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# 两个 4,4′-二甲基-2,2′-联吡啶锰(Ⅱ)配合物切割 DNA 的动力学研究

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**摘要**:对 2 个 4,4′-二甲基-2,2′-联吡啶锰(Ⅱ)配合物在生理条件及 $H_2O_2$ 的存在下对 DNA 切割的动力学进行了研究。结果表明,这 2 个配合物分别存在下的 DNA 切割反应具有相似的动力学反应特征。其中对超螺旋 DNA 切割成缺口 DNA 步骤,均表现为三级反应,即反应速率分别与底物 DNA 的浓度、配合物的浓度和 $H_2O_2$ 的浓度的一次方成正比;同时得到了 2 个反应的速率常数、活化能($E_a$)、活化焓($\Delta H^{\neq}$)和活化熵($\Delta S^{\neq}$)等动力学参数,并根据这些结果提出了一个可能的氧化切割反应机理。

**关键词**:4,4′-二甲基-2,2′-联吡啶锰(Ⅱ)配合物;DNA 切割;动力学;切割机理
**中图分类号**:O614.7;O643.1 **文献标识码**:A **文章编号**:1001-4861(2008)07-1094-04

## Kinetics of DNA Cleavage by Two 4,4′-dimethyl-2,2′-pyridyl Manganese(Ⅱ) Complexes

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**Abstract:** The kinetics of DNA cleavage, especially the conversion of supercoiled to nicked DNA, by two 4,4′-dimethyl-2,2′-pyridyl (dmbpy) manganese(Ⅱ) complexes, $Mn(dmbpy)_2(OCN)_2$(**1**) and $Mn(dmbpy)_2(dca)_2$(**2**), was investigated. Results show that there is similar kinetic characterization for complex **1**-mediated DNA cleavage and **2**-mediated DNA cleavage. For the conversion of supercoiled to nicked DNA, the overall reaction is three orders, i.e., the reaction rate is proportional to $C_{\text{DNA substrate}}$, $C_{\text{complexes}}$ and $C_{H_2O_2}$, respectively. The rate constants are $(2.54\pm0.36)\times10^4$ and $(2.51\pm0.45)\times10^4$ $L^2\cdot mol^{-2}\cdot s^{-1}$ at 37 ℃ for **1** and **2**-mediated DNA cleavage, respectively. The temperature dependence of the rate constants results in the comparable activation energies($E_a$), activation enthalpies($\Delta H^{\neq}$) and activation entropies ($\Delta S^{\neq}$) for **1** and **2**-mediated reactions, which suggests that the two reactions have similar organized transition states. Based on the above results, a mechanism for the Mn(Ⅱ) complexes-mediated DNA cleavage was proposed.

**Key words:** 4,4′-dimethyl-2,2′-pyridyl manganese(Ⅱ) complex; DNA cleavage; kinetics; cleavage mechanism

The studies on the chemical nucleases have attracted considerable interest over the last few decades for their potential applications in biotechnology, therapeutic approaches and the study of nucleic acid conformations[1~4]. The transition metal complex may be a good candidate and the oxidative and/or hydrolytic cleavage of supercoiled plasmid DNA by various metal complexes has been extensively studied[5~7]. Two 4,4′-dimethyl-2,2′-pyridyl (dmbpy) manganese (Ⅱ) complexes, $Mn(dmbpy)_2(OCN)_2$(**1**) and $Mn(dmbpy)_2(dca)_2$(**2**) (scheme **1**), were reported to be able to convert supercoiled DNA to nicked DNA then linear DNA in a sequential manner in

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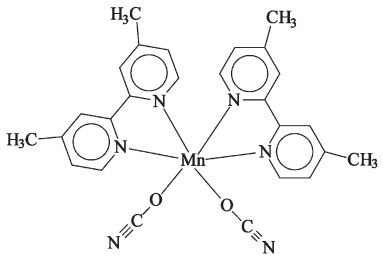
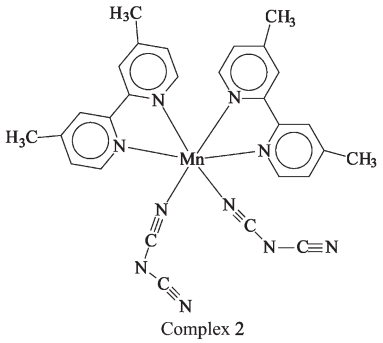


Complex 1



Complex 2

Scheme 1

the presence of $H_2O_2$ under physiological conditions and the hydroxyl radical might be involved in the cleavage mechanism by our group[8]. Here we report the detailed investigation of the kinetics of the plasmid DNA cleavage by the two complexes and the discussion on the cleavage mechanism.

## 1 Experimental

### 1.1 Materials

The two manganese(Ⅱ) complexes was synthesized using our previously reported procedure[8]. Supercoiled plasmid DNA (pBR 322 DNA) was purchased from Dingguo Ltd.(Beijing, P.R. China). Other material and solvents were purchased commercially and without further purification before use unless otherwise noted.

### 1.2 DNA cleavage

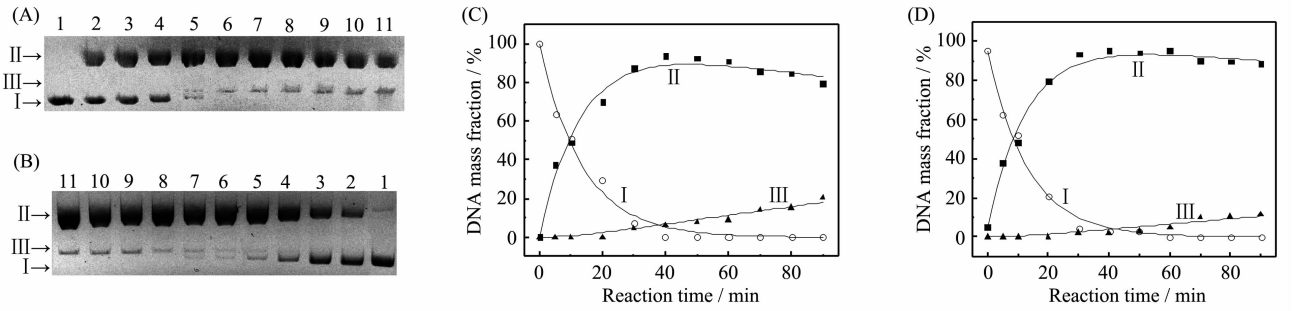
The reaction mixture (25 μL total volume) contained 280 ng of pBR 322 DNA, 10 mmol·$L^{-1}$ Tris-HCl buffer(pH=7.0), 80 mmol·$L^{-1}$ NaCl, 30 μmol·$L^{-1}$ individual Mn(Ⅱ) complexes and 1.5 mmol·$L^{-1}$ $H_2O_2$. The reaction mixture was incubated at 37 ℃ for 1 h, and then quenched by the addition of 5 μL loading buffer. 20 μL of samples 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 of 1.3, based on average literature estimate of lowered binding of ethidium to this structure[9].

## 2 Results and discussion

### 2.1 Kinetic studies of DNA cleavage

Both **1** and **2** are capable of converting supercoiled DNA (Form Ⅰ) to nicked DNA (Form Ⅱ) and linear DNA (Form Ⅲ) in the presence of $H_2O_2$ under physiological conditions(pH value of 7.0, 37 ℃)[8]. The kinetic studies of the Mn(Ⅱ) complexes-mediated DNA cleavage were also carried out at physiological pH value and temperature. Firstly, using 30 μmol·$L^{-1}$ individual Mn(Ⅱ) complexes and 1.5 mmol·$L^{-1}$ $H_2O_2$, the experiment to explore time dependence of the Mn(Ⅱ) complexes-mediated DNA cleavage was carried out. The fragments produced in the presence of each complex were analyzed by electrophoresis (Fig.1A and 1B), and plots of the percentage of either DNA form versus reaction time were obtained by densitometric quantitation of the electrophoresis data (Fig.1C and 1D). From Fig.1, we can find that there is similar kinetic characterization for complex **1**-mediated DNA cleavage and **2**-mediated DNA cleavage. Both of the two Mn(Ⅱ) complexes can convert supercoiled DNA to nicked DNA. After treatment with the either complex for 30 min, linear DNA is also observed. Supercoiled DNA completely disappears after 40 min. That is to say, the Mn(Ⅱ) complexes can convert supercoiled DNA to nicked DNA, then linear DNA in a sequential manner. From Fig.1C and 1D, one can see that the treatment of plasmid DNA with either complex **1** or complex **2** results in exponential conversion of supercoiled DNA to nicked DNA and this is a typical pesudo-first-order consecutive reaction, which is consistent with the general model for enzyme-mediated reactions[10]. This means that the manganese(Ⅱ) complexes may act as the chemical nucleases in the presence of $H_2O_2$ as the redox cofactors. The experimental




(A) Gel electrophoresis results of complex **1**-mediated DNA cleavage. (B) Gel electrophoresis results of complex **2**-mediated DNA cleavage. The reaction time for lane 1-11 was 0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 min, respectively. $C_1$ or $C_2$=30 μmol·L$^{-1}$, $C_{H_2O_2}$=1.5 mmol·L$^{-1}$. (C) Densitometric quantitative results of the gel electrophoresis of complex **1**-mediated DNA cleavage. (D) Densitometric quantitative results of the gel electrophoresis of complex **2**-mediated DNA cleavage. Scatter: experimental data, line: fitting curves.

Fig.1 Reaction time dependence of complex **1** or **2**-mediated DNA cleavage

data in Fig.1C and 1D can be fitted well with the first-order consecutive kinetic equations as follows:

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

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

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

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

Where $C_t^{\text{Ⅰ}}$, $C_t^{\text{Ⅱ}}$ and $C_t^{\text{Ⅲ}}$ represent the concentration of supercoiled DNA, nicked DNA and linear DNA, respectively; $C_0^{\text{Ⅰ}}$, $C_0^{\text{Ⅱ}}$, $C_0^{\text{Ⅲ}}$ 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.

For complex **1**-mediated DNA cleavage, the observed rate constants ($k_{obsd}$) of 4.57±0.05 h$^{-1}$ ($k_{obsd1}$) and 0.079±0.006 h$^{-1}$ ($k_{obsd2}$) for the conversion of supercoiled to nicked DNA and nicked to linear DNA are obtained, respectively. For complex **2**-mediated DNA cleavage, the values of $k_{obsd1}$ and $k_{obsd2}$ are 4.46 ±0.07 h$^{-1}$ and 0.149±0.008 h$^{-1}$, respectively. Since the rate constant $k_{obsd1}$ is much larger than $k_{obsd2}$, the Mn(Ⅱ) complexes may be said to be the single strand cleavage agents, and the cleavage is nonspecific[11].

The concentration of $H_2O_2$ was kept constant at 1.5 mmol·L$^{-1}$ and the concentration of the Mn(Ⅱ) complexes was varied from 10 to 40 μmol·L$^{-1}$. At each concentration, time dependencies of DNA cleavage were analyzed, and the cooresponding $k_{obsd1}$ values were obtained. From the plot of $k_{obsd1}$ value vs. the concentration of each complex, we found that the cleavage reaction was first order to complexes in the range of complex concentrations we used (Fig.2A). Similarly, first order $H_2O_2$ dependence is expected when the concentration of each complex was kept constant at 30 μmol·L$^{-1}$ and the concentration of $H_2O_2$ was varied from 0.6 to 1.8 mmol·L$^{-1}$ (Fig.2B). Therefore, the rate law of the Mn(Ⅱ) complexes-mediated DNA cleavage may be described as:

$$\text{rate} = kC_{DNA}C_{complex}C_{H_2O_2}$$

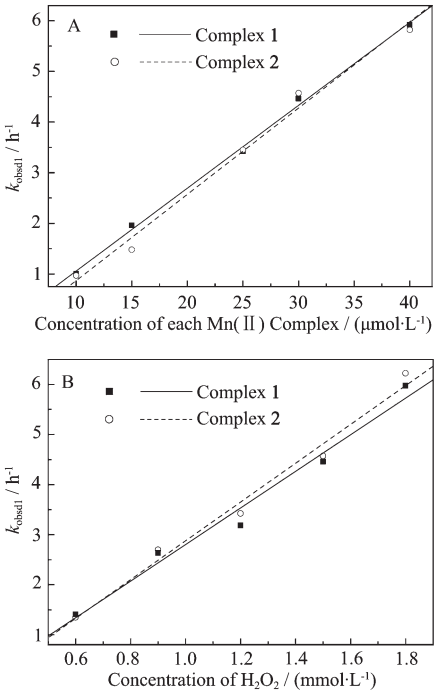



Fig.2 Observed rate constant $k_{obsd1}$ as a function of the concentration of the Mn(Ⅱ) complexes(A) and $H_2O_2$(B)

From Fig.2, the rate constants($k$) were obtained as $(2.54\pm0.36)\times10^4$ and $(2.51\pm0.45)\times10^4$ $L^2\cdot mol^{-2}\cdot s^{-1}$ for complex **1** and **2**-mediated DNA cleavage, respectively.

### 2.2 Activation parameters for DNA cleavage

The rate constants were also determined for the Mn(Ⅱ) complexes-mediated DNA cleavage at other temperatures, such as 30 ℃ and 45 ℃. The activation energies, $E_a$, were calculated according to the Arrehenius equation. For complex **1** and **2**-mediated DNA cleavage, the values of $E_a$ were determeined to be 82.37±1.84 and 81.40±2.17 $kJ\cdot mol^{-1}$, respectively(Fig.3). Using transition state theory, other activation parameters, such as activation enthalpy($\Delta H^{\neq}$) and activation entropy($\Delta S^{\neq}$) were also determined. For complex **1**-mediated DNA cleavage, the values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ are 79.79 ± 1.84 $kJ\cdot mol^{-1}$ and 96.36±5.94 $J\cdot mol^{-1}\cdot K^{-1}$, respectively. For complex **2**-mediated DNA cleavage, the values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ are 78.82±2.17 $kJ\cdot mol^{-1}$ and 93.14± 6.99 $J\cdot mol^{-1}\cdot K^{-1}$, respectively. The similar activation parameters for the two Mn(Ⅱ) complexes-mediated DNA cleavage suggest that the two reactions have similarly organized transition states[12].

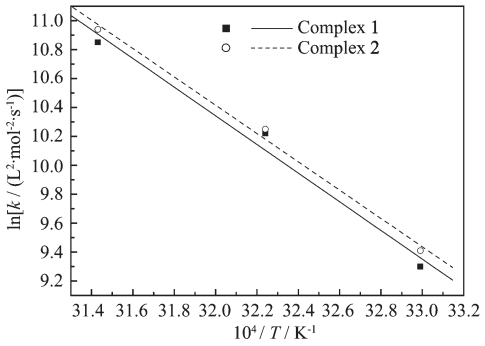
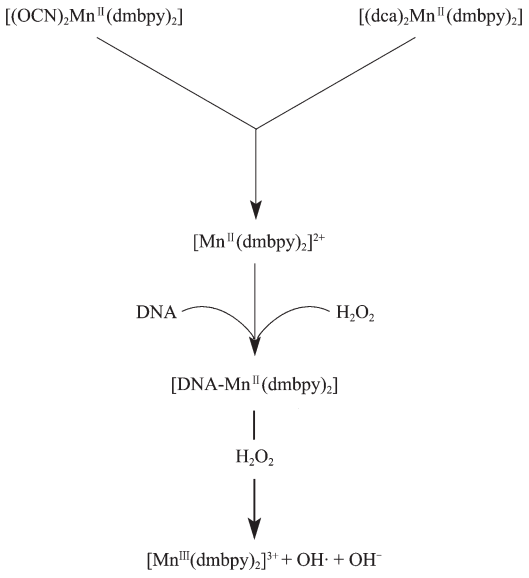



Fig.3　Plot of ln$k$ to 1/$T$

### 2.3 Mechanism of DNA cleavage

Upon the basis of the observation mentioned above and the results that we have reported[8], it would be expected that the Mn(Ⅱ) complexes have different chemical structrues in solution and in the solid state. In aquaous solutions, the Mn(Ⅱ) complexes dissociate and present as$[Mn(dmbpy)_2]^{2+}$. In the presence of DNA and $H_2O_2$, the two vacant sites of the central Mn(Ⅱ) ion may be occupied by DNA and $H_2O_2$, and then OH is formed via a Fenton-like reaction (Scheme 2)[13]. $OH^-$ is an anionic ligand, it may also coordinate to manganese and blocks the coordination site on manganese that is involved in radical formation. This may be the reason why the two Mn(Ⅱ) complexes have low DNA cleavage activities in alkaline solutions[8].




Scheme 2

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