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A Trinuclear Copper(II) Complex of 2,4,6-Tris(di-2-pyridylamine)-1,3,5-triazine Shows Prominent DNA Cleavage Activity

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A highly water soluble 3:2 complex of copper(II) and 2,4,6-tris(di-2-pyridylamine)-1,3,5-triazine (TDAT) has been synthesized and structurally characterized. The complex crystallized in a triclinic *P*1 space group with a molecular formula of $[Cu_3(TDAT)_2Cl_3]Cl_3 \cdot 2H_2O$ (**1**), where each copper ion is coordinated by four pyridine nitrogen atoms and an apical chloride. The trinuclear complex is stable at physiological relevant conditions. It can bind to DNA through electrostatic attraction and cleave efficiently the supercoiled pBR322 DNA into its nicked and linear forms at micromolar concentrations. Active oxygen intermediates such as hydroxyl radicals and singlet oxygen generated in the presence of **1** may act as active species for the DNA scission.

Introduction

There is a huge scope in the designs of DNA cleavage reagents that have many potential applications in molecular biology, biotechnology, and medicine.[1−3] Since the discovery of the first chemical nuclease,[4] considerable efforts have been made to design metal complexes as nuclease mimics because of their advantages in electronical and structural diversities.[1c,5,6] The redox properties of the metal center(s) in complexes are essential to generate reactive oxygen species (ROS) for DNA cleavage.[1a,7] Moreover, the smaller size of synthetic nucleases as compared with natural ones facilitates their reach to sterically hindered regions of DNA.[8] Copper complexes have been extensively studied and documented because of their biologically accessible redox potential and relatively high affinity for nucleobases.[1a,7,9] For example, bis(1,10-phenanthroline)-copper(II) complex was proved to have high nucleolytic efficiency and has been widely used as a footprinting reagent of nucleic acids and proteins.[10] Recent studies demonstrated that some multinuclear copper(II) complexes can efficiently promote DNA cleavage by selectively oxidizing deoxyribose or nucleobase moieties.[11] The

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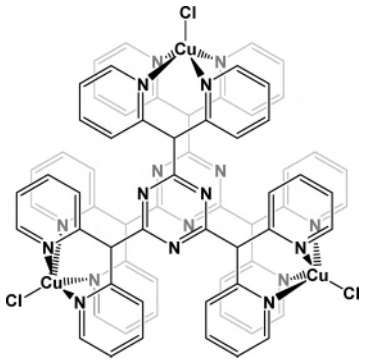
design of ligand plays an important role in achieving the selectivity.[9b] Polypyridylamine-derived ligands have received particular interest for their ability to coordinate metal ions, and an array of multinuclear complexes of these ligands has been developed. Many of them displayed versatile DNA cleavage properties in the absence or presence of a redox agent.[8,9b,g,11,12] In some multinuclear copper complexes, the copper centers were found to act synergistically in DNA cleavage to realize higher efficiency or selectivity.[11,12b,13]

Derivatives of 1,3,5-triazine have exhibited promising potential as antitumor agents,[14] enzyme inhibitors,[15] and other bioactive agents.[16] However, their metal complexes received little attention for biological applications partly due to their poor solubility in both water and common organic solvents.[17] Recently, trinuclear copper(II) complexes of 2,4,6-tris(di-2-pyridylamine)-1,3,5-triazine (TDAT) were used as building blocks for metallo-supramolecular assemblies,[18] but the biological properties of these compounds remain unknown. We previously reported a polypyridyl-based trinuclear copper(II) complex that displayed an efficient oxidative ability in DNA cleavage in the presence of ascorbate.[19] However, poor water solubility of the complex impedes its further biological investigations. More recently, we synthesized bi- and trinuclear copper complexes of bis(2-pyridylmethyl)-amine linked by *m*- and *p*-xylene and mesitylene, which are water soluble and show structurally dependent DNA cleavage activities.[20] As a part of these studies, we report herein a TDAT-based trinuclear copper(II) complex (Chart 1) that shows significant DNA cleavage activity in the presence of different redox agents.

Chart 1. Chemical Structure of the Cation of Trinuclear Copper(II) Complex **1**



Experimental Section

Materials and Measurements. Plasmid pBR322 was purchased from MBI Fermentas and purified before use according to the published method.[12c] The disodium salt of calf thymus DNA (CT-DNA), tris(hydroxymethyl)aminomethane (Tris), ethidium bromide (EB), methyl green, and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma. The purity of DAPI was checked by ^{1}H NMR, and its concentration was measured by UV−vis spectroscopy (ϵ_{340} 2.7 × 10^4 M^{-1} cm^{-1}).[21] Other chemicals and solvents such as methanol, dichloromethane, and anhydrous diethyl ether were of analytical or chromatographical grade and were used without further purification. Double distilled water was used to prepare solutions for DNA measurement and scission experiment. The concentration of nucleotide and the purity of CT-DNA were determined by UV−vis spectroscopy.[22,23]

Infrared spectra were recorded on a Bruker VECTOR22 spectrometer in KBr pellets over a range of 400−4000 cm^{-1}. Elemental analyses were performed on a Perkin-Elmer 240C analytic instrument. Electrospray mass spectra were recorded using an LCQ electron spray mass spectrometer (ESMS, Finnigan). The isotopic distribution patterns for the complex were simulated using the Isopro 3.0 program.[24] UV−vis spectra were recorded on a Perkin-Elmer Lambda 35 spectrometer. Circular dichroism (CD) spectra were recorded on a Jasco J-810 spectropolarimeter in a 1 cm path length cylindrical quartz cell at room temperature. Fluorescence spectra were recorded using an AMINCO Bowman series 2 luminescence spectrometer. Electronic paramagnetic resonance (EPR) spectra were taken at the X- and Q-band frequencies at room temperature with a Bruker ELEXSYS spectrometer. The pH measurements were carried out on a PHS-3C pH meter equipped with a Phonix Ag−AgCl reference electrode that was calibrated with standard pH buffer solutions. The gel imaging was assessed using Labworks Imaging and Analysis Software (UVP, Inc., Upland, CA).

Syntheses. The ligand TDAT was prepared according to the procedure described in the literature.[25] The trinuclear copper complex was prepared as follows: A solution of $CuCl_2 \cdot 2H_2O$ (174 mg, 1.02 mmol) in methanol (15 mL) was added dropwise to a stirred solution of TDAT (200 mg, 0.34 mmol) in dichloromethane

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(25 mL) at room temperature. Dark blue crystalline solid was formed after one week, which was filtered and washed with cold methanol/diethyl ether (yield: 302.6 mg, 55%). This solid was recrystallized in water/methanol (v/v, 5:2), resulting in dark blue crystals suitable for X-ray crystallographic analysis. ES-MS: *m*/*z* (calcd.), 497.3 (497.46), $[Cu_3(C_{33}H_{24}N_{12})_2Cl_6 - 3Cl^- + H_2O]^{3+}$; 754.7 (754.9), $[Cu_3(C_{33}H_{24}N_{12})_2Cl_6 - 2Cl^-]^{2+}$; 1545.3 (1545.2), $[Cu_3(C_{33}H_{24}N_{12})_2Cl_6 - Cl^-]^+$. Elemental analysis; Found (Anal. Calcd) for $C_{66}H_{52}Cl_6Cu_3N_{24}O_2$ (**1**): C, 48.83 (49.03); H, 3.18 (3.24); N, 20.47 (20.79). FT-IR spectra (ν, cm^{-1}): 3447.9 (br, s), 1606.5 (m), 1552.9 (m), 1481.9 (m), 1467.8 (m), 1486.4 (s), 1301.5 (w), 773.1 (w).

Crystal Structure Determination. The single crystal of **1** was mounted on glass fibers, and the X-ray diffraction intensity data were measured at 293 K on a Bruker Smart Apex CCD area detector fitted with graphite-monochromated Mo Kα radiation (0.71073 Å) by a 4-ω scan. The collected data were reduced using the SAINT program, and an empirical absorption correction was carried out using the SADABS program. The structure was solved by direct methods using the SHELXTL-XS program. Refinement was conducted with the full-matrix least-squares method on F^2 using the SHELXTL-XL program for all data with anisotropic thermal parameters for non-hydrogen atoms and isotropic parameters for hydrogen atoms.[26]

Crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Center, CCDC No. 259834. Any inquiries relating to the data can be e-mailed to deposit@ccdc.cam.ac.uk.

Interaction of Complex 1 with DNA. CT-DNA was dissolved in buffer solution (5 mM Tris/40 mM NaCl, pH 7.40) as stock solution, which was stored at 4 °C and used within 4 days. The solution gave a UV absorbance ratio (A_{260}/A_{280}) of ca 1.8, indicating that CT-DNA was sufficiently protein-free.[22] The concentration of CT-DNA solution was determined by UV absorbance at 260 nm after proper dilution with water, taking 6600 M^{-1} cm^{-1} as the extinction coefficient (ϵ_{260}).[23] Complex **1** was resolved in the above buffer solution (2.71×10^{-5} M) and titrated with CT-DNA aqueous solution (2.5×10^{-3} M). The changes in absorbance at 285.5 nm for **1** with increasing amounts of CT-DNA were recorded, and the intrinsic binding constant (K_b) for the reaction was determined by the following equation:[27]

$$[DNA]/(\epsilon_a - \epsilon_f) = [DNA]/(\epsilon_b - \epsilon_f) + 1/K_b(\epsilon_b - \epsilon_f)$$

The CD spectra were recorded in the range of 220−320 nm with increasing molar ratio of **1**/CT-DNA (0−0.14, [CT-DNA] = 1.033×10^{-4} M). The average of three independent scans was taken as the final CD spectrum of each sample after the buffer background was subtracted.

The fluorescence spectra were recorded at room temperature ($\lambda_{ex} = 526$ nm, $\lambda_{em} = 600$ nm). The experiment was carried out by titrating complex **1** (2.68×10^{-3} M, 5 mM Tris-HCl/40 mM NaCl, pH 7.40) into EB−CT-DNA (1:1, 2.68×10^{-5} M, 3 mL) aqueous solution.

DNA Cleavage by Complex 1. A typical cleavage assay was carried out as follows: pBR322 DNA (1 μL, 100 ng/μL), buffer (8 μL, 5 mM Tris-HCl/40 mM NaCl, pH 7.40), and **1** (8 μL, 10 μM) in the same buffer were mixed with H_2O_2 (3 μL, 2.65 mM). The sample was incubated at 310 K for an appropriate time and then quenched by addition of sodium diethyldithiocarbamate trihydrate and loading buffer (0.25% bromphenol blue, 50% glycerol). The solution was then subjected to electrophoresis on 0.7% agarose gel in TAE buffer (40 mM Tris acetate/1 mM EDTA) at 100 V. The electrophoresis bands were visualized by EB staining under UV light. In all cases, the background fluorescence was subtracted. A correction factor of 1.47 was used for supercoiled DNA (Form I) assessment since the intercalation between EB and Form I DNA is relatively weak compared with that of nicked (Form II) and linear DNA (Form III).[9c] All results are the average of triplicate experiments. The dioxygen-dependent DNA scission was conducted as described above except that solutions were degassed by bubbling with prepurified nitrogen for 15 min before mixing. The mixture was kept under nitrogen throughout the DNA scission process.

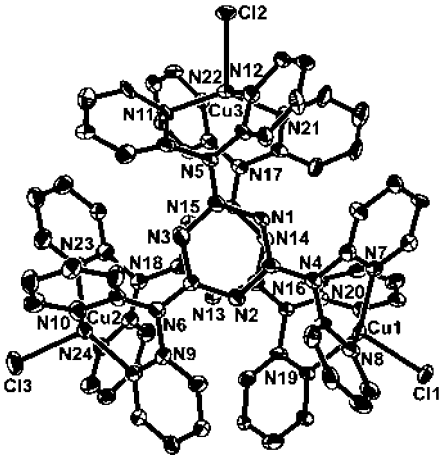

Figure 1. ORTEP plot (30%) for the cationic core of complex **1**. The carbon atom labelings and hydrogen atoms are omitted for clarity.

DNA Cleavage in the Presence of Radical Scavenger. Scavenging agent DMSO (6 μL, 20 mM), *tert*-butyl alcohol (10 μL, 20 mM), potassium iodide (10 μL, 20 mM), or sodium azide (10 μL, 20 mM) was added respectively to the solution of supercoiled DNA (1 μL, 100 ng/μL) prior to the addition of **1** (4 μL, 20 μM) and H_2O_2 (3 μL, 2.65 mM) in the buffer (5 mM Tris-HCl/40 mM NaCl, pH 7.40). The mixture was diluted with the buffer to a total volume of 20 μL. The reaction was initiated, quenched, and analyzed according to the procedures described above.

Results and Discussion

Crystal Structure of Complex 1. The trinuclear copper complex of TDAT was prepared by mixing copper(II) chloride with TDAT in 3:1 molar ratio in methanol/dichloromethane at room temperature. The structural analysis revealed that **1** crystallizes in a triclinic *P*1 space group with a molecular formula of $[Cu_3(TDAT)_2Cl_3]Cl_3 \cdot 2H_2O$. An ellipsoid plot for the crystal structure of the cationic core in **1** is shown in Figure 1. The detailed crystal data and refinement parameters are listed in the Supporting Information, Table S1. The selected bond lengths and angles of the complex are given in the Supporting Information, Table S2.

In the core of **1,** two TDAT ligands are stacked in parallel and linked together by three Cu(II) ions, and two triazine centroids are rotated by ca. 30° with respect to each other. The arrangement of **1** is somewhat different from the perfect face-to-face mode in its analogue $[Cu_3(TDAT)_2Cl_3][CuCl_4]$-Cl reported previously.[18a] The distance between the two

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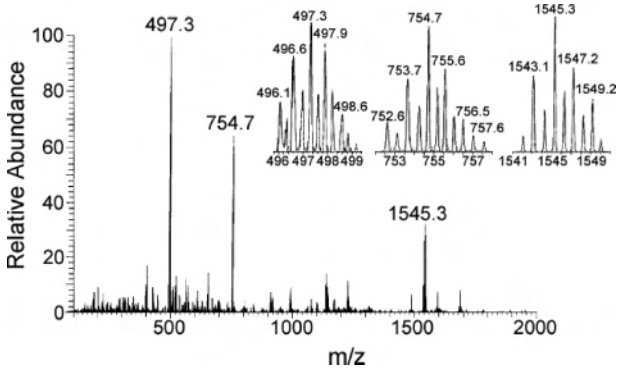

Figure 2. Electrospray mass spectra and the isotope distribution patterns for complex **1**. Attributions: 497.3, $[Cu_3(C_{33}H_{24}N_{12})_2Cl_6 - 3Cl^- + H_2O]^{3+}$; 754.7, $[Cu_3(C_{33}H_{24}N_{12})_2Cl_6 - 2Cl^-]^{2+}$; 1545.3, $[Cu_3(C_{33}H_{24}N_{12})_2Cl_6 - Cl^-]^+$.

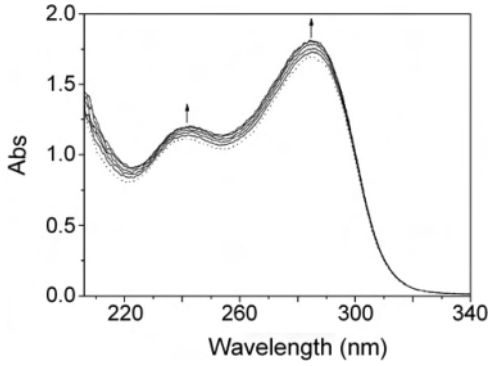

Figure 3. Variations of UV−vis absorption for **1** (2.71×10^{-5} M) in the absence (dashed line) and presence (solid line) of CT-DNA (2.5×10^{-3} M, 10 μL per scan) in buffer (5 mM Tris-HCl/40 mM NaCl, pH 7.40) at room temperature.

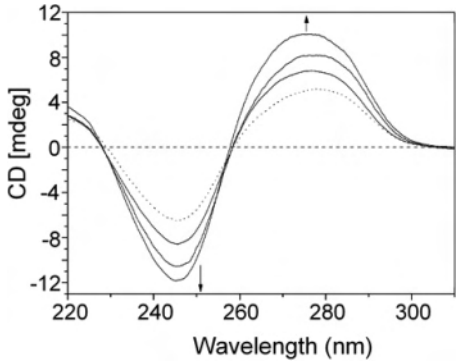

Figure 4. CD spectra of CT-DNA (1.033×10^{-4} M) in the absence (dashed line) and presence (solid line) of **1** ($0-1.4 \times 10^{-5}$ M) in buffer (5 mM Tris-HCl/40 mM NaCl, pH 7.40) at room temperature.

triazine planes in **1** is 3.67 Å, which is larger than that found for aromatic stacking (3.5 Å) but smaller than that in $[Cu_3(TDAT)_2Cl_3][CuCl_4]Cl$ (3.78 Å).[18a] Each Cu(II) center is coordinated by an apical chloride and four pyridine nitrogen atoms from two ligands, taking on a square-pyramidal geometry. Additionally, each asymmetrical unit cell of **1** contains two disordered water molecules that occupy five different positions with occupancies of 0.38466, 0.40558, 0.42187, 0.38754, and 0.40031, respectively. The EPR spectrum and magnetic susceptibility of complex **1** (Supporting Information, Figures S2 and S3) are in accordance with the crystallographic structure characteristics.

Solution Properties of Complex 1. Complex **1** exhibits a high solubility in water; in contrast, most metal-TDAT analogues have poor solubility in common solvents.[17] The water solubility may facilitate the investigations of **1** in aqueous solutions. As shown in Supporting Information, Figure S1, there are two intense $\pi \rightarrow \pi^*$ or charge-transfer transitions at 285.5 nm ($\epsilon = 6.28 \times 10^4$ dm^3 mol^{-1} cm^{-1}) and 242 nm ($\epsilon = 3.65 \times 10^3$ dm^3 mol^{-1} cm^{-1}) in the UV−vis spectra of complex **1** in buffer solution (1.72×10^{-5} M, 5 mM Tris-HCl/40 mM NaCl, pH 7.40). These characteristic bands and their absorption intensities remain unchanged for 8 h, which may suggest that the complex is stable in aqueous solution. The stability of **1** in aqueous solution was also supported by the ES-MS results (Figure 2), where three Cu(II) and two TDAT species existed as a whole entity.

Interaction between DNA and Complex 1. UV−vis and CD spectroscopy were employed to study the interaction between **1** and CT-DNA. As demonstrated in Figure 3, upon addition of CT-DNA ($0-3.34 \times 10^{-5}$ M) to the aqueous solution of **1** (2.71×10^{-5} M), a moderate hyperchromicity was observed in the UV spectra, which is similar to that observed in other trinuclear copper(II) complexes interacting with DNA.[19] The hyperchromicity implies that some interaction other than intercalation occurred between **1** and DNA, because intercalation would lead to hypochromism and bathochromism in UV absorption spectra.[28] The absorption plateaued when the concentration of DNA reached 3.34×10^{-5} M and remained unchanged within 8 h. The intrinsic binding constant (K_b) for **1** to CT-DNA was calculated to be 7.8×10^3 M^{-1}, a value significantly lower than those obtained for typical intercalators (e.g., EB−DNA, $\sim 10^6$ M^{-1}),[28,29] indicating the affinity of **1** for DNA is relatively low. Judging by the changing tendency of the UV spectra and the binding constant, the intercalative binding mode appears unlikely.

Ligand TDAT or Cu(II) ion alone did not show any sign of binding to CT-DNA in the UV−vis spectra under the same conditions.

In the CD spectra, the addition of **1** to the solution of CT-DNA induced an increase in intensity for both positive and negative ellipticity bands (Figure 4), suggesting that the stacking mode and the orientation of base pairs in DNA were disturbed.[27,30] However, the B-form character of CT-DNA was still maintained and, hence, the disturbance is mild. Again, intercalative interaction with DNA is unlikely because this binding mode would cause a characteristic decrease in both positive and negative bands.[31]

An EB replacement assay was carried out to further exclude the possibility of the intercalative binding mode. EB is an intercalator that gives a significant increase in fluorescence emission when bound to DNA, and its displacement

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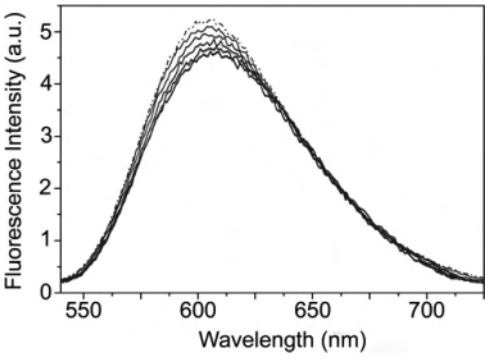

Figure 5. Fluorescence emission spectra of the EB−CT-DNA system (1: 1, 2.68×10^{-5} M) in the absence (dashed line) and presence (solid line) of complex **1** (2.68×10^{-3} M, 10 μL per scan).

from DNA results in a decrease in fluorescence intensity.[32] As shown in Figure 5, the addition of **1** to the EB−DNA system did not induce notable fluorescence changes at 605 nm, which indicated that EB molecules could not have been replaced by complex **1**, and therefore, the intercalative binding mode can be ruled out.

Many properties of metal complexes, such as size, charge, shape, and chirality could influence the binding mode and modification extent to DNA.[33] Taking the above UV−vis, CD, and fluorescence spectroscopic data together, a covalent binding to DNA appears unlikely for **1**. In fact, cationic complexes involving ligands with extended hydrophobic regions or surfaces usually bind to DNA noncovalently.[27,34] External static electronic effects, intercalation, and groove binding are three major noncovalent binding modes for small molecules interacting with DNA.[9h,12c] The cationic core of **1** could exert a strong electrostatic attraction to the anionic phosphate backbone of DNA; thus, the electrostatic binding mode is highly possible. This inference will be further demonstrated in the following section.

DNA Cleavage by Complex 1. The DNA cleavage ability of **1** was studied by agarose gel electrophoresis using supercoiled pBR322 plasmid DNA as a substrate. The activity of **1** was assessed by the conversion of DNA from Form I to Form II or Form III. A concentration-dependent DNA cleavage by **1** was first performed. Briefly, pBR322 DNA was mixed with different concentrations of **1** in aqueous buffer solution (5 mM Tris-HCl/40 mM NaCl, pH 7.40) using H_2O_2 as an activator, and the mixture was incubated at 310 K for 30 min. With the increase of concentration of **1**, DNA was converted from Form I to Form II and then to Form III. Direct double-strand DNA cleavage was not observed in this case. The concentration-dependent cleavage activity of **1** is shown in Figure 6. The amounts of Form I DNA decreased whereas those of Form II increased with the increase of concentration (lanes 3 and

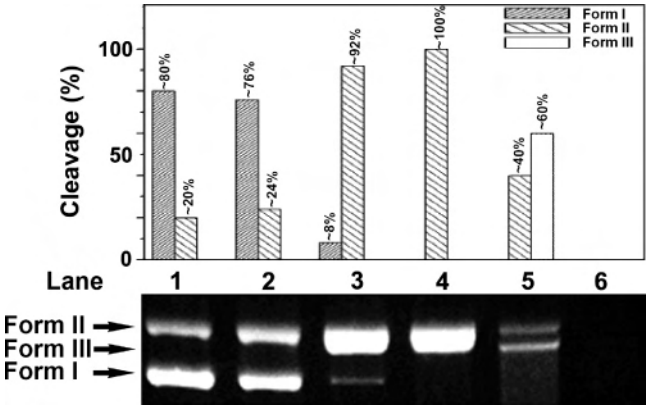

Figure 6. The cleavage patterns of the agarose gel electrophoresis and the corresponding cleavage extent (%) for pBR322 plasmid DNA (100 ng) by **1** in buffer (5 mM Tris-HCl/40 mM NaCl, pH 7.40) at 310 K after 30 min of incubation. Lane 1, DNA control; lane 2, DNA + 0.4 mM H_2O_2; lanes 3−6 represent the DNA cleavage status at 2, 4, 6, and 8 μM of **1** in the presence of 100-fold excess of H_2O_2, respectively.

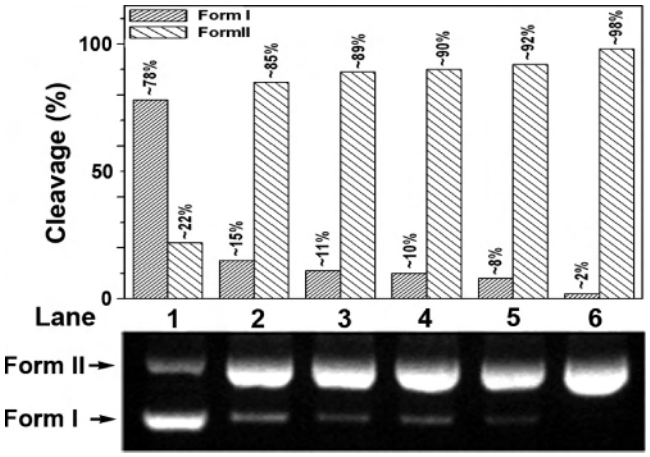

Figure 7. Time-dependent agarose gel electrophoresis patterns and histogram representation for the pBR322 plasmid DNA (100 ng) cleavage by **1** (4 μM) over a period of 30 min in the presence of H_2O_2 (0.4 mM) at 310 K (5 mM Tris-HCl/40 mM NaCl; pH 7.40). Lane 1, DNA control; lanes 2−6, DNA + **1** + H_2O_2. Incubation times of lanes 2−6 equal 5, 10, 15, 20, and 30 min, respectively.

4). The conversion of DNA from Form I to Form II was almost completed (ca. 100%) at 4 μM, and Form III (ca. 60%) appeared at 6 μM (lane 5). When the concentration of **1** reached 8 μM, Form I was totally degraded into undetectable minor fragments (lane 6). Complex **1** or H_2O_2 alone did not exhibit DNA scission activity in the control experiments.

A time-dependent cleavage of DNA by **1** (4 μM) was also observed under similar conditions. As shown in Figure 7, the amounts of Form I DNA decreased and those of Form II DNA increased gradually with time until Form I DNA almost disappeared after incubation for 30 min.

The nuclease efficiency of copper(II) complexes is usually dependent on activators.[35−37] Thus, besides H_2O_2, other

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activators such as ascorbate (Asc), 3-mercaptopropionic acid (MPA), and glutathione (GSH) were also used to investigate the DNA cleavage activity of complex **1**. As shown in Supporting Information, Figure S4, the cleavage activity of **1** was significantly enhanced by these activators. Their activating efficacy follows the order of Asc ≈ MPA > GSH, which is similar to the observations on the nuclease activity of bis(*o*-phenanthroline)copper(II).[35] In a word, complex **1** exhibited a remarkable DNA cleavage activity with different activators.

Minor groove binding agent DAPI[38] and major groove binding agent methyl green[39] were used to probe the potential interacting site of complex **1** with supercoiled plasmid DNA. The supercoiled DNA was treated with DAPI or methyl green prior to the addition of **1**. The patterns presented in the Supporting Information, Figure S5, demonstrated that neither DAPI nor methyl green affected the DNA cleavage activity of **1**, suggesting that neither major nor minor grooves are the preferred reacting sites for the complex. In these circumstances, complex **1** may interact directly with exterior phosphates of DNA via electrostatic attraction. This assumption is in accord with the above proposed binding mode for complex **1** to DNA. The cationic core of **1** in aqueous solution could bind to the anionic phosphate backbone of DNA by electrostatic attraction. Such general affinity would enhance local concentration of **1** around DNA[12b] and consequently enhance the DNA cleavage activity.

Reactive Oxygen Species Responsible for DNA Cleavage. ROS generated during the interaction between copper complexes and dioxygen or redox reagents are believed to be a major cause of DNA damage.[9g,33b,40,41] To probe the potential mechanism of DNA cleavage mediated by complex **1**, some standard radical scavengers were used prior to the addition of **1** to DNA solution. The involvement of ROS was investigated using dimethyl sulfoxide (DMSO),[42] *tert*-butyl alcohol,[43] potassium iodide[44] as a hydroxyl radical scavenger, and azide[45] as a singlet oxygen scavenger. Diffusible hydroxyl radicals (·OH) are the most common reactive species that induce DNA cleavage by copper complexes. To examine whether ·OH is the key factor responsible for DNA cleavage in this case, experiments with DMSO were first carried out in detail under conditions similar to those described in the caption of Figure 7. As demonstrated in Figure 8, the DNA cleavage activity of **1** was reduced dramatically upon addition of DMSO in the presence of dioxygen (lane 5), indicating that ·OH may be involved in the cleavage process.

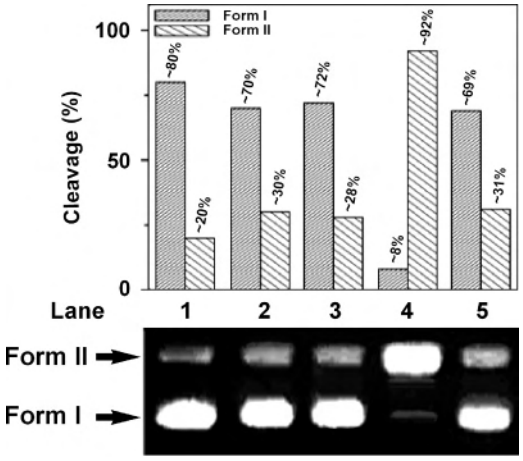

Figure 8. Agarose gel electrophoresis patterns and histogram representation for the cleavage of pBR322 plasmid DNA (100 ng) by **1** (4 μM) in the presence of standard radical scavenger DMSO (6 mM) in 5 mM Tris-HCl/40 mM NaCl buffer (pH 7.40) at 310 K after incubation for 30 min. Lane 1, DNA control; Lane 2, DNA + 0.4 mM H_2O_2; Lane 3, DNA + 0.4 mM H_2O_2 + DMSO; lane 4, DNA + 4 μM **1** + 0.4 mM H_2O_2; lane 5, DNA + 4 μM **1** + 0.4 mM H_2O_2 + DMSO.

Reactions in the absence of dioxygen and/or hydrogen peroxide were performed simultaneously. DNA was scarcely cleaved by **1** under such conditions (Supporting Information, Figure S6, lane 2, 3). The results indicated dioxygen is an indispensable cofactor for DNA scission and a hydrolytic cleavage mode is not involved.

Other hydroxyl radical scavengers such as *tert*-butyl alcohol, potassium iodide, and singlet oxygen scavenger sodium azide were also used to capture the active oxygen species involved in the cleavage process. In all these cases, the DNA cleavage activity of complex **1** was significantly inhibited (Supporting Information, Figure S7). The results further demonstrated that ·OH is the active oxygen species involved in the cleavage process and singlet oxygen or some singlet oxygen-like entities may also be the active oxygen intermediates responsible for the activity.

On the basis of the above observations, the mechanism of DNA cleavage mediated by complex **1** may be similar to that proposed for other multinuclear copper complexes.[11,12] In the mechanism, Cu(II) centers are initially reduced to Cu(I) species and subsequently react with dioxygen to form a peroxodicopper(II) derivative, which could generate active oxygen species needed for cleavage.

Conclusion

The trinuclear copper(II) complex derived from 1,3,5-triazine exhibits an effective DNA cleavage activity near physiologically relevant conditions in the presence of different activators. Synergistic effects of three copper(II) centers of complex **1** may contribute to the significant DNA cleavage activity. The cationic property of the complex in aqueous solution may be another factor that contributes to the high activity. Active oxygen intermediates such as hydroxyl radicals and singlet oxygen may play an important

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role in the cleavage mechanism. The complex discussed in this work appears to be the first example of a 1,3,5-triazine-based metal complexe showing such properties in aqueous solution. Further investigations into the cleavage mechanism are needed to develop more effective nucleases for this class of compounds.

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Supporting Information Available: X-ray crystallographic file in CIF format; EPR spectrum; plot of variable-temperature magnetic susceptibility; agarose gel electrophoresis patterns in the presence of major and minor binding agents, different activators, or standard radical scavengers; agarose gel electrophoresis patterns in the absence of dioxygen and/or H_2O_2. This material is available free of charge via the Internet at http://pubs.acs.org.

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