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N-邻苯二甲酰甘氨酸双核铜(Ⅱ)配合物的合成、晶体结构及与 DNA 的作用

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摘要:合成了新的双核铜(Ⅱ)配合物:$[Cu_2(DMSO)_2(ODPA)_4]\cdot 2DMSO$(其中,DMSO=二甲基亚砜,ODPA=*N*-邻苯二甲酰甘氨酸)。通过 X-射线单晶衍射测定了该配合物的结构。配合物晶体属于三斜晶系,空间群 $P\bar{1}$,晶胞参数:a=1.036 1(2) nm,b=1.127 3(2) nm,c=1.212 6(2) nm,α=84.084(2)°,β=81.765(3)°,γ=82.163(2)°;晶胞体积 V=1.383 6(4) nm^3,晶胞结构基元数 Z=1,D_c=1.508 g·cm^{-3},最后的残差因子:R=0.075 0,wR_2=0.192 7。用紫外和荧光光谱等方法研究了配合物与 DNA 的相互作用。结果表明,配合物以部分插入方式与 DNA 作用,凝胶电泳实验结果表明,该配合物能够切割 pBR322 DNA 成 Form Ⅱ。

关键词:双核铜(Ⅱ)配合物;晶体结构;质粒 DNA;小牛胸腺 DNA

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Synthesis, Crystal Structure and DNA-Binding Properties of Binuclear Copper(Ⅱ) Complex with *N*-Phthaloylglycinate

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Abstract: A new copper(Ⅱ) complex $[Cu_2(DMSO)_2(ODPA)_4]\cdot 2DMSO$ (DMSO=dimethyl sulfoxide, ODPA=*N*-Phthaloylglycinate) was synthesized and characterized by single-crystal X-ray diffraction. The complex crystallizes in the triclinic system, space group $P\bar{1}$, with cell parameters: a=10.361(2) nm, b=11.273(2) nm, c=12.126(2) nm, β=81.765(3)°, cell volume; V=1 383.6(4) nm^3, number of molecules inside the cell: Z=1, D_c=1.508 g·cm^{-3}, the final R=0.075 0 and wR_2=0.192 7. The interaction of the complex with DNA was studied by UV spectroscopy, EtBr fluoescent probe and DNA cleavage. The results indicate that the complex could interact with DNA by partial intercalative mode. The agarose gel electrophoresis studies revealed that the complex can cleave pBR322 DNA to Form Ⅱ. CCDC: 937398.

Key words: binuclear copper(Ⅱ) complex; crystal; PBR322DNA; CT-DNA

0 Introduction

The interaction of DNA and transition metal complexes has been a hot topic in the field of bioinorganic chemistry over these decades[1]. Transition metal complexes could be used as probes of DNA structure, SOD mimics, chemical nucleases, and potential therapeutic agents[2-6].

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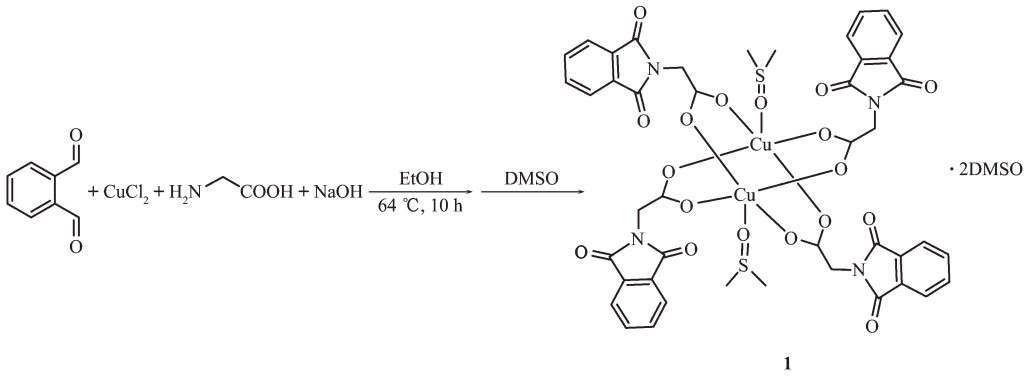

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Scheme 1 Synthesis of the title complex **1**

The influence of metal carboxylates complexes, especially transition metal carboxylates and their derivatives have received considerable attention owing to their synthetic and effective wide range of biological activity [7-8]. Phthalimide derivatives were important in medicinal chemistry and always were used as antimicrobial reagents[9-10]. *N*-phthaloylglycinate is one of the *N*-phthaloyl derivatives of amino acids. *N*-phthaloylglycinate ligand can coordinate the metal ion through one carboxylate group and two carbonyl moieties[11-12].

Copper is one of essential trace elements of life and copper complexes exist abundantly in the life system. Because of variable valence, copper can participate in a series of electron transfer and redox in life, resulting its special biological activities[13].

In the present studies, a new complex complexes of *N*-phthaloylglycine with copper(Ⅱ) was obtained and its biological properties was studied in respect to the interaction between DNA. The preliminary results showed that the complex is expected to be used as a new type of DNA secondary structure probe.

1 Experimental

1.1 Materials and apparatus

All materials and reagents are of analytical grade and were purchased from commercial source. Ethidium bromide (EB) and agarose were purchased from Sigma. Tris-HCl buffer solution was prepared with double distilled water. pBR322 DNA was purchased from MBI Fermentas and calf thymus DNA from the Sigma Biotechnology Company. UV-Vis and fluorescence spectra were obtained respectively on Pharmacia 4000 UV-Vis and Hitachi F-4500 spectrophotometers at room temperature, and the diagram of electrophoresis was obtained via Peiqing JS-780 sensi ansys electrophoresis image analysis system, respectively. IR spectra were taken with a Nicolet FT-IR 5DX spectrometer.

1.2 Solution preparation of DNA

Calf thymus DNA was dissolved in 5 mmol·L^{-1} Tris-HCl buffer (50 mmol·L^{-1} NaCl, pH=7.2. Calf thymus DNA was checked spectroscopically at 260 and 280 nm on UV-Vis spectrophotometer and resulted a ratio greater than 1.84, which indicated the DNA was sufficiently free of protein[14]. The concentration of CT-DNA was measured from the band intensity at 260 nm with a known ε value of 6 600 L·mol^{-1}·cm^{-1} [15].

1.3 Synthesis of complex 1

A solution of *o*-phthalaldehyde (20 mmol) in 120 mL 95% ethanol was added drop wise to an ethanol solution of copper(Ⅱ) chlorides (3.409 6 g, 20 mmol, 30 mL). The reaction mixture was stirred at room temperature for 1 h. An aqueous solution of glycinate (40 mmol) and NaOH (40 mmol) in 12 mL was added and stirred at 64 ℃ for over 10 h. The resulting solution was evaporated under reduced pressure. Then the solid was filtered and washed with large amount of water and ethanol. The crude product was dissolved in dimethyl sulfoxide (DMSO) in a glass tube, and a solution of petroleum ether was carefully added to the glass tube for one week. The green crystal precipitation was generated in the interface of DMSO and petroleum ether. The crystal was was collected, dried (m.p. 278~280 ℃). IR in cm^{-1} (KBr): ν=3 386

Table 1　Crystal data of the complexes

Empirical formula	$C_{48}H_{48}Cu_2N_4O_{20}S_4$	D_c / (g·cm^{-3})	1.508
Formula weight	1 256.22	F(000)	646
Crystal system	Triclinic	Absorption coefficient μ / mm^{-1}	0.997
Temperature / K	296(2)	Crystal size / mm	0.15×0.12×0.10
λ / nm	0.710 73	θ range for data collection / (°)	1.83 to 25.25
Space group	$P\bar{1}$	Index ranges	$-12 \leqslant h \leqslant 12, -12 \leqslant k \leqslant 13, -14 \leqslant l \leqslant 14$
a / nm	1.036 1(2)	Reflections collected	9 968
b / nm	1.127 3(2)	Independent reflection	4 950
c / nm	1.212 6(2)	Rint	0.048
α / (°)	84.084(2)	Refinement method	Full-matrix least-squares on F^2
β / (°)	81.765(3)	Goodness. of-fit on F^2	1.017
γ / (°)	82.163(2)	Final R indices ($I>2\sigma(I)$)	0.075 0
V / nm^3	1.383 6(4)	R indices (all data)	0.132 9
Z	1	$\Delta\rho_{max}$, $\Delta\rho_{min}$ / (e·nm^{-3})	1 314, −758

water (O-H), 1 769 and 1 700 keto (C=O), 1 587 and 1 423 carboxylic (COO).

1.4　Crystal structure determination

For X-ray Crystallographic analysis, the X-ray diffraction intensities and the unit cell parameters were determined on a Brucker SMART APEX Ⅱ CCD diffractometer employing graphite-monochromated (Mo $K\alpha$) radiation (λ =0.071 073 nm) and operating in the ω scan mode. The SMART program was used for data acquisition, and the SAINT$^+$ software for data extraction. Absorption correction was carried out using the SADABS program[16]. The structure was solved and refined by full-matrix least-squares method using the SHELX system of programs[17]. All non-hydrogen atoms refined anisotropically. The hydrogen atoms were placed in calculated positions and refined using a riding mode. Details of the crystal parameters, data collection and refinements are listed in Table 1.

CCDC: 937398.

1.5　DNA binding

The property of the copper (Ⅱ) complex for binding DNA was investigated using electronic absorption and fluorescence spectroscopy, respectively. The electronic absorption spectra were collected within the 200 to 400 nm range with DMSO and Tris-HC1/NaCl buffer solution as reference. Different concentrations of CT-DNA solution with the same volume were sequentially added to the complex solution in the same concentration.

Fluorescence spectra titration experiments were performed with fixed concentration of CT-DNA while gradually increasing the complex concentration. Fluorescence spectra were recorded with excitation at 525 nm and emission at 610 nm.

1.6　DNA cleavage

The cleavage of pBR322 DNA by the complex was examined by gel electrophoresis. Supercoiled pBR322 DNA (1 μL, 0.25 μg·μL^{-1}) was treated with different concentration of complex (7.5 μL) in MOPS buffer (40 mmol, pH=7.4). After mixing, the DNA solutions were immersed in constant temperature bath at 37 ℃ for 2 h. Then, sterilized solution (3.3 μL) was added to quench the reaction and analyzed by electrophoresis at 70 V for 70 min on agarose gel in TAE buffer (40 mmol·L^{-1} Tris-base, 40 mmol·L^{-1} acetic acid and 1 mmol·L^{-1} EDTA, pH=7.38). The gel was stained with EB (15 μL) for 0.5 h after electrophoresis and then photographed.

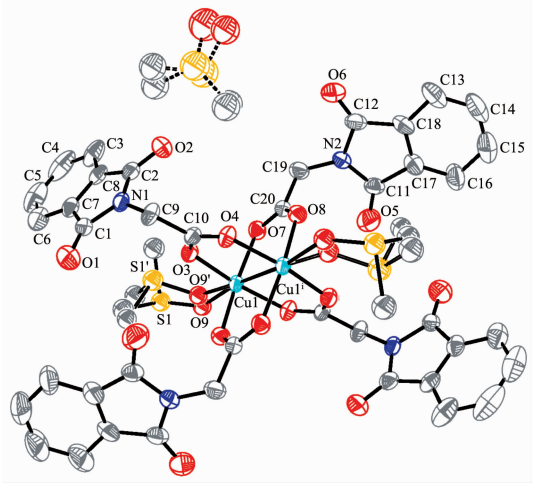
2　Results and discussion

The title complex is insoluble in water and in common organic solvents like methanol and petroleum ether except for DMSO. So, all relevant solution of the complex was prepared using DMSO-containing solvents. The reaction of (opa) with amino acids produced an immensely fluorescent product, which

although apparently proceeding initially via a Schiff base rearranged to a fluorescent stable cyclic *N*-substituted isoindolin-1-ones derivative [18]. When we added copper (Ⅱ) chlorides to the reaction mixture before glycinate, we found that a new binuclear copper(Ⅱ) complex was synthesized.

2.1 Crystal structure

The selected bond lengths and angles are summarized in table 2. The coordination environment of central ion Cu(Ⅱ) is show in Fig.1. In the crystal structure, the title binuclear Cu(Ⅱ) complex exhibit the typical neutral paddlewheel structure. The Cu(Ⅱ) is coordinated by O(3), O(7), O(4A), O(8A), the four acetate groups each bridge a pair of Cu^{II} atoms. Each copper atom was further accommodated a DMSO molecular. The Cu1-O (carboxylic) distance is shorter than the Cu1-O(9) (DMSO) distance. All the O-Cu(1)-Cu(1A) bond angles in the complex are range from 81.79(14) to 85.22(14). Moreover, the Cu-O distances ranging from 0.196 3~0.216 9 nm are comparable with those reported in literature[19-20]. The Cu-Cu distance is 0.268 03(15) nm and each metal atom exhibits a Jahn-Teller-distorted octahedral geometry.

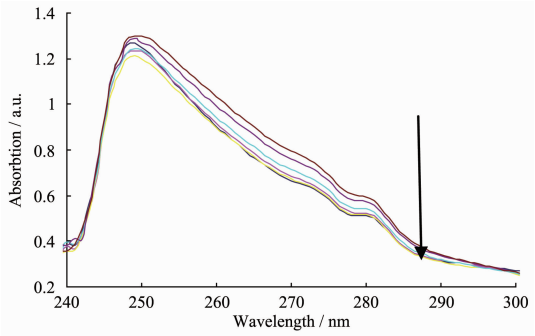


Hydrogen atoms are omitted for clarity; Symmetry transformations used to generate the equivalent atoms: ^{i} $-x$, $1-y$, $1-z$

Fig.1 ORTEP view of the title complex with ellipsoid drawn at 30% probability

2.2 UV spectroscopic studies

The absorption spectra of the title complex in the absence and presence of CT-DNA are shown in Fig.2. The absorption spectrum of the title complex shows a very strong absorption at maximum of 249 nm and a week absorption at 280 nm. The strong band around 249 nm is attributed to π-π^* transitions in complex. Upon addition of an increasing amount of DNA to the complex solution, about 1 nm of red shiftand a 6.8%



$c_{complex}$=33.3 μmol·L^{-1}, Lane 1~6 c_{DNA}=0, 8.33, 12.33, 20.32, 32.33, 40.12 μmol·L^{-1}

Fig.2 UV-Vis spectra of the complex in presence of increasing CT-DNA

Table 2 Selected bond lengths (nm) and angles (°) for $[Cu(DMSO)_2(Gly)_2(OPA)_2]_2$

O9-Cu1	0.216 9(11)	Cu1-Cu1^{i}	0.268 03(15)	Cu1-O8^{i}	0.196 3(5)
O9′-Cu1	0.210 9(11)	Cu1-O3	0.197 2(4)		
Cu1-O7	0.195 7(5)	Cu1-O4^{i}	0.197 2(4)		
O7-Cu1-O8^{i}	166.95(18)	O8-Cu1^{i}-O9	90.6(3)	O4-Cu1^{i}-O3	167.22(18)
O7-Cu1-O3	89.07(19)	O3-Cu1-O9	100.3(3)	O8-Cu1^{i}-O9′	97.2(3)
O7-Cu1-O9′	95.7(3)	O8-Cu1^{i}-Cu1^{i}	81.79(14)	O4-Cu1^{i}-O9′	100.1(3)
O3-Cu1-O9′	92.6(3)	O9-Cu1-Cu1^{i}	171.6(3)	O7-Cu1-O9	102.5(3)
O9′-Cu1-Cu1^{i}	175.6(3)	O7-Cu1-O4^{i}	89.1(2)	O4-Cu1^{i}-O9	92.4(3)
O7-Cu1-Cu1^{i}	85.22(14)	O8-Cu1^{i}-O4^{i}	90.75(19)	O9′-Cu1-O9	10.3 (4)
O3-Cu1-Cu1^{i}	83.06(13)	O8-Cu1^{i}-O3	88.23(19)	O4-Cu1^{i}-Cu1^{i}	84.19(14)

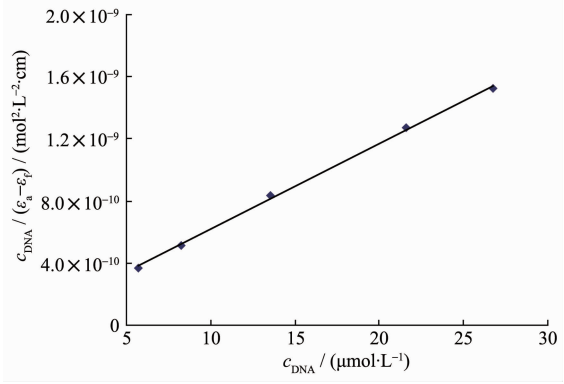
Symmetry code: ^{i} $-x$, $1-y$, $1-z$.

hypochromism were observed.

An equation[21] was used to determine the intrinsic binding constant K_b of the complex with CT-DNA.

$$c_{DNA}/(\varepsilon_a-\varepsilon_f)=c_{DNA}/(\varepsilon_b-\varepsilon_f)+1/K_b(\varepsilon_b-\varepsilon_f)$$

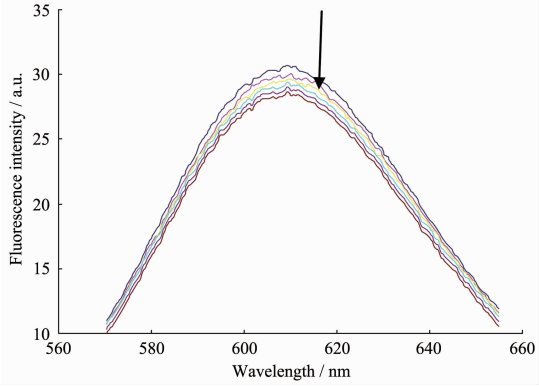
Where ε_a, ε_f and ε_b respectively corresponds to $A_{obsd}/c_{complex}$, the extinction coefficient for the free complex, and extinction coefficient for the complex in the fully bound form. In the plot of $c_{DNA}/(\varepsilon_a-\varepsilon_f)$ versus c_{DNA}, K_b is given by the ratio of the slope to the intercept in Fig. 3. The intrinsic binding constant K_b is calculated to be 1.5 L·mol^{-1}, which is much smaller than [Cu(H_2O)(Gly)(PZTA)]ClO_4[22]. The results suggested that the binding of the complex to DNA is very week. Also the results may be explained by the fact that the severe steric constraints near the core of Cu (Ⅱ) of the binuclear copper (Ⅱ) hinders intercalation of the complex into the DNA base pairs.



2.3 Fluorescence spectroscopic studies

Fluorescence spectroscopy has been widely used to study the interactions between the complex and DNA. Ethidium bromide (EB) is a conjugate plane molecule, emitting weak fluorescence, can selectively insert inside the DNA base pairs, resulting its fluorescence enhancement. When complex is added, the fluorescence intensity of EB-DNA was reduced, which may be caused by the complex intercalation into the DNA helix. The degree of EB-DNA system fluorescence quenching can be used to describe the interaction of complex with DNA.

The emission spectra of EB bound to CT-DNA in the absence and the presence of the complex are given in Fig.3. The addition of the complex to CT-DNA pretreated with EB caused appreciable reduction in emission intensity at 610 nm, indicating that some EB molecules were repelled to solution after an exchange with the complex, which resulted in the fluorescence quenching of EB.



c_{EB}=10.35 μmol·L^{-1}; c_{DNA}=10.01 μmol·L^{-1}; Line 1~6: $c_{complex}$=0, 40, 60, 80, 100, 120 μmol·L^{-1}

Fig.3　Effects of the complex on the fluorescence spectra of EB-DNA system

In order to describe the binding strength of the complex with DNA, Stern-Volmer equation[23] can be used: $I_0/I=1+K_{sq}r$. I_0 is the fluorescence intensities of EB-DNA in the absence of complex; I is the fluorescence intensities of EB-DNA in the presence of complex; r is the ratio of the concentration of complex to DNA.

From the Fig.4, the obtained K_{sq} value for the complex is 0.004 7, is smaller than that of complex [Cu(H_2O)(Gly)(PZTA)]ClO_4[22], which indicated that the title complex is difficult to insert the DNA molecule due to its large steric hindrance. The result is in agreement with those obtained in UV experiments.

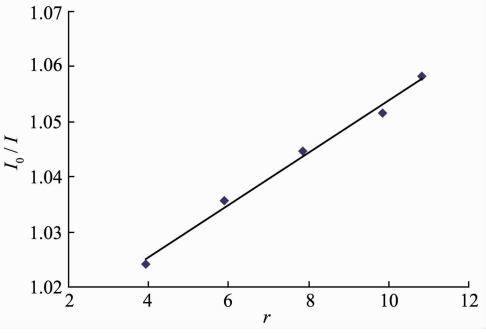
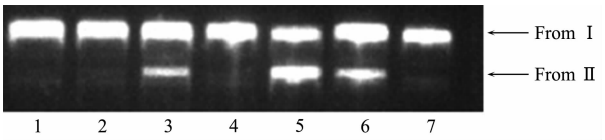


Fig.4　Stern-Volmer quenching plot of EB bound to DNA by complex

2.4 DNA cleavage

Agarose gel electrophoresis is an important

method to study the effect of a complex to cleave DNA. There are three kinds of configuration of the pBR322 DNA, supercoiled circular (From Ⅰ), nick circular(From Ⅱ) and linear (From Ⅲ). The Agarose gel electrophoresis of supercoiled pBR322 DNA in the different the title complex was depicted in Fig.5.



Agarose gel electrophoresis patterns for the cleavage pBR322 plasmid DNA (0.02 μg·μL^{-1}) by complex $[Cu_2(DMSO)_4(Gly)_4(OPA)_4]$ (8.5 μL) was incubated for 2 h in MOPS buffer (40 mmol, pH=7.4) at 37 ℃. Lane 7: DNA control (DNA+DMSO); Lane 1~6: DNA+complex ($c_{complex}$=6.25, 12.5, 25, 37.5, 50,75 μmol·L^{-1})

Fig.5 Effect of complex on gel electrophoresis of plasmid pBR322DNA

As shown in Fig.5, low concentration of the complex can not cleave the pBR322DNA while an obvious cleavage effect was observed with the increase of the concentration, displaying a concentration-dependent cleavage. No Form Ⅲ was observed in the electrophoresis when the concentration of the complex reached 50 μmol·L^{-1}.

3 Conclusions

In conclusion, a new binuclear copper(Ⅱ) complex has been synthesized and characterized by single crystal X-ray diffraction. The binding of the complex to CT-DNA were studied by UV-Vis and fluorescence spectroscopies. Because of the large steric hindrance of the complex, the binding strength of complex with DNA molecule is very weak. Gel electrophoresis assay further reveals the weak binding effect of the complex with DNA molecule. The further relationship between structure of the complex and biological activates is still in progress in our lab.

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