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Oxidative DNA Strand Scission Induced by a Trinuclear Copper(II) Complex

Chao Tu,[†] Ying Shao,[†,‡] Ning Gan,[†] Qiang Xu,[§] and Zijian Guo*[,†]

State Key Laboratory of Coordination Chemistry, Coordination Chemistry Institute, Nanjing University, 210093 Nanjing, P. R. China, Department of Chemistry, Ningbo University, 315211 Ningbo, P. R. China, and State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, 210093 Nanjing, P.R. China

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A novel trinuclear copper(II) complex, Cu_3-L (L = *N*,*N*′,*N*′,*N*′′,*N*′′-hexakis(2-pyridyl)-1,3,5-tris(aminomethyl)benzene), exhibited efficient oxidative strand scission of plasmid DNA. The solution behavior of the complex has been studied by potentiometric titration, UV spectroscopy, and cyclic voltammetry. The data showed that there are three redox-active copper ions in the complex with three types of bound water. The complex demonstrated a moderate binding ability for DNA. Cu_3-L readily cleaves plasmid DNA in the presence of ascorbate to give nicked (form II) and then linear (form III) products, while the cleavage efficiency using H_2O_2 is less than by ascorbate, suggesting that the cleavage mode of the trinuclear complex is somewhat different from the traditional Fenton-like catalysis. Meanwhile, Cu_3-L is far more efficient than its mononuclear analogue Cu-DPA (DPA = 2,2′-dipyridylamine) at the same [Cu^{2+}] concentration, which suggests a possible synergy between the three or at least two Cu(II) centers in Cu_3-L that contributes to its relatively high nucleolytic efficiency. Furthermore, the presence of standard radical scavengers does not have clear effect on the cleavage efficiency, suggesting the reactive intermediates leading to DNA cleavage are not freely diffusible radicals.

Introduction

Selective cleavage of DNA is of paramount interests in medicine and biotechnology.[1−4] Transition metal complexes stand out as candidates for artificial nucleases due to their diverse structural features and reactivities.[1] The redox properties of the metal center and dioxygen are utilized to generate reactive oxygen species that damage DNA by direct strand scission or base modification.[2]

The exploration of copper complexes as chemical nucleases is well documented because they possess biologically accessible redox potential and relatively high nucleobase affinity.[2] The most well-known and best studied example is $[Cu(OP)_2]^{2+}$ (OP = 1,10-phenanthroline),[3] which has been used extensively as a footprinting reagent for DNA, RNA, and important cellular processes.[4−6] In the presence of a standard reductive reagent such as 3-mercaptopropionic acid, it is reduced to Cu(I) and then binds to the minor groove of DNA and induces direct strand scission in the presence of hydrogen peroxide.[7] The reaction is initiated by the reduction

* Author to whom correspondence should be addressed. E-mail: zguo@nju.edu.cn. Tel: 86-25-83594549. Fax: 86-25-83314502.
† State Key Laboratory of Coordination Chemistry, Nanjing University.
‡ Ningbo University.
§ State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University.

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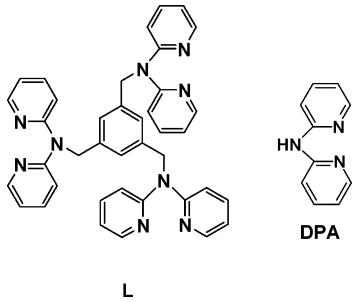
Chart 1. Schematic Drawing of Ligands Used

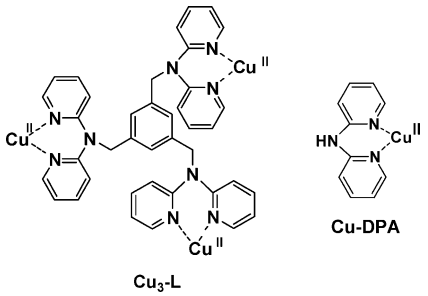


of $[Cu(OP)_2]^{2+}$ to $[Cu(OP)_2]^{+}$, the suggested binding form to the minor groove of DNA. The proposed mechanism for this process requires 2 mol equiv of Cu(II): one bound to the minor groove of DNA and the other remaining free[8] to produce a nondiffusible intermediate equivalent to hydroxyl radical to cleave one strand of the duplex.[1a,3] Because of this, formation of a dimer requires another 1 equiv of Cu(II) remains unbound in solution. If all available complexes are bound to DNA, the reactive intermediates such as peroxide, hydroperoxide, or hydroxyl radical cannot be generated. A similar process may occur with other mononuclear complexes.

Polynuclear copper complexes are attracting much attention currently,[9] because they offer the potential to form reactive intermediates without the need of an extra 1 equiv of the complex. They may also be capable of efficient intramolecular activation of bound O_2 and selective binding to particular conformations of nucleic acid.[9] In addition, incorporation of more than one Cu(II) center in a single complex produces enhanced electrostatic interactions to the anionic DNA phosphate backbone and facilitates its binding to DNA.[10] The Cu(II) centers in close proximity may undergo multielectron reductive cleavage of O_2 to generate an equivalent amount of hydroxyl radicals.[10] These features were excellently demonstrated by examples reported by Karlin et al. using polynuclear Cu(II) complexes.[10]

In this work we report the nuclease activity of a Cu(II) complex (Cu_3-L) with a multidentate ligand L (Charts 1 and 2) designed by our laboratory for the synthesis of polynuclear metal complexes.[11] The nuclease activity of mononuclear Cu(II) complex of 2,2′-dipyridylamine (DPA, Chart 1) is also studied to assess the potential synergistic effect of multicopper centers in Cu_3-L (Chart 2).

Chart 2. Schematic Drawing of Mono- and Trinuclear Copper Complexes



Experimental Section

Materials and Physical Methods. Plasmid pBR322 and Hind III nuclease were purchased from MBI Fermentas, and calf thymus (CT) DNA was from Sigma. The 2,2′-dipridylamine (DPA, Chart 1) was obtained from Fluka and ligand L (Chart 1) was synthesized as described previously.[11,12] All other chemicals were used as supplied. Solutions of the Cu(II) complexes and other reagents used for strand scission were prepared freshly in double distilled water before use. The preparation and nuclease activity of mononuclear analogue Cu-DPA[13] (Chart 2) was examined under conditions identical with those described for reactions of Cu_3-L[13] (Chart 2). The UV−vis spectra were recorded on a UV-3100 spectrometer. The potentiometric titration was performed on a PHS-3C pH meter, and the cyclic voltammetry of Cu_3-L was carried out on a BAS 100 electrochemical analyzer. The Gel Imaging and Documentation DigiDoc-It System (version 1.1.23, UVP, Inc. Unpland, CA) was assessed using Labworks Imaging and Analysis Software (UVP, Inc., Unpland, CA).

Potentiometric Titration of Cu_3-L. To an aqueous solution of Cu_3-L (1.0×10^{-4} M) which containing 1% DMF was added dropwise the solution of 0.1 M NaOH. Each data point was obtained by adding 2−20 μL of NaOH solution at 25 °C. The solution starts to turn turbid at pH above 8.

Electrochemistry. The redox potentials of Cu_3-L were determined by cyclic voltammetry method using conventional three-electrode system. A glass carbon electrode and a platinum wire were used as the working electrode and the counter electrode, respectively. A saturated calomel electrode (SCE), separated from the test solution by the electrolytic solution sandwiched between two fritted disks and calibrated using the ferrocene/ferrocenium redox couple, was used as the references electrode. The tetra-*n*-butylammonium bromide solution (TBAB, 0.1 M) was used as supporting electrolyte. The experiments were carried out in dried *N*,*N*-dimethylformamide solution of Cu_3-L (1 mM) under nitrogen atmosphere at room temperature.

Electronic Absorption Titration of Cu_3-L Binding to DNA. The solutions of CT DNA in 50 mM NaCl/5 mM Tris-HCl (pH = 7.3) gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of 1.8−1.9, indicating that the DNA was sufficiently free of protein.[14] The concentrated stock solution of CT DNA (stored at 4 °C and used not more than 4 days) was prepared in 5 mM Tris-HCl/50 mM NaCl in water, pH = 7.3, and the concentration of DNA was determined by UV absorbance at 260 nm after 1:100

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dilutions. The molar absorption coefficient of CT DNA was taken as 6600 M^{-1} cm^{-1}.[15]

A solution of 1.0×10^{-3} M Cu_3-L[13] in 10% DMF and 90% buffer (5 mM Tris-HCl/50 mM NaCl) was incubated for 2 h at 25 °C before DNA solution was added. The concentration of Cu_3-L was kept constant and eventually reduced to 10^{-5} M with the addition of increasing amounts of DNA (10^{-3} M), and the final volume of the solutions was fixed to 3 mL. The binding constant was determined using the following equation:[16]

$$[\mathrm{DNA}]/(\epsilon_A - \epsilon_B) = [\mathrm{DNA}]/(\epsilon_B - \epsilon_F) + 1/K_b(\epsilon_A - \epsilon_F), \quad (1)$$

Here ϵ_A, ϵ_F, and ϵ_B correspond to A_{obsd}/[Cu], the extinction coefficient for the free copper complex, and the extinction coefficient for the copper complex in the fully bound form, respectively. A plot of [DNA]/($\epsilon_A - \epsilon_F$) versus [DNA] gives K_b as the ratio of the slope to the intercept.

Cleavage of pBR322 by the Cu(II) Complexes. A typical reaction was carried out by mixing 1 μL of pBR322 (0.335 μg/μL, 0.1185 μM), 4.5 μL of 3-morpholinopropanesulfonic acid (MOPS) buffer (pH = 7.4), and 8.5 μL of Cu(II) complex solution (20 μM) in 40 mM MOPS buffer (pH = 7.4, 4% DMF) with 3 μL of ascorbate at a 100-fold molar excess relative to the complex to yield a total volume of 17 μL. 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% bromphenol blue, 50% glycerol). Then the solution was subjected to electrophoresis on 0.7% agarose gel in TAE buffer (40 mM Tris acetate/1 mM EDTA) at 80 V and visualized by ethidium bromide staining.

Cleavage of pBR322 in the Presence of Standard Radical Scavengers and Reaction Inhibitors. Scavengers of reactive oxygen intermediates, ethanol, D-mannitol, and dimethyl sulfoxide (DMSO) (6 mM) were added alternatively to the reaction mixtures. Cleavage was initiated by the addition of Cu_3-L and quenched with 3 μL of loading buffer (0.25% bromphenol blue, 25% glycerol, 1 mM EDTA). Further analysis was conducted using the standard procedures described above.

Results and Discussion

Formation and Characterization of Cu_3-L. The UV-absorption change upon complexation was employed as a quantitative probe to verify the formation of Cu_3-L. Figure 1 shows UV-absorption changes of L with the increase of the concentration of $Cu(ClO_4)_2$. With the increase of Cu(II) concentration, the absorbance at 306 and 255 nm decreased gradually while a new band at 296 nm appeared. When the absorbance at 306 nm is plotted as a function of the ratio of Cu(II) to L (Figure 1, inset), a 3:1 ratio between Cu(II) and L was evident.

According to the data from the potentiometric titration (Figure 2), the pK_a values of the metal-bound water ligands in the Cu_3-L complex are 6.8, 7.4, and 7.8, respectively. These values are close to each other and comparable with the corresponding values for analogous Cu(II) complexes,[17] which suggests that three Cu(II) ions in Cu_3-L are likely bound in a similar environment and the metal centers act independently in the pH titrations. The pK_a values of the water bound to the Cu(II) ion in the bipyridine and terpyridine complexes are 7.0 and 8.08, respectively.[18] The Cu(II) centers in Cu_3-L are likely bound by two nitrogen atoms from pyridyl and two oxygen atoms from water, although the direct structural data are currently unavailable yet. A binuclear Cu(II) complex formed by two DPA and two hydroxyl bridges has been crystallized and will be published elsewhere.

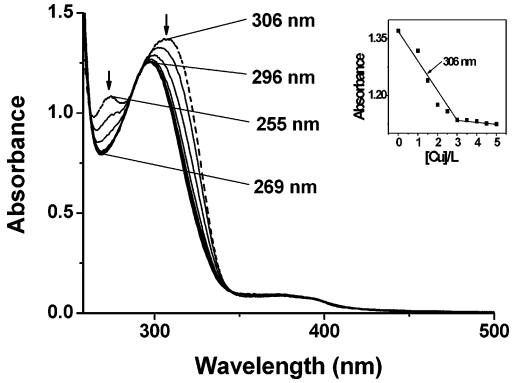

Figure 1. UV absorption changes of L (3.5×10^{-5} M) at various concentrations of $Cu(ClO_4)_2$ (6.4×10^{-3} M, 10 μL/scan) in DMF−H_2O (20:80) at 25 °C. The dashed line indicates the free L. Inset: Plot of absorbance at 306 nm against the ratio of Cu^{2+} to L. The plateau indicates the formation of Cu_3-L.

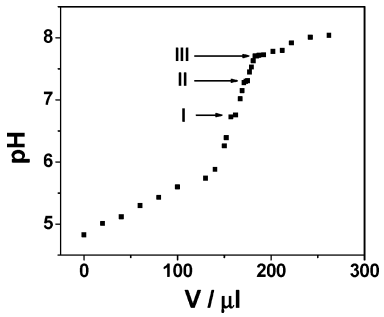

Figure 2. Potentiometric titration of Cu_3-L (1.0×10^{-4} M) with an increasing amount of NaOH (0.1 M). I−III show three turning points upon addition of NaOH, which indicates the presence of three types of bound water.

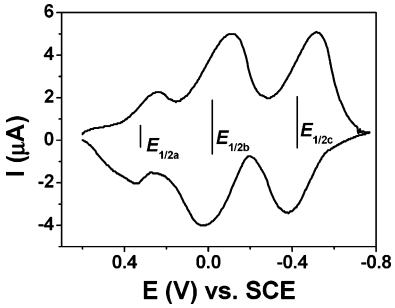

Figure 3. Cyclic voltammogram of Cu_3-L (0.3 mM) in DMF containing 0.1 M TBAP (scan rate, 100 mV·s^{-1}).

Electrochemistry. Figure 3 shows the cyclic voltammogram of Cu_3-L. The CV of the complex shows three quasi-

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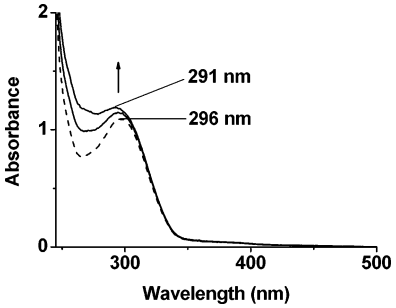

Figure 4. Absorption spectra of Cu_3-L (3.3×10^{-5} mol·L^{-1}) in the absence (dashed line) and presence (solid line) of increasing amounts of CT DNA ($0-6.7 \times 10^{-5}$ mol·L^{-1}) at room temperature.

reversible redox waves at $E_{1/2a} = +0.29$ V, $E_{1/2b} = -0.05$ V, and $E_{1/2c} = -0.45$ V (vs SCE), corresponding to the Cu(II,II,II)/Cu(II,II,I), Cu(II,II,I)/Cu(II,I,I), and Cu(II,I,I)/Cu(I,I,I) redox couples, respectively, which also confirms that there are three copper ions in the complex. The separation between the anode peak potential E_{pa} and the cathode peak potential E_{pc} ($\Delta E_p = E_{pa} - E_{pc}$) are 0.09, 0.12, and 0.13 V, respectively, that are comparable to the reported literature values.[19]

Copper(II)−DNA Interaction. DNA binding is the critical step for DNA cleavage; therefore, the potential DNA binding ability of Cu_3-L was studied by UV spectroscopy by following the intensity changes of the intraligand $\pi-\pi^*$ transition band at 296 nm. Upon addition of an increasing amount of DNA (from 10^{-5} to 10^{-4} M) to the complex (10^{-5} M), a 9% hyperchromism and a slight blue shift (5 nm) were observed, which suggested possible interactions between Cu_3-L and DNA (Figure 4). A similar hyperchromism was previously observed for Cu(II) complexes,[20] which was attributed to the dissociation of complex aggregates and the breakage of intermolecular hydrogen bonds when bound to DNA. Even though the Cu(II) complex has several labile coordination site, its binding to heterocyclic nitrogen of the DNA base is very unlikely, since a marked change in the ligand field (LF) transition of the complex would be expected for such a binding. However, no change in the energy of the LF transition was observed in the presence of DNA. From the plot of [DNA]/($\epsilon_A - \epsilon_F$) versus [DNA], the intrinsic binding constant of the complex with DNA was calculated to be $(7.0 \pm 0.02) \times 10^4$ M^{-1}. The moderate binding for this complex is comparable to those observed for many copper and ruthenium complexes[21] but smaller than the classical intercalators and metallointercalators whose binding constants were in the order of 10^7 M^{-1}.[22] Taken together, it is likely that Cu_3-L binds to DNA through intercalation of one DPA group or through general hydrophobic interactions between the ligand and the minor groove of DNA; nevertheless, electrostatics and hydrogen bonding interactions could not be ruled out.

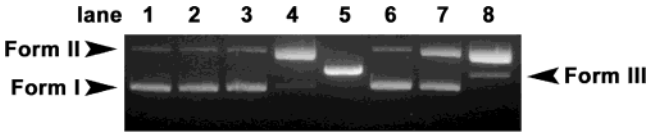

Figure 5. Agarose gel electrophoresis patterns for the cleavage of pBR322 plasmid DNA (0.02 μg/μL) by Cu-DPA and Cu_3-L in the presence of 100-fold excess of ascorbate for 25 min in MOPS buffer (40 mM, pH 7.4) at 37 °C. Key: lane 1, DNA control; lanes 2−4, DNA and 6, 12, or 18 μM of Cu-DPA; lane 5, DNA + Hind III; lane 6−8, DNA and 2, 4, or 6 μM of Cu_3-L.

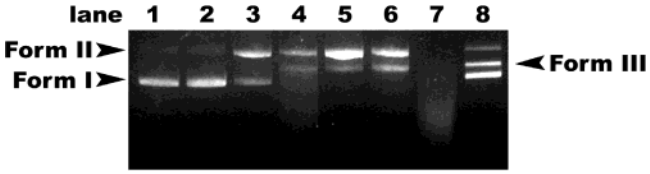

Figure 6. Agarose gel electrophoresis patterns for the cleavage of pBR322 plasmid DNA (0.02 μg/μL) in MOPS buffer (40 mM, pH 7.4) at 37 °C. Key: lane 1, DNA control; lane 2, Cu_3-L (10 μM) + DNA, without ascorbate; lane 3, $Cu(ClO_4)_2$ (30 μM) + DNA + 100-fold excess of ascorbate, for 40 min; lanes 4−7, Cu_3-L (10 μM) + DNA + 100-fold excess of ascorbate, after 10, 20, 30, and 40 min, respectively; lane 8, DNA + Hind III.

Reactions with Plasmid pBR322 in the Presence of Ascorbate or H_2O_2. The strand scission of plasmid pBR322 by Cu_3-L was assayed in the presence of ascorbate or H_2O_2. As can be seen from Figure 5, in the absence or at low concentrations of Cu_3-L (2 μM) and ascorbate, the plasmid DNA remained primarily supercoiled (form I, lanes 1 and 6). As the concentration of Cu_3-L was doubled to 4 μM, the amount of nicked (single-strand break; form II) DNA increased (Figure 5, lane 7). Linearized DNA (two subsequent and proximate single-strand breaks; form III) was not observed under these conditions, which suggests that cleavage may occur randomly over the DNA since a significant portion of the plasmid already converted to form II without the concurrent formation of form III. Additional increase of concentrations of Cu_3-L and ascorbate resulted in the complete conversion of form I to forms II and III (Figure 5, lane 8). Above this concentration, linearized products are further degraded (to smaller undetectable fragments) as indicated by a smear on the gel (Figure 6, lane 7).

The time dependence of the cleavage reaction by Cu_3-L was also examined (Figure 7). The quantified data for different forms of DNA produced are listed in Table 1. The reaction was monitored over the course of 1 h at 6 μM of Cu_3-L, which was chosen to avoid degradation of the linear products at longer time. Obviously, the amounts of linear forms of the DNA were increased gradually with time.

Owing to the similarities of the copper coordination environments of Cu_3-L and Cu-DPA, these complexes might

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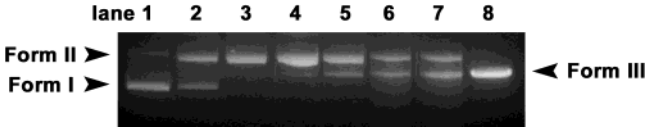

Figure 7. Agarose gel electrophoresis patterns for the cleavage of pBR322 plasmid DNA (0.02 μg/μL) by Cu_3-L (6 μM) in the presence of 100-fold excess of ascorbate for 5−60 min in MOPS buffer (40 mM, pH 7.4) at 37 °C. Key: lane 1, DNA control; lanes 2−7, DNA and Cu_3-L, after 5, 10, 20, 30, 40, and 60 min, respectively; lane 8, DNA + Hind III.

Table 1. Time-Dependent Cleavage of PBR322 by Cu_3-L in the Presence of 100-Fold Excess of Ascorbate[a]

	% form of DNA		
reacn time (min)	I	II	III
60 (DNA alone)	97	3	0
5	35	65	0
10	0	100	0
20	0	91	9
30	0	76	24
40	0	53	47
60	0	49	51

[a] Reaction conditions: 6 μM Cu_3-L, pH 7.4, 37 °C.

Table 2. Cleavage of PBR322 by Cu_3-L and Cu-DPA at Different Concentrations in the Presence of 100- Fold Excess of Ascorbate[a]

		% form of DNA		
complex	complex concn (μM)	I	II	III
none	0	88	12	0
Cu-DPA	6	84	16	0
Cu-DPA	12	77	23	0
Cu-DPA	18	6	94	0
Cu_3-L	2	81	19	0
Cu_3-L	4	45	55	0
Cu_3-L	6[b]	0	87	13

[a] Reaction conditions: pH 7.4, 37 °C, 40 mM MOPS buffer; reaction time 25 min. [b] Above these concentrations, form III DNA was further degraded as indicated by a smear on the gel.

have been expected to exhibit comparable reactivity with DNA, especially if the metal-binding units of the trinuclear complex were functioning independently. Therefore, the reaction of Cu-DPA and plasmid was conducted for comparison. In contrast to Cu_3-L, Cu-DPA was not capable of mediating conversion of supercoiled DNA to its linear form (III) at any of the concentrations tested (Figure 5, lanes 2−4). At concentration lower than 12 μM, the plasmid DNA remained primarily supercoiled. However, the amount of form II did increase with addition of 18 μM Cu-DPA, at which time the majority of the plasmid has been converted into form II (Figure 5, lane 4). The amounts of supercoiled, nicked, and linear forms of the DNA were quantified by densitometry and are listed in Table 2.

The comparisons of reactivity at equivalent copper ion concentrations revealed that Cu_3-L was considerably more reactive than its mononuclear analogue Cu-DPA. The complex Cu_3-L at 18 μM [Cu^{2+}] effected complete degradation of the plasmid to yield small linear fragments (Figure 5, lane 8), whereas equimolar [Cu^{2+}] in Cu-DPA only mediated conversion of the supercoiled plasmid to its open circular form (Figure 5, lane 4). This result suggests a possible synergistic effect between the three or at least two copper ions in Cu_3-L may exist and contribute to its relatively high nucleolytic efficiency. Moreover, the higher electrostatic attraction of Cu_3-L than Cu-DPA for DNA may add extra force to drive the Cu(II) centers toward DNA.

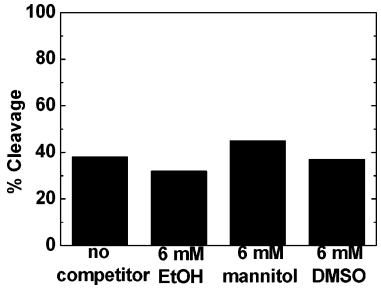

Figure 8. Histogram representing overall strand scission (linear form) of pBR322 plasmid DNA by 6 μM Cu_3-L and 100-fold excess of ascorbate in the presence of standard radical scavengers (6 mM). The cleavage percentage was calculated as described in the caption of Table S1.

Unlike the reactions conducted in the presence of ascorbate, the incubation of 6 μM Cu_3-L with plasmid DNA in the presence of H_2O_2 and subsequent electrophoresis did not show efficient DNA cleavage (Figure S1, lane 2). When 6 μM Cu_3-L was incubated with plasmid DNA for 35 min in the presence of 100-fold excess of H_2O_2, only 80% of form II was observed and no linear form appeared (Table S1). These data suggest that the behavior of Cu_3-L is somewhat unlike the traditional Fenton-like catalysis,[1a,7] such as that observed for $[Cu(OP)_2]^{2+}$.

Several control reactions have been carried out to ensure that Cu_3-L is responsible for the observed cleavage of pBR322. Hydrogen peroxide and ascorbate themselves do not show any cleavage of pBR322 beyond background. Furthermore, 30 μM $Cu(ClO_4)_2$, alone or in combination with ascorbate does not show efficient cleavage of pBR322 after 40 min (Figure 6, lane 3), a period during which Cu_3-L can cleave pBR322 to smaller undetectable fragments as indicated by a smear on the gel (Figure 6, lane 7). These experiments ruled out the possibility of free copper(II) induced DNA cleavage.

Reactions with Plasmid pBR322 in the Presence of Standard Radical Scavengers. The presence of a diffusible radical species can be diagnosed by monitoring the quenching of DNA cleavage in the presence of alternative H-donors which would scavenge radicals (such as $^{\bullet}OH$) in solution. To this end, standard radical scavengers were added to the reaction of Cu_3-L prior to initiation with ascorbate. The individual addition of ethanol, D-mannitol, and dimethyl sulfoxide (DMSO) (6 mM) was found to have little effect on DNA cleavage, which remained constant at 40 ± 5% of form III (Figure 8, Table S1). The reactive intermediates leading to DNA cleavage were thus not freely diffusible radicals that were subject to the quenching of hydrogen-atom donors. This rules out an uncoordinated hydroxyl radical such as that involved in DNA cleavage mediated by EDTA-Fe(II).[23] However, a metal-bound species similar to that proposed for $[Cu(OP)_2]^{2+}$, which is often formulated as $[CuOH]^{2+}$ or $[CuO]^{+}$, remains a possibility.[1a,24] A cupric superoxo ($Cu^{II}-O_2^{-}$) or cupric hydroperoxo ($Cu^{II}-OOH$)

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such as that suggested for peptidylglycine monooxygenase and dopamine β-hydroxylase may also serve as the active O_2-derived species.[25] Further experiments will be necessary to determine the exact identity of the intermediates leading to DNA strand scission.

Reactions with Plasmid pBR322 in the Absence of H_2O_2 or Ascorbate. Cu_3-L is also observed to cleave pBR322 in the absence of H_2O_2 or ascorbate to give nicked products. However, the process is much less efficient and requires higher concentrations of complex ($\gg$10 μM) and either longer reaction time (24 h) or elevated temperature (data not shown). We noted that $Cu(ClO_4)_2$ does not effect detectable cleavage under these conditions. Since the trinuclear complex Cu_3-L possesses several exchangeable labile coordination sites, it can be speculated that a hydrolytic mechanism could also be operative here since the copper-bound water could easily deprotonate to produce Cu(II)-bound hydroxyl group which might serve as a nucleophilic agent to attack DNA. It has been reported previously that a phosphate-ester bond can be hydrolytically cleaved by Cu(II) complexes with N-containing ligands.[26] We are currently unable to rule out that the reaction under these conditions, lacking exogenously added H_2O_2 or ascorbate, may still proceed in an oxidative manner, because endogenous reductants (e.g. amine buffers[27]) or O_2 may still exist under the reaction conditions described here and thus have some effects on the nuclease activity.

Summary and Conclusions

A novel trinuclear copper(II) complex, Cu_3-L, has been shown to exhibit efficient oxidative strand scission of DNA. The Cu(II) ion and the multidentate ligand L is shown to form Cu_3-L almost quantitatively in solution. Potentiometric titration of Cu_3-L with an increasing amount of NaOH shows three turning points, which indicates the presence of three types of bound water. The cyclic voltammogram of Cu_3-L also shows three quasi-reversible redox waves at $E_{1/2a} = +0.29$ V, $E_{1/2b} = -0.05$ V, and $E_{1/2c} = -0.45$ V (vs SCE), corresponding to the Cu(II,II,II)/Cu(II,II,I), Cu(II,II,I)/Cu(II,I,I), and Cu(II,I,I)/Cu(I,I,I) redox couples, respectively, which also confirms that there are three copper ions in the complex. A moderate binding ability for DNA was observed for this complex.

The trinuclear complex readily cleaves plasmid DNA in the presence of ascorbate to give nicked (form II) and then linear (form III) products. Interestingly, the cleavage efficiency using H_2O_2 is less than by ascorbate, suggesting that the cleavage mode of the trinuclear complex is somewhat different from the traditional Fenton-like catalysis. Meanwhile, Cu_3-L is far more efficient than its mononuclear analogue Cu-DPA at the same [Cu^{2+}] concentration and hence suggests a possible synergy between the three or at least two Cu(II) centers in Cu_3-L that contributes to its relatively high nucleolytic efficiency. This work may lead to rational design and development of additional compounds with high nucleolytic efficiency in the future. Furthermore, the presence of standard radical scavengers does not have a clear effect on the cleavage efficiency, suggesting the reactive intermediates leading to DNA cleavage are not freely diffusible radicals.

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Supporting Information Available: Quantified outcome for the reaction of DNA and Cu_3-L (6 μM) in the presence of 100-fold excess of ascorbate and potential inhibitors or in the presence of 100-fold H_2O_2 and agarose gel electrophoresis patterns for the cleavage of pBR322 plasmid DNA (0.02 μg/μL) by Cu_3-L (6 μM) in the presence of 100-fold ascorbate or H_2O_2 and standard radical scavenger for 35 min in MOPS buffer (40 mM, pH 7.4) at 37 °C (pdf). This material is available free of charge via the Internet at http://pubs.acs.org.

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