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Efficient DNA Cleavage by Copper(Ⅱ) Complex Derived from 1, 1′-Dimethyl-2, 2′-biimidazole Ligand

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Abstract: The cleavage of DNA (pBR322) by $[Cu(Dime\text{-}biim)_2(H_2O)](ClO_4)_2$ (Dime-biim=1, 1′-dimethyl-2, 2′-biimidazole) was investigated. The results showed that the complex could cleave DNA efficiently at pH=8.0 and 37℃.

Keywords: Copper(II) complex, DNA, efficient cleavage, biimidazol derivative.

Artificial enzymes are non-protein molecules that are simpler than natural enzymes, but they also possess high efficiency and specificity. Artificial metallonucleases have been proven to be an efficient tools for the footprinting and sequencespecific targeting of nucleic acid[1, 2]. In recent years, the study of enzyme model is one of the most active fields, especially chemical nuclease[3]. The binding of copper ions to DNA is well established as they have ability to cleave DNA, particularly when Cu(II) is complexed with certain metal ion chelators. One commonly used oxidative cleavage agent is the bis(1,10-phenantholine-N^1, N^{10})copper(I) cation $[(OP)Cu^+]$ which has been used as a footprinting reagent and as a probe of DNA and RNA secondary structure[3,4]. Copper(II)-L-histidine complexes effectively promote the cleavage of plasmid DNA and dideoxynucleotide dApdA at physiological pH and temperature[5]. The presence of an imidazole moiety in biological molecules has the encouraged studies of H_2biim-containing transition metal complexes[6]. In this paper, we report our work that $[Cu(Dime\text{-}biim)_2(H_2O)](ClO_4)_2$ could effectively cleave plasmid DNA. To our knowledge, this is the first paper to study DNA cleavage by containing-H_2biim metal complexes.

Cu/Dime-biim are capable of cleaving double-stranded DNA at pH=8.0 and 37℃. When plasmid pBR322 DNA was incubated with Cu/Dime-biim, the supercoil DNA was degraded from form I (supercoiled) to form II (nicked) and then slowly to form III (lineared). DNA cleavage products were analyzed by agarose gel electrophoresis in syngene gel documentation and analysis system.

Cleavage reactions of plasmid DNA (pBR322) by Cu/Dime-biim were investigated in different concentrations of complex (**Figure 1**) in the presence of AH_2 (ascorbic acid).

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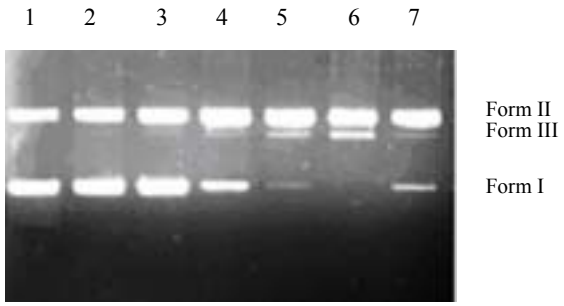
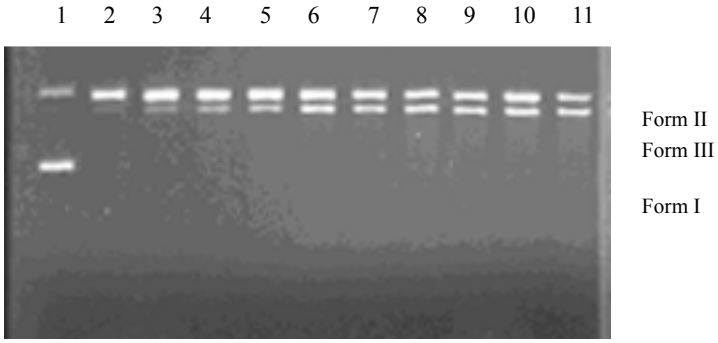
Figure 1 Agarose gel electrophoresis of cleavage reaction of plasmid pBR322 DNA by different concentrations of Cu(II) complex (ethidium bromide staining)



Scission conditions: 0.014 mg/mL DNA; 1.25×10^{-4} mol/L AH_2; 10 mmol/L Tris-HCl buffer (containing 5 mmol/L NaCl); pH=8.0; 37℃ for 1.5 h. Lane 1: DNA control. Lane 2: DNA+AH_2. Cu(II) complex concentration: Lane 3-7: 5×10^{-5} mol/L, 7.5×10^{-5} mol/L, 1.0×10^{-4} mol/L, 2.5×10^{-4} mol/L, 5×10^{-4} mol/L.

Figure 2 Agarose gel electrophoresis of cleavage reaction of plasmid pBR322 DNA by different reaction time of Cu(II) complex (ethidium bromide staining)



Scission conditions: 0.014 mg/mL DNA; 2.5×10^{-4} mol/L Cu(II) complex; 1.25×10^{-4} mol/L AH_2; 10 mmol/L Tris-HCl buffer (containing 5 mmol/L NaCl); pH=8.0; 37℃. Lane 1: DNA control. Lane 2-11: reaction after 10, 20, 30, 50, 70, 90, 120, 150, 240, 480 min, respectively.

Under the same conditions, free AH_2 produced no cleavage of pBR322 (Lane 2). All supercoiled (form I) DNA was cleaved to form the mixture of form II and form III in the concentration of 2.5×10^{-4}mol/L (lane 6). The conversion of form I to form II and form III were observed with the increase in concentration of the complex when concentration was lower than 2.5×10^{-4}mol/L, the effect of cleavage was the best when the concentration was 2.5×10^{-4}mol/L (Lane 6), and the effect of cleavage was better than that of the complexes as 5×10^{-4}mol/L (lane 7). The formation of form III began to appear in the presence of the complexes as 7.5×10^{-5}mol/L (lane 4).

Higher concentrations of Cu(II) complex led to precipitation of the plasmid DNA as a white solid(lanes not shown), due to charge neutralization caused by extensive binding of the Cu/Dime-biim. A single cut or nick on strand of supercoiled DNA relaxes the supercoiling and leads to form II. A second cut on the complementary strand, within approximately 12 base pairs[7] of the original cut site, linearizes the DNA to form III DNA.

Therefore, Cu/Dime-biim must cut the DNA at least twice to convert to form III DNA from form I.

The DNA strand scission chemistry with Cu/Dime-biim has been kinetically characterized by quantitation of supercoiled, nicked and linear DNA(**Figure 2)**. The observed distribution of supercoiled, nicked and linear DNA in the agarose gel provides a measure of the extent of the reaction in each plasmid DNA and we used these data to perform simple kinetic analysis. **Figure 3(A)** shows the mass fractions of DNA species present during reaction under mild conditions and the mass fraction of DNA species was determined by using the volume quantitative method in Gene Tools software. **Figure 3(B)** is a time course plot of form III formation during cleavage by Cu/Dime-biim. Values for k_{obs} were obtained under single-turnover conditions. The increase of form III also fitted to a single exponential curve[8]. From these curve fits, k_{obs} at complexes concentration of 2.5×10^{-4} mol/L was estimated to be 1.01 h^{-1} (R=0.9726) at 37℃ for the increase of form III.

Figure 3 (A) Mass fractions of DNA species during cleavage reaction of pBR322 DNA with Cu(II) complex: (b) form II nicked DNA; (c) form III linear DNA. (B) Time course of the cleavage reaction on form III linear DNA. Data were fit to either a single-exponential equation.

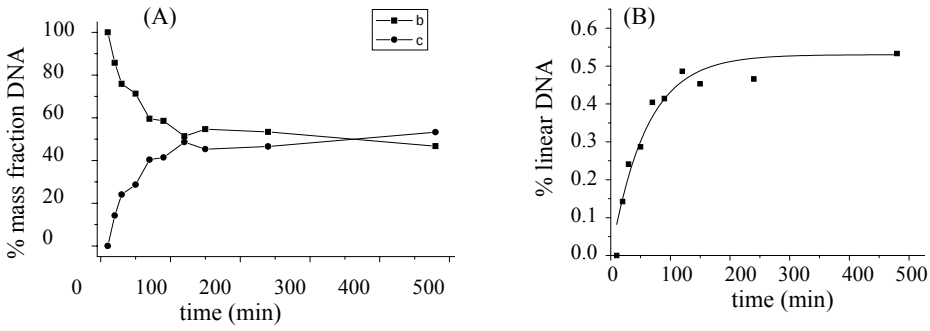
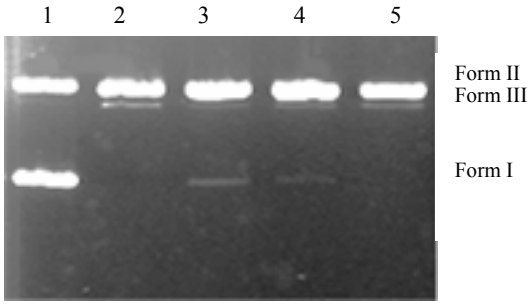


Figure 4 Agarose gel electrophoresis of cleavage reaction of plasmid pBR322 DNA by Cu(II) complex in the absence or presence of radical scavengers(ethidium bromide staining)



Scission conditions: 0.014 mg/mL DNA; 2.5×10^{-4} mol/L Cu(II) complex; 1.25×10^{-4} mol/L AH_2; 10 mmol/L Tris-HCl buffer (containing 5 mmol/L NaCl); pH=8.0; 37℃ for 1.5 h. Lane 1: DNA control. Lane 2: Cu(II) complex+AH_2. Lane 3: Cu(II) complex+AH_2+0.4 mol/L DMSO. Lane 4: Cu(II) complex+AH_2+0.4 mol/L glycerol. Lane 5: Cu(II) complex+AH_2+2.5 mol/L MeOH.

Cu(II)-Dime-biim-mediated DNA cleavage mechanism was considered. When pBR322 DNA was incubated with the complex in the presence of either 0.4 mol/L DMSO, 0.4 mol/L glycerol or 2.5 mol/L MeOH as hydroxyl radical scavengers, only slight inhibition of the DNA cleavage was observed (**Figure 4**). These observations suggested that Cu(II)-Dime-biim-mediated cleavage reaction did not proceed *via* radical cleavage.

In summary, $[Cu(2Me\text{-}biim)_2(H_2O)](ClO_4)_2$ is able to perform an efficient cleavage of DNA. Further studies on the reaction mechanism as well as on sequence selectivity of the copper(II) complex are in progress.

Acknowledgments

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