 mM EDTA). Further analysis was conducted using the standard procedures described above.

## 2 Results and Discussion

### 2.1 Synthesis

The reactions of 1,10-phenanthroline and 2,6-pyridinedicarboxyl acid with  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  resulted in the dinuclear copper(II) complex,  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ . It is interesting that the perchlorate ions do not appear in the crystal structure of the complex (see Crystal Structure) although the product of the complex was isolated from the reaction mixture by  $\text{NaClO}_4 \cdot \text{H}_2\text{O}$ . This phenomenon is also demonstrated on the IR spectra in which no bands of  $\text{ClO}_4^-$  are presented. The broad band in 3 422  $\text{cm}^{-1}$  in the IR spectra of the copper complex may attribute to the stretching vibration  $\nu(\text{O}-\text{H})$  of the water molecule. The IR spectra of the complex shows the asymmetric  $\nu_{\text{as}}(\text{COO}^-)$  and the symmetric stretching vibration  $\nu_{\text{s}}(\text{COO}^-)$  fall in 1 648 and 1 431  $\text{cm}^{-1}$ , respectively. The bands at 1 521 and 1 431  $\text{cm}^{-1}$  can be attributed to the ring stretching frequencies  $[\nu(\text{C}=\text{C})$  and  $\nu(\text{C}=\text{N})]$  of 1, 10-phenanthroline.

### 2.2 Crystal Structure

The X-ray structure of  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$  is shown in Fig.1. Details of the crystal parameters, data collection and refinements are listed in Table 1 and selected bond lengths and angles are given in Table 2. The structure of the complex consists of two Cu(II) centers which are bridged by the carboxylate group of one dipic ligand. The Cu(1) ion is situated in a distorted six-coordinated octahedron. The

two approximately planar ONO ligands coordinated with Cu (1) ion are mutually perpendicular to each other. The equatorial plane consists of two oxygen atoms (O(5) and O(7)) and one nitrogen atom (N(2)) from one dipic ligand and one nitrogen atom (N (1)) from another dipic ligand. The apical positions are occupied by oxygen donors from dipic ligand. The mean Cu(1)-O and Cu(1)-N bond lengths are similar to those found in the reported copper complex  $[\text{Cu}(\text{dipic})(\text{H}_2\text{dipic})] \cdot \text{H}_2\text{O}$ <sup>[26]</sup>. The N(1)-Cu(1)-O(1) and N(1)-Cu(1)-O(3) angles are 77.53(13) $^\circ$  and 74.22(13) $^\circ$ , respectively, which are much less than the ideal value of 90 $^\circ$ . At the same time, the opposite are 107.47(13) $^\circ$  and 100.99(12) $^\circ$ , respectively, indicating a coordination through a distorted rhombically octahedron. The Cu(2) ion is situated in a distorted square-pyramidal. In the equatorial plane, the copper ion is bonded to two nitrogen atoms (N(3) and N(4)) of the phen ligand, an oxygen atom (O(4)) of the dipic group and an oxygen atom (O(9)) of a water molecule with bond lengths Cu(2)-N(3)=0.200 3(4), Cu(2)-N(4)=0.199 8(4), Cu(2)-O(4)=0.196 5(3) and Cu(2)-O(9)=0.196 4(3) nm, respectively. The apical position of the square-pyramidal is occupied by another water molecule with the bond

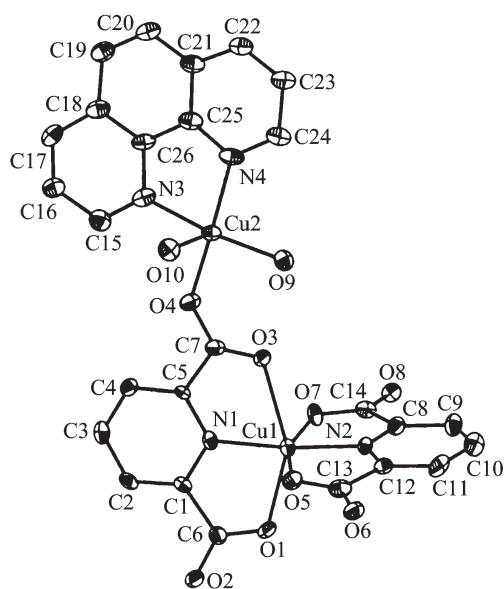


Fig.1 Crystal structure of  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$  (two water molecules are co-crystallized with the complex and are omitted in the ORTEP drawing for clarity)

**Table 1** Crystal data and structure refinement for  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ 

Empirical formula	$\text{C}_{26}\text{H}_{22}\text{Cu}_2\text{N}_4\text{O}_{12}$	<i>Z</i>	2
Formula weight	709.56	Calculated density / ( $\text{g} \cdot \text{cm}^{-3}$ )	1.552
<i>T</i> / K	293(2)	Absorption coefficient / $\text{mm}^{-1}$	1.467
Crystal size / mm	0.3×0.2×0.2	<i>F</i> (000)	720
Crystal habit, color	prism, light-blue	$\theta$ range for data collection / ( $^\circ$ )	1.46 to 25.02
Crystal system	Triclinic	Limiting indices	$0 \leq h \leq 10, -15 \leq k \leq 15, -16 \leq l \leq 17$
Space group	$P\bar{1}$	Reflections collected / unique( $R_{\text{int}}$ )	5747 / 5338(0.022 2)
<i>a</i> / nm	0.846 0(17)	Absorption corrections	Empirical
<i>b</i> / nm	1.289 5(3)	Data / restraints / parameters	5338 / 0 / 418
<i>c</i> / nm	1.452 7(3)	Goodness-of-fit on $F^2$	1.013
$\alpha$ / ( $^\circ$ )	77.42(3)	Final <i>R</i> indices [ $I > 2\sigma(I)$ ] <sup>a</sup>	$R_1=0.055\ 4, wR_2=0.147\ 1$
$\beta$ / ( $^\circ$ )	79.11(3)	<i>R</i> indices (all data) <sup>a</sup>	$R_1=0.065\ 7, wR_2=0.151\ 6$
$\gamma$ / ( $^\circ$ )	87.08(3)	Largest diff. Peak, hole / ( $\text{e} \cdot \text{nm}^{-3}$ )	711, -726
<i>V</i> / $\text{nm}^3$	1.518 8(6)		

$$^a R_1 = \sum \|F_o\| - |F_c| / \sum \|F_o\|; wR_2 = [\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2]^{1/2}$$

**Table 2** Selected bond distances (nm) and angles ( $^\circ$ ) of  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ 

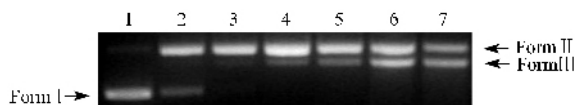
Cu(1)-O(1)	0.223 1(3)	Cu(2)-N(3)	0.200 3(4)	Cu(1)-O(3)	0.239 9(3)
Cu(2)-N(4)	0.198 8(4)	Cu(1)-O(5)	0.208 1(3)	Cu(2)-O(4)	0.196 5(3)
Cu(1)-O(7)	0.212 6(3)	Cu(2)-O(9)	0.196 4(3)	Cu(1)-N(1)	0.196 4(3)
Cu(2)-O(10)	0.219 7(3)	Cu(1)-N(2)	0.193 2(3)		
N(1)-Cu(1)-N(2)	172.27(14)	O(3)-Cu(1)-O(7)	89.97(12)	N(1)-Cu(1)-O(1)	77.53(13)
O(5)-Cu(1)-O(7)	158.16(12)	N(1)-Cu(1)-O(3)	74.22(13)	N(3)-Cu(2)-N(4)	82.94(18)
N(1)-Cu(1)-O(5)	106.28(13)	N(3)-Cu(2)-O(4)	89.45(15)	N(1)-Cu(1)-O(7)	95.54(13)
N(3)-Cu(2)-O(9)	169.96(15)	N(2)-Cu(1)-O(1)	107.47(13)	N(3)-Cu(2)-O(10)	90.00(14)
N(2)-Cu(1)-O(3)	100.99(12)	N(4)-Cu(2)-O(4)	165.54(15)	N(2)-Cu(1)-O(5)	79.95(14)
N(4)-Cu(2)-O(9)	92.05(17)	N(2)-Cu(1)-O(7)	78.22(13)	N(4)-Cu(2)-O(10)	96.77(15)
O(1)-Cu(1)-O(5)	89.59(13)	O(4)-Cu(2)-O(9)	93.51(14)	O(1)-Cu(1)-O(7)	96.20(13)
O(9)-Cu(2)-O(10)	99.25(13)	O(1)-Cu(1)-O(3)	151.53(11)	O(4)-Cu(2)-O(10)	95.50(14)
O(3)-Cu(1)-O(5)	94.92(12)				

length Cu(2)-O(10)=0.219 7(3)nm.

### 2.3 Nuclease Activity

The ability of the dinuclear copper complex to perform DNA cleavage has been studied by gel electrophoresis using supercoiled pBR322 DNA in MOPS buffer (pH=7.4). High DNA cleavage activity of the complex was obtained (Fig.2 and 3) in the presence of 50 fold excess of  $\text{H}_2\text{O}_2$  at 37  $^\circ\text{C}$ . The concentration dependence of the DNA cleaving efficiency (Fig.2) was studied for 10 min reaction time, and quantified data are shown in Table 3. As can be observed from Fig.2, supercoiled DNA(form I) decreased significantly with the increase of the concentrations of the copper complex. At the concentration of 5  $\mu\text{mol} \cdot \text{L}^{-1}$ ,

the complex completely converted supercoiled DNA into nicked DNA(form II). Linear DNA (form III) was found at 7.5  $\mu\text{mol} \cdot \text{L}^{-1}$ , and its content increased to 36% at 12.5  $\mu\text{mol} \cdot \text{L}^{-1}$ . Increasing the concentration of



**Fig.2** Concentration dependence of the cleavage of pBR322 plasmid DNA by  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$  in the presence of  $\text{H}_2\text{O}_2$ . Conditions: 0.007  $\mu\text{mol} \cdot \text{L}^{-1}$  DNA (or 0.02  $\mu\text{g} \cdot \mu\text{L}^{-1}$ ; 50 fold excess of  $\text{H}_2\text{O}_2$ ; 40  $\text{mmol} \cdot \text{L}^{-1}$  MOPS buffer; pH 7.4; at 310 K for 10 min. Lane 1, DNA control. Lane 2-7, DNA +  $\text{H}_2\text{O}_2$  + complex (2.5  $\mu\text{mol} \cdot \text{L}^{-1}$ , 5  $\mu\text{mol} \cdot \text{L}^{-1}$ , 7.5  $\mu\text{mol} \cdot \text{L}^{-1}$ , 10  $\mu\text{mol} \cdot \text{L}^{-1}$  and 12.5  $\mu\text{mol} \cdot \text{L}^{-1}$ , respectively)

**Table 3** Data of pBR322 DNA cleavage at different concentrations of  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ 

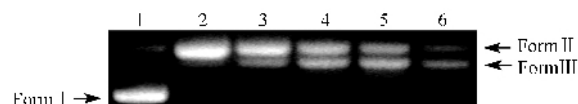
Serial No. <sup>A</sup>	Reaction condition	Form / %		
		I	II	III
1	DNA control	97	3	
2	DNA + $\text{H}_2\text{O}_2$ + $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ ( $2.5 \mu\text{mol} \cdot \text{L}^{-1}$ )	82	18	
3	DNA + $\text{H}_2\text{O}_2$ + $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ ( $5.0 \mu\text{mol} \cdot \text{L}^{-1}$ )		100	
4	DNA + $\text{H}_2\text{O}_2$ + $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ ( $7.5 \mu\text{mol} \cdot \text{L}^{-1}$ )		94	6
5	DNA + $\text{H}_2\text{O}_2$ + $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ ( $10.0 \mu\text{mol} \cdot \text{L}^{-1}$ )		78	22
6	DNA + $\text{H}_2\text{O}_2$ + $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ ( $12.5 \mu\text{mol} \cdot \text{L}^{-1}$ )		64	36

<sup>A</sup>The serial number is the same as the lane number shown in Fig.2.

the complex to  $15 \mu\text{mol} \cdot \text{L}^{-1}$ , some smears corresponding to multi-fragmented DNA was observed, which indicated that the optimum concentration showing most efficient cleavage was about  $12.5 \mu\text{mol} \cdot \text{L}^{-1}$ .

Fig.3 illustrated the influence of the reaction time on the cleavage of pBR322 DNA. An increasing of reaction time produced a significant increase of the cleaved DNA. When the time varied from 5~20 min, the supercoiled DNA disappeared completely and the content of linear DNA increased from 0 to 55% (Table 4). Many smears have been observed after 30 min

which suggested that the optimum reaction time may be 15~20 min.



**Fig.3** Time dependence of the cleavage of pBR322 DNA ( $0.02 \mu\text{g} \cdot \mu\text{L}^{-1}$ ) by  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$  ( $10 \mu\text{mol} \cdot \text{L}^{-1}$ ) in the presence of 50-fold excess of  $\text{H}_2\text{O}_2$  in MOPS buffer, pH 7.4,  $37^\circ\text{C}$ . Lane 1, DNA control; lane 2-6, DNA + complex +  $\text{H}_2\text{O}_2$ , after 5, 10, 15, 20 and 30 min, respectively.

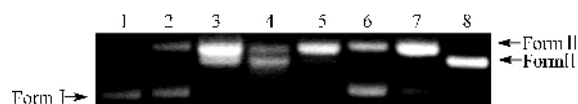
**Table 4** Data of pBR322 DNA cleavage at different reaction time

Serial No. <sup>A</sup>	Reaction condition	Form / %		
		I	II	III
1	DNA control	96	4	
2	DNA + $\text{H}_2\text{O}_2$ + $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ (5 min)		100	
3	DNA + $\text{H}_2\text{O}_2$ + $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ (10 min)		76	24
4	DNA + $\text{H}_2\text{O}_2$ + $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ (15 min)		56	44
5	DNA + $\text{H}_2\text{O}_2$ + $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$ (20 min)		45	55

<sup>A</sup>The serial number corresponds to the lane number shown in Fig.3.

We have also compared the nucleolytic activity of the title dinuclear complex with mononuclear complex,  $[\text{Cu}(\text{dipic})(\text{dipicH}_2)](\text{H}_2\text{O})$  (see lanes 2 and 3 of

Figure 4). At the same concentration of copper ion ( $20 \mu\text{mol} \cdot \text{L}^{-1}$ ), the dinuclear complex converted the initial supercoiled DNA to 75% nicked DNA and 25% linear



**Fig.4** Cleavage of pBR322 DNA ( $0.02 \mu\text{g} \cdot \mu\text{L}^{-1}$ ) by mononuclear  $[\text{Cu}(\text{dipic})(\text{dipicH}_2)](\text{H}_2\text{O})$  ( $20 \mu\text{mol} \cdot \text{L}^{-1}$ ) for line 2 and by  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$  ( $10 \mu\text{mol} \cdot \text{L}^{-1}$ ) using different inhibitors for lines 4-7 in the presence of 50-fold excess of  $\text{H}_2\text{O}_2$  in MOPS buffer, pH 7.4,  $37^\circ\text{C}$ . Lane 1, control; lane 2, DNA +  $[\text{Cu}(\text{dipic})(\text{dipicH}_2)](\text{H}_2\text{O})$  ( $20 \mu\text{mol} \cdot \text{L}^{-1}$ ); lane 3, DNA +  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$  ( $10 \mu\text{mol} \cdot \text{L}^{-1}$ ); lane 4, DNA +  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$  ( $10 \mu\text{mol} \cdot \text{L}^{-1}$ ) +  $\text{NaN}_3$ ; lane 5, DNA +  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$  ( $10 \mu\text{mol} \cdot \text{L}^{-1}$ ) +  $\text{D}_2\text{O}$ ; lane 6, DNA +  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$  ( $10 \mu\text{mol} \cdot \text{L}^{-1}$ ) + DMSO; lane 7, DNA +  $[\text{Cu}_2(\text{phen})(\text{dipic})_2(\text{H}_2\text{O})_2] \cdot 2\text{H}_2\text{O}$  ( $10 \mu\text{mol} \cdot \text{L}^{-1}$ ) + *t*-butyl alcohol; lane 8, Hind III.

**Table 5** Data of pBR322 DNA cleavage by [Cu(dipic)(dipicH<sub>2</sub>)](H<sub>2</sub>O) and by [Cu<sub>2</sub>(phen)(dipic)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>]·2H<sub>2</sub>O using different inhibitors (50 fold fold H<sub>2</sub>O<sub>2</sub>)

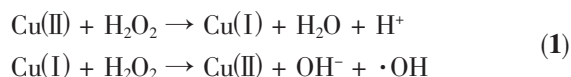
Serial No. <sup>A</sup>	Reaction condition	Form / %		
		I	II	III
1	DNA control	97	3	
2	DNA + H <sub>2</sub> O <sub>2</sub> + [Cu <sub>2</sub> (phen)(dipic) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] (20 μmol·L <sup>-1</sup> )	52	48	
3	DNA + H <sub>2</sub> O <sub>2</sub> + [Cu <sub>2</sub> (phen)(dipic) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]·2H <sub>2</sub> O (10 μmol·L <sup>-1</sup> )		75	25
4	DNA + H <sub>2</sub> O <sub>2</sub> + [Cu <sub>2</sub> (phen)(dipic) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]·2H <sub>2</sub> O (10 μmol·L <sup>-1</sup> ) + NaN <sub>3</sub>		33	67
5	DNA + H <sub>2</sub> O <sub>2</sub> + [Cu <sub>2</sub> (phen)(dipic) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]·2H <sub>2</sub> O (10 μmol·L <sup>-1</sup> ) + D <sub>2</sub> O		82	18
6	DNA + H <sub>2</sub> O <sub>2</sub> + [Cu <sub>2</sub> (phen)(dipic) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]·2H <sub>2</sub> O (10 μmol·L <sup>-1</sup> ) + DMSO	60	40	
7	DNA + H <sub>2</sub> O <sub>2</sub> + [Cu <sub>2</sub> (phen)(dipic) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ]·2H <sub>2</sub> O (10 μmol·L <sup>-1</sup> ) + <i>t</i> -butyl alcohol	19	81	

<sup>A</sup>The serial number corresponds to the lane number shown in Fig.4.

DNA, while for the mononuclear complex, only 48% conversion of supercoiled DNA to nicked DNA was realized. The results showed that the possible synergy between the two copper ions might contribute to the relative high nucleolytic efficiency of the studied dinuclear complex compared with the mononuclear complex.

The preliminary mechanism of pBR322 DNA cleavage by the title complex was also studied using inhibiting reagents, such as NaN<sub>3</sub>, D<sub>2</sub>O, DMSO and *t*-butyl alcohol (Fig.4). Quantification of the gel afforded the data listed in Table 5. Obviously both NaN<sub>3</sub> and D<sub>2</sub>O had no effect on the cleavage reaction (lines 4 and 5), suggesting that <sup>1</sup>O<sub>2</sub> is not involved in the reaction. The hydroxyl radical scavengers, especially DMSO (line 6), diminished significantly the nuclease activity of the complex, which is indicative of the involvement of the hydroxyl radical in the cleavage process. These results suggested that in this case the active oxygen species involved in the DNA cleavage might be ·OH. Generally, the DNA damage produced by Cu(II) involves initial reduction to Cu(I) by an exogenous reducing agent, and then the Cu(I) species binds to DNA and reacts with H<sub>2</sub>O<sub>2</sub> to generate some active oxygen species, such as hydroxyl radical or copper-oxo species which result in the DNA strand scission. In this study, the efficient cleavage of DNA by the title Cu(II) complex and H<sub>2</sub>O<sub>2</sub> without addition of reducing agent may also proceed through Cu(I) intermediate, which may be formed by direct reduction

of the Cu(II) complex by H<sub>2</sub>O<sub>2</sub><sup>[31]</sup>. The Cu(I) species then reacts with H<sub>2</sub>O<sub>2</sub> again to generate ·OH, the active oxygen species in this case that leads to the cleavage reaction (Eq.1)<sup>[32]</sup>.



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