The construction and activity of a cerium (III) complex in DNA hydrolytic cleavage

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ABSTRACT

A new biomimetic catalyst(ML) is formed by the combination of Ce (III, M) and an azacrown ether (1,10-Dioxa-4,7,13,16-tetraaza-cyclooctadecane) in the aqueous solution. The interaction of this complex and DNA is studied with the measurements of fluorescence spectroscopy and gel electrophoresis. The results indicate that ML and calf thymus DNA interacts by the electrostatic interaction mode. Based on gel electrophoresis analysis, the study shows that ML can cleave the circular plasmid pUC19 DNA into nicked DNA under the presence of free radical scavenger, and the cleavage of DNA is a model of hydrolyzed cleavage.

Key words: azacrown ether; cerium; complex; DNA; cleavage
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INTRODUCTION

The study of the nucleic acid cleavage agents is one of the most active leading edges in the chemistry and biochemistry[1-2]. Metal complexes as nucleic acid mimic enzymes will cause biological effects on DNA and then they have attracted more and more attention in the study of mimetic enzyme[3-4]. The investigation into the binding mode and mechanism of metal complex and DNA is very important to exploring the applications of metal complexes in anti-cancer drugs, molecular biochemistry, biotechnology and so on[5-8]. Due to the unique structure and property, aza-macrocyclic metal complex has been widely employed to the study of the nucleic acid mimic enzyme[9-11]. Generally, the binding mode can be determined by the optical spectroscopy of interactions between small molecule and DNA, which is favorable for understanding the interaction mechanism of drug from molecular level and providing theoretical basis for new drug development.
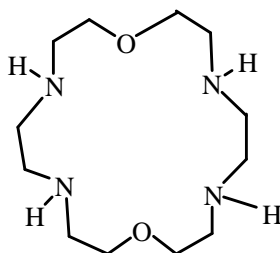
Scheme 1. The structure of ligand L

Among numerous metal complexes studied for the catalyst of cutting DNA, the complex of the rare earth metal ions has been attracted much attention due to these metal ions unique properties, such as their extremely strong Lewis acidity, the conjunction of higher charge density, coordination number, and rapid ligand exchange rates[12-13],

which showed a remarkable promotion in phosphodiester cleavage of DNA[14].In order to further study the interactions between the rare earth metal complexes and DNA, and design efficient artificial nucleases model for DNA hydrolytic cleavage, in this work, a new biomimetic catalyst(ML) is formed by the combination of Ce (III, M) and an azacrown ether (1,10-Dioxa-4,7,13,16-tetraaza-cyclooctadecane) was used in the study of DNA binding and cutting, which was not reported so far.

EXPERIMENTAL SECTION

Materials and instrumentation
The fluorescence spectra were recorded on a Cary Eclipse fluorescence spectrometer (Agilent Technologies Co. Ltd, USA). The electrophoresis bands were examined by a Quantum ST4 automatic gel imaging analysis system (Vilber Lourmat Co. Ltd, France). A Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific Inc.USA) was used for spectrophotometric study.

PUC19 DNA(2300bp) was obtained from Shanghai Yansheng Biochemical Reagents Industrial Co. Ltd. 50×TAE, Goldview dyes and Agarose (Agarose G-10) were purchased from Biowest. Double distilled water was used to prepare buffer ($5×10^{-3}$ mol dm^{-3} Tris (three hydroxyl methyl amino methane)–HCl, 0.05mol dm^{-3} NaCl, pH = 7.3). N_2 (99.99 percent) was purchased from Chongqing Jinhua Special Gas Co. Ltd. All other chemicals were of analytical grade or better quality and used as received. All solution was prepared in double distilled water.

Synthesis of the ligand 1,10-Dioxa-4,7,13,16-tetraaza-cyclooctadecane (L, $C_{16}H_{40}N_4O_2$) [15]
4,7,13,16-four tosyl-1,10-two oxygen-4,7,13,16-four aza-18-C-6.
A mixture of N, N '- two tosyl ethylenediamine (33.3g), K_2CO_3(18 g), dichloroethvl ether (11.9 ml) and mL N, N '- two methyl formamide (40.0 ml) was heated at 170" (oil bath temp) stirring for 10 h. The reaction mixture was cooled for 20 h at 5°C, and then filtrated. The precipitate was collected and washed with acetone and water. Yield 5.5g (15%). m.p. 242°C. After recrystallization from boiliig DMF it had m.p. 245°C. (Found C, 54.8; H, 6.14. Calc for C,H,,N,O,& C, 54.8; H, 5.98%).

1,10-Dioxa-4,7,13,16-tetraaza-cyclooctadecane.
4,7,13,16-four tosyl-1,10-two oxygen-4,7,13,16 -four aza-18-C-6(15g) in 37.0 mL of concentrated sulfuric acid, was heated at 100°C oil bath stirring for 3 d. The solution was then treated with excess concentrated NaOH solution, to adjust the pH value to 12 or so, and the product extracted with CHCl$_3$. The organic layer was dried (Na$_2$SO$_4$) and the filtrated, and then the filtrate was concentrated by the method of rotary evaporation and the white solid was obtained. The residue was crystallized from heptane. Yield, 2.90g (19.33%), m.p. 60-61°C. Anal . calcd for $C_{12}H_{28}N_4O_2$: C 54.46 , H10.62 , N 20.92 ; found C 54.56 , H 10.46 , N 20.95.

Fluorescence spectra studies
Luminescence experiments for metal complex were carried out at a fixed metal Ce(III) concentration ($5×10^{-5}$ mol dm^{-3}) to which increments of a stock L solution ($5×10^{-4}$ mol dm^{-3}) was added to keep the molar ratio [M]:[L]= 1:0, 2:1, 1:1, 1:2, 1:3, respectively. After addition of L, the resulting solution was allowed to equilibrate at 25°C in the dark for 10 min before being excited and scanned. Luminescence experiments for DNA titration was performed at a fixed [ML] concentration ($5×10^{-5}$ mol dm^{-3}) to which increments of a stock CT-DNA solution was added. After addition of CYC, the resulting solution was allowed to equilibrate at 25°C in the dark for 5 min before being excited by 254 nm lights at voltage 750 V and slit 5 nm, and the emission was observed between 300 and 490 nm.

Gel electrophoresis studies
A certain amount of supercoiled pUC19 DNA was treated with cerium (III) complex ML to give a mixture solution and then incubated at 37°C for a certain time. After this, the reaction was quenched by adding 6×loading buffer, and then the resulting solutions were loaded on a 1% agarose gel containing 0.5% gold view dye. Electrophoresis was carried out at 100 V for a certain time in 1×TAE buffer in a mini-gel electrophoresis unit. Subsequently, the plasmid bands were visualized by viewing the gel under a trans illuminator and photographed.

RESULTS AND DISCUSSION

Formation of the complex as catalyst
Fluorescent technique was employed to study the interaction between metal cerium ion and ligand L in this work. The increase or decrease of the fluorescent intensity of the system indicates that the interaction between metal ion and ligand occurs, which is ascribed to the energy transfer and lead to the change of the fluorescent intensity [16-17]. The fluorescent spectra was obtained at a fixed concentration of metal cerium ion and shown in figure 1. From this figure, it can be seen that the fluorescent intensity of the metal cerium ion decreases apparently upon the addition of

ligand L, which indicates that there must have interactions between metal ion and ligand. From the curve of figure 1, it can be also seen that characteristic spectra of the complex does almost no change in [ligand]:[Ce^{3+}] > 1:1. Because of the coordination number of the Ce^{3+} ion is 8 or 9 usually, and the two molecules of the ligand can supply only 6 donor atoms to Ce^{3+} ion, the complex CeL is containing at least two water molecules directly coordinated to the Ce^{3+} ion. Therefore, the complex as catalyst can combine aqueous complex and form the aqueous complex $CeL(H_2O)_2$.
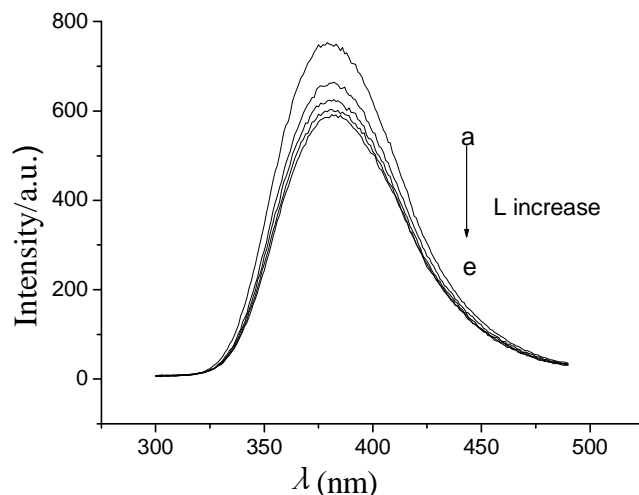
Fig. 1. Plot of the characteristic spectrum increasing amounts of ligand
pH=7.00, [M] =5×10^{-5} mol dm^{-3}, t=25ºC, λ$_{ex}$=254 nm; a-e, [M]: [L]= 1:0, 2:1, 1:1, 1:2, 1:3.

Binding action between ML and DNA
DNA binding is the critical step for DNA cleavage in most cases. Therefore, the binding of the catalytic system to DNA were studied in this work. Fluorescent technique can also be used to investigate the interaction between metal complex and nucleic acid and the change of the fluorescent spectra is related with interactions and the magnitudes of the change depend on the strength of the interaction. Therefore, the fluorescent spectra of the cerium complex was recorded in the absence and presence of CT-DNA and shown in Figure.2.
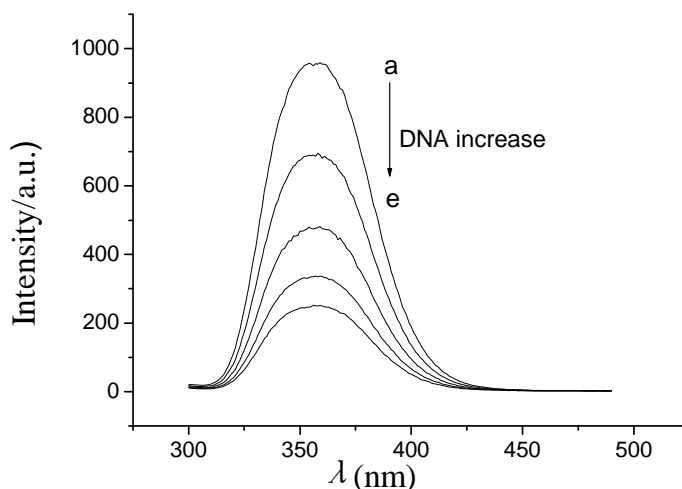

Fig. 2. Fluorescence spectra of the complex with increasing amounts of DNA
[L]=5.0×10^{-5} mol dm^{-3}, pH=7.00, t=20ºC, λex=254 nm, [DNA]=5.5×10^{-3} mol dm^{-3}; a-e: V$_{DNA}$=0, 5μl, 10μl, 20μl, 30μl

As shown in figure 2, upon addition of CT-DNA, the fluorescent intensity decreases apparently compared with that of the complex alone. The results obtained in this work is different from the studies that the fluorescent intensity increase when the metal complex intercalate to the DNA [18] and this may be due to the following reason: when some molecules interact with DNA by the mode of electrostatic interaction between metal cerium ion and negatively charged phosphate backbone of DNA, which cause the charge transfer between the complex and DNA, and will reduce the fluorescent intensity of the complex[19=20].

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DNA cleavage and DNA cleavage mechanism

Supercoiled pUC19, a widely used DNA cleavage substrate, is a circular double stranded DNA. In gel electrophoresis, supercoiled DNA usually moves faster towards to anode than relaxed DNA because it is tightly coiled and hence more compact, while nicked DNA (open circular form) moves more slowly at the same conditions. Therefore, gel electrophoresis can be employed to study the intercalation of metal complex into DNA.

In order to explore the DNA cleavage mechanism, all reactions were performed under anaerobic conditions or in the presence of the hydroxyl radical scavenger (DMSO, glycerol, MeOH) and the result was shown in figure 3. In this figure, complex ML can efficiently cleave DNA under aerobic and anaerobic conditions (lane 2 and 3). The efficacy of DNA cleavage was almost the same in the presence of hydroxyl radical scavenger DMSO in lane 4, glycerol in lane 5 and MeOH in lane 6. Therefore, the DNA cleavage by cerium complex ML was carried out most probably via hydrolytic pathway.
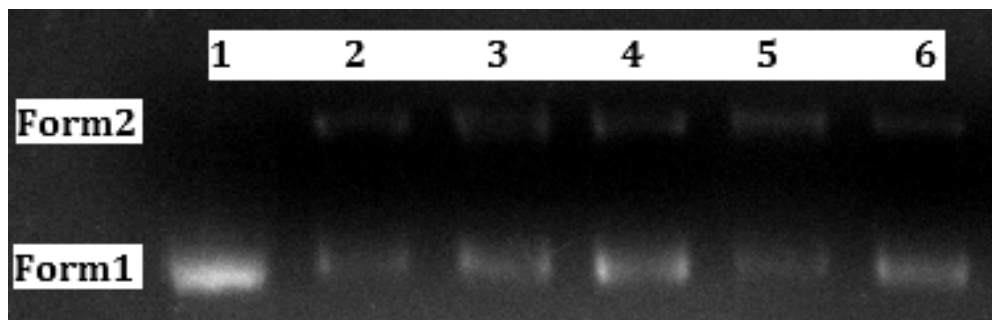

Fig.3. DNA cleavage under the presence of the complexes and different reagent
(Lane 1: DNA alone; Lane 2: complex; Lane 3: complex, fill nitrogen; Lane 4: complex+DMSO; Lane 5: complex+glycerol; Lane 6: complex+ MeOH. 37°C for 1 h, pH=7.80, [ML]=7.5×10⁻⁴ mol dm⁻³

DNA binding assays indicate that the combination of the catalytic system with DNA is a mode of electrostatic binding through phosphodiester linkages of DNA. DNA hydrolytic cleavage usually proceeded via nucleophilic substitution and the hydrolytic mechanism of DNA cleavage was proposed as shown in the following figure 4. Figure 4 indicates that: at the step (I), that the positive cerium ion in metal complex attracts negatively charged oxygen in the DNA phosphate group by electrostatic interaction, which was illustrated by fluorescent quenching observed in this work; the intramolecular metal hydroxide as a nucleophile attacks the electropositive P atom of the DNA molecule and the cleavage agent converted the supercoiled plasmid to nicked DNA with non-selective single-strand breaks at the step (II) and the step (III); the catalyst is regenerated with phosphate molecules released rapidly at the step (IV).
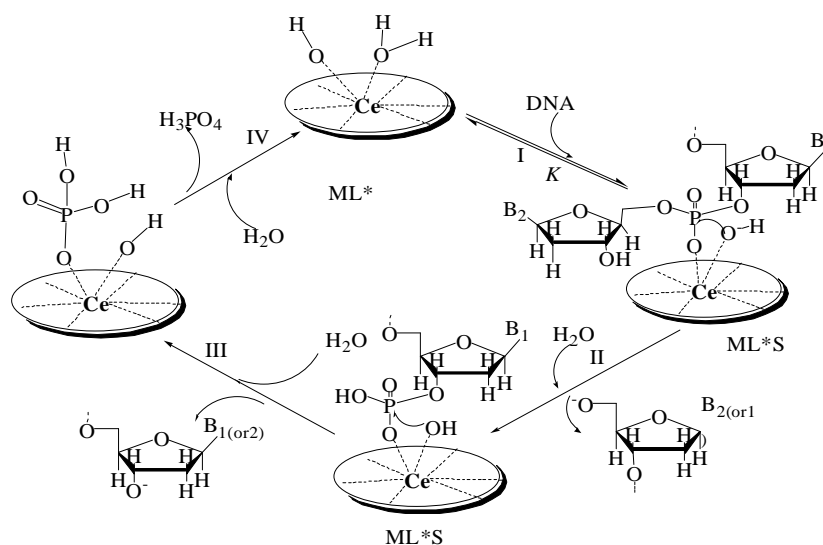

Fig. 4. pUC 19 DNA cleavage mechanism catalyzed by the complex

CONCLUSION

The interaction between cerium(III) complex (ML) made of cerium ion (III, M) and an azacrown ether has been

studied in this work. The fluorescent results indicate that the metal complex interacts with CT-DNA by the mode of electrostatic interaction. In addition, the cerium complex (ML) can efficiently cleave supercoiled pUC19 DNA into nicked form DNA under certain conditions. The DNA cleavage was almost the same in the presence of hydroxyl radical scavengers (DMSO, glycerol and MeOH), which indicates that the DNA cleavage by ML was most likely the hydrolytic pathway.

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