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Synthesis and DNA cleavage properties of ternary Cu(II) complexes containing histamine and amino acids

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Abstract—The ternary complexes of $[Cu^{II}(Hist)(Tyr)]^+$ **1** and $[Cu^{II}(Hist)(Trp)]^+$ **2** have been synthesized, structurally characterized and their DNA binding and cleavage abilities probed. The intrinsic binding constants (K_b) for complexes/CT-DNA were also determined ($K_b = 2.7 \times 10^2$ for complex **1** and $K_b = 2.2 \times 10^2$ for complex **2**). These complexes exhibit their nuclease activity on plasmid DNA, which seems to depend on the nature of the aromatic moiety. The DNA hydrolytic cleavage rate constants were also determined for complexes **1** and **2**, which are 0.91 and 0.79 h^{-1}, respectively.

The good stability of phosphate diesters to hydrolysis under physiological conditions is an essential feature of the chemistry of nucleic acids and is intrinsic to life itself. Nucleases that cleave nucleic acids are well known. Restriction endonucleases, which recognize specific DNA sequences bind to them and cleave both DNA strands.[1]

There are also small molecules that carry out similar phosphate diester hydrolysis. A thoroughly studied DNA cleavage reagent is the glycopeptide antibiotic and anticancer drug bleomycin A_2. A number of closely related antibiotics have been discovered with slight modifications in different regions of the bleomycin molecule.[2]

Synthetic metal complexing reagents such as porphyrin derivatives and 1,10-phenanthroline metal complexes have also been found to bind to DNA and cause strand cleavage.[3] The methidium propyl-EDTA derivative (MPE) designed by Moser and Dervan[4] is now widely used as a foot-printing reagent because of its relatively non-selective cleavage of DNA. Unlike the highly specific proton abstraction observed with the natural antibiotics, synthetic cleavage reagents produce diffusible species, presumably a hydroxyl radical, which can react at several nucleic acid sites. The species is short-lived and attacks the deoxyribose groups of DNA in close proximity to the intercalated ring of the metal complexes. Shape-selective recognition units, which bind to nucleic acid sites that are complementary to the shape and symmetry of the metallo species, have also been designed.[3] It was reported[5] that strong binding of the reagent is not essential, but highly selective binding is required. The metallo-reagent, tris(4,7-diphenyl-1,10-phenanthroline) rhodium(III), gives highly specific cleavage at base-pair mismatches in A-form RNA but not at unperturbed sections of A-form duplex. However, the structural rationale for these highly specific cleavage events has not been determined.

Recognition of different structures can be achieved by variation of the metal and the ligands. Therefore, one of the most exciting areas on synthetic cleavage reagents involves the design of highly specific artificial nucleases.

A survey of the literature revealed that the most efficient cleavage agents that involve a hydrolytic mechanism happen to be mononuclear complexes[6–13] in comparison to dinuclear,[14–19] trinuclear,[20–23] and multinuclear complexes.[24,25] Enhancements of rate constants in the range of 0.09–0.25 h^{-1} for DNA hydrolysis by metal complexes are considered impressive,[26,27] even though they are still a long way from the rate enhancements produced by natural enzymes (between 40 and 1.4×10^4 h^{-1}).[28–30]

In order to develop artificial nucleases, our group has designed two new copper-containing metallo-reagents

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and probed their DNA binding and cleavage abilities. Here we report the preparation, characterization, and DNA binding and cleavage abilities of [Cu(II)(Hist)-(Tyr)] **1** and [Cu(II)(Hist)(Trp)] **2**. The hydrolysis rate constants are impressive considering the nature of the ligands involved in this investigation.

The ligands (histamine and tyrosine for complex **1** and histamine and tryptophan for complex **2**) were dissolved in doubly distilled water (30 cm^3) and added dropwise to a solution of $CuCl_2$ dissolved in the same solvent (30 cm^3). The resulting slurry was refluxed with stirring under an inert atmosphere. The solution was filtered to eliminate excess unreacted $CuCl_2$. The precipitate was washed with methanol and air dried.[31] (Yield: 62% for complex **1** and 64% for complex **2**).

The experimental details and reaction set-up are available as supplementary information.

The broad peak at 3408 cm^{-1} in the IR spectrum of complex **1** is due to a combination of the amine N–H and phenol O–H stretches. The absorptions at 2933 and 1263 cm^{-1} are the C–H and C–N stretching frequencies of the aliphatic amine, the peaks at 2100 and 1219 cm^{-1} are the result of asymmetric NH_2 bending and C–O stretching vibrations in phenol, respectively. The non-ligand bands observed in the far IR region of the spectra were assigned to Cu–O and Cu–N stretching vibrations.[32]

The peak at 3027 cm^{-1} for complex **2** is due to the amine N–H stretching. The carboxylate peak at 1681 cm^{-1} in the free ligand was shifted to 1626 cm^{-1} in the complex, indicating involvement of the COO^- group in metal coordination. The peaks at 456 and 559 cm^{-1} were assigned to Cu–O and Cu–N stretching vibrations, respectively.[32]

The electronic spectra of the Cu(II) complexes exhibited absorption bands at 17,211 cm^{-1} for complex **1** and 17,574 cm^{-1} for complex **2** due to the $^2B_1g \rightarrow {}^2Eg$ transition, which corresponds to a square planar configuration.[33] No absorption band was observed below 10,000 cm^{-1} in both the complexes ruling out the possibility of a tetrahedral geometry.[34,35]

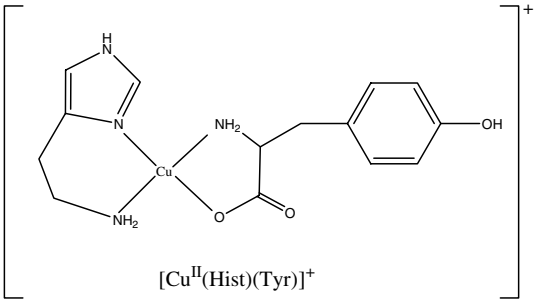
The active chemical species of the complexes were identified by means of electrospray ionization mass spectrometry (ESI-MS). The peaks at *m/z* 355 and 377 are the molecular ion peaks for complexes **1** and **2**, respectively. In addition, the peak at *m/z* 174 is due to the $[Cu(Hist)]^+$ ion in both complexes. The peak at *m/z* 181 is due to the tyrosine of complex **1** and the peak at *m/z* 204 corresponds to tryptophan in complex **2**.

It was reported[36] that histamine binds to copper through N–N coordination and the amino acids, tyrosine and tryptophan, coordinate in a glycine-like manner forming mononuclear complexes. The phenolic oxygen of tyrosine and the pyrrole-like proton of the indole ring in tryptophan do not appear to take part in coordination at pH < 10. Crystal structure evidence also does not show any copper–phenol interactions in tyrosine.[37] These findings consistently support the view that the copper coordinates through amino and imidazole nitrogens of histamine and amino nitrogen and carboxylate oxygen of tyrosine or tryptophan. Structure 1 is proposed for complex **1**.

The absorption spectra of complexes **1** and **2** in the absence and presence of calf thymus DNA are illustrated in Figure 1. In the presence of DNA, a decrease in absorption intensities (hypochromism) with a slight increase of wavelength (bathochromism) was observed for **1** and **2**. Hypochromism and bathochromism were suggested to arise due to the interaction between the electronic state of the intercalating chromophore and that of the DNA bases.[38–42] These spectral changes are consistent with the intercalation of complexes **1** and **2** into the DNA base stack. The intrinsic binding constants (k_b) of the complexes with CT DNA were determined using the following function equation.[43]

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f) \quad (1)$$

where [DNA] is the concentration of DNA in base pairs, ε_a, ε_f and ε_b correspond to A_{obsd}/[Cu], the extinction



$[Cu^{II}(Hist)(Tyr)]^+$

Structure 1. Proposed structure for complex **1**.

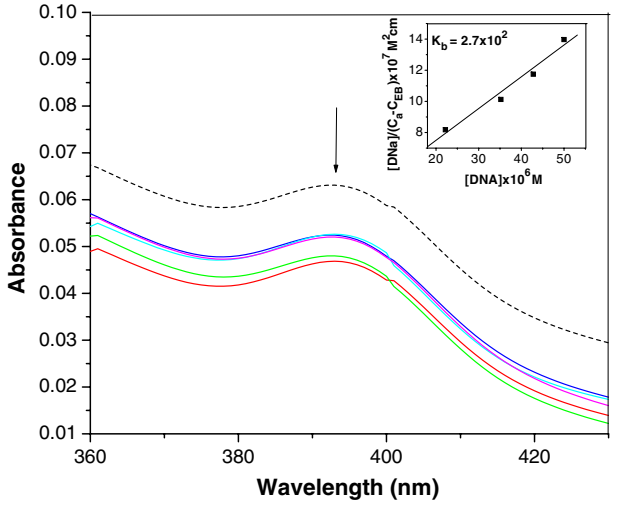


Figure 1. Absorption spectra of complex **1** in the absence (---) and presence (—) of increasing amounts of DNA. [Cu] = 50 μM. Arrow (↓) shows the absorbance changes upon increasing DNA concentration.

coefficient for the free copper complex, and the extinction coefficient for the copper complex in the fully bound form, respectively. In plots of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA], is given k_b as the ratio of the slope to the intercept. The intrinsic binding constants K_b of **1** and **2** were determined to be 2.7×10^2 and 2.2×10^2, respectively.

The thermal denaturation profiles of DNA in the absence and presence of complexes **1** and **2** are provided in Figure 2. An increase of 5 °C was observed in the T_m profiles of complexes as compared to free DNA. These results provide evidence for intercalative binding of the Cu^{II} complexes with DNA. It seems that the first interaction between DNA and **1** and **2** is due to an electrostatic interaction with the unipositively charged complexes and the negatively charged DNA-phosphate backbone followed by an intercalative mode of binding.

Ethidium bromide (EB) strongly fluoresces in the presence of DNA due to complete intercalation between adjacent DNA base pairs, a process that can be reversed by addition of a competing molecule (fluorescence quenching).[44] The emission spectra of EB bound to DNA in the absence and presence of **1** or **2** are shown in Figure 3. The addition of the complexes to DNA-EB caused a reduction in fluorescence intensity, indicating binding of the complexes to DNA.

Fluorescence scatchard plots for the binding of EB to calf thymus DNA in the presence of metal complexes were obtained as described by Lepecq and Paoletti,[45] and r_{EB} values were determined. The term $r_{EB} = [EB]_b/$DNA is the concentration ratio of bound EB to total DNA; and $[EB]_f$ is the concentration ratio of free EB (i.e., $[EB] = [EB]_b + [EB]_f$). As can be seen from the plots (Fig. 4), a decrease of slope and intercept resulted upon the addition of complexes **1** and **2** indicating an intercalative and covalent binding of the complexes with DNA.

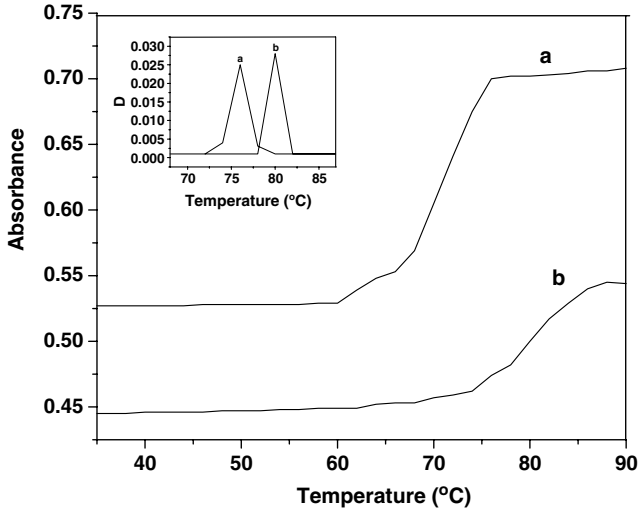


Figure 2. Thermal denaturation profiles and differential melting curves (insets a and b) of calf thymus DNA before (a) and after (b) addition of complex **1** (75 μM), DNA concentration (75 μM). *D* is the difference in absorbance.

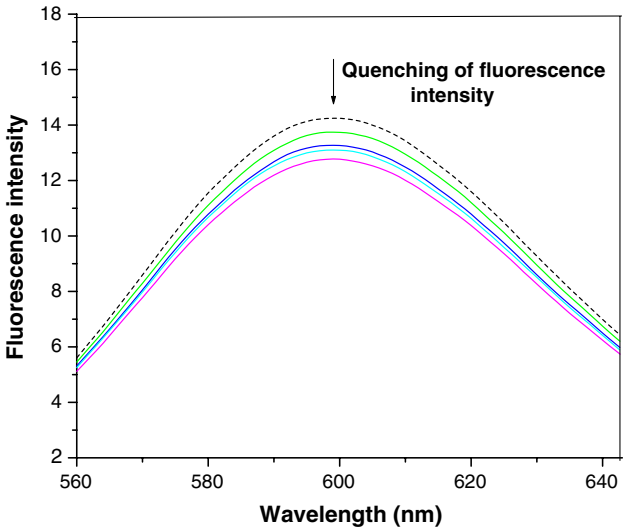


Figure 3. Emission spectra of EB bound to DNA in the absence (...) and presence (—) of complex **1**. $\lambda_{ex} = 540$ nm, in tris buffer (aqueous medium).

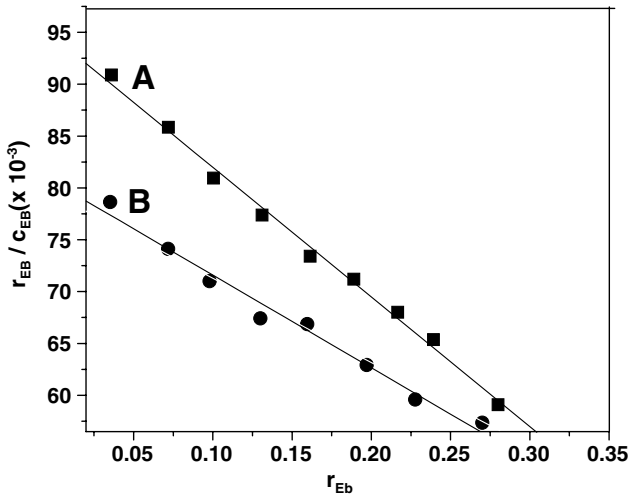


Figure 4. Fluorescence scatchard plots for EB to CT-DNA in the absence (A) and presence (B) of [Cu^{II}(Hist)(Tyr)] (**1**). The term r_{EB} is the concentration ratio of bound EB to total DNA, and c_{EB} is the concentration of free EB.

The cleavage reaction on plasmid DNA was monitored by agarose gel electrophoresis. When circular plasmid DNA was subjected to electrophoresis, relatively fast migration was observed for the intact supercoiled DNA (type I). If scission occurs on one strand (nicking), the supercoiled DNA will relax to generate a slower moving open circular form (type II). If both strands are cleaved, a linear form (type III) that migrates between type I and type II will be generated.[46,47] The conversion of type I (supercoiled) to type II (nicked circular) was observed with increase in the concentrations of complexes **1** and **2** (Fig. 5a and b). It is important to note here that even at 1.0 mM concentration of free Cu(II) and independent free ligands, there was no significant DNA cleavage activity. The extent of DNA cleavage was quantified via fluoroimaging (Table 1). The possible reasons for the degradation of DNA are

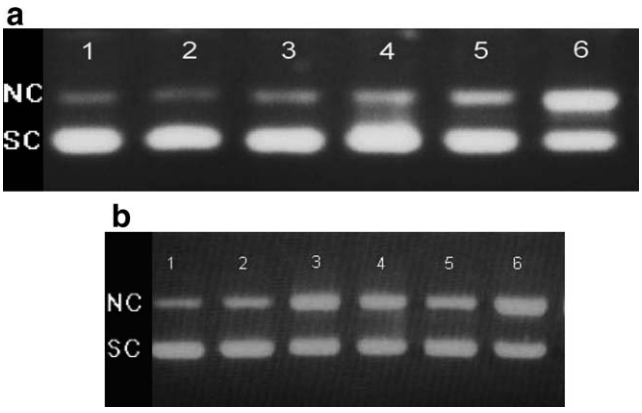


Figure 5. Agarose gel electrophoresis results for the cleavage of pUC19 DNA by (a) [Cu^{II}(Hist)(Tyr)] and (b) [Cu^{II}(Hist)(Trp)]. (a) Lane 1, DNA control; lane 2, **1** (125 μM); lane 3, **1** (250 μM); lane 4, **1** (375 μM); lane 5, **1** (500 μM); lane 6, **1** (625 μM). (b) Lane 1, DNA control; lane 2, **2** (125 μM); lane 3, **2** (250 μM); lane 4, **2** (375 μM); lane 5, **2** (500 μM); lane 6, **2** (625 μM).

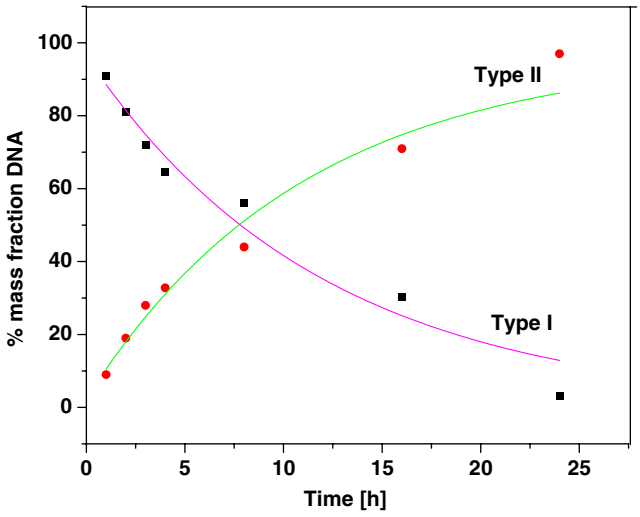


Figure 6. Time course of DNA cleavage by complex **1** at 37 °C (■) Type I (supercoiled) (●) Type II (nicked circular).

(a) formation of a three-center hydrogen bond involving the NH_2 group of guanine, (b) the lone pair of electrons on the pyrrole nitrogen of imidazole, and (c) the hydroxyl group of phenol in tyrosine.

Although these complexes did not require the addition of an external agent, we were keen to discount the possibility that the DNA cleavage occurred via a hydroxyl radical-based depurination pathway. When pUC 19 DNA was incubated with complexes **1** and **2** in the presence of 0.4 M DMSO or glycerol, only a slight change in the extent of DNA cleavage was observed. This suggests that the complex **1** or **2** mediated cleavage in the absence of exogenous coreactants does not proceed via either diffusible hydroxyl radicals or free superoxide.[48]

In the investigation of the kinetics of the reaction, the observed distribution of supercoiled (type I) and nicked (type II) DNA, as determined by agarose-gel electrophoresis, provides a measure of the extent of hydrolysis of phosphodiester bonds in plasmid DNA. These data were used to perform a simple kinetics analysis (Fig. 6). The relative fractions of type I and type II DNA are plotted as a function of time for complexes **1** and **2**. The decrease and increase of type I and type II DNA were found to fit well to single-exponential curves, respectively. By curve fitting, the hydrolysis rates for the supercoiled DNA at 37 °C in tris buffer at fixed complex concentrations of 375 μM were the following: 0.91 h^{-1} (**1**; $R = 0.941$) and 0.79 h^{-1} (**2**; $R = 0.971$). The high rate constant for complex **1** may be due to the presence of the phenol group.

Table 1. Cleavage of pUC 19 DNA by complexes **1** and **2** at different concentrations

Entry	Conc (μM)	Type I (%)		Type II (%)	
		1	**2**	**1**	**2**
1	0	93	90	7	10
2	125	88	79	12	21
3	250	79	65	21	35
4	375	68	58	32	42
5	500	55	51	45	49
6	625	44	47	56	53

Among the aromatic amino acids, tyrosine is unique in the sense that its phenol group can be involved in binding and cleavage of DNA. Planar copper(II) complexes with aromatic groups in the ligands often intercalate to DNA. The intercalation of planar aromatic molecules to DNA causes an increase in the melting temperature.[49] These results indicate that the hydroxyl group in the amino acid has a potential function in the binding of the metal complex to DNA and also its cleavage.[50]

The hydrolysis rate constant for [Cu^{II}(Hist)(Tyr)], which has high nuclease activity, gives a 2.52×10^7 fold rate enhancement over unhydrolyzed double stranded DNA. This is impressive considering the nature of the ligands involved.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.08.033.

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