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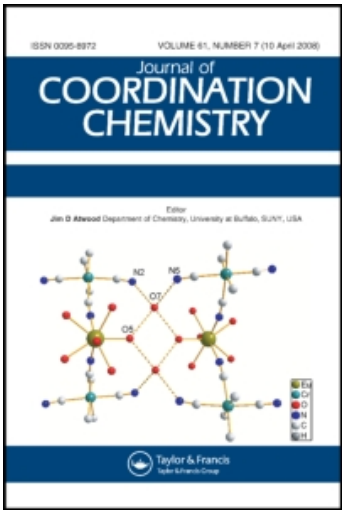

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Synthesis, characterization and nucleolytic property of *bis*(*N*,*N*'-dimethylglycinato)copper(II)

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Synthesis, characterization and nucleolytic property of *bis*(*N*,*N*′-dimethylglycinato)copper(II)

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Aquabis(*N*,*N*-dimethylglycinato)copper(II) dihydrate, $[Cu(dmg)_2(H_2O)] \cdot 2H_2O$, has been synthesized and characterized by elemental analysis, FT-IR, magnetic data, thermal analysis, solution UV-visible spectroscopy, X-ray crystal structure analysis and electrospray ionization mass spectroscopy. The square pyramidal copper(II) complex crystallizes in the orthorhombic space group $P2_12_12_1$. The complex cleaves circular plasmid DNA (pBR322) in the presence of H_2O_2. The extent of cleavage varies with the concentration range of the complex, the type of buffer used, pH of buffer and the concentration of H_2O_2. Hydroxyl radical scavenger can inhibit the nucleolytic ability of this complex.

Keywords: Copper(II) complex; DNA cleavage; Amino acid; *N*,*N*-dimethylglycine; Hydroxyl radical

1. Introduction

It has been our long-term interest to study Aldol-type condensation and Mannich aminomethylation reactions of copper(II) and other transition metal complexes of amino acids; we have found the reactive centers to be at the amino nitrogen and α-carbon of the chelated amino acids, and the type and number of the α-carbon substituents of the chelated amino acids affect the reaction pathway and type of products formed [1–3]. We unsuccessfully attempted to investigate such reactions involving *bis*(*N*,*N*-dimethylglycinato)copper(II), a very soluble complex. Copper(II) complexes of amino acids have also attracted attention due to SOD-mimetic properties, i.e. able to inhibit the harmful effect of superoxide anion [4].

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However, this SOD-mimetic study did not involve N-substituted amino acids where the N-substituents can cause distortion and/or change in the geometry of the complex through steric effects. In spite of increasing numbers of publications of copper(II) complexes with the ability to cleave DNA in the presence of H_2O_2, such complexes usually involve non-amino acid ligands [5–7]. Consequently, we decided to carry out a similar study on *bis*(*N*,*N*-dimethylglycinato)copper(II) trihydrate. The capacity of this complex to cleave DNA is detailed in this study along with its crystal structure and other characterization data.

2. Experimental

2.1. *Reagents, materials and methods*

Supercoiled plasmid pBR322 was purchased from BioSyn Tech (Fermentas). Sodium azide, t-butanol, D-mannitol, dimethyl sulfoxide (DMSO) and potassium iodide were used as supplied. The electrophoresis experiments were performed on a horizontal gel electrophoresis system.

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2.2. *Synthesis of* $[Cu(dmg)_2(H_2O)]\cdot 2H_2O$

The complex was synthesized from the reaction of *N*,*N*-dimethylglycine (also called 2,2-dimethylglycine) and freshly prepared copper hydroxide. The reagents, in a 2 : 1 ratio, were heated in a small volume of water until they dissolved completely; large lilac crystals separated from the filtered solution in 21% yield. Elemental analysis: found C, 29.95; H, 6.85; N, 8.53%. Calculated for $CuC_8H_{22}N_2O_7$: C, 29.86; H, 6.89; N, 8.70. IR (KBr): 3489vs, 3390s, 3256s, 1690s, 1664s, 1616vs, 1469m, 1398s, 1376s, 1321m, 1263w, 1185m, 1150w, 1030m, 1020m, 986m, 932m, 867s, 746m, 669m, 619m, 527w, 504w, 424w cm^{-1}.

2.3. *Physical measurements*

C, H and N microanalysis was carried out with a Perkin-Elmer 2400 CHN analyser. Thermal analysis was performed using a Mettler Toledo Star System on 2–5 mg samples with a heating rate of $10^{\circ}C\,min^{-1}$ under nitrogen gas flowing at $20\,mL\,min^{-1}$ in the temperature range 50–800°C. Infrared spectra of all compounds were recorded as KBr pellets using a Perkin-Elmer FT-IR spectrometer in the frequency range 4000–400 cm^{-1}. The magnetic susceptibility measurement at room temperature was obtained with a Sherwood Magnetic Susceptibility Balance MSB Mk1 which was calibrated with $Hg[Co(SCN)_4]$. An electrospray ionisation mass spectrum of an aqueous solution of the complex was obtained by using a MAT LCQ bench top quadrupole ion trap mass spectrometer. UV-visible spectroscopic measurements were carried out on a Shimadzu UV-160 spectrophotometer; for studies on aqueous solutions, distilled water was the reference while those on buffered solutions (such as complex-DNA interaction studies), the corresponding buffer was the reference material.

Table 1. Crystal data and structure refinement.

Formula	$C_8H_{22}N_2O_6Cu$
Formula weight	321.82
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
V (Å^3)	1461.3(3)
D_c (g cm^{-3})	1.463(3)
$F(000)$	676
μ (mm^{-1})	1.520
Index ranges	$-6 \le h \le 8$; $-9 \le k \le 8$; $-40 \le l \le 37$
Data [$F^2 > 2\sigma(F^2)$]	2732
R [$F^2 > 2\sigma(F^2)$]	0.0311
R_w	0.0755
S	1.002
Flack parameter	0.01(2)
a (Å)	6.5200(8)
b (Å)	7.0477(9)
c (Å)	31.800(4)
α (°)	90.00
β (°)	90.00
λ (°)	90.00
T (K)	298(2)
Z	4
λ (Å)	0.71073
θ range (°)	2.56–27.03
Reflections refined	3175
Goodness-of-fit on F^2	1.002
R (all data)	0.0379
R_w	0.0730

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2.4. *X-ray crystallography*

Diffraction data were collected on a Siemens CCD area-detector diffractometer at 298(2) K on a crystal of size $0.48 \times 0.37 \times 0.32$ mm using the ω-scan technique over the range $2.56 < \theta < 27.03°$ with Mo-Kα radiation ($\lambda = 0.71073$ Å). The intensity data were collected over the range $\theta = 2.56$–$27.03°$ for 8617 reflections. Lorentz-polarization and absorption corrections were applied. Crystal structure data and refinement are tabulated in table 1.

The structure was solved and refined by using the SHELX system of programs [8]. The final R ($F^2 > 2\sigma(F^2)$) and $R_w(F^2)$ values were 0.0311 and 0.0755. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were treated by a mixture of independent and constrained refinement. The weighting scheme was $w = 1/[\sigma^2(F_o^2) + (0.0455P)^2$ where $P = (F_o^2 + F_c^2)/3$. The perspective view of the molecule was obtained using ORTEP [9]. Selected bond distances and angles are listed in table 2.

2.5. *DNA cleavage experiments*

Agarose gel electrophoresis experiments were carried out on supercoiled plasmid DNA pBR322 (4.4 kb). All samples in TBE buffer (90 mM *Tris*-borate, 2.5 mM EDTA; pH 8.3; *Tris* = *Tris*(hydroxymethyl)aminomethane; EDTA = disodium salt of ethylene-diaminetetra-acetic acid) were incubated at 37°C for 2 h, and then electrophoresed on 1% agarose gel for 2.5 h at 80 V. Essentially, samples with increasing concentration

Table 2. Selected geometric parameters.

Bond lengths and angles (Å, °)			
Cu1–O1	1.945(2)	O1–Cu1–O1w	98.5(1)
Cu1–O3	1.953(2)	O1–Cu1–N2	94.0(1)
Cu1–O1w	2.239(2)	O3–Cu1–N2	83.4(1)
Cu1–N1	2.026(2)	O1w–Cu1–N1	94.9(1)
Cu1–N2	2.023(2)	N1–Cu1–N2	168.0(1)
O1–Cu1–O3	162.1(1)	O3–Cu1–O1w	99.4(1)
O1–Cu1–N1	83.8(1)	O1w–Cu1–N2	97.2(1)
O3–Cu1–N1	95.1(1)		
Hydrogen bonds (Å)			
O1w···O2w	2.730(3)	O2w···O2	2.823(3)
O1w···O3w	2.721(3)	O3w···O4	2.774(3)
O2w···O2	2.814(4)	O3w···O3	2.809(3)

of complex with $0.0125\,\mu g\,\mu L^{-1}$ DNA for each sample were incubated in the presence of a specified concentration of H_2O_2. The resultant DNA bands after the electrophoresis step for each set of experiments were stained with ethidium bromide before being photographed under UV light using a Syngene Bio Imaging system; the digital image was viewed with Gene Flash software.

In addition, the effect of the use of EDTA-containing buffer (TBE pH 8.3 mentioned above) and non EDTA-containing buffer was investigated. The latter buffer was *Tris*-NaCl (100 mM Tris, 150 mM NaCl; abbreviated as TN buffer); TN buffer pH 7 and pH 8.3 were used. The effect of varying H_2O_2 concentration (change from 0.5 to 2 mM) was also undertaken. Samples with increasing concentration of complex were similarly treated with DNA ($0.0125\,\mu g\,\mu L^{-1}$) in the presence of H_2O_2 and each electrophoresis experiment was similarly carried out as detailed in the preceding paragraph. Additionally, the effect of hydroxyl radical scavengers (sodium azide, t-butanol, D-mannitol and DMSO) and hydrogen peroxide scavenger (KI) on the nucleolytic activity of $Cu(dmg)_2$ was investigated. For each experiment, a 20 μL mixture consisting of the appropriate volumes of stock solutions of DNA, complex dissolved in buffer, additional buffer and aqueous H_2O_2 (last component added) was incubated at 37°C for 2 h before electrophoresis. Each 20 μL mixture in experiments involving scavengers was similarly prepared; H_2O_2 solution was added as the last component after addition of quencher.

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3. Results and discussion

3.1. *Structural analysis of crystalline complex*

In the crystal structure of $[Cu(dmg)_2(H_2O)]\cdot 2H_2O$ (figure 1), the copper atom is five-coordinate in a slightly distorted square-pyramidal geometry, and is displaced 0.258(1) Å out of the N_2O_2 square plane in the direction of the coordinated water. The distortion from trigonal bipyramidal (TBP) to square pyramidal (SP) geometry along the Berry pseudorotation pathway is 92%; alternately, the SP geometry has a 10% trigonal distortion based on a TBP component τ ($\tau = 0\%$ for ideal SP, whereas $\tau = 100\%$ for ideal TBP) [10]. The two *N*,*N*-dimethylglycinate anions bonded in the basal plane are in a *trans*-configuration. The coordinated water molecule

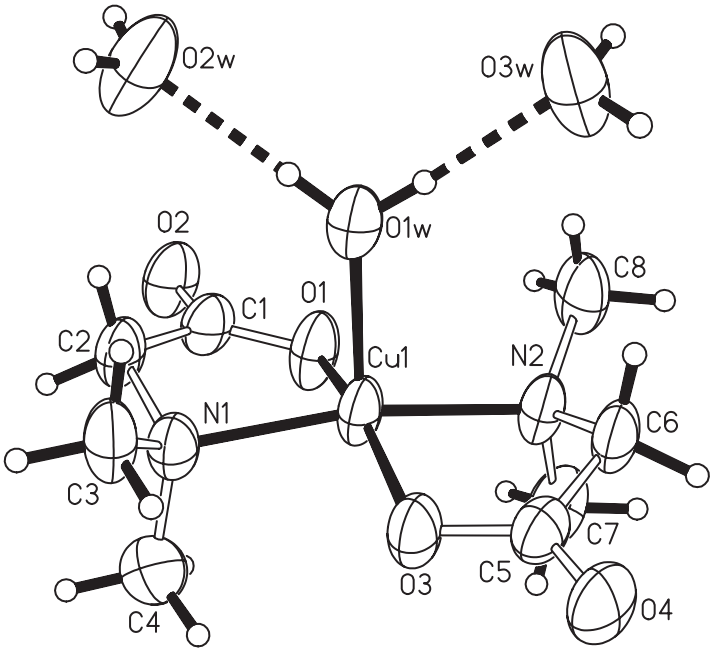

Figure 1. 50% Probability ORTEP plot illustrating the geometry of the copper atom in the complex. H atoms are drawn as spheres of arbitrary radii.

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is hydrogen-bonded to two lattice water molecules, and these interact with neighbouring carboxylate oxygen atoms to furnish a three-dimensional structure. Opposite the apical position, lies one methyl group of each *N*,*N*-dimethylglycinate; the other methyl group is approximately in the basal plane. Thus, steric obstruction precludes a sixth bond with the copper. Consistent with this square-pyramidal geometry is the experimental magnetic moment value of 1.93 BM [11].

The thermal decomposition of the complex shows three steps, i.e. at 66.89°C (8.46% weight loss), 132.83°C (8.08% weight loss) and 248.80°C (64.12% weight loss). The first two steps correspond to a total loss of three water molecules (calculated, 16.80%; experimental, 16.54%).

3.2. *UV-visible spectral data of complex in aqueous and buffered solutions*

An aqueous solution of the complex has a λ_{max} value of 610 nm (molar extinction coefficient, $\epsilon = 90\,M^{-1}\,cm^{-1}$) due to d–d transition. This λ_{max} value in the visible region shifts towards higher wavelength in the following buffer solutions: 625 nm (TN buffer pH 7), 619 nm (TN buffer pH 8.3), 652 nm (TBE buffer pH 8.3). Aqueous solution of *bis*(aminoacidato)copper(II) typically shows λ_{max} values of $\sim$620 nm with two nitrogen atoms coordinated to copper [12]. In the copper(II)-L-Histidine system, the aqueous solution has a λ_{max} value of 630 nm and electrospray ionisation mass spectra (abbreviated as ESI-MS) data show the major species in solution to be $[Cu(L\text{-}His)_2]$, in which two L-histidine ligands are coordinated to each copper atom [13]. As the λ_{max} values for the present complex in various buffers are still far from that

of $[Cu(H_2O)_6]^{2+}$ (aqueous $CuCl_2$) at 819 nm, the major species in these buffer solutions is most likely to be $[Cu(dmg)_2]$ or $[Cu(dmg)_2(H_2O)]$ or $[Cu(dmg)_2(H_2O)_2]$. ESI-MS data of an aqueous solution of $[Cu(dmg)_2(H_2O)] \cdot 2H_2O$, show positive ion peaks of 219.8 (36%), 267.7 (100%) and 285.0 (55%) attributed to $[Cu(dmg)]^+$, $[Cu(dmg)_2]$ and $[Cu(dmg)_2(H_2O)]$, respectively. Thus, the solid state five-coordinate, square-pyramidal species persist in solution. In addition, the stability of the $Cu(dmg)_2$ complex is quite high ($\log \beta_2 = 13.76$; $\log K_1 = 7.21$; $\log K_2 = 6.56$) [14] and it is only slightly lower than other non-N-substituted common *bis*(α-aminoacidato)copper(II) complexes which have $\log \beta_2$ values of ~14.0–15.0 [15]. Furthermore, our extensive studies on aldol-type and Mannich aminomethylation reactions of *bis*(α-aminoacidato)copper(II) complexes give quite high yields of the product containing both chelated amino acid moieties, suggesting the presence of a substantial amount of undissociated *bis*(α-aminoacidato)copper(II) in the aqueous reaction mixtures.

The UV spectrum of the aqueous solution of the complex shows a higher intensity charge transfer band at 244 nm ($\epsilon = 4370\ M^{-1}\,cm^{-1}$) attributed to amino acid ligand to metal charge transfer (LMCT) [16]. This λ_{max} value shifts to slightly longer wavelength in different buffers: 248 nm (Tris-NaCl pH 7), 247 nm (Tris-NaCl pH 8.3) and 262 nm (TBE buffer pH 8.3). Increasing the concentration of complex shifts the UV band to slightly longer wavelength for TN buffers (pH 7 and pH 8.3); this trend is reversed for TBE buffer (pH 8.3). As the λ_{max} of the DNA lies at 260 nm, there is substantial overlap of these two bands hindering determination of the binding constant of the complex with DNA in the various buffers.

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3.3. *DNA cleavage studies with Cu(dmg)$_2$*

3.3.1. Cleavage of DNA in TN buffer pH 8.3. The reaction of 1 mM copper(II) complex with DNA in the presence of 0.5 mM H_2O_2 (figure 2, lane **6**) resulted in complete conversion of supercoiled plasmid to nicked and linear forms. The control

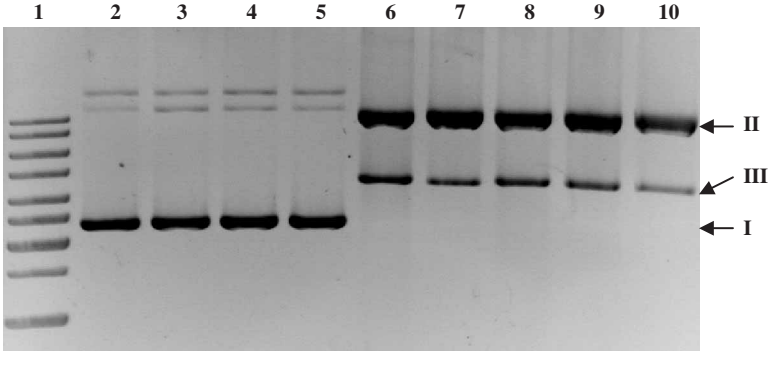

Figure 2. Agarose (1%) gel electrophoretic banding patterns of **plasmid pBR322** ($0.0125\ \mu g\ \mu L^{-1}$) treated with various concentration of $Cu(dmg)_2$ in the presence of **0.5 mM H_2O_2**, and **TN buffer pH 8.3**. Lane **1**, Gene ruler 1 kb DNA ladder; Lane **2**, pBR322 control; lane **3**, pBR322 + $Cu(dmg)_2$ (3.0 mM); lane **4**, pBR322 + 0.5 mM H_2O_2; lane **5**, pBR322 + 1.8 mM $CuCl_2$; lane **6**, pBR322 + 1.8 mM $CuCl_2$ + 0.5 mM H_2O_2; lanes **7**–**10**, plasmid pBR322 + $Cu(dmg)_2$ in which the complex concentration is 1.00 mM, 1.10 mM, 1.20 mM, and 1.80 mM, respectively. Form **II** represents nicked circular (nc) pBR322, Form **I** represents supercoiled or closed circular pBR322 (cc) and Form **III** represents the linear pBR322.

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experiments (lanes **1**–**5**), viz. (i) incubating DNA alone, or (ii) the incubation of the DNA with either the complex or $CuCl_2$ or only H_2O_2, showed no apparent DNA cleavage or variation in the band intensity. The necessity of both the copper(II) complex and H_2O_2 for DNA scission suggests an oxidative mechanism. This requirement contrasts with copper(II) complexes which need a coreductant, such as ascorbate and mercaptoethanol, for oxidative cleavage of DNA [5, 6, 17–19]. In the latter studies, it was suggested/established that the copper(I) complex (formed from reduction of the copper(II) complex) reacted with H_2O_2 to yield the hydroxyl free radicals ($^{\bullet}OH$) which cause DNA scission. Similar activation of H_2O_2, *via* a Fenton-like mechanism, occurs for copper(II) complexes of oligopeptides containing histidyl residue in the second or third position; a reductant (ascorbic acid or cysteine) is necessary for these complexes to cause scission of DNA in the presence of H_2O_2 [20]. However, $[Cu(en)_2]^{2+}$ is known to directly react with H_2O_2 to yield $^{\bullet}OH$ radicals [21], as can copper(II) complexes of histidine-containing peptides [22]; thus, both are potential nucleases. In addition, copper(II) complexes of aminoglycosides can cleave RNA and DNA in the presence of H_2O_2 alone; here, the processes yielding $^{\bullet}OH$ radicals proceed *via* copper(II) oxidation to copper(III) species [23]. Although oxidative cleavage of DNA by copper(II) complexes may not require H_2O_2 [24–27], a reductant may be necessary for strand scission as is observed for a trinuclear copper(II) complex where it is suggested that an active oxidant is formed by reduction of copper(II) and its subsequent reaction with dioxygen [28]. Thus, always attributing production of $^{\bullet}OH$ radicals to a Fenton-type mechanism may not be reasonable unless evidence is obtained. On the other hand, DNA cleavage by a copper(II) chelated amino acid can occur *via* a hydrolytic mechanism, as found for a copper(II)-L-histidine complex [13]. This hydrolytic cleavage of DNA is not likely for, $Cu(dmg)_2$, as H_2O_2 is necessary, and because it has been recently reported that copper ions bound to amino acids can interact with H_2O_2 to produce $^{\bullet}OH$ radicals [29].

Increasing the concentration of complex from 1 mM to 1.8 mM (figure 2, lanes **7**–**10**) did not seem to show appreciable increase in DNA cleavage as evidenced by the intensity of both the nicked and linear bands. However, extensive cleavage of DNA was observed when the H_2O_2 concentration was increased from 0.5 to 2 mM (figure 3).

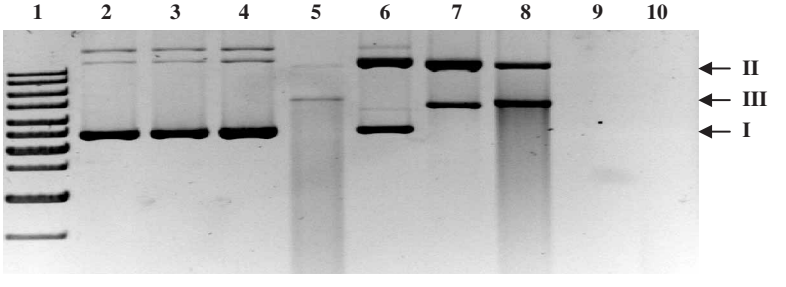

Figure 3. Agarose (1%) gel electrophoretic banding patterns of **plasmid pBR322** ($0.0125\,\mu g\,\mu L^{-1}$) treated with various concentration of $Cu(dmg)_2$ in the presence of **2 mM H_2O_2**, and **TN buffer pH 8.3**. Lane **1**, Gene ruler 1 kb DNA ladder; Lane **2**, pBR322 control; lane **3**, pBR322 + $Cu(dmg)_2$ (3.0 mM); lane **4**, pBR322 + 2 mM H_2O_2; lane **5**, pBR322 + 3 mM $CuCl_2$ + 2 mM H_2O_2; lanes **6**–**10**, plasmid pBR322 + $Cu(dmg)_2$ in which the complex concentration is 10 μM, 50 μM, 100 μM, 300 μM and 3.0 mM, respectively. Form **II** represents nicked circular (nc) pBR322, Form **I** represents supercoiled or closed circular pBR322 (cc) and Form **III** represents the linear pBR322.

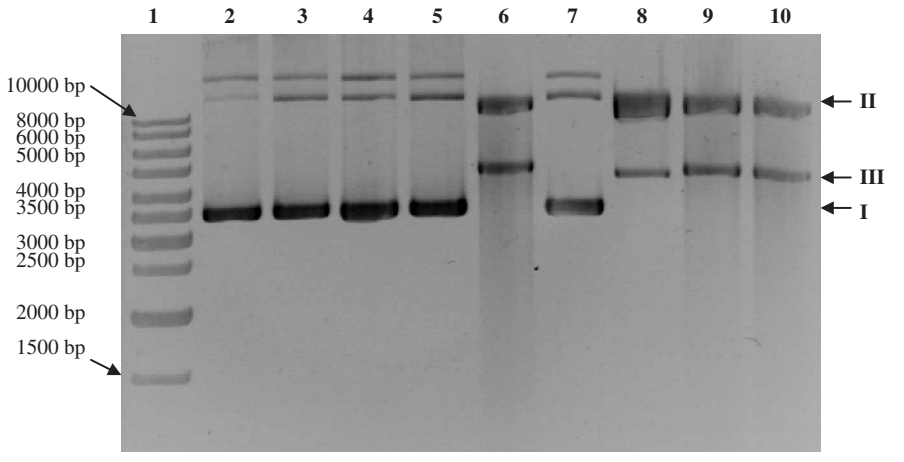

Figure 4. Agarose (1%) gel electrophoretic banding patterns of **plasmid pBR322** ($0.0125\,\mu g\,\mu L^{-1}$) treated with various concentration of $Cu(dmg)_2$ in the presence of **0.5 mM H_2O_2**, and TBE buffer pH 8.3. Lane **1**, Gene ruler 1 kb DNA ladder; lane **2**, pBR322 control; lane **3**, pBR322 + $Cu(dmg)_2$ (3.0 mM); lane **4**, pBR322 + 0.5 mM H_2O_2; lane **5**, pBR322 + 1.8 mM $CuCl_2$; lane **6**, pBR322 + 1.8 mM $CuCl_2$ + 0.5 mM H_2O_2; lanes **7**–**10**, plasmid pBR322 + $Cu(dmg)_2$ in which the complex concentration is 1.00, 1.10, 1.20, and 1.80 mM, respectively. Form **II** represents nicked circular (nc) pBR322, Form **I** represents supercoiled or closed circular pBR322 (cc) and Form **III** represents the linear pBR322.

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The complex, at a concentration of 300 μM, caused total degradation of the DNA (figure 3, lane **9**). In addition, complete conversion of supercoiled plasmid to nicked and linear forms could now be effected at a much lower concentration of complex (figure 3, lane **6** at 50 μM), and 10 μM of complex could caused appreciable single strand cleavage of the double-stranded supercoiled plasmid DNA. Using this higher concentration of H_2O_2, increase in concentration of complex showed more clearly increasing cleavage of DNA.

The above results demonstrate the effect of both the concentrations of the complex and H_2O_2 on the nucleolytic property of the complex. Insufficient concentration of copper(II) complex may be the reason for the inability of [Cu(N-quinoli-8-yl-*p*-toluenesulfonamidate)$_2$] to cleave DNA in the presence of H_2O_2, with the complex concentration in the range 100–500 μM [30]. Within this concentration range, $CuCl_2$ is a potent DNA nuclease on DNA plasmid pUC18 [30].

3.3.2. Effect of the type of buffer and pH. When 0.5 mM H_2O_2 was used, change of TN buffer pH 8.3 (figure 2) to TBE buffer pH 8.3 (figure 4) did not show any distinct difference in DNA cleavage by the copper(II) complex. The cleavage study of copper(II) complex on the DNA (figure 5) was then repeated with TN buffer pH 7, TN buffer pH 8.3 and with TBE buffer pH 8.3 in the presence of 2 mM H_2O_2 to investigate any effect due to a change in pH and to ascertain the effect due to the type of buffer (non-EDTA and EDTA-containing buffers). TBE buffer contains the disodium salt of ethylenediaminetetra-acetic acid (now, abbreviated as Na_2H_2Y, where Y^{4-} is the ethylenediaminetetra-acetate anion). At each complex concentration tested, the copper(II) complex (concentration used: 10, 50, and 100 μM) caused greater cleavage of plasmid pBR322 in the presence of H_2O_2 (2 mM) in TN buffers at both pH 7 and pH 8.3 compared to that when TBE buffer (pH 8.3) was used (figure 5).

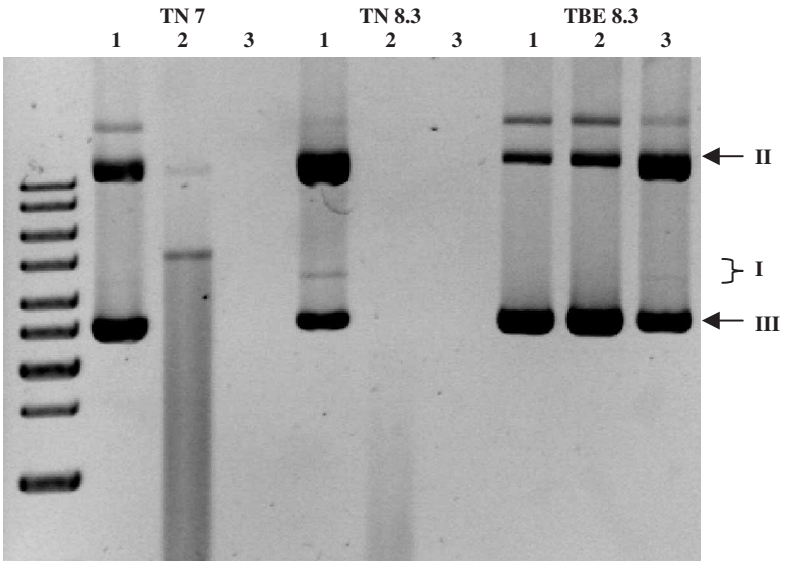

Figure 5. Effect of **different buffers and pH** on pBR322 ($0.0125\,\mu g\,\mu L^{-1}$) cleavage by increasing concentration of complex in presence of H_2O_2 (2 mM): (a) TN buffer pH 7: lane **1** (10 μM $Cu(dmg)_2$), lane **2** (50 μM complex), lane **3** (100 μM $Cu(dmg)_2$); (b) TN buffer pH 8.3: lane **1** (10 μM $Cu(dmg)_2$), lane **2** (50 μM $Cu(dmg)_2$), lane **3** (100 μM $Cu(dmg)_2$); (c) TBE buffer pH 8.3: lane **1** (10 μM $Cu(dmg)_2$), lane **2** (50 μM $Cu(dmg)_2$), lane **3** (100 μM $Cu(dmg)_2$). Unlabelled lane is Gene ruler 1 kb DNA ladder.

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In TBE buffer, there was no apparent cleavage or less cleavage by the complex at 10 and 50 μM (refer to lanes **1** and **2** for each buffer). 100 μM of the complex could totally degrade the DNA in the TN buffers at both pH's while the same concentration of complex in TBE buffer merely nicked some of the supercoiled plasmid DNA (figure 5, lane **3**). The apparent quenching effect of TBE buffer may be attributed to complexation of the complex by hexadentate Y^{4-} anion to yield the negatively charged ternary complex, which cannot bind to negatively charged DNA. Thus comparison of cleavage properties of different complexes should take into account the type of buffer. Finally, the complex at 50 μM in TN buffer pH 8.3 totally degraded the supercoiled plasmid while the same concentration of complex in TN buffer pH 7 had lower nucleolytic efficiency as both the nicked and linear bands were clearly visible (figure 5, lane **2**). The effect of different pH values on the nucleolytic property of the complex is thus clearly demonstrated.

3.3.3. Possible mechanism. The postulated mechanism for cleavage by the $Cu(dmg)_2$ complex must consider the fact that its nuclease activity is dependent on the presence of both the complex and H_2O_2. This fact alone suggests reaction of the complex with H_2O_2 to generate reactive oxygen species for DNA cleavage, in contrast to hydrolytic cleavage by copper(II)-L-histidine complex [13]. Nevertheless, the results of the preceding section, although not exhaustive, clearly demonstrate that the extent of cleavage is influenced by the type of buffer (TN or TBE), and the concentration of complex. For example, the cleavage of DNA in TN buffer pH 8.3 in the presence of 2 mM H_2O_2 increases with increasing concentration of complex.

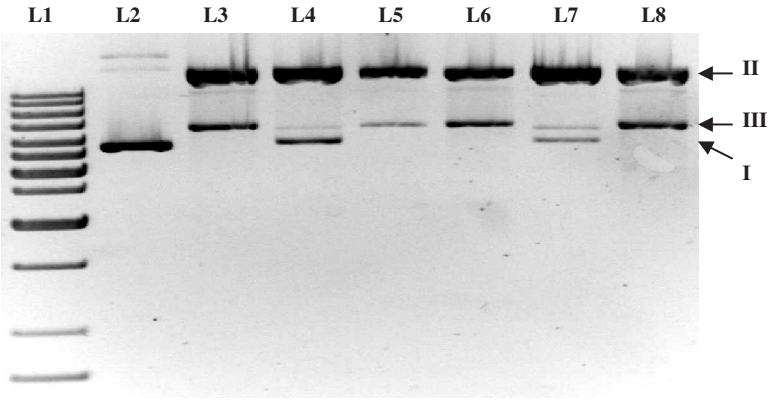

Figure 6. Effect of **hydroxyl radical scavengers** on the cleavage of pBR322 ($0.0125\,\mu g\,\mu L^{-1}$) by $Cu(dmg)_2$ ($50\,\mu M$) in the presence of H_2O_2 (2 mM) in TN buffer pH 8.3. Lane **1**, Gene Ruler 1 kb DNA ladder; lane **2** DNA alone; and lane **3**, pBR322 + $Cu(dmg)_2$ + 2 mM H_2O_2. Reaction of complex with DNA and H_2O_2 in the presence of various scavengers; lane **4**, 2.8 M DMSO; lane **5**, 4 μL ter-BuOH (2.2 M); lane **6**, 5 mM NaN_3; and lane **7**, 5 mM KI; lane **8**, 5 mM Mannitol.

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Consistent with previous studies, the $Cu(dmg)_2$ needs to bind to the DNA and react with H_2O_2 to yield an active species for cleaving the DNA on site. Earlier ESR studies by Chikira *et al.* have provided evidence for binding of other copper(II) amino acid complexes to DNA [30]. Such binding for our complex can thus be reasonably assumed. To verify the role of $^{\bullet}OH$ radicals, some radical scavengers were used to inhibit the cleavage of pBR322 by the $Cu(dmg)_2$ complex in the presence of H_2O_2.

Among the $^{\bullet}OH$ radical quenchers (t-butanol, sodium azide, D-mannitol and DMSO), DMSO at 2.8 M apparently induced the greatest inhibition (figure 6). The H_2O_2 quencher, KI, at a concentration of 5 mM prevented total nicking of supercoiled DNA and inhibited conversion of nicked DNA to linear DNA. An earlier investigation also revealed that copper ions bound to other amino acids could react with H_2O_2 to produce $^{\bullet}OH$ radicals [31], and thus a similar radical-based mechanism may operate for the DNA cleavage by the $Cu(dmg)_2$ in the presence of H_2O_2.

Supplementary material

Detailed crystallographic data for the crystal structure analysis have been deposited with the Cambridge Crystallographic Data Center, CCDC No. 210052.

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