Chinese Chemical Letters Vol. 10, No. 5, pp. 383–386, 1999

DNA Cleavage by Copper (II) Complexes Without added Coreactants

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Abstract: The cleavage activity of complexes between copper (II) and four different amino acid or amino acid methyl ester on DNA was tested at physiological pH and temperature.. It was found that Cu (II)-L-His and Cu (II)-L-His methyl ester complexes could cleave DNA. The extent of DNA cleavage is largely dependent on the metal ion-to-ligand ratio. The cleavage of double-stranded DNA mediated by Cu (II)-L-His complexes occurs *via* a hydrolytic mechanism.

Keywords: Copper (II) complexes; DNA; hydrolytic cleavage.

The development of reagents which hydrolytically cleave nucleic acids under mild conditions is currently attracting great interest in the field of artificial nucleases[1-3], While many reagents have been successfully applied to RNA hydrolysis[4], there have been fewer successes with DNA[5] due to its relatively high hydrolytic stability[6] (the pseudo-first-order rate constant for the nonenzymatic hydrolysis of phosphodiesters is extropolated to be 2×10^{-10} h^{-1} at 24℃ and pH 7.4[7]). The exceptional stability of phosphodiesters has been suggested as one reason that nucleic acids evolved as genetic material[6]. Small metal complexes that promote the hydrolysis of DNA could be useful not only in molecular biology and drug design but also in elucidating the precise role of metal ions in enzyme catalysis.

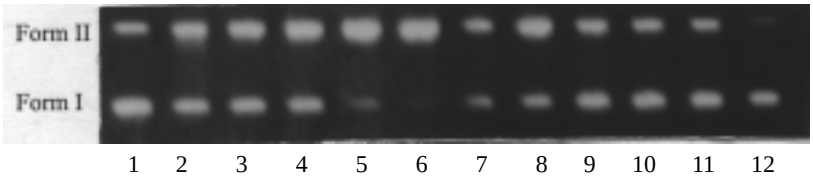
It is known that in many metal dependent phosphoryl transfer enzymes catalysis is supported by amino acid side chains which act as acids, bases or nucleophiles. The NH-acidic groups are represented by the positively charged residues Arg-guanidinium, His-imidazolium and Lys-ammonium. These groups stabilize the pentacoordinate transition state (intermediate, respectively) of the nucleophilic substitution reaction at phosphorus by hydrogen bonding or proton transfer. Metal ion promoted phosphate monoester hydrolysis is supported by Arg-guanidinium or His-imidazolium residues in alkaline phosphatase from *E.coli*[8] and purple acid phosphatase from *kidney beans*[9]. It was reported[10] that neutralization of the charged carboxylate residue by methyl ester formation results in greatly increased DNA binding. So we chose Arg, His and Lys three kinds of amino acids and L-histidine methyl ester for the DNA cleavage studies. In this paper we report our finding that copper (II)-L-histidine and copper (II)-L-histidine

methyl ester complexes could effectively promote the hydrolytic cleavage of plasmid DNA at physiological pH and temperature.

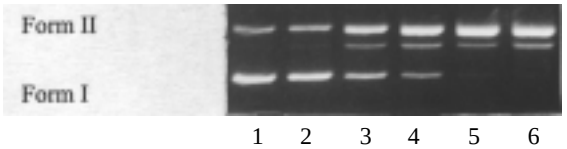
Figure 1 (A) shows agarose gel electrophoresis patterns for the cleavage of plasmid pBR322 DNA after treatment with 0.5 mmol /L of Cu (II)-L-amino acid (or Cu (II)-L-amino acid methyl ester) complexes at pH 7.0 (5 mmol /L Tris-HCl, 5 mmol /L NaCl) and 37℃ for 4h. The initial concentration of DNA was set at 42 μ mol /L base pairs (lane 1: DNA control). The conversion of form I (supercoiled) to form II (nicked) were observed in the systems of Cu (II)-L-His and Cu (II)-L-His methyl ester (lanes 2, 4, 6, 8: 0.5 mmol /L $CuCl_2$ to L-His complexes (molar ratios of 1:1, 1:2, 1:3, 1:4) in turn; lanes 3, 5, 7, 9: 0.5 mmol /L $CuCl_2$ to L-His methyl ester complexes (molar ratios of 1:1, 1:2, 1:3, 1:4) in turn), and the extent of DNA degradation (or the rate of DNA hydrolysis) depends on the metal ion-to-ligand ratio. The minimum rate was observed at molar ratio of 1:1 (lanes 2, 3), and the maximum rate was at molar ratio of 1:3 [Cu (II)-L-His] (lane 6). However, 0.5 mmol /L Cu (II)-L-Arg, 0.5 mmol /L Cu (II)-L-Lys, 0.5 mmol /L $CuCl_2$ were mostly ineffective under the conditions employed (lanes 10-12), These results indicated that the extent of DNA cleavage is largely dependent on the metal ion-to-ligand ratio and Cu (II)-L-His (molar ratio of 1:3) has a high potential for DNA hydrolysis. (see **Figure 1 (A)**). So we further investigated Cu (II)-L-His (1:3) system in detail. **Figure 1 (B)** shows agarose gel electrophoresis patterns for the cleavage of plasmid pBR322 DNA after treatment with 1:3 molar ratio of $CuCl_2$ to L-His complexes at pH 7.0 (5 mmol /L Tris-HCl, 5 mmol /L NaCl) and 37℃ for 4 h. The initial concentration of DNA was set at 42 μ mol /L base pairs (lane 1: DNA control), and the concentration of Cu (II)-L-His complexes was varied from 0 to 1.08 mmol /L (lanes 2-6: 0.048, 0.24, 0.48, 0.96, 1.08 mmol /L of Cu (II)-L-His complexes (molar ratio of 1:3) in turn). The conversion of form I (supercoiled) to form II (nicked) and form III (linearized) were observed with the increase in concentration of Cu (II)-L-His complexes, and the formation of form III began to appear in the presence of 48 μ mol /L

Figure 1. Cleavage of pBR322 DNA by Cu (II)-amino acid (or Cu (II)-His methyl ester) complexes (**A**) and Cu (II)-L-His complexes (molar ratio of 1:3) (**B**).

(A)



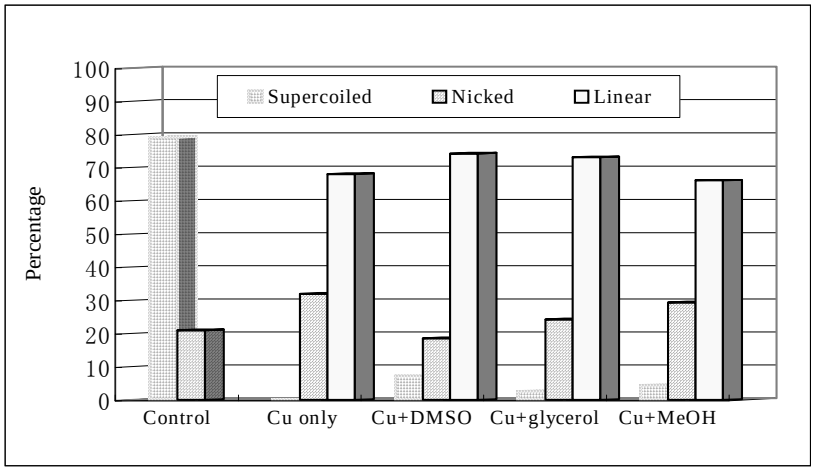
(B)



Cu (II)-L-His (lane 2). The form I was not almost observed in lane 6 (1.08 mmol /L Cu (II)-L-His). Higher concentrations (2.0-3.0 mmol /L) of the Cu (II)-L-His complexes, led to band broadening and slower migration of the plasmid DNA, indicative of binding of the complexes to the DNA (lanes not shown). 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 (II)-L-His complexes. At the concentrations used in our studies, we find that free copper (II) ions produce almost no cleavage of pBR322 DNA at concentrations below 1.08 mmol / L copper (II) ions under the employed conditions. From these results, it is concluded that Cu (II)-L-His complexes are reagents responsible for the degradation of DNA.

Although Cu (II)-L-His cleavage system 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. In control experiments, the addition of radical scavengers such as 0.4 mol /L DMSO, glycerol or 2.5 mol /L MeOH to the reaction mixtures had only slight influence of the efficiency of DNA cleavage[11] (**Figure 2**), however, these radical scavengers almost completely inhibited the oxidative degradation

Figure 2. Cleavage of pBR322 DNA by Cu (II)-L-His complexes in the presence of radical scavengers determined *via* fluorimaging.



of **DNA by Fe (EDTA)$^{2-}$/H_2O_2**. Furthermore, incubation of the reactants under 'anaerobic' conditions[12] shows only slight inhibition (approximately 5% decrease in the extent of DNA degradation relative to aerobic reactions at the same metal complex concentration), thus suggesting that oxidation products are hardly involved. Taken together these results suggest that the double-stranded cleavage mediated by Cu (II)-L-His complexes does not occur by an oxidative mechanism. Furthermore, an enzymatic assay was performed by using T4 DNA ligase to determine whether the cleavage reaction afforded exclusively 3'-OH and 5'-OPO_3 ends, as found for hydrolysis mediated by restriction enzymes, treatment of Cu (II)-L-His-linearized plasmid DNA

with T4 DNA ligase resulted in its quantitative conversion to closed circular plasmid and concatemers of pBR322, Pst I-linearized plasmid treated in the same manner afforded the same results. The efficiency of religation after cleavage by Cu (II)-L-His complexes was about 50%. Barton and coworkers noted that religation of the hydrolytic species would not be detected if a mixture of hydrolytic and redox cleavage products occurred on the same circle[5c]. The assay therefore indicated that cleavage should be through a hydrolytic path. Taken together these results suggest that Cu (II)-L-His mediated cleavage reaction in the absence of exogenous reagents occurs *via* a hydrolytic mechanism.

Acknowledgments

The authors acknowledge the support of the National Natural Science Foundation of China and Provincial Natural Science Foundation of Shanxi.

References and Notes

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11. Densitometric quantitation of the gels was performed using a UVP GDS 8000 complete gel documentation & analysis system equipped with Gelworks 1D version 3.00 software.
12. Reaction included incubation in a nitrogen filled glove-bag using degassed buffers.

Received 29 July 1998
Revised 21 March 1999