A Simple Copper(II)−L-Histidine System for Efficient Hydrolytic Cleavage of DNA

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Copper(II)−L-histidine complexes effectively promote the cleavage of plasmid DNA and dideoxynucleotide dApdA at physiological pH and temperature. Studies of the mechanism of plasmid DNA cleavage by added radical scavengers, using rigorously anaerobic experiments, analyses for malondialdehyde-like products, religation assays, and HPLC analyses, indicate that DNA cleavage mediated by Cu(L-His) occurs via a hydrolytic path. The hydrolytic cleavage rate constants at 37 °C are estimated to be 0.76 h^{-1} for the decrease of form I and 0.25 h^{-1} for the increase of form III. The phosphoimager picture reveals that Cu(L-His) cleaves DNA with a certain sequence specificity (preferentially at 5′-GT-3′). The dinucleotide hydrolysis shows, with [Cu(L-His)] = 0.8 mM, rate enhancement factors of $>10^8$. Interestingly, histidine−metal ion interactions (with Cu(II), Ni(II), Zn(II), etc.) have been used for various applications, e.g., protein purification, cross-linking, and targeting proteins to lipid bilayers. Our findings may provide the basis for developing new applications and new ways to design more effective and useful catalysts for DNA cleavage. Cu(L-His) is one of only a few well-defined metal complexes demonstrated to hydrolytically cleave dideoxynucleotides and DNA.

Introduction

Artificial metallonucleases have been proven to be efficient tools for the footprinting and sequence-specific targeting of nucleic acids.[1] The development of reagents which hydrolytically cleave nucleic acids under mild conditions is currently attracting great interest in the field of artificial metallonucleases.[2,3] While many reagents have been successfully applied to RNA hydrolytic cleavage, [3a,d,4] there have been fewer successes with DNA[5] because of its relatively high hydrolytic stability.[6] This stability is a result of the repulsion between the negatively charged phosphodiester backbone and potential nucleophiles. Consequently, much of the literature on phosphodiester degradation by small molecules has focused on either hydrolysis of activated substrates or oxidative degradation of DNA. A number of oxidative cleavage reagents have been utilized with great success for DNA footprinting,[7] for locating base mismatches and loop regions,[8] for locating conformational variations in DNA,[9] and as chemotherapeutic agents.[10] These oxidative cleavage agents require the addition of an external agent (e.g., light or hydrogen peroxide) to initiate cleavage and are thus limited to in vitro applications. Because these processes are radical-based [7,11] and deliver products lacking 3′- or 5′-phosphate groups[12] that are not amenable to further enzymatic manipulation, the use of these reagents has been limited in the field of molecular biology and their full therapeutic potential

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has not been realized. Hydrolytic cleavage agents do not suffer from these drawbacks. They do not require coreactants and therefore could be more useful in drug design. Also, they produce fragments that may be religated enzymatically. Metal ions play important roles in many enzyme- and all ribozyme-mediated examples of these scission reactions,[13] and yet their precise role in the hydrolytic mechanism is unclear.[13e] Small metal complexes that promote the hydrolytic cleavage of DNA therefore could be useful not only in molecular biology and drug design but also in elucidating the precise role of metal ions in enzyme catalysis.

The binding of copper ions to DNA is well established as is their ability to cleave DNA, particularly when Cu(II) ions are complexed with certain metal ion chelators. One commonly used oxidative cleavage agent is the bis(1,10-phenanthroline-N^1,N^{10})copper(I) cation [$(OP)_2Cu^+$].[9] In the presence of H_2O_2 or O_2 plus a reducing agent, this chelate efficiently cleaves B-DNA in the minor groove. [$(OP)_2Cu^+$] has been used as a footprinting reagent and as a probe of DNA and RNA secondary structures.[1c,9] Numerous other ligands have been also been complexed with copper ions to promote the oxidative degradation of DNA. These ligands include tambjamine,[14a] *o*-quinacridines,[14b] Clip-phen,[14c] 2,2′-bipyridine,[15a] the tripeptide Gly-Gly-His,[15b] and various hydroxamic acids.[16] Because hydrolytic cleavage of DNA is so difficult compared to oxidative degradation of DNA, most studies of hydrolytic reactions utilize activated phosphodiesters. Various lanthanides, either as free ions[17] or as macrocyclic complexes,[18] have been used to catalytically hydrolyze activated phosphate esters. The coordination chemistry of lanthanides, however, is not as well studied as that of transition metals, and many lanthanide complexes decompose in water because of their extreme lability.[4b,19] Furthermore, because the coordination numbers of lanthanide complexes are difficult to determine, the detailed mechanism of lanthanide-promoted hydrolysis of phosphodiesters is still unknown. Substitutionally labile amine complexes of Cu(II)[15a,20,21] and Zn(II),[15a,22] although usually slower hydrolytic agents than lanthanide complexes, provide both catalytic turnover and stable, well-characterized metal complexes. Recently, rapid phosphodiester hydrolysis by an ammonium-functionalized Cu(II) complex and Cp_2MoCl_2 has been reported.[23] Hydrolysis of phosphodiesters by zinc complexes of a histidine-containing peptide,[24a] dinuclear Zn(II),[24b,25a] Cu(II),[24b] Ni(II),[24b] and Co(II)[25b] complexes, and a trinuclear zinc complex[26] has also proven to be promising, and there is currently much interest in developing polynuclear cleavage agents. Hydrolysis of DNA itself has remained elusive.

It is perhaps remarkable that there are a few of examples of hydrolytic cleavage of DNA in the literature. It was reported that $(Ru(DIP)_2macro)^{n+}$ complexes, where $Ru(DIP)_2$ binds to DNA via intercalation and macro is a chelating ligand, hydrolyze DNA in the presence of certain divalent metal cations.[5c] Lanthanum and cerium ions and their complexes have been reported to hydrolyze dinucleotides, short oligonucleotides, and supercoiled plasmid DNA.[5a,d,27,28] A diiron species rapidly hydrolyzes supercoiled DNA in the presence of H_2O_2 or O_2 plus a reducing agent.[5e] These are conditions under which iron species typically oxidatively degrade DNA, but the products observed were consistent with a hydrolytic mechanism. Finally, it was recently reported that Cu(II) macrocyclic complexes promote the hydrolytic cleavage of plasmid DNA[29a] and that a Cu(II) complex with *cis*,*cis*-1,3,5-triaminocyclohexane effectively promotes the hydrolytic cleavage of phage DNA.[30a] Cobalt(III) polyamine complexes[31] and zirconium(IV)[2e] as catalysts for the hydrolysis of DNA have been reported.

It is known that basic amino acid residues are used in various regulatory proteins, such as zinc figure proteins, for the recognition of the specific base sequences through hydrogen bond formation with the nucleic bases in DNA.[32a] Histidine is a biochemically important ligand for copper(II) binding in many biological systems. It plays a major role as a nucleophilic site of biological macromolecules.[32b] We are interested in this ligand

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because imidazole is an efficient catalyst of ester hydrolysis at neutral pH and catalyzes hydrolysis of RNA and its various derivatives (poly(u), UpU, ApA)[33] and the histidine residues are often involved in natural hydrolytic metalloenzyme active centers (e.g., alkaline phosphatase, ribonuclease, and carboxypeptidase A) and have potential to bind specifically to DNA. On the other hand, it is clear that divalent ions play a major role in DNA hydrolysis.[34] Although copper(II) is not a natural cofactor of phosphodiesterase enzymes, various studies on the hydrolysis of activated phosphodiesters and of RNA and DNA by copper(II) complexes have contributed to the general understanding of metal-promoted phosphodiester hydrolysis.[4a,21c,29,30] Therefore, we designed the Cu(II)−L-histidine system described herein. Interestingly, histidine−metal ion interactions (with Cu(II), Ni(II), Zn(II), etc.) have been used for various applications, e.g., protein purification,[35] cross-linking,[36] and targeting proteins to lipid bilayers.[37] In this paper, we report our finding that Cu(II)−L-histidine complexes effectively promote hydrolytic cleavage of plasmid DNA and a dideoxynucleotide at physiological pH and temperature.

Experimental Section

Instrumentation and Reagents. An LCQ electrospray mass spectrometer (ESMS, Finnigan) was employed for the investigation of charged metal complex species in aqueous solutions, and pH determinations were performed using a Beckman Φ 50 pH meter. UV−vis spectra were recorded on a Shimadzu UV-365 spectrophotometer, HPLC spectra were acquired with an HP1050 HPLC system in combination with a reversed-phase BDS C-18 column (5 μm Hypersil at 40 °C). Plasmid cleavage products were quantitated and analyzed with a UVP GDS 8000 complete gel documentation and analysis system. The phosphoimager picture was obtained by using the STORM 860 imaging system (Molecular Dynamics, Inc.).

All chemicals were of reagent grade and were used without further purification unless otherwise noted. Calf thymus (CT) DNA and plasmid pBR322 DNA were purchased from Sino-American Biotechnology. Agrose, T4 DNA ligase, and a Wizard PCR Preps DNA purification system were purchased from Promega. Ethidium bromide was obtained from Fluka, and 2′-deoxyadenylyl-(3′→5′)-2′-deoxyadenosine ammonium salt, 2′-deoxyadenosine 5′-monophosphate sodium salt, 2′-deoxyadenosine 3′-monophosphate sodium salt, 2′-deoxyadenosine 3′,5′-cyclic monophosphate sodium salt, 2′-deoxyadenosine, and adenine were purchased from Sigma. A T7 Sequenase protocol was obtained from Life Technology. CT DNA was purified by standard procedures. A UV−vis spectrophotometer was employed to check DNA purity (A_{260}: $A_{280} > 1.9$) and concentration ($\epsilon = 6600$ dm^3 mol^{-1} cm^{-1} at 260 nm).

For the experiments, Cu(II)−L-histidine systems were prepared in aqueous solutions by mixing $CuCl_2$ and L-histidine in molar ratios of 1:3 to maintain some level of unsaturated in coordination in these labile complexes, which were then allowed to deposit for more than 5 days at room temparature. The pH of the solutions was 5.4. All solutions were prepared using doubly distilled deionized water.

Electrospray Ionization MS Measurements. Typically, a 1.0 μL portion of Cu(L-His) solution, loaded into the injection valve of the LCQ unit, was injected into the mobile-phase solution (50% aqueous methanol; not containing acetic acid) and then carried through the electrospray interface into the mass analyzer at a rate of 60 μL min^{-1}. The potential employed at the electrospray was 4.5 kV, and the capilliary was heated to 200 °C. The positive-ion mass spectra was then obtained. Determination of the charge of the species was made by using the isotopic pattern.

DNA Scission Conditions. Electrophoresis experiments were performed with pBR322 DNA; all other experiments were performed with calf thymus DNA. The cleavage of pBR322 by Cu(L-His) was accomplished by mixing (in order) 16 μL of 5 mM Tris−HCl (pH 7.0; containing 5 mM NaCl) buffer, varying concentrations of Cu(L-His) complexes (0.2−0.42 μL), and 0.58 μL of pBR322 (0.8 μg/μL; 10 mM Tris−HCl, pH 8.0). After mixing, the DNA solutions were incubated at 37 °C. At the appropriate times, the reactions were quenched by the addition of EDTA and bromphenol blue and the mixtures were analyzed by gel electrophoresis.

In order to examine if hydroxyl radicals were present, HO• scavengers such as DMSO, glycerol, or methanol were introduced to afford a final concentration of 0.4 or 2.5 M before Cu(II)−L-His addition.

Oxidative cleavage of DNA by Fe(EDTA)$^{2-}$/DTT was accomplished by incubating the DNA with 10 mM DTT and 0.1 mM Fe(EDTA)$^{2-}$ for 1 h at 37 °C. All other reaction conditions were the same as those listed for the cleavage of DNA by Cu(L-His).

Determination of malondialdehyde-like products was accomplished by adding 0.6% 2-thiobarbituric acid to the calf thymus DNA cleavage reaction solution, heating the sample in boiling H_2O for 20 min, and analyzing the sample spectrophotometrically at 532 nm.

Anaerobic Reactions. Deoxygenated water was prepared by four freeze−pump−thaw cycles. Before the final two cycles, the water was equilibrated with nitrogen to aid in the deoxygenation process. The deoxygenated water was stored under a nitrogen atmosphere prior to use. All anaerobic stock solutions were prepared in a nitrogen-filled glovebag using the deoxygenated water. Reaction mixtures were prepared by the addition of the appropriate volumes of stock solutions to the reaction tubes and incubation in a glovebag.

Ligation of Linearized pBR322. pBR322 linearized by Cu(L-His) was recovered from an agarose low melting point gel by cutting off a gel fragment and subjecting it to the Wizard PCR Preps DNA purification system and then dissolving the sample in doubly distilled water. Overnight ligation reactions were performed as follows: 2 μL of Promega 10X ligation buffer, 16.5 μL of cleaved pBR322 (0.1 μg/μL), and 1.5 μL of T4 DNA ligase (4.5 units) were mixed and incubated for 14−16 h at 16 °C. The ligation mixtures were run on 1% agarose gels in TAE (40 mM Tris acetate/1 mM EDTA), and the levels of religation were quantitated via fluoroimaging.

Product Analysis and Quantitation. The extent of cleavage was determined via densitometric analysis of ethidium bromide-stained agarose gels. The 1% agarose gels were run at 80 V for 2−3 h in TAE. Following electrophoresis, the gels were stained with ethidium bromide solution at 0.5 μg/mL to observe the cleaved DNA products. The extent of DNA cleavage was determined by using the volume quantitation method in Gelworks 1D version 3.00. For reactions involving supercoiled DNA, a correction factor of 1.42 was utilized to account for the decreased ability of ethidium bromide to intercalate into form I DNA versus forms II and III. The relative amounts of the different forms of DNA were determined by dividing the fluorescence intensity for a particular band by the sum of the fluorescence intensities for each band in that lane.

Cleavage of dApdA. Cleavage reactions of dideoxynucleotide dApdA were conducted at 37 °C and pH 6.0 (5 mM Tris−HCl buffer containing 5 mM NaCl) and were monitored by HPLC using the HP1050 HPLC system in combination with a reversed-phase BDS C-18 column (5 μm Hypersil at 40 °C). Detection of the peaks was performed online with an HP detection system at 260 nm. In a typical run, 1 μL of the reaction mixture was injected onto the C-18 reversed-phased

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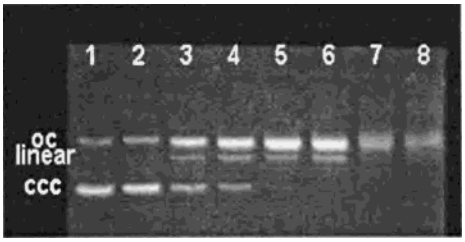

Figure 1. Agarose gel electrophoresis patterns for the cleavage of pBR322 DNA by various concentrations of Cu(L-His). Lane 1: DNA control. Lane 2: DNA + 48 μM Cu(L-His). Lane 3: DNA + 0.24 mM Cu(L-His). Lane 4: DNA + 0.48 mM Cu(L-His). Lane 5: DNA + 0.96 mM Cu(L-His). Lane 6: DNA + 1.08 mM Cu(L-His). Lane 7: DNA + 2.0 mM Cu(L-His). Lane 8: DNA + 3.0 mM Cu(L-His).

column (5 μm Hypersil at 40 °C) and eluted for 5 min with $NH_4H_2PO_4$ (0.2 M, pH 5.5), followed by a 0−50% linear gradient of $NH_4H_2PO_4$ (0.2 M, pH 5.5) and methanol/water (3:2) solutions over 12 min with a flow rate of 0.85 mL/min.

Sequence Selectivity Analysis. All DNA sequencing was done according to the T7 Sequenase protocol (Life Technology Inc., U.S.A.). The location of cleavage sites was determined on a 6% denaturing polyarylamide electrophoresis gel. All experiments were performed at least in triplicate.

Results

Hydrolytic Cleavage of Double-Stranded DNA. Cu(II)−L-histidine (Cu(L-His)) complexes are capable of cleaving double-stranded DNA at physiological pH and temperature. When plasmid pBR322 DNA was incubated with Cu(L-His), the supercoiled DNA was degraded from form I (supercoiled) to form II (relaxed circular) and then slowly to form III (linear). Figure 1 shows agarose gel electrophoresis patterns for the cleavage of plasmid pBR322 DNA after being treated with a 1:3 molar ratio of $CuCl_2$ to L-histidine at pH 6.0−7.0 (5 mM Tris−HCl, 5 mM NaCl) and 37 °C for 4 h. The initial concentration of DNA was set at 9.8 nM, or 42 μM base pairs, and the concentration of Cu(L-His) was varied from 0 to 1.08 mM. The conversions of form I (supercoiled) to form II (nicked) and to form III (linearized) were observed with the increase in concentration of Cu(L-His), and form III began to appear in the presence of 48 μM Cu(L-His) (lane 2). Form I was barely observable in lane 6 (1.08 mM Cu(L-His)). At higher concentrations (2.0−3.0 mM) of Cu(L-His), band broadening and slower migration of the plasmid DNA were observed, indicative of binding of the complexes to the DNA (lanes 7 and 8). Still higher concentrations led to precipitation of the plasmid DNA as a white solid, due to charge neutralization caused by extensive binding of the Cu(L-His). A single cut or nick on a strand of supercoiled DNA relaxes the supercoiling and leads to form II. A second cut on the complementary strand, within approximately 12 base pairs[38,39] of the original cut site, linearizes the DNA to form III. Therefore, Cu(L-His) must have cut the DNA at least twice to convert it from form I to form III. The extent of DNA cleavage was quantitated via fluoroimaging (Table 1). Fluorescence intensities were corrected for the different quantum yields of forms I−III as described in the Experimental Section. However, under the same conditions, Cu(II)−histamine complexes and Cu(II)−imidazole complexes produced no cleavage of pBR322 DNA at concentrations below 3 mM. Free Cu(II) ions produced hardly any cleavage of pBR322 DNA at concentrations below 1.08 mM, and $Cu(NO_3)_2$ was less effective than $CuCl_2$ at identical concentrations in this cleavage system. L-Histidine was also inactive. These results suggest that the Cu(L-His) complexes are the reagents responsible for the degradation of DNA. The carboxyl group in L-histidine plays a key role in the cleavage of plasmid pBR322 DNA. It should be noted that Cl^- competes as a ligand to a low degree with L-histidine and OH^- for Cu(II).

Table 1. Cleavage of pBR322 by Cu(L-His)

Cu(L-His) concn[a] (mM)	% DNA form		
	supercoiled	nicked	linear
control	83 ± 1	17 ± 1	0 ± 0
0.048	75 ± 2	24 ± 2	3 ± 3
0.24	40 ± 2	49 ± 1	11 ± 5
0.48	28 ± 6	63 ± 0	14 ± 2
0.96	10 ± 6	80 ± 2	16 ± 6
1.08	3 ± 2	71 ± 5	29 ± 7

[a] 0.048−1.44 mM Cu(L-His) refer to C_{CuCl_2} = 0.048−1.44 mM and $n(CuCl_2)$:n(L-histidine) = 1:3.

Table 2. Effect of Additives on the Extent of pBR322 Cleavage by Cu(L-His)[a]

conditions	% form I	% form II	% form III
control	79	21	0
Cu(L-His)	8	53	39
+0.5 μL of DMSO	7	49	44
+0.5 μL of glycerol	0	48	52
+1.9 μL of MeOH	0	42	58
aerobic	8	56	36
anaerobic	7	61	32

[a] Reactions were run at pH 6.0 (5 mM Tris−HCl, 5 mM NaCl) using 1 mM Cu(L-His) and 42 μM base pair concentration of pBR322; 1 mM Cu(L-His) refers to C_{CuCl_2} = 1 mM and $n(CuCl_2)$:n(L-histidine) = 1:3.

Although Cu(L-His) does not require addition of external agents, we were keen to discount the possibility that DNA cleavage occurred via a hydroxyl radical-based depurination pathway. Table 2 shows the effect of various additives on the extent of DNA cleavage by Cu(L-His). When pBR322 DNA was incubated with Cu(L-His) in the presence of 0.4 M DMSO or glycerol or 2.5 M MeOH as an $HO^\bullet$ radical scavenger, only slight inhibition of the DNA cleavage was observed. This suggests that the Cu(L-His)-mediated cleavage reaction in the absence of exogenous coreactants does not proceed via either diffusible hydroxyl radicals or free superoxide. To probe the effects of molecular oxygen on the degradation of DNA by Cu(L-His), reactions were also performed under rigorously anaerobic conditions. Cu(L-His) still effectively cleaved pBR322 DNA; only slightly inhibition was observed (approximately a 5% decrease in the extent of DNA degradation relative to aerobic reactions at the same metal complex concentration). These results suggest that oxidation products are hardly involved. It should be pointed out that while the anaerobic conditions we employed were sufficient to stop any visible oxidation and Tris is known as a singlet oxygen scavenger,[40] it would be difficult to exclude the presence of a small amount of oxidation, and since the experiments utilize low concentrations of substrate (9.8 nM DNA, or 42 μM base pairs), residual O_2 could potentially account for a significant amount of cleavage in the anaerobic experiments. The likelihood of residual O_2-dependent cleavage in the anaerobic reactions was assessed by using the oxidative cleaving system $Fe(EDTA)^{2-}$/DTT. In the presence

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(39) (a) Basile, L. A.; Barton, J. K. *J. Am. Chem. Soc.* **1987**, *109*, 7548. (b) Kishikawa, H.; Jiang, Y. P.; Goodisman, J.; Dabrowiak, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 5434.
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of O_2, this system rapidly degrades supercoiled DNA by producing diffusible hydroxyl radicals via the Fenton reaction. Under the anaerobic conditions described in the Experimental Section, however, DNA incubated with $Fe(EDTA)^{2-}$/DTT for over 20 h did not exhibit any degradation. Thus, we conclude that the cleavage of DNA by Cu(L-His) in anaerobic experiments is oxygen-independent. O_2 does not appear to be the limiting reagent under aerobic conditions. In addition, analysis for malondialdehyde-like products was performed by adding 0.6% 2-thiobarbituric acid to this reaction system. After calf thymus DNA had been treated with Cu(L-His) at 37 °C for 24 h, malondialdehyde-like products were not detected.[5e] Taken together, these data suggest that the double-stranded cleavage mediated by Cu(L-His) does not occur by an oxidative mechanism.

To determine whether the cleavage reaction afforded exclusively 3′-OH and 5′-OPO_3 ends, as found for hydrolysis mediated by restriction enzymes, Cu(L-His)-linearized plasmid was treated with T4 DNA ligase. It was found that plasmid DNA linearized by Cu(L-His) can be converted into the closed circular plasmid and concatemers of pBR322, as detected by a shift in the electrophoresis bands. We performed ligation experiments the pBR322 linearized by Cu(L-His) several times. The efficiencies of religation differed, with the highest efficiency of religation after cleavage by Cu(L-His) being about 55%. This indicates that the cleavage probably afforded 3′- and 5′-phosphate products. Less than quantitative religation efficiency may be expected if the bound and free Cu(L-His) complexes inhibited ligase to a certain extent. It should be noted that it would be difficult to exclude the presence of a small amount of Cu(L-His), and because the assay measures ligation by the conversion of the Cu(L-His)-linearized plasmid to the closed circular plasmid and concatemers of pBR322, if a mixture of hydrolytic and redox cleavage products occurred on the same circle, religation of the hydrolytic species would not be detected; the assay therefore indicated only if cleavage occurred solely through a hydrolytic path.[5c] Further evidence for the hydrolytic path was obtained from HPLC of the DNA dinucleotide 2′-deoxyadenylyl-(3′→5′)-2′-deoxyadenosine (dApdA).

Hydrolysis of the Dinucleotide dApdA. The hydrolytic nature of a dinucleotide cleavage should also be reflected in the undamaged constituents one would expect from such a mechanism. The typical products of a radical degradation of the sugar part would be the nucleobase alone (without the sugar) and denatured nucleobase derivatives, if any.[1c] Optimization of HPLC separations on reversed-phase columns with phosphate buffer/methanol gradients allowed separations of all possible components in blind runs. The dinucleotide dApdA (5 mM) was reacted with 0.8 mM Cu(L-His) at pH 6.0 and 37 °C for up to 72 h, which allowed the reaction to be followed to up to 25% completion (Figure 2). The detected products were dA (2′-deoxyadenosine) and 5′-dAMP, and no byproducts such as adenine (formed by the oxidative cleavage of the ribose) were found. Thus, the cleavage is totally hydrolytic. Trace amounts of 3′-dAMP and 3′,5′-dcAMP (2′-deoxyadenosine 3′,5′-cyclic monophosphate) were detected although their concentrations did not accumulate during the cleavage reaction. This indicated that 3′,5′-dcAMP as the intermediate was rapidly hydrolyzed to 3′- and/or 5′-dAMP. 3′-dAMP and 5′-dAMP also undergo rapid cleavage under the above conditions, and their concentrations do not accumulate appreciably during the course of dApdA cleavage. These products indicate that two processes are occurring: the cyclic ester 3′,5′-dcAMP is the result of an intramolecular attack by either the 3′- or the 5′-OH. The possible

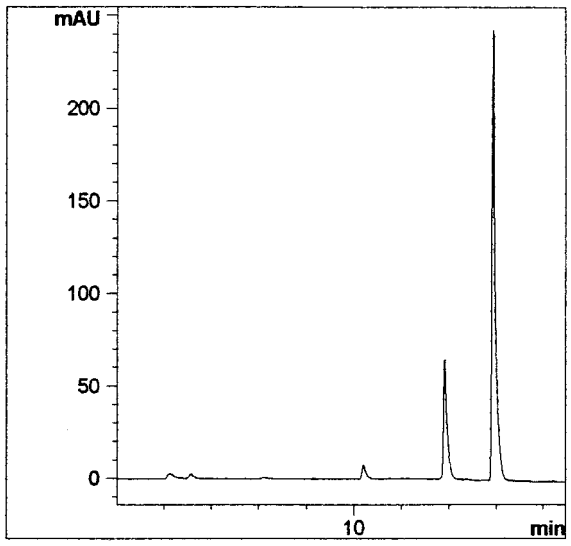
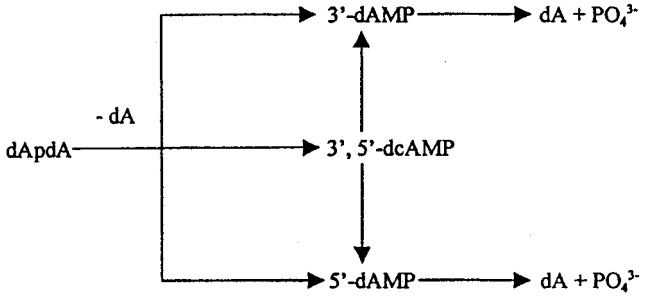


Figure 2. HPLC chromatograms showing the cleavage of dApdA (5 mM) by Cu(L-His) (0.8 mM) at pH 6.0 (5 mM Tris−HCl buffer containing 5 mM NaCl) and 37 °C for 72 h. Retention times: 5′-dAMP, 10.8 min; dApdA, 16 min; dA, 14.1 min; Cu(L-His), 2.3 min and 3.4 min separately.

Scheme 1. Possible Reaction Products from dApdA Hydrolysis with Cu(L-His)



pathways (Scheme 1) lead partially to the same products, in particular to the "normal" DNA-type hydrolysis products 3′- and 5′-dAMP. The pseudo-first-order rate constant of dApdA cleavage was obtained by following the initial rate of decrease in the concentration of dApdA ($k_{obs} = 7.94 \times 10^{-7}$ s^{-1}). Chin et al.[41a] have derived an estimated value of $k = 1 \times 10^{-16}$ s^{-1} at 25 °C on the basis of a linear free energy correlation between the log k values for diesters of phosphoric acid and the pK values of the leaving groups. If we allow a limiting factor of 10 for the temperature difference between 25° and 37 °C, the uncatalyzed hydrolysis at 37 °C should proceed with $k = 10^{-15}$ s^{-1}. The rate acceleration factor then is $>10^8$ for [Cu(L-His)] = 0.8 mM. However, under the same conditions, similar to the case of the cleavage of plasmid DNA, Cu(II)−histamine and Cu(II)−imidazole complexes do not produce cleavage of dApdA to a measurable extent. Both $CuCl_2$ and L-histidine are also inactive. These results further indicate that the carboxyl group in L-histidine plays a key role in the cleavage of the phosphate diester backbone of DNA. Bruice et al.,[42] in their study of bis-(2-carboxyphenyl) phosphate, found that carboxyl groups could participate in phosphodiester hydrolysis. Our findings support

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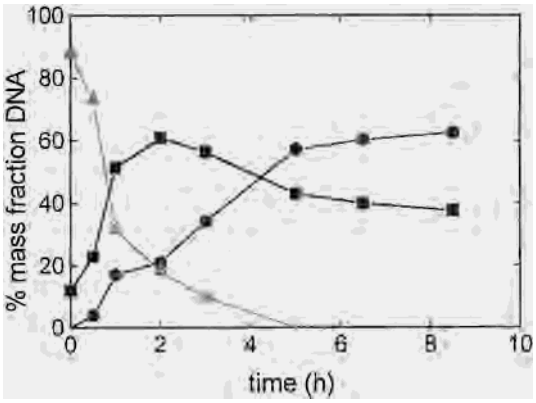

Figure 3. Mass fractions of DNA species during the cleavage reaction of pBR322 DNA with 1 mM Cu(L-His): (▲) form I supercoiled DNA; (■) form II nicked DNA; (●) form III linear DNA.

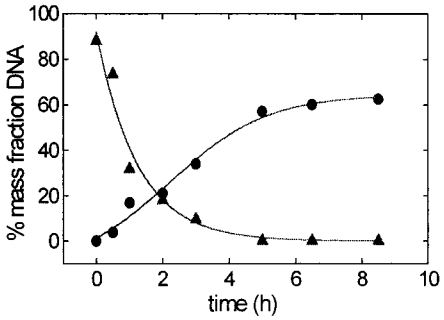

Figure 4. Time course of DNA cleavage by 1 mM $CuCl_2$−L-histidine (molar ratio 1:3) at 37 °C; (5 mM NaCl in 5 mM Tris−HCl buffer, pH 6.0): (▲) form I; (●) form III.

this, and detailed mechanisms will be investigated. This is also consistent with the observation that metals are much more efficient at promoting hydrolysis than are organic buffers of similar pK_a values.[30c,33]

Kinetic Studies of Double-Strand DNA Scission Chemistry. The chemistry of DNA strand scission by Cu(L-His) has been kinetically characterized by quantitation of supercoiled, nicked, and linear DNA. The observed distribution of supercoiled, nicked, and linear DNA in an agarose gel provides a measure of the extent of hydrolysis of the phosphodiester bond in each plasmid DNA, and we used these data to perform simple kinetic analyses. Figure 3 shows the mass fractions of DNA species present during a reaction under mild conditions. Figure 4 is a time course plot of form I decrease and form III formation during cleavage by Cu(L-His). As shown, the decrease of form I fits well to a single-exponential decay curve. The increase of form III also fits to a single-exponential curve, although the deviation for form III is somewhat larger than that for form I. From these curve fits, the hydrolysis rate constants at 37 °C and at complex concentrations of 1 mM were estimated to be 0.76 h^{-1} ($R = 0.984$) for the decrease of form I and 0.25 h^{-1} ($R = 0.968$) for the increase of form III. Linear least-squares fits of the data beyond the induction period give half-lives of 0.907 h for form I decrease and 2.81 h for form III formation. These $t_{1/2}$ values may not be experimentally distinct, given the degree of scatter of the points. Similarly, the hydrolysis rate constants for pBR322 DNA in the presence of 48 μM, 0.24 mM, 0.48 mM, 0.72 mM, and 1.44 mM Cu(L-His) were obtained and are given in Table 3.

Ionic Strength and pH Dependence of Double-Strand Scission Chemistry. To assess the ionic strength dependence of strand scission chemistry, plasmid pBR322 DNA was incubated with Cu(L-His) under conditions of increasing ionic strength brought about by adding NaCl. As shown in Figure 5, lower ionic strength has almost no effect on DNA strand scission until it reaches 50 mM. Higher ionic strength influences the efficiency of DNA cleavage. Two separate ionic strength dependencies for nicking and linearization of plasmid DNA are apparent. The process of linearization is more sensitive to increasing ionic strength than is nicking. Virtually no linear DNA forms above 100 mM added NaCl, while substantial amounts of nicking continue to occur. The process of DNA nicking becomes inhibited at high ionic strengths and stops at 300 mM added NaCl. The patterns of these data may reflect two separate binding events that have different electrostatic components, or the lack of linear DNA at higher ionic strengths may be a kinetic consequence of a lower degree of nicking at higher ionic strengths, which would deplete the substrate pool for the formation of linear DNA. It should be noted that NaCl was chosen as the background electrolyte because Cl^- competes as a ligand to a low degree with L-histidine and OH^- for Cu(II).

Table 3. Rate Constants for the Cleavage of pBR322 by Cu(L-His)

Cu(L-His) concn[a] (mM)	decrease of form I	increase of form III
0.048	0.0139	0
0.24	0.138	0.194
0.48	0.257	0.233
0.72	0.581	0.244
1.0	0.764	0.247
1.44	0.841	0.300

[a] 0.048−1.44 mM Cu(L-His) refer to $C_{CuCl_2} = 0.048-1.44$ mM and $n(CuCl_2):n$(L-histidine) = 1:3.

DNA scission vs pH was also investigated. As shown in Figure 6, in the low to nearly neutral pH range, where the imidazole group of the complex was certainly protonated (the imidazole pK_a of L-histidine is 6.16[44d]), the linearization process was observed. At pH values above this range, deprotonation of the imidazole group began and linearization was still observed. The formation of significant amouts of linear DNA in the high-pH region, where we believe Cu(L-His) is deprotonated (leading to a total charge on the deprotonated complex of 0; in the pH 7.0−9.0 region, major species are ML_2 and $MH_{-1}L_2$ in the Cu(L-His) system[44a]), suggests that electrostatic contributions to the DNA binding are minor compared to the nucleophilic displacement reaction and other contributions. This would lead to a relative insensitivity of scission to ionic strength, as is observed (Figure 5). Thus, the data in Figure 5 may represent a situation in which the formation of substantial amounts of nicked DNA even at high ionic strength reflects a weak electrostatic contribution to binding and not a strong electrostatic interaction. This would explain why the binding event associated with linearization, which supposedly occurs at a negatively charged nick gap, appears to have greater sensitivity to ionic strength than the binding event associated with nicking in Figure 5.

Specificity of DNA Cleavage. To determine whether the cleavage reaction displayed any form of sequence selectivity, we used the primer extension method to locate the cleavage sites. Plasmid pUC18 DNA was cleaved with Cu(L-His) in a buffer (5 mM Tris−HCl buffer containing 5 mM NaCl) at 37 °C for 8 h and then purified by precipitation with EtOH. The experiments with pUC 18 DNA as the control were performed

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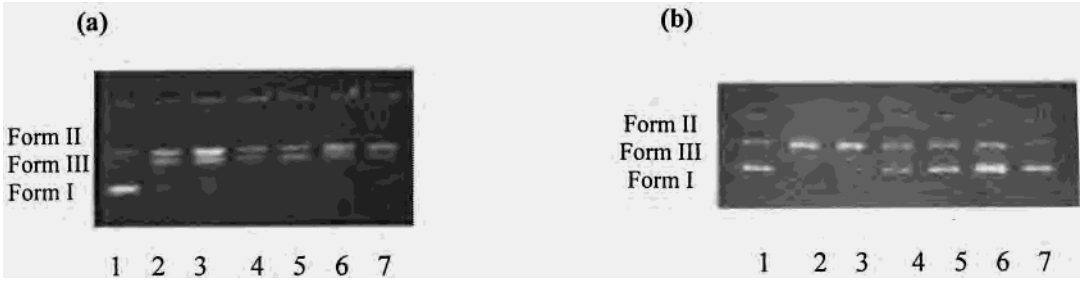

Figure 5. Ionic strength dependence of pBR322 DNA cleavage by Cu(L-His). (a) Lane 1: control DNA. Lanes 2−7: 0, 2.5, 5, 10, 20, and 50 mM NaCl added to cleavage buffer. (b) Lane 1: control DNA. Lanes 2−7: 50, 100, 200, 300, 400, and 500 mM NaCl added to cleavage buffer. Cleavage conditions: 42 μM DNA base pairs; 1 mM $CuCl_2$−L-histidine (molar ratio 1:3); 5 mM Tris−HCl buffer, pH 6.0; 37 °C for (a) 5.5 and (b) 3.5 h.

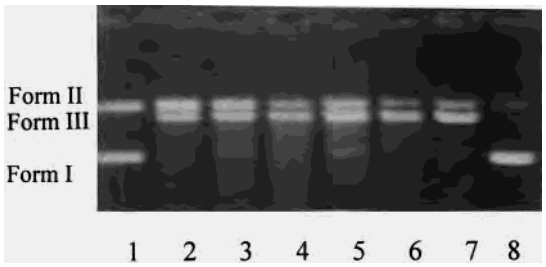

Figure 6. DNA strand scission by Cu(L-His) as a function of pH: lane 1, control DNA at pH 5.48; lane 2, pH 5.48; lane 3, pH 5.88; lane 4, pH 6.28; lane 5, pH 7.59; lane 6, pH 8.43; lane 7, pH 8.59; lane 8, control DNA at pH 8.59. Cleavage conditions: 42 μM DNA base pairs; 1 mM $CuCl_2$−L-histidine (molar ratio 1:3); 5 mM Tris−HCl buffer; 37 °C for 5 h.

under identical conditions. The ^{32}P-labeled, M13/pUC sequencing primer (−40) (CAGCACTGACCCTTTTG) was annealed with the denatured purified plasmid DNA and extended with T7 DNA polymerase along the plasmid templates. The location of cleavage sites was analyzed on a 6% denaturing polyarylamide electrophoresis gel (Figure 7). As shown in Figure 7, Cu(L-His) cleaved DNA with a certain sequence specificity, the new cleavage sites (5′→3′) were GTCG and GTG. All cleavage sites were almost exclusively located at the 3′-thymine of the 5′-GT-3′ sequence of the target (lane 2); however, the sites of damage of control DNA such as CCTA and CTT did not exist in the Cu(L-His)-mediated DNA cleavage system (lane 1). This further proved that Cu(L-His) cleaved DNA with a certain sequence specificity.

The selective DNA strand scission by Cu(L-His) is possibly due to the presence of a group that recognizes a specific base sequence such as the imidazole moiety of L-His. It is known that L-His chelates the Cu(II) ion with both its nitrogen and carboxyl functional groups.[44] Therefore, one possible explanation for G, T recognition is the formation of a three-center hydrogen bond involving the NH_2 group of guanine, the lone pair of electrons on the pyridine nitrogen of imidazole, and the carbonyl of L-His. This explanation is also consistent with the fact that Cu(II)−histamine and Cu(II)−imidazole complexes do not produce any cleavage of pBR322 DNA.

Investigation of Chemical Species in the Cu(L-His) System. To obtain information regarding the active chemical species that effect DNA damage, we identified the simultaneously existing multiple species in the Cu(L-His) aqueous solution system by means of electrospray ionization mass spectrometry (ESI-MS) (Figure 8). As Figure 8 shows, in the ES mass spectrum of Cu(L-His) in aqueous solution, the peak at $m/z = 372.2$ is the most intense signal; this is identified as $[Cu(L\text{-}His)_2 - H]^+$ ($[CuL_2H]^+$; HL = L-histidine). The characteristic peak at $m/z = 527.0$ is typical for $[Cu(L\text{-}His)_3 - 2H]^+$ ($[CuL_3H]^+$). Furthermore, the signal shows a characteristic isotope pattern that is mainly due to the different isotopes of the Cu atom. The isotopic peaks separated by 1.0 m/z unit are attributed to a singly charged cationic complex. The $m/z = 156.2$ peak is assigned to [L-His + H]$^+$. Two other peaks can also be assigned: an L-His dimerization product ($m/z = 311.3$) and a dimerization product of $[CuL_2H]^+$ ($m/z = 744.9$). These observations suggest that the main species existing in aqueous solution is $[CuL_2H]^+$, with $[CuL_3H]^+$ being minor.[43] The interaction of copper(II) and L-histidine has been extensively studied,[44,45] and it has been concluded that the major species are CuL_2 and $[CuL_2H]^+$ for a freshly prepared solution in the pH 4.5−7.0 region, the minor species being CuL and CuHL.[44a,b,45b] Unexpectedly, ESI-MS indicated the formation of $[CuL_3H]^+$ species in the system. This may be explained by the fact that $[CuL_3H]^+$ is formed with a prolonged mixing time because of the excessive amount of L-histidine ligand employed and the formation of $[CuL_3H]^+$ is slower than that of $[CuL_2H]^+$. Further, optimization of HPLC separations on reversed-phase C-18 columns with water/methanol gradients allowed separations of all possible components in our Cu(L-His) solutions used in blind runs. We collected two main components, freeze-dried them under vacuum, and obtained their IR spectra. The IR data indicated that the imidazole amino group is coordinated to Cu(II) along with the carboxyl group. It was reported[45f,g] that Cu(II) prefers to coordinate with histidine via the amino N and the carboxyl O atoms in the lower pH range (3−5) and via the imidazole and the amino N atoms in the higher pH range (>4.8). Considering the geometry of $[CuL_3H]^+$ that must satisfy the steric and electrostatic requirements for the complexation and combining this with our experimental ESI-MS, visible absorption, and IR results, we suggest the possible structure for $[CuL_3H]^+$ (**II**) at pH ≥5 formulated in Chart 1. The structure of $[CuL_2H]^+$ shown in Chart 1 is that proposed by Kruck and Sarkar[44b] and is also consistent with our experimental results.

The structures of the copper(II)−L-histidine complexes are of considerable interest because of their importance in blood plasma[45c] and because of the complexities introduced by combining a metal ion favoring four-coordination with a potentially tridentate ligand. Depending on the pH, histidine molecules may act as (a) bidentate ligands (LL) bound to copper(II) through the amino N and carboxyl O atoms (glycine-

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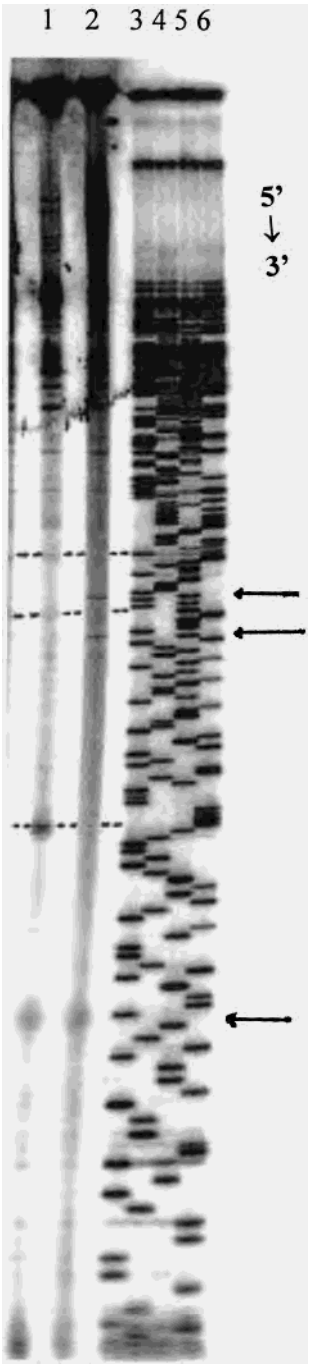


Figure 7. Determination of the location of the Cu(L-His) cleavage site. Lane 1: pUC 18 plasmid DNA as control incubated in 5 mM Tris−HCl buffer (containing 5 mM NaCl) at 37 °C for 8 h. Lane 2: pUC 18 plasmid DNA incubated with 1 mM Cu(L-His). Lanes 3−6: G, A, T, C. The arrows show the sites of damage in Cu(L-His), and the dotted lines show the sites of damage in the control DNA, except in lane 2.

like bonding), (b) bidentate ligands (LL′) bound to copper(II) through the amino and imidazole N's, or (c) tridentate ligands with the N's of the amino and imidazole in a square plane and the carboxyl oxygen chelated axially.[45a] However, until now, the X-ray diffraction solid-state structures of the physiologically important complexes CuL_2 and $[CuL_2H]^+$ have not been available and the structures of copper(II)−L-histidine complexes are far from being elucidated. It is quite possible that $[CuL_3H]^+$ possesses various kinds of structures in solution, so this is still an open question.

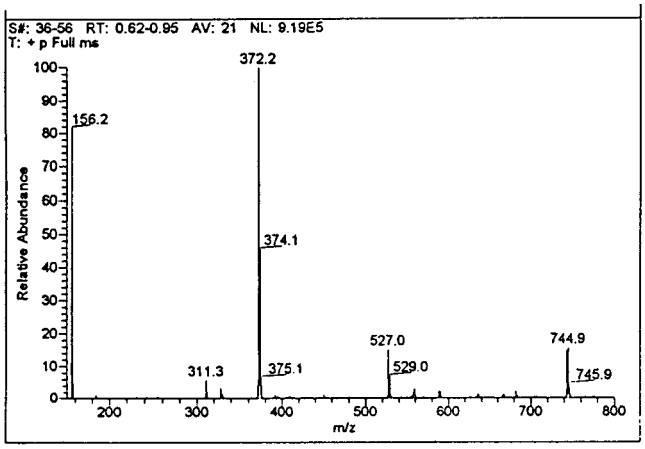
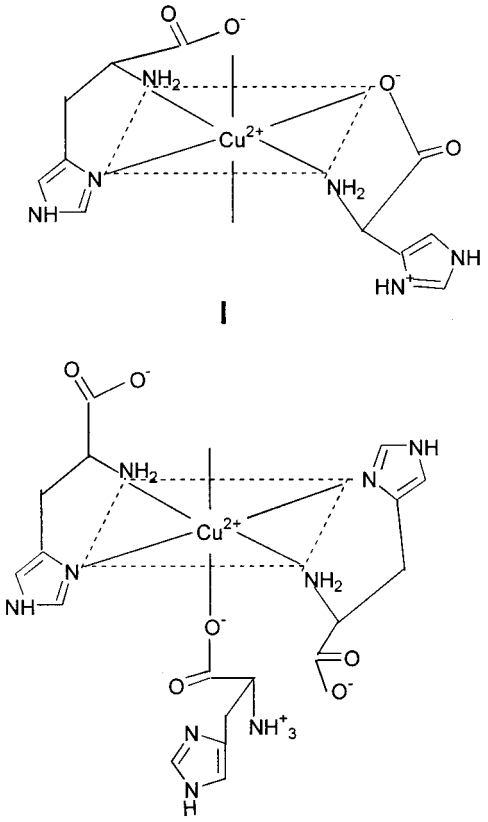


Figure 8. ESI mass spectrum of Cu(L-His) in aqueous solution.

Chart 1. Proposed Structures of the Species in the Prepared Cu(L-His) Solutions: **I**, $[CuL_2H]^+$; **II**, $[CuL_3H]^+$ [a]



[a] The diagrams are not to be taken as representing absolute steric configurations.

It should be pointed out that a prepared solution of copper(II)−L-histidine complexes was more effective than a freshly prepared solution for cleavage of DNA; thus $[CuL_3H]^+$ participation in DNA phosphate diester hydrolysis cannot be excluded. Further investigations are being carried out.

Visible spectra of 1:3 Cu(L-His) (4 mM) were also recorded (pH 6.0, Tris−HCl buffer) in the absence and the presence of DNA. As shown in Figure 9, the spectrum of Cu(L-His) is typical for Jahn−Teller-distorted copper(II) complexes, with λ_{max} for these species at 630 nm. In the presence of DNA, a marked increase in intensity coupled with a blue shift in λ_{max}

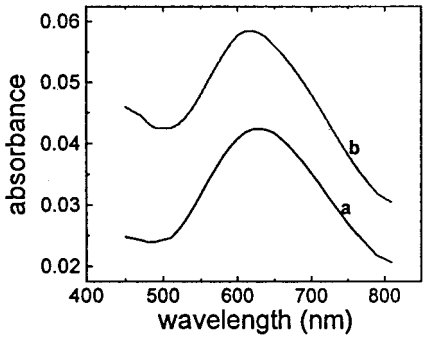


Figure 9. Visible spectra of 1:3 Cu(L-His) complexes (4 mM) in 5 mM Tris−HCl buffer (containing 5 mM NaCl; pH 6.0): (a) no DNA; (b) CT DNA added, [DNA-phosphate]:[Cu] = 1:20.

to 620 nm occurs. This provides evidence for apical coordination of DNA to Cu(L-His). A blue shift of the absorption maximum for the Cu(L-His)−DNA system indicates partial deprotonation of the pyrrole nitrogens of the coordinated histidine molecules.[44c] This implies that $[CuL_2H]^+$ and $[CuL_3H]^+$ facilitate binding with DNA.

Discussion

Hydrolytic Cleavage Efficiency. To evaluate the efficiency of Cu(L-His)-catalyzed DNA hydrolytic cleavage, it is necessary to compare the literature rate constants for metal-promoted DNA phosphate diester hydrolysis with those measured in this study. Burstyn and co-workers reported that copper(II)−macrocyclic triamine complexes promote the hydrolytic cleavage of plasmid DNA; however, the rate constant was estimated to be 0.04−0.09 h^{-1} at 50 °C from the half-life of the hydrolysis.[29a] The stable Co(III) complexes $[(en)_2Co(OH)(OH_2)]^{2+}$ (**1**), $[(cyclen)Co(OH)(OH_2)]^{2+}$ (**2**) and $[(tamen)Co(OH)(OH_2)]^{2+}$ (**3**) promote hydrolysis of the DNA phosphodiester. The approximate pseudo-first-order rate constants for the disappearance of form I DNA and appearance of form II DNA at a complex concentration of 1 mM and at 37 °C are $\ll 3.6 \times 10^{-3}$ h^{-1} for hydrolysis promoted by **1**, 3.6×10^{-2} h^{-1} for hydrolysis promoted by **2**, and 0.18 h^{-1} for hydrolysis promoted by **3**.[5b] The hydrolytic cleavage of plasmid ds-DNA by lanthanoid cations and their complexes is characterized by saturation kinetics; the accessible pseudo-first-order rate constant at the catalyst concentration used for the Michaelis−Menten analysis with native DNA is 0.25 h^{-1}, which could be realized experimentally with [Eu(III)] = 2.5 mM.[5a] Faster systems have been reported, e.g., a Co(III)−polyamine system for the decrease of pBR322 RF I, with $k = 0.82$ h^{-1} at 1 mM catalyst concentration and 37 °C,[31] and a Cu(II)−1,3,5-triaminocyclohexane system, with $k = 4.3$ h^{-1} at 37 °C.[30a] The rate constants for DNA hydrolysis by Cu(L-His) complexes ([Cu(L-His)] = 1 mM) are estimated to be 0.76 h^{-1} for the decrease of form I and 0.25 h^{-1} for the increase of form III, so it is concluded that the Cu(L-His) system has a higher potential for DNA hydrolytic cleavage. DNA is enormously resistant to hydrolysis, with the pseudo-first-order rate constant for the nonenzymatic hydrolysis of phosphodiesters extrapolated to be 2×10^{-10} h^{-1} at 24 °C and pH 7.4.[41b] The exceptional stability of phosphodiesters has been suggested as one reason that nucleic acids evolved as genetic material.[46] In view of the need to cleave only one or two out of 8800 nucleotide bonds in the plasmid DNA in order to observe the disappearance of the form I or the appearance of the form III, the limiting rate would be $k = 2 \times 10^{-10}$ h^{-1} at 24 °C and pH 7.4. Comparison of the catalytic rate constant for Cu(L-His)-mediated DNA hydrolytic cleavage with the rate constant for nonenzymatic hydrolysis indicates an approximate rate enhancement factor of 10^8 for DNA hydrolysis by the Cu(L-His) system (after correction by a factor of about 10 for the temperature difference (25 °C vs 37 °C)).[31a]

It should be pointed out that until now only a few metal complexes were demonstrated to hydrolytically cleave phosphodiester bonds of DNA dideoxynucleotides.[2a,e,5d,27,28,31b] Komiyama et al.[27,28] using HPLC have obtained intact thymidine (T) by from reactions of the dinucleotide TpT with trivalent lanthanides, but at conversion rates of only 0.2%. Hettich and Schneider[31b] have been able to cleave TpT with 5 mM Eu(III), at a conversion rate of 25%. Eu(III) accelerates the hydrolyses of DNA dinucleotides at 70 °C by factors of about 3×10^7. dApdA (5 mM) cleavage by 0.8 mM Cu(L-His) reaches 25% completion, with a rate acceleration factor of about 7×10^8 at pH 6.0 and 37 °C. Zr(IV) also has a higher hydrolysis capacity.[2e] The Ce(IV) ion is best for DNA hydrolysis, with a rate acceleration factor of $>10^{11}$ for [Ce(IV)] = 20 mM.[5d] However, a disadvantage of Ce(IV) compounds for applications in molecular biology is their sensitivity to reduction (formation of Ce(III)). It is known that enzymes can use some transition metals, Mg(II), or Ca(II) to cleave phosphate esters. These nonlanthanide ion catalysts, if available, should be useful for various practical applications (especially in vivo). Consequently, there is considerable interest in developing simple transition metal, Mg(II), or Ca(II) complexes that can efficiently hydrolyze the phosphate diester bonds of DNA and RNA. Cu(L-His) represents, by far, the more reactive transition metal complex reported to date for hydrolyzing DNA.

DNA Scission Chemistry. In this paper, we establish that Cu(L-His) complexes are capable of cleaving double-stranded DNA and dApdA. Supercoiled plasmid DNA (form I) incubated with Cu(L-His) at physiological pH and temperature is nicked to form relaxed circular DNA (form II) and slowly linearized to form linear DNA (form III). The extent of DNA degradation depends on both the metal complex concentration and the time of incubation, and it is concluded that the Cu(L-His) complexes are the reagents responsible for the degradation of DNA.

Anaerobic reactions exhibit an approximately 5% decrease in the extent of DNA degradation relative to aerobic reactions at the same metal complex concentration. The presence of the radical scavengers DMSO, glycerol, and MeOH does not significantly reduce the efficiency of DNA cleavage. These results rule out the possibility of forming diffusible hydroxyl radicals;[7] however, they do not completely rule out the possibility of an oxidative cleavage mechanism. Another aspect of the Cu(L-His)-promoted cleavage of DNA is the amount of activity retained in the absence of O_2: approximately 95% of the cleavage activity is retained when the reactions are performed under rigorously anaerobic conditions with Tris as the buffer (known to be a singlet oxygen scavenger).[40] This result suggests that oxidation products are not involved to a significant extent and rules out the possibility of an oxidative cleavage mechanism. Analysis for malondialdehyde-like products further supports the conclusion that the reaction does not occur by an oxidative mechanism. Furthermore, treatment of Cu(L-His)-linearized plasmid DNA with T4 DNA ligase results in its conversion to the closed circular plasmid and concatemers of pBR322. These observations suggest that the double-stranded cleavage mediated by Cu(L-His) occurs via a hydrolytic path. Further support for a hydrolytic path is obtained from an HPLC experiment with 2′-deoxyadenylyl-(3′→5′)-2′-deoxyadenosine

(46) Westheimer, F. H. *Science* **1987**, *235*, 1173.

(dApdA). The evidence presented here suggests that the structures of $[CuL_2H]^+$ and $[CuL_3H]^+$ facilitate their binding at nicked sites on DNA, in addition to binding at the intact DNA surface. The highest yields of linear DNA are found in ~1.7:1 linear:nicked DNA product mixtures (Figure 3); since base-hydrolyzable sites form in a 1:1 ratio with double-strand cleavage sites, $[CuL_2H]^+$ and $[CuL_3H]^+$ can produce complementary lesions in about two-thirds of the DNA. We attribute the efficiency of double-strand cleavage by $[CuL_2H]^+$ and $[CuL_3H]^+$ to their charged, carboxyl group, and imidazole heterocyclic aromatic recognition elements.

Nicked DNA in general has a structure very similar to that of B-DNA, with perturbations due to a nick only occurring at the nick position (on both strands) and at the flanking base pairs.[47,48] Changes of approximately 1 Å are estimated to occur in this region.[47] The site is likely to have 3′- and 5′-phosphate end groups at the nick. The nick gap probably allows greater access to the hydrophobic interior than normally found along a DNA surface. Given that a nick provides a negatively charged gap in close proximity to an accessible region of the DNA interior, $[CuL_2H]^+$ and $[CuL_3H]^+$ recognize nicked DNA by electrostatic and nucleophilic displacement reactions.

One of the most interesting and remarkable observations of this study is that such a simple Cu(II)−L-histidine system can not only promote hydrolytic cleavage of DNA but also efficiently hydrolyze the phosphate diester bonds of DNA. Many transition metal complexes can efficiently hydrolytically cleave plasmid DNA, but they cannot efficiently cleave dinucleotides or oligonucleotides, e.g., Co(III) complexes[31] Cu(II)−macrocyclic triamine complexes.[29a]

(47) Snowden-Ifft, E. A.; Wemmer, D. E. *Biochemistry* **1990**, *29*, 6017.
(48) Pieters, J. M. L.; Mans, R. M. W.; van den Elst, H.; van der Marel, G. A.; van Boom, J. A.; Altona, C. *Nucleic Acids Res.* **1989**, *17*, 4551.

Conclusion

In summary, we have designed a simple copper(II)−L-histidine system that can effectively promote the hydrolytic cleavage of plasmid DNA with a rate constant of 0.76 h^{-1} at pH 6.0 (5 mM Tris−HCl, 5 mM NaCl) and 37 °C. The cleavage reaction displays a certain sequence selectivity (preferentially at 5′-GT-3′), and the dinucleotide hydrolysis shows, with [Cu(L-His)] = 0.8 mM, rate enhancement factors of $>10^8$. This is one of only a few well-defined metal complex systems demonstrated to hydrolytically cleave dideoxynucleotides and DNA.

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Supporting Information Available: Photographs showing the cleavage of pBR322 DNA by Cu^{II}(histamine), Cu^{II}(imidazole), L-His, and Cu(II), the cleavage of pBR322 DNA by Cu(L-His) in the presence of radical scavengers, and the cleavage of the pBR322 DNA by 1 mM Cu(L-His) at various time intervals and figures showing the concentrations of dApdA, dAMP, dcAMP, and dA as functions of time in the Cu(L-His)-mediated cleavage of dApdA, along with ES-MS spectra of $[Cu(L\text{-}His)_2 - H]^+$ and $[Cu(L\text{-}His)_3 - 2H]^+$. This material is available free of charge via the Internet at http://pubs.acs.org.

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