第 24 卷第 11 期 无 机 化 学 学 报 Vol.24 No.11
2008 年 11 月 CHINESE JOURNAL OF INORGANIC CHEMISTRY 1890~1894

席夫碱大环铜配合物的化学核酸酶活性研究

孔德明*,[1] 郑文英[1] 王光宇[2] 朱莉娜[2] 李孝增[2]
([1]南开大学化学院分析科学研究中心,天津 300071)
([2]天津大学理学院化学系,天津 300072)

摘要:对 3 种结构相近的席夫碱四氮大环草酰胺铜配合物(CuL^{1-3})的化学核酸酶活性进行比较研究。结果表明:这类配合物的化学核酸酶活性与中心金属离子的类型、配体的结构、溶液的 pH 值、离子强度及配合物的浓度等都有关系。3 种配合物表现出来的化学核酸酶活性顺序为 $CuL^3>CuL^2>CuL^1$。CuL^3 的 DNA 切割反应表现为典型的假一级连续反应。在 80 μmol·L^{-1} CuL^3 和 2 mmol·L^{-1} H_2O_2 的存在下,就超螺旋 DNA 向切口开环型 DNA 进而向线型 DNA 的转化而言,反应速率常数分别为 0.044 0±0.001 5 min^{-1}(k_1)和 0.003 52±0.000 18 min^{-1}(k_2)。

关键词:席夫碱大环铜配合物;化学核酸酶;pBR 322 DNA;DNA 切割
中图分类号:O614.7;O643.1 **文献标识码**:A **文章编号**:1001-4861(2008)11-1890-05

Chemical Nuclease Activities of Schiff Base Macrocyclic Copper(Ⅱ) Complexes

KONG De-Ming*,[1] ZHENG Wen-Ying[1] WANG Guang-Yu[2] ZHU Li-Na[2] LI Xiao-Zeng[2]
([1]*Research Centre for Analytical Sciences, Department of Chemistry, Nankai University, Tianjin* 300071)
([2]*Department of Chemistry, School of Science, Tianjin University, Tianjin* 300072)

Abstract: The chemical nuclease activities of three closely related Schiff base tetraazamacrocyclic oxamido copper(Ⅱ) complexes (CuL^{1-3})were compared and the result shows that their chemical nuclease activities follow the order of $CuL^3>CuL^2>CuL^1$. Where complexes CuL^{1-3} are [5,6-d][13,14-d]dibenzo-1,4,8,11 -tetraazacyclotetradecane-dione-2,3-diene-7,11-di-ethylcarboxylate-7,12 Copper(Ⅱ), [5,6-d][13,14-d]dibenzo-10-methyl-1,4,8,11-tetraazacyclo-tetradecane-dione-2,3-diene-7,11-di-ethylcarboxylate-7,12 Copper (Ⅱ) and [5,6-d] [13,14-d]dibenzo-[9,10-d] -cyclehexano-1,4,8,11-tetraazacyclotetradecane-dione-2,3-diene-7,11-di-ethylcarboxylate-7,12 Copper(Ⅱ), respectively. The interactions of CuL^3 with DNA were studied in detail, and the results show that the chemical nuclease activity of this complex is affected by central metal ions, structure of ligands, pH value, ion strength and complex concentration. DNA cleavage reaction in presence of the copper(Ⅱ) complexes is a typical pesudo-first-order consecutive reaction, and the rate constants of 0.044 0±0.001 5 min^{-1} (k_1) and 0.003 52±0.000 18 min^{-1} (k_2) for the conversion of supercoiled to nicked DNA and nicked to linear DNA are obtained in presence of 80 μmol·L^{-1} CuL^3 and 2 mmol·L^{-1} H_2O_2, respectively.

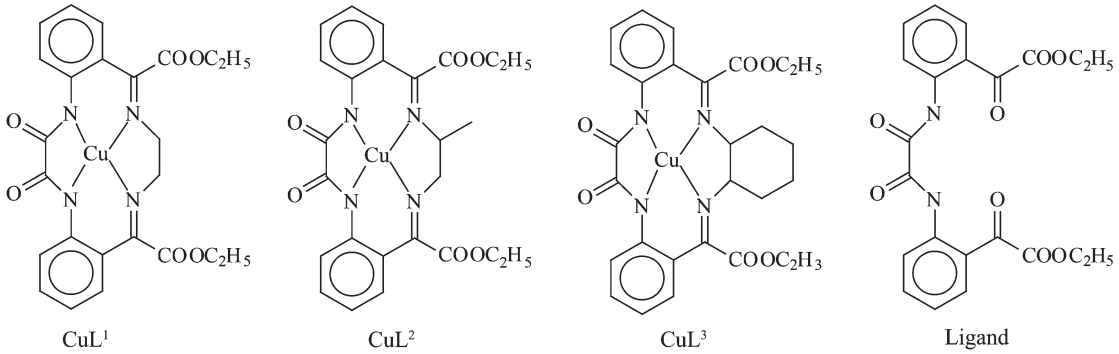
Key words: Schiff base macrocyclic copper(Ⅱ) complexes; chemical nuclease; pBR 322 DNA; DNA cleavage

Identifying small molecules capable of binding and cleaving DNA has attracted considerable interest over the last few decades[1]. Such inexpensive small-molecule catalysts would potentially be valuable tools in biotechnology, nanotechnology, therapeutic approaches and the study of nucleic acid conformations. In this regard,

收稿日期:2008-06-30。收修改稿日期:2008-09-14。
国家自然科学基金(No20675041)及天津市应用基础及前沿技术研究计划(No.08JCZDJC21200)资助项目。
*通讯联系人。E-mail:kongdem@nankai.edu.cn
第一作者:孔德明,男,33 岁,博士,副教授;研究方向:生物分析化学。

metal complexes have been found to be particularly useful because of their potential to bind DNA via a multitude of interactions and to cleave the duplex[2]. The hydrolytic and oxidative cleavage of supercoiled plasmid DNA by various metal complexes have been extensively reported[3,4].

Schiff base complexes present suitable biometric properties that can mimic the structural features of the active sites, and they have been widely used in various fields such as illness treatment, biochemical reaction and biological regulator[5]. Recently, the oxidative DNA cleavage by six schiff base tetraazamacrocyclic oxamido nickel(Ⅱ) complexes has been reported by us[6]. The results show that all of these complexes can promote the oxidative cleavage of plasmid DNA at physiological pH value and temperature in the presence of H_2O_2, and the reactivity is highly dependent upon the macrocyclic ligand employed. In this paper, to investigate the influence of central metal ions on the DNA cleavage activities of the complexes, we report the synthesis of three Schiff base tetrazamacrocyclic oxamido copper(Ⅱ) complexes and the cleavage behavior of these complexes toward pBR 322 DNA(Scheme 1).



Scheme 1　Schematic structures of the Schiff base tetraazamacrocyclic oxamido copper(Ⅱ) complexes and the corresponding ligand

1　Experimental

1.1　Materials and general methods

Supercoiled plasmid DNA (pBR 322 DNA) was purchased from Dingguo Ltd. (Beijing, P.R. China). Other materials and solvents were purchased commercially and without further purification before use unless otherwise noted. Elemental analyses of C, H and N were carried out on a Perkin-Elmer 240 analyzer. IR spectra were recorded as KBr discs on a BIO-RAD 3000 infrared spectrophotometer in the 4 000~400 cm^{-1} region.

1.2　Synthesis of complexes

The ligand diethyl 2,2-(oxalyldiimino)bis(phenylglyoxylate) and complex CuL^1 and CuL^2 were prepared according to the literature method[7,8].

The complex CuL^3 was synthesized by the same method. To a suspension of the ligand (2 mmol) in ethanol(30 mL), $Cu(OAc)_2 \cdot H_2O$(2 mmol), Et_3N(0.7 mL) and ±-1,2-cyclohexanediamine (70% , 2 mmol) were successively added. The mixture was refluxed for 8 h under continuous stirring. The resulted solid was collected by filtration. Then the solid was recrystallized in ethanol and the brown microcrystal of CuL^3 was obtained. Anal. Calcd. for $C_{28}H_{28}N_4O_6Cu$ (M=580.09): C 57.97, H 4.87, N 9.66; Found: C 57.81, H 4.95, N 9.36%. Main IR bands(KBr, cm^{-1}): 1 727s, δ(C=O, ester); 1 655s, δ(C=O, oxamido); 1 603s and 1 577s, δ(C=N).

1.3　DNA cleavage studies

The reaction mixtures (25 μL total volume) contained 10 ng·μL^{-1} of supercoiled plasmid (pBR 322 DNA), 10 mmol·L^{-1} NaH_2PO_4-Na_2HPO_4 buffer(pH value of 7.0), 10 mmol·L^{-1} NaCl, 80 μmol·L^{-1} individual metal complexes and 2 mmol·L^{-1} H_2O_2. Reaction mixtures were incubated at 37 ℃ for 2 h, and then quenched by the addition of 5 μL loading buffer(0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol, 10 mmol·L^{-1} EDTA). Samples(20 μL) were loaded onto a 1% agarose gel containing ethidium bromide (1 μg·mL^{-1}) in TBE buffer (90 mmol·L^{-1} Tris-borate, pH 8.0, 20 mmol·L^{-1} EDTA). The gel was run at 120 V for 1

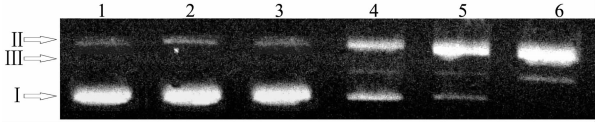
h and photographed under UV light. Quantitation of cleavage products was performed by Glyko BandScan software, Version 4.30. Supercoiled plasmid DNA values were corrected by a factor 1.3, based on average literature estimate of lowered binding of ethidium to this structure[9].

2 Results and discussion

2.1 DNA Cleavage activity comparison of the three copper(Ⅱ) complexes

In order to assess the competence of the three copper(Ⅱ) complexes for DNA strand scission, pBR 322 DNA was incubated with individual copper(Ⅱ) complexes under identical reaction conditions. The cleavage reaction can be monitored by gel electrophoresis. When circular pBR 322 DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact supercoiled form (Form Ⅰ). If scission occurs on one strand (nicking), the supercoiled form will relax to generate a slower-moving nicked form (Form Ⅱ). If both strands are cleaved, a linear form (Form Ⅲ) that migrates between Form Ⅰ and Form Ⅱ will be generated.

As shown in Fig.1, all of the examined complexes are capable of promoting oxidative damage of DNA in the presence of H_2O_2 under physiological conditions (pH value of 7.0, 37 ℃), but display different cleavage activities. CuL^3 exhibits the highest DNA cleavage activity. Under the conditions used, it can completely convert supercoiled DNA (Form Ⅰ) to nicked DNA (Form Ⅱ) and linear DNA (Form Ⅲ). CuL^1 and CuL^2 can also convert supercoiled DNA to nicked and linear DNA, but the supercoiled form is still seen. The results show that the chemical nuclease activity of the three complexes follows the order: CuL^3>CuL^2>CuL^1, which is consistent with the nickel(Ⅱ) complexes having the corresponding ligands. But the copper(Ⅱ) complexes display higher chemical nuclease activity than the corresponding nickel(Ⅱ) complexes. At the same concentrations of metal complexes and H_2O_2 ($c_{complex}$=80 μmol·L^{-1}, $c_{H_2O_2}$ = 2 mmol·L^{-1}), nearly 100% supercoiled DNA can be converted to nicked or linear forms in the presence of CuL^3, but corresponding nickel(Ⅱ) complexes can only convert around 6% supercoiled DNA to nicked form[6]. Under the same condition, both copper(Ⅱ) ion and the ligand show little DNA cleavage activities. The different cleavage behavior of the copper(Ⅱ) complexes and copper(Ⅱ) ion illustrates that the cooperativity between central copper(Ⅱ) ion and appropriate ligand can increase the DNA cleavage ability of copper(Ⅱ) ion. Considering the highest DNA cleavage activity of CuL^3 in the three copper(Ⅱ) complexes, the DNA cleavage behavior of CuL^3 was studied in detail in subsequent experiments.

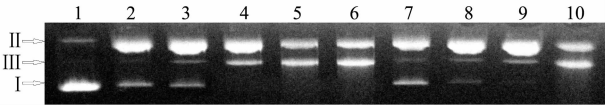


Lane 1: DNA control; lane 2: Cu^{2+}; lane 3: ligand; lane 4: CuL^1; lane 5: CuL^2; lane 6: CuL^3

Fig.1 Cleavage of pBR 322 DNA by different metal complexes

2.2 Effects of pH buffer and pH value on CuL^3-mediated DNA cleavage

The pH-dependence of CuL^3-mediated DNA cleavage was investigated in two different buffers (Tris-HCl buffer and NaH_2PO_4-Na_2HPO_4 buffer). From Fig.2, one can see that no matter what pH buffer is used, the DNA cleavage activity of CuL^3 increases with the increasing of pH value. In addition, the DNA cleavage activity of CuL^3 in NaH_2PO_4-Na_2HPO_4 buffer is a little higher than that in Tris-HCl buffer. Though CuL^3 could display higher DNA cleavage activity in basic conditions, NaH_2PO_4-Na_2HPO_4 buffer with pH value of 7.0 was used in subsequent experiments to investigate the chemical nuclease activity of CuL^3 at physiological pH value and temperature.

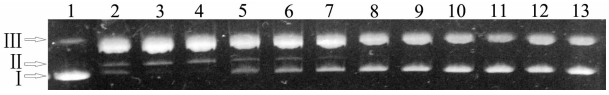


Lane 1: DNA control; lane 2~6: Na_2HPO_4-NaH_2PO_4 buffer; lane 7~10: Tris-HCl. Lane 2: pH=5.0; lane 3, 7: pH=6.0; lane 4, 8: pH=7.0; lane 5, 9: pH=8.0; lane 6, 10: pH=9.0

Fig.2 Buffer and pH value dependence of CuL^3-mediated DNA cleavage

2.3 Effects of ion strength on CuL^3-mediated DNA cleavage

Fig.3 shows the results of an ionic strength study in which pBR 322 DNA is treated with CuL^3 under con-



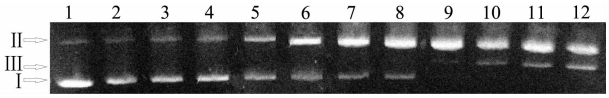
Lane 1: DNA control; lane 2~13, 0, 5, 10, 15, 20, 40, 60, 80, 100, 120, 140, 160 mmol·L^{-1} NaCl added to the reaction mixture

Fig.3　Ionic strength dependence of CuL^3-mediated DNA cleavage

ditions of increasing ionic strength by addition of NaCl. From this figure one can see that the process of DNA cleavage is sensitive to the change of ionic strength. CuL^3 displays the highest cleavage activities when appropriate amount of NaCl (about 5~10 mmol·L^{-1}) is added. Further increasing the NaCl concentration, both nicked and linear DNA decrease, and no linear DNA can be observed above 60 mmol·L^{-1} NaCl. These results indicate that appropriate ion strength will benefit to DNA cleavage activity of CuL^3. Therefore, 10 mmol·L^{-1} NaCl was added to the reaction mixtures in subsequent experiments.

2.4　Effects of complex concentration on CuL^3-mediated DNA cleavage

As shown in Fig.4, the cleavage reaction exhibits obviously metal complex concentration dependence. With the increase of CuL^3 concentration, the amount of supercoiled DNA decreases and that of nicked DNA increases at the same time. When the concentration of CuL^3 exceeds 140 μmol·L^{-1}, the supercoiled DNA disappears completely and linear DNA can be observed. In order to avoid over-degradation of DNA into smaller fragments, the reaction time is set at 30 min.

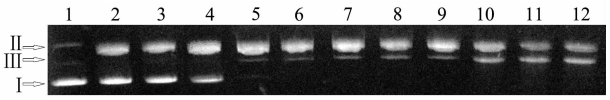


Lane 1: DNA control; lane 2~12, 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200 μmol·L^{-1} CuL^3 added to the reaction mixture

Fig.4　Complex concentration dependence of CuL^3-mediated DNA cleavage

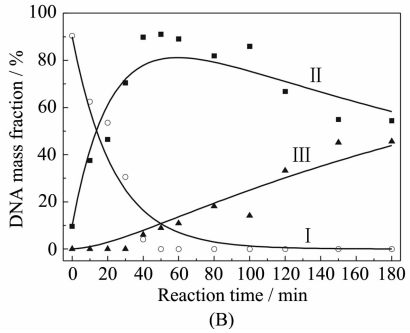
2.5　Kinetics of CuL^3-mediated DNA cleavage

The experiment to explore time dependence of CuL^3-mediated DNA cleavage was also carried out at physiological pH value and temperature. The fragments produced in the presence of CuL^3 were analyzed by electrophoresis(Fig.5A) and a plot of the percentage of either DNA form versus reaction time is obtained by



(A)

The reaction time for lane 1~12 is 0, 10, 20, 30, 40, 50, 60, 80, 100, 120, 150, 180 min, respectively



(B)

Scatter: experimental data; line: fitting curves

Fig.5　Reaction time dependence of CuL^3-mediated DNA cleavage (A) Gel electrophoresis results; (B) Densitometric quantitative results of the gel electrophoresis

using the electrophoresis data(Fig.5B). From these figures, we can find that treatment of plasmid DNA with CuL^3 results in exponential conversion of supercoiled DNA to nicked DNA. After treatment with CuL^3 for 40 min, linear DNA is also observed. Supercoiled DNA completely disappears after 50 min. This means that CuL^3 can convert supercoiled DNA to nicked DNA , then to linear DNA in a sequential manner. The experimental data in Fig.5B can be fitted well with the first-order consecutive kinetic equations as follows[10]:

$$\text{Form } \mathrm{I} \xrightarrow{k_1} \text{Form } \mathrm{II} \xrightarrow{k_2} \text{Form } \mathrm{III}$$

$$\text{Form } \mathrm{I}: C_t^{\mathrm{I}} = C_0^{\mathrm{I}} e^{-k_1 t}$$

$$\text{Form } \mathrm{II}: C_t^{\mathrm{II}} = C_0^{\mathrm{I}} \frac{k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) + C_0^{\mathrm{II}} e^{-k_2 t}$$

$$\text{Form } \mathrm{III}: C_t^{\mathrm{III}} = C_0^{\mathrm{I}}\left(1 - \frac{k_2}{k_2 - k_1} e^{-k_1 t} + \frac{k_1}{k_2 - k_1} e^{-k_2 t}\right) + C_0^{\mathrm{II}}(1 - e^{-k_2 t}) + C_0^{\mathrm{III}}$$

Where C_t^{I}, C_t^{II}, and C_t^{III} represent the concentration of supercoiled DNA, nicked DNA and linear DNA, respectively; C_0^{I}, C_0^{II}, C_0^{III} represent the initial concentration of supercoiled DNA, nicked DNA and linear DNA, respectively; k_1 and k_2 represent the rate constant of conversion of supercoiled DNA to nicked DNA and nicked DNA to linear DNA, respectively.

The rate constants of 0.044 0±0.001 5 min^{-1}(k_1) and

0.003 52±0.000 18 min^{-1}(k_2) for the conversion of supercoiled to nicked DNA and nicked to linear DNA are obtained in presence of 80 μmol·L^{-1} CuL^3 and 2 mmol·L^{-1} H_2O_2, respectively. Since the rate constant k_1 is much larger than k_2, the CuL^3 complex may be said to be the single strand cleavage agents, and the cleavage is nonspecific[11].

3 Conclusions

DNA cleavage abilities of three closely related Schiff base macrocyclic oxamido copper(Ⅱ) complexes were compared. The agarose gel electrophoresis studies show that all of these complexes can promote the oxidative cleavage of plasmid DNA at physiological pH value and temperature in the presence of H_2O_2, but the reactivity is highly dependent upon the macrocyclic ligand and central metal ions employed. The DNA cleavage behavior of CuL^3, which displays the highest DNA cleavage activity in the three complexes, has been studied in detail. The results indicate that the chemical nuclease activity of this complex is affected by pH value, ion strength and complex concentration.

References:

[1] Dhara K, Ratha J, Manassero M, et al. *J. Inorg. Biochem.*, **2006**,**101**:95~103

[2] Arounaguiri S, Easwaramoorthy D, Ashokkumar A, et al. *Proc. Indian. Acad. Sci.*(*Chem. Sci.*), **2000**,**112**:1~17

[3] Zhou D-M, Taira K. *Chem. Rev.*, **1998**,**98**:991~1026

[4] Pogozelski W K, Tullius T D. *Chem. Rev.*, **1998**,**98**:1089~1108

[5] Muller J G, Chen X, Dadiz A C, et al. *J. Am. Chem. Soc.*, **1992**,**114**:6407~6411

[6] Kong D M, Wang J, Zhu L N, et al. *J. Inorg. Biochem.*, **2008**,**102**:824~832

[7] Black D S C, Moss G I. *Aust. J. Chem.*, **1987**,**40**:129~142

[8] Gao E Q, Yang G M, Liao D Z, et al. *Transition Met. Chem.*, **1999**,**24**:244~246

[9] Pamatong F V, Detmer C A, Bocarsly J R. *J. Am. Chem. Soc.*, **1996**,**118**:5339~5345

[10]ZHOU Qing-Hua(周庆华), YANG Pin(杨 频). *Chinese J. Inorg. Chem.*(*Wuji Huaxue Xuebao*), **2005**,**21**:960~964

[11]Googisman J, Krik C, Dabrowiak J C. *Biophys. Chem.*, **1997**, **69**:249~268