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Study on the binding mode of Mg(Sal$_2$trien) with DNA

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Abstract In this study the complex Mg(Sal$_2$trien) was synthesized for the first time, the binding mode of which with CT DNA was studied by the methods of UV spectra, fluorescence spectra, viscosity and CV (cyclic voltammetry). It was found that after the complex acted with CT DNA, the Abs of UV spectra rose obviously; the fluorescence intensity of EB-DNA was almost not changed; viscosity decreased. Determination of cyclic voltammetry showed that DNA made the MgL's formal potential negatively shift. Scatchard plot showed that the addition of the binding mode of the complex to EB was uncompetitive inhibition compared with EB to DNA. So the binding mode of the complex with CT DNA was stable-electricity binding. Then the interaction of the complex with pBR322 was studied by the method of gel electrophoresis. The result showed that the complex could cleave pBR322 DNA effectively.

Keywords: Mg(Sal$_2$trien), calf thymus DNA, Scatchard plot.

Polyamide could not only combine with DNA outside but also inhibit expressing of gene[1]. At the same time, polyamide could coordinate many molecules. Ren *et al.*[2] found that $[Mg^{II}(dien)(OH)]^+$ can cleave pBR322DNA effectively in 2004. Liu *et al.*[3,4] found that polyamide combining with glucose shows great promise as new therapeutics to treat both acquired and inherited diseases. These polyamide compounds that promote the hydrolytic cleavage of DNA therefore could be useful not only in molecular biology and drug design but also in elucidating the precise role of metal ions in enzyme catalysis[5]. In this study, a new compound [Mg(Sal$_2$trien)] was synthesized, and determined by UV spectra, fluorescent spectra, viscosity and CV (cyclic voltammetry). We can conclude that the complex binding to DNA is essentially electrostatic bound to DNA. Furthermore the interaction of it with pBR322DNA has been investigated using gel electrophoresis. The experimental results show that the compounds can cleave pBR322DNA effectively. This work may be usefully applied to elucidating the mechanisms of natural nucleases and drug design.

1 Experimental

1.1 Materials and instruments

Elemental analysis was performed on a Perkin-Elmer 2400 instrument, ^{1}H NMR spectra were obtained on a Bruker DRX-300 spectrometer, absorbance spectra were recorded on a Hewlett-Packard HP-8453 Chemstation spectrometer, and fluorescence measurements were made with a Perkin-Elmer LS-50B fluorescence spectrophotometer. The viscometric measurement was made with an Ubbelodhe viscometer, and pH determination was made using a Beckman Φ50 pH meter. Plasmid DNA cleavage products were analyzed with a UVP GDS8000 complete gel documentation and analysis system. IR spectra were obtained on a Shimadzu-FfIR-8300 spectrometer. Cyclic voltammetry spectra were obtained at a CHI660 electrochemical working station.

Calf thymus DNA was obtained from Sino-American Biotechnology Company, and UV-Vis spectrometer was employed to check DNA purity (A_{260}:A_{280}>1.80) and concentration (ε = 6600 L·mol^{-1}·cm^{-1}at 260 nm). All the measurements about interaction of the complex with CT DNA were conducted using solutions of the complex in Tris-HCl buffer (pH 7.2) containing 0.1 mol·L^{-1} Tris and 0.1 mol·L^{-1} HCl. Ethidium bromide was purchased from Beijing Superior Chemicals Co. Ltd. All other reagents were reagent-grade and used without further purification.

1.2 Synthesis

Ligand (Hsal)$_2$ trien was synthesized according to ref. [6] and the pale yellow solid was collected (see Fig. 1). ^{1}H NMR ($DCCl_3$, 300 MHz)δ: 8.65(s, 2H), 7.62(m, 2H), 7.53(m, 2H), 7.23(m, 2H), 7.17(m, 2H), 5.02(s, 2H), 4.04(t, 4H), 3.01(t, 4H), 2.89(s, 4H), 2.47(s, 2H); Anal Calc. for $C_{20}H_{26}N_4O_2$: C 67.77, H 7.39, N 15.81%; found C 67.46, H 7.21, N 15.39%.

To a solution of 1.770 g (5 mmol) in methanol (30 mL) was added dropwise a methanol solution (30 mL) of 0.54 g (10 mmol) sodium methoxide, and then added dropwise a methanol solution (30 mL) of 1.282 g (5

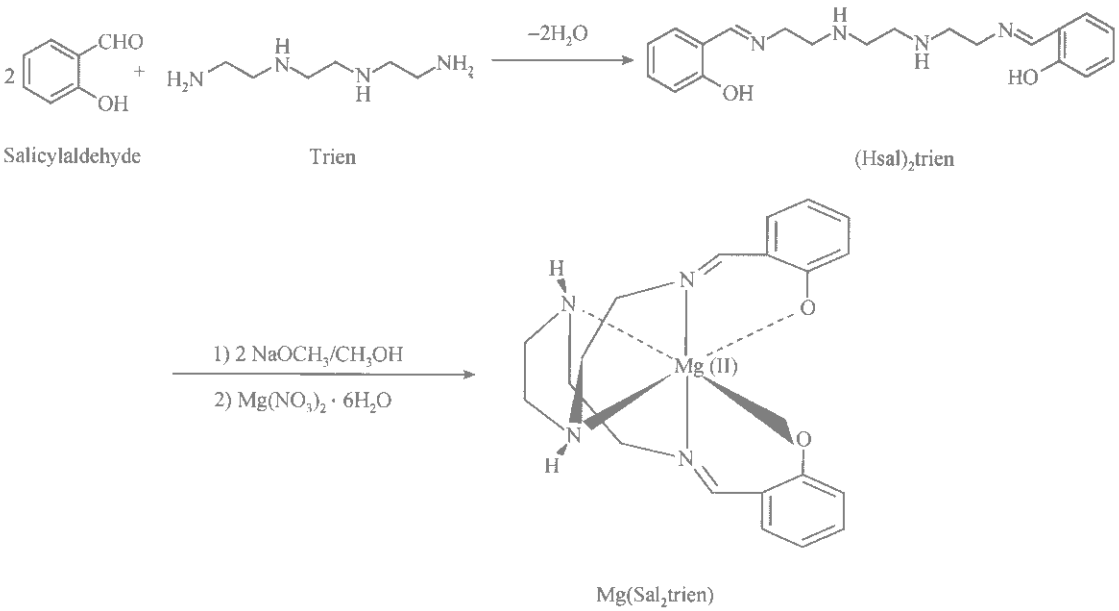

Fig. 1. Synthesis of complex.

mmol) $Mg(NO_3)_2 \cdot 6H_2O$. The solution turned yellow, and was stirred for 30 min at room temperature, and then filtered. The precipitate was collected and dissolved in 200 mL warm water. The solution was stored at 4℃ in the refrigerator overnight after being stirred, and filtered. The solid was recrystallized from 125 mL warm water after water was washed, and dried in vacuo. The last product was deep yellow solid. Yield: 0.4512 g, 24.0%. IR(KBr)v: 3310.58, 3019.35, 2856.4, 1638.42, 1598.4, 1474.48, 1455.19, 1397.3, 1340.0, 1188.6, 1149.0, 1122.98, 1026.5, 930.10, 899.7, 852.96, 758.0, 740.1 cm^{-1}. Anal Calc. for $Mg(Sal_2trien)$: C 63.70, H 6.37, N 14.86%; found C 63.28, H 6.19, N 14.32%.

1.3 MgL-DNA interaction

The MgL was dissolved in Tris-HCl buffer (pH 7.2) at a concentration of 0.002 $mol \cdot L^{-1}$. The absorption titrations were performed by keeping the concentration of MgL (15 $\mu mol \cdot L^{-1}$) constant while varying the CT DNA concentration, (R = [DNA]/[MgL]=0, 0.1, 0.3, 0.5, 0.7). The absorption was recorded after each addition of the CT DNA. The fluorescent spectra ((λ_{ex} = 520 nm) were also recorded at room temperature. All solutions were allowed to equilibrate thermally for about 30 min before measurements were made.

Thermodenaturation determination was made by heating DNA-EB solution (cDNA/cEB=20) and DNA-EB-Mg (cDNA/cEB=20, cEB=cMgL) solution from 25℃ to 98℃, and determining the solution fluorescence intensity.

Viscosity measurements were made on an Ubbelodhe viscometer immersed in a thermostatic waterbath maintained at 28±0.1℃. Fixed amounts of calf thymus DNA (1 $mmol \cdot L^{-1}$) were titrated with increasing amounts of complex, followed by bubbling with nitrogen to ensure mixing. Data were presented as $(\eta/\eta_o)^{1/3}$ versus r, where η is the viscosity of DNA in the presence of complex, η_o is the viscosity of DNA alone and r is the binding ratio.

In the electrochemistry supporting electrolyte was 50 $mmol \cdot L^{-1}$ NaCl, 5 $mmol \cdot L^{-1}$Tris, (pH=7.2); scan rate: 100 $mV \cdot s^{-1}$; working electrode was glassy carbon electrode, reference electrode was saturated calomel electrode (SCE), and auxiliary electrode was platinum electrode.

The cleavage of pBR322 DNA by MgL was carried out with 10 μL reaction mixture containing 5 mmol/L Tris-HCl (pH 7.4 containing 5 mmol/L NaCl) buffer, varying concentrations of complex, 0.5 μL of pBR322 (0.5 μg/μL). After mixing, the DNA solutions were incubated at 37℃ for 4 h. The reactions were quenched by the addition of EDTA and bromophenol blue. The gel was stained with EB (ethidium bromide) for 0.5 h after electrophoresis, and then photographed.

2 Results and discussion

2.1 UV spectroscopy

Fig. 2 shows the absorption spectra of the complex

upon interaction with DNA from free complex to R= [DNA]/[MgL] = 0.7. In the presence of DNA, the electronic absorption of MgL showed strong increases in the peak intensity. Hypochromism was suggested to be due to a strong interaction between the electronic state of the intercalating chromophore and that of the DNA bases. Hyperchromism was suggested to be due to an electrostatic binding to the phosphate group of DNA backbone[7].

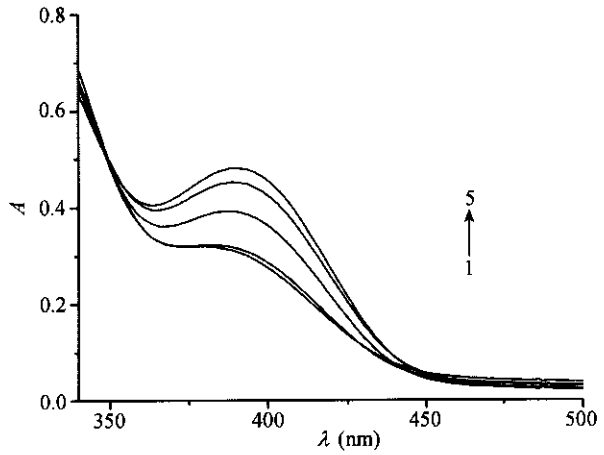

Fig. 2. Absorption spectra of MgL with increasing concentration of DNA. R = [DNA]/[MgL] =0(1), 0.1(2), 0.3(3), 0.5(4), 0.7(5), respectively.

2.2 Fluorescence spectroscopy

(i) Effect of complex on the fluorescence spectra of DNA-EB complex. In order to investigate the mode of the MgL binding to DNA, the competitive binding experiment has been carried out. The fluorescent emission of EB bound to DNA in the absence and presence of complex is shown in Fig. 3. EB is a conjugate planar molecule. Its fluorescence intensity is very weak, but it is greatly increased when EB is specifically intercalated into the double-stranded base pairs. When EB is free from DNA, the fluorescence of DNA-EB complex is quenched evidently. Therefore EB can be used as a probe for DNA structure detection[8]. MgL itself does not show appreciable fluorescence in the spectral region studied, either free from or bound to DNA. As shown in Fig. 3, the emission band at 600 nm of the DNA-EB system intensity was nearly constant with increasing MgL concentration. It may be due to the electrostatic effect of the MgL and DNA.

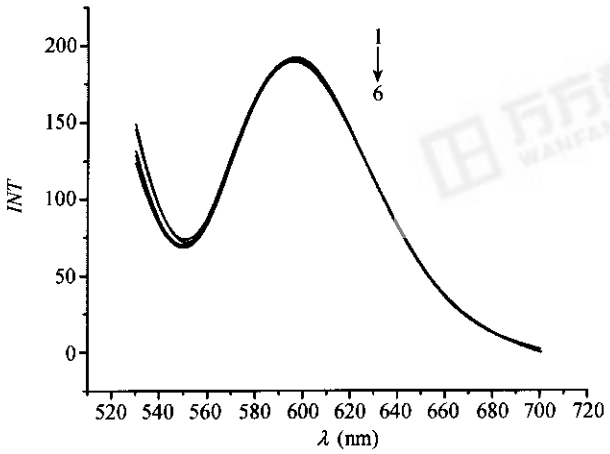

Fig. 3. Fluorescence emission spectra. From 1 to 6: [MgL]/[DNA-EB] = 0(1), 0.1(2), 0.3(3), 0.5(4), 0.7(5), 0.9(6).

(ii) Scatchard plots. To get a better insight into the nature of complex-DNA binding, we have carried out a fluorescence study of EB to DNA in the presence of a competing metal complex. The characteristics of the binding of EB to DNA can be expressed by Scatchard equation[9].

$$r/C_f = K(n-r).$$

Here r is the ratio of bound EB to total nucleotide concentration, C_f is the concentration of free EB, n is the number of binding sites per nucleic acid, and K is the intrinsic binding constant for EB. Fluorescence Scatchard plot is given in Fig. 4. K is constant and n is change, which indicates that the complex binds DNA by an uncompetitive inhibition, and they are electrostatic binding style.

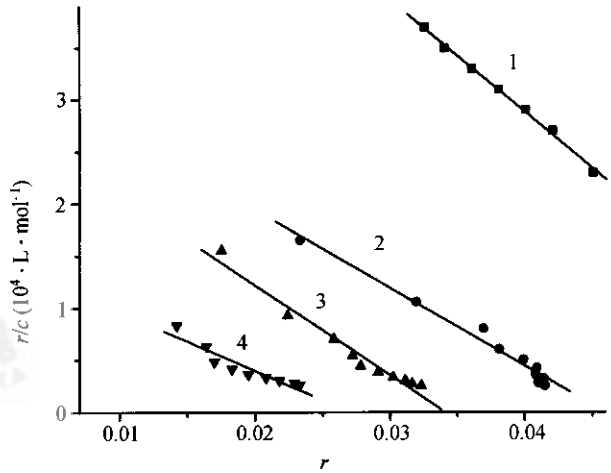

Fig. 4. Fluorescence Scatchard plot of the binding of EB to CTDNA in the MgL. [DNA] = 2.5 μmol·L^{-1}. From right to left: [MgL]/[DNA] = 0 (1), 0.25 (2), 0.5 (3), and 1.0 (4).

2.3 CT-DNA denatured temperature

EB is a conjugate planar molecule. It can increase the stability of double DNA and increase the denatured temperature (t_m)[10,11] of DNA. We can differentiate the interaction style of complex with DNA (intercalation or outside binding) by the denatured temperature (t_m).

When complex intercalates into DNA, t_m will increase sharply; when complex binds DNA outside, t_m will increase lightly[12]. Fig. 5 shows that t_m of DNA-EB is 82.5℃; and when MgL interacts with DNA-EB, t_m is 84℃. The t_m increased lightly, so we can conclude that complex binds DNA outside.

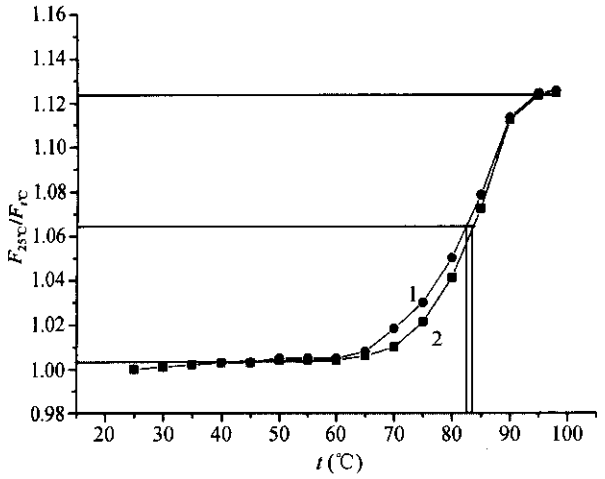

Fig. 5. Plot of DNA denatured temperature. 1, Curve of DNA-EB denaturation; 2, curve of DNA-EB-Mg denaturation.

2.4 *Viscosity*

To further explore the binding, we carried out viscosity studies. Hydrodynamic measurements sensitive to length change (i.e. viscosity and sedimentation) are regarded as the least ambiguous and most critical tests of a binding mode in solution in the absence of crystallographic structural data[13,14]. The classical intercalation mode demands that the DNA helix lengthens as base pairs are separated to accommodate the bound ligand, leading to the increase of DNA viscosity.

Fig. 6 shows that the complex decreases the relatively specific viscosity. From this observation, we can conclude that the complex is not bound by classical intercalation. The MgL^{2+} may be electrostatic bound to the phosphate group of DNA backbone.

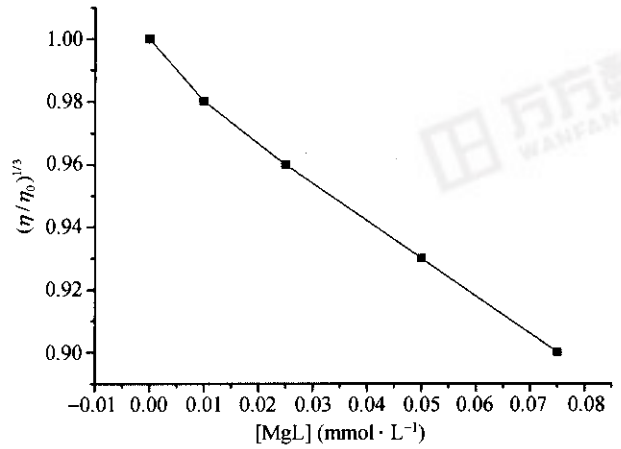

Fig. 6. Effect of the increasing concentration of bound ligand on the relative viscosity of DNA. [MgL]=0, 0.01, 0.025, 0.05, 0.075 mmol·L^{-1}.

2.5 *Cyclic voltammogram*

We can determine the interaction mode of small molecule with DNA from the shifting of formal potential[15]. When small molecule intercalated into DNA, the formal potential of it shifted to more positive values. When small molecule electrostatically bound the phosphate group of DNA backbone, the formal potential of it shifted to more negative values. As shown in Fig. 7, anodic potential (E_{pa}) of MgL was –0.4089 V and cathodic potential (E_{pc}) of MgL was –0.4991 V, peak potential phase difference was 90.2 mV, and the formal potential was –0.4540 V. After MgL interacted with DNA, anodic potential (E_{pa}) of MgL was –0.4507 V and cathodic potential (E_{pc}) of MgL was –0.5396 V, peak potential phase difference was 88.9 mV, and the formal potential was –0.4952 V. The formal potential shifted to more negative values. So we think that MgL electrostatically bound the phosphate group of DNA backbone.

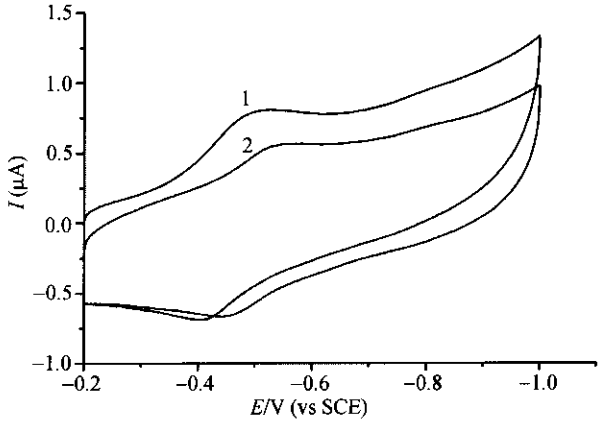

Fig. 7. Cyclic voltammogram. 1, MgL (1.0×10^{-5} mol·L^{-1}); 2, MgL+DNA (1.0×10^{-4} mol·L^{-1}).

2.6 *Electrophoretic analysis*

Further insight into the interaction between the MgL and DNA was obtained using gel electrophoresis. As shown in Fig. 8, the conversion of form I to form II was observed with the increasing concentration of MgL. Form 1 was barely observed and form III was observed in lane 8 (10×10^{-5} mol·L^{-1}). It is indicated that the complexes can effectively cleave pBR322DNA.

It was known from previous study that MgL maybe electrostatically bound the phosphate group of DNA backbone. One possible mechanism is that MgL can

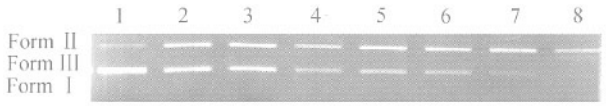

Fig. 8. Electrophoretic analysis of pBR322 mixed with different amounts of MgL. Lanes 1—8: [MgL] = 0, 0.1, 0.5, 1, 2, 3, 5, 10×10^{-5} mol·L^{-1} respectively.

catalyze DNA phosphate diester hydrolysis. The study of its mechanism will be reported in the next paper.

3 Proposed mechanism for the hydrolysis of DNA by proposed MgL

In ref. [2], we have supposed the following action mode similar to Mg-dien with DNA. As shown in Fig. 9, it appears plausible that Mg^{II}-bound hydroxide ion of $[Mg^{II}(dien)(OH)]^+$ attacks the phosphorus atom to form a pentacovalent phosphorane intermediate, further, the presence of Mg-NH_2…phosphate H-bonding, Mg^{II} ion of $[Mg^{II}(dien)(OH)]^+$ acts as a Lewis acid in facilitating the leaving of the 3′ oxyanion and could play an additional role in stabilizing the transient pentacovalent species, ultimately, leading to P-O bond cleavage.

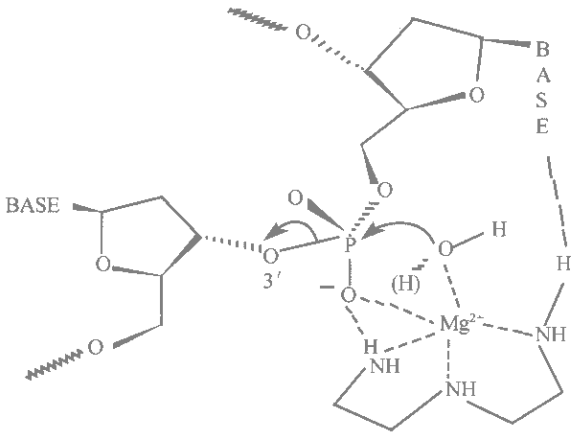

Fig. 9. Proposed mechanism for the hydrolysis of DNA by the Mg^{II}-dien complex.

The results from studying the thermodenaturation, viscosity, cyclic voltammetry by spectra, electrophoresis method showed that Mg(Sal_2trien) can only electrostatically bind DNA, and its cleavage-DNA-reactivity was possibly similar to this mode. Certainly, these proposals need to be proved by several methods.

4 Conclusion

In this study, the complex Mg(Sal_2trien) was synthesized for the first time. The interaction of Mg(Sal_2trien) with the plasmid DNA was studied by spectra, viscosity, cyclic voltammetry, electrophoresis, etc. The results showed that the binding points of the complex with the DNA were uncompetitive type compared with EB, and this complex can cleave pBR322 effectively into nick and linear, and its cleavage mechanism was possibly the hydrolysis cleavage action to the phosphate diester. This result provided available information for exploration of man-made nuclease and discussion of the application of complex in medicine exploration and molecular-biology from molecular level.

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