J. Cent. South Univ. (2015) 22: 1619−1625
DOI: 10.1007/s11771-015-2679-3

Springer

Synthesis, structure, DNA binding and cleavage activity of a new copper(II) complex of bispyridylpyrrolide

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Abstract: A copper−bispyridylpyrrolide complex [Cu(PDP_H)Cl] (PDP_H = 2,5-bis(2′-pyridyl)pyrrole) was synthesized and characterized. The complex crystallizes in the orthorhombic system with space group *Pccn*, a = 0.9016(3) nm, b = 1.0931(4) nm, c = 2.5319(8) nm, and V = 2.4951(15) nm^3. The copper center is situated in a square planar geometry. The interaction of the copper(II) complex with calf thymus DNA (CT-DNA) was investigated by electronic absorption, circular dichroism (CD) and fluorescence spectra. It is proposed that the complex binds to CT-DNA through groove binding mode. Nuclease activity of the complex was also studied by gel electrophoresis method. The complex can efficiently cleave supercoiled pBR322 DNA in the presence of ascorbate (H_2A) via oxidative pathway. The preliminary mechanism of DNA cleavage by the complex with different inhibiting reagents indicates that the hydroxyl radicals were involved as the active species in the DNA cleavage process.

Key words: copper complex; bispyridylpyrrolide; DNA-binding; DNA cleavage

1 Introduction

In recent years, tremendous interests have been focused on the interactions between transition metal complexes and DNA due to the general thought that the target of the powerful anticancer drug cisplatin is cellular DNA [1]. Metal complexes capable of binding to and cleaving DNA under physiological conditions have the potential as structural probes in nucleic acids chemistry and as therapeutic agents [2−3]. Therefore, a large number of complexes have been prepared and explored for their biological activities, such as nuclease and anticancer activities [4−7].

Copper is a bio-essential element and plays an important role in biological processes. Copper complexes are the preferred molecules for bringing about DNA binding and cleavage among the numerous metal complexes since copper ions have been observed in the active site of many nucleases [8−9]. In 1979, SIGMAN et al [10] first reported that bis(1, 10-phenanthroline) copper complex was able to efficiently mediate DNA strand scission in the presence of thiol and hydrogen peroxide. Since then, many other copper complexes with various structural ligands exhibiting effective DNA binding and cleavage activities have also been studied, such as copper complexes with N, N-donor heterocyclic bases [11−12], thiosemicarbazones [13−14], schiff bases [15−16], and macrocyclic ligands[17−18].

It is commonly thought that the planarity of the aromatic chelate ligands may facilitate them stack between DNA bases and then lead to enhanced binding ability of the complexes to DNA [19−20]. In our previous studies, several copper(II) complexes of planar phenanthroline or its derivatives have been prepared, and these complexes were shown to bind to CT-DNA and cleavage supercoiled DNA efficiently [21−23]. Besides, some copper(II) complexes of planar terpyridine ligands (Cu-terpy) have also been studied recently, and these Cu-terpy systems were able to interact with DNA by intercalation or groove binding mode [24−26].

In this work, the synthesis and characterization of a new copper(II) complex of bispyridylpyrrolide ligand which is structurally analogous to terpyridine are reported. The interaction between the complex and DNA has also been studied by spectroscopy and gel electrophoresis. The results suggest that the copper(II) complex can interact with DNA by groove binding mode and cleavage DNA via a oxidative process.

2 Experimental

2.1 Materials and instrumentation

All reagents and chemicals were purchased from

Foundation item: Project(21001118) supported by National Natural Science Foundation of China; Project(12JJ3016) supported by Natural Science Foundation of Hunan Province, China
Received date: 2014−04−07; **Accepted date:** 2014−10−20
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commercial sources. CT-DNA, Agrose, ethidium bromide (EB), tris(hydroxyl-methyl)aminomethane (Tris) and ascorbate (H_2A) were purchased from Sigma. Supercoiled pBR322 DNA was purchased from Takara Biotechnology (Dalian) Co., Ltd. All other chemicals are of analytical grade and double distilled water was used for all the experiments. 2,5-bis(2'-pyridyl) pyrrole (PDP_H) was prepared according to Ref. [27].

The infrared spectra were recorded on a Bruker VEC-TOR22 spectrometer as KBr pellets (4000−500 cm^{-1}), and elemental analysis was performed on a Perkin-Elmer 240 C analytical instrument. The electronic absorption spectra measurement was made on a Shimadzu UV-2450 spectrometer. Fluorescence measurement was recorded on a Hitachi F4600 luminescence spectrometer. The CD spectra were carried out on a Jasco J−815 automatic recording spectropolarimeter. DNA cleavage experiments were made on a DYY6C gel electrophoresis instrument and using Fluorchem Fc2AIC software for data analysis.

2.2 Synthesis of [Cu(PDP_H)Cl]

To a mixture of PDP_H (330 mg, 1.5 mmol) and CuCl (148.5 mg, 1.5 mmol) in THF (20 mL), Et_3N (151.8 mg, 1.5 mmol) was added. The suspension solution became to clear, and color was changed to red-orange immediately. After continuing to stir for 1 h, the reaction mixture was exposed in air for 1 h, during which color of solution was changed to green. Recrystallization from THF-hexane afforded the needle green crystals which were suitable for the X-ray diffraction study. Yield: 0.406 g, 85 %. Anal. Calcd for $C_{14}H_{10}N_3CuCl$: C, 52.58; H, 3.13; N, 13.15%. Found: C, 51.36; H, 3.35; N, 12.91%. IR (KBr)(ν_{max}/cm^{-1}): 3,451(br, w), 1,599 (vs), 1,502 (s), 1,458 (w), 1,435 (s), 1,402 (w), 1,371 (s), 1,341 (w), 1,269 (w), 1,146 (m), 1,042 (m), 996 (w), 887 (w), 844 (w), 781 (w), 752 (vs), 710 (w), 652 (w), 563 (w).

2.3 X-ray crystallography

Diffraction data of [Cu(PDP_H)Cl] were recorded on a Bruker CCD diffractometer with monochromatized Mo−K radiation (λ=0.71073 Å). The collected frames were processed with the software SAINT. The absorption correction was treated with SADABS [28]. Structures were solved by direct methods and refined by full-matrix least-squares on F2 using the SHELXTL software package [29]. Atomic positions of non-hydrogen atoms were refined with anisotropic parameters. All hydrogen atoms were introduced at their geometric positions and refined as riding atoms. Basic crystal data, description of the diffraction experiment, and details of the structure refinement are given in Table 1. Selected bond distances and bond angles are listed in Table 2.

Crystallographic data for the structure of [Cu(PDP_H)Cl] have been deposited with the Cambridge Crystallographic Data Centre (CCDC). These data can be obtained free of charge from the CCDC (CCDC: 982233).

2.4 DNA binding experiments

All the DNA binding experiments were carried out in a mixture solvent of DMSO and tris-HCl buffer (5 mmol/L tris-HCl/50 mmol/L NaCl, pH 7.4). A solution of CT-DNA in the tris-HCl buffer gave a ratio of UV absorbance at 260 and 280 nm of about 1.9, indicating that the CT-DNA was sufficiently free of protein [30]. The CT-DNA concentration was determined by employing extinction coefficient of 6600 L/(mol·cm) at 260 nm [31]. [Cu(PDP_H)Cl] was dissolved in a solvent of a little of DMSO first and then diluted with tris-HCl buffer at concentration 3.0×10^{-5} mol/L. The amount of DMSO was kept less than 5% (by volume) for each set of experiment and has no effect on any experimental results. An equal amount of CT-DNA was added to both the compound solution at increasing DNA/complex ratio (r=0.0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35) when measuring the absorption spectra. Each sample solution was scanned in the range of 200−600 nm and the absorption spectra were recorded. The intrinsic binding constant k_b for the interaction of the copper(II) complex with DNA was calculated from the following equation [32]:

$$[DNA]/(\varepsilon_a-\varepsilon_f)=[DNA]/(\varepsilon_b-\varepsilon_f)+1/k_b(\varepsilon_b-\varepsilon_f) \quad (1)$$

where [DNA] is the concentration of DNA in base pairs; the apparent absorption coefficient ε_a, ε_f and ε_b correspond to A_{obsd}/[Cu]. The ratio of slope to intercept in the plot of [DNA]/(ε_a–ε_f) versus [DNA] gives the value of k_b.

Table 1 Crystal and structure refinement data for complex [Cu(PDP_H)Cl]

Empirical formula	Relative molecular mass	Crystal system	Space group	a/nm	b/nm	c/nm	α/(°)	β/(°)	γ/(°)	Volume/nm^3
$C_{14}H_{10}ClCuN_3$	319.24	Orthorhombic	Pccn	0.9016(3)	1.0931(4)	2.5319(8)	90	90	90	2.4951(15)
Z	ρ_{calc}/ (g·cm^{-3})	T/K	μ/mm^{-1}	F(000)	No. of refln.	No. of indep. refln.	R_{int}	[a]GoF	[b]R_1, [c]wR_2 [$I>2\sigma(I)$]	R_1, wR_2 (all data)
8	1.700	2962	1.951	1288	10839	2147	0.0665	1.010	0.0394, 0.0920	0.0616, 0.1015

[a] GoF = $[\Sigma w(|F_o|-|F_c|)^2/(N_{obs}-N_{param})]^{½}$; [b] $R_1=\Sigma||F_o|-|F_c||/\Sigma|F_o|$; [c] wR_2 $[(\Sigma w|F_o|-|F_c|)^2/\Sigma w^2|F_o|^2]^{½}$.

Table 2 Selected bond distances and angles of titled complex

Bond	Distance/nm	Angel/(°)
Cu(1)—N(1)	0.1862(3)	
Cu(1)—N(2)	0.2141(3)	
Cu(1)—N(3)	0.2148(3)	
Cu(1)—Cl(1)	0.2222(11)	
N(1)—Cu(1)—N(2)		76.81(11)
N(1)—Cu(1)—N(3)		77.25(12)
N(1)—Cu(1)—Cl(1)		174.32(9)
N(2)—Cu(1)—Cl(1)		102.69(8)
N(3)—Cu(1)—Cl(1)		102.67(8)
N(2)—Cu(1)—N(3)		153.67(12)

CD spectra measurement was carried out at increasing complex/DNA ratio (r=0.0, 0.05, 0.10, 0.15). Each sample solution was scanned in the range of 220−320 nm. Each CD spectrum was collected after averaging three scans using a scan speed of 500 nm/min, and the buffer background had been subtracted. The concentration of DNA was 1.0×10^{-4} mol/L.

The fluorescence spectra were recorded at room temperature with excitation at 530 nm and emission at 610 nm. The experiment was carried out by titrating the solution of [Cu(PDP_H)Cl] (6.2×10^{-4} mol/L) into samples containing 1.0×10^{-4} mol/L DNA and 1.0×10^{-5} mol/L EB.

2.5 DNA cleavage experiments

The cleavage of plasmid DNA in the presence of activating agents ascorbate (H_2A) was determined by agrose gel electrophoresis. In this experiment, supercoiled pBR322 DNA (0.2 μg) in 5% DMSO-tris buffer (5 mmol/L tris-HCL/50 mmol/L NaCl, pH 7.4) was treated with the copper(II) complex. The concentration of complex was varied while keeping the concentration ratio of 1:50 of complex to ascorbate in a total volume of 10 μL. All the samples were incubated for 60 min at 310 K before 6×DNA loading buffer containing 30 mmol/L EDTA, 36% (v/v) glycerol and 0.05% (w/v) bromophenol blue were added.

In the inhibition experiment, additives like DMSO (3 μL), histidine (100 μmol/L), tryptohan (100 μmol/L) were added to the supersoiled DNA and the incubation carried out for 15 min at 310 K pior to the addition of the complex and ascorbate. Finally, all samples were performed on 1% agarose gel containing 0.5 μg/mL EB. The gels were run at 100 V for 90 min in tris-acetic acid−ethylene diaminetetraacetic acid (TAE) buffer. The resulting bands were visualized by UV light and photographed in a gel documentation system. The relative amounts of closed circular, nicked, and linear DNA were quantified by the Fluorchem Fc2AIC.

3 Results and discussion

3.1 Synthesis and crystal structure

Previously, we reported the reaction of PDP_H and CuCl in the presence of NaH under nitrogen atmosphere yielded sodium−copper(I) complex [Cu(PDP_H)$_2$Na(thf)$_2$] [33]. This sodium−copper(I) is air-sensitive, is easily oxidated to green untractable oily product. When PDP_H was treated with CuCl in the presence of Et_3N, orange solution was formed immediately. This resulting mixture was also air-sensitive, and was exposed in air to readily form stable copper(II) complex [Cu(PDP_H)Cl] in high yield. In comparison, PDP_H directly reacted with $CuCl_2$ only to produce the mixture of the titled complex [Cu(PDP_H)Cl] and another complex (PDP_H)$_2$Cu. [Cu(PDP_H)Cl] was characterized by IR spectrum. The stretching frequencies at 3008−3088 cm^{-1} and 1420−1600 cm^{-1} are assigned to aromatic C—H and C═C bonds in-plane vibrations, respectively.

The structure of [Cu(PDP_H)Cl] was studied by X-ray single crystal diffraction method. The copper(II) complex was crystallized in the orthorhombic space group *Pccn*. As shown in Fig. 1, [Cu(PDP_H)Cl] is mononuclear. Its structure is well analogous with that of known bispyridylpyrrolide metal complexes, such as [M(PDP_H)Cl] (M=Pd, Pt) [34]. Cu atom is four-coordination in the square planar geometry. Three interplanar angles among two side rings and the pyrrole ring are close to zero (in the range of 2.2°−9.5°). The total bond angel around Cu is 359.42°, which is close approximately to 360°, indicating a planar structure. The copper atom is out of the plane by 0.01044 nm. Cu—N (pyridine) distances (0.2141(3) or 0.2148(3) nm) is shorter than that of Cu—N (pyrrole) (0.1862(3) nm), indicating that pyrrole N atom behaves as π-acceptor responding to the metal site π-bonding properties. Cu—Cl bond distance is 0.2222(11) nm. Figure 2 shows the packing plot of [Cu(PDP_H)Cl]. It is clearly displayed that choloride atoms act as a bridging ligand to link the neighbouring copper atom with Cu1C−Cl1A distance of 0.2841 nm. If this longer Cu—Cl distance is not ingored,

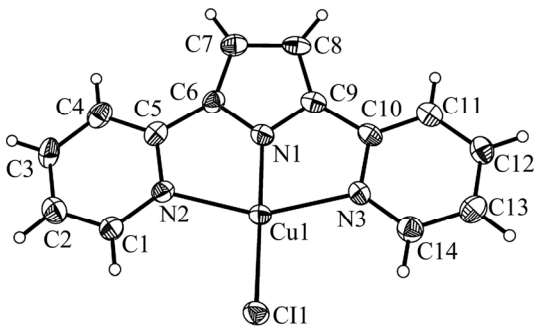

Fig. 1 Molecular structure of [Cu(PDP_H)Cl] (Probability of ellipsoids of 50% is shown)

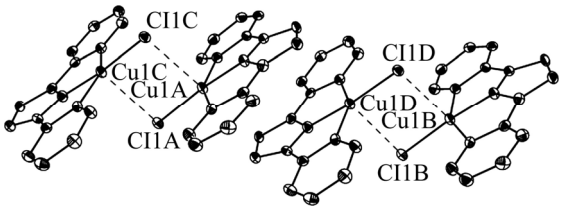

Fig. 2 Packing plot of [Cu(PDP$_H$)Cl] (Probability of ellipsoids of 50% is shown. Hydrogen atoms are omitted for clarity)

structure of [Cu(PDP$_H$)Cl] could be modeled as dimer. Weak intermolecular face-to-face π−π stacking is found within the pyridine rings in the dimer. Its centroid-centroid distance is 0.3824 nm, which is well comparable with the reported bispyridylpyrrolide metal complexes [33].

3.2 DNA binding studies

The interactions between [Cu(PDP$_H$)Cl] and CT-DNA can be monitored by electronic absorption spectra, CD spectra and fluorescence spectra. The absorption spectra of the copper(II) complex in the absence and presence of CT-DNA are shown in Fig. 3. Addition of increasing amounts of CT-DNA results in moderate hypochromism (15.6% at 400 nm) but no apparent bathochromism. These observations indicate that there are interactions between DNA and the copper(II) complex via a non-classical intercalation, such as groove binding since complexes binding to DNA through classical intercalation usually result in distinct hypochromism and some bathochromism [35−36]. The intrinsic binding constant (k_b) for the copper(II) complex has been calculated from Eq. (1) is 2.26×10^5 L/mol, which is using the absorption at 400 nm. The k_b value obtained here is lower than that observed for typical intercalators [37]. These results suggest that the copper(II) complex has a weaker binding of DNA than the classical intercalator and it is likely that the complex binds to CT-DNA via groove binding. This binding mode has also been found in the interactions of some Cu-terpy systems with DNA [25−26].

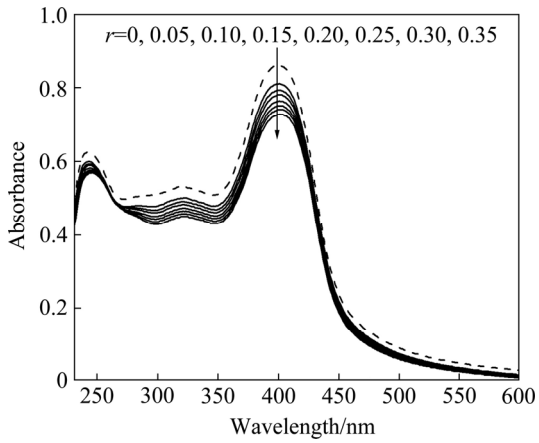

Fig. 3 Electronic absorption spectra of 3.0×10^{-5} mol/L [Cu(PDP$_H$)Cl] in the absence (dashed line) and presence (solid line) of increasing amounts of CT-DNA at the ratio r=0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35

The conformational changes of DNA induced by [Cu(PDP$_H$)Cl] was investigated by CD spectroscopy. The CD spectra of CT-DNA in the absence and presence of the copper(II) complex are shown in Fig. 4. The positive band at 275 nm is due to base stacking and the negative band at 245 nm is due to helicity, which is characteristic of B-DNA [38]. Upon addition of the copper(II) complex to the solution of CT-DNA, there is a decrease in the intensity of the positive band while the negative band is relatively less perturbed. The observed results indicate a non-intercalative binding mode and support the groove-binding nature of the copper (II) complex [39−40].

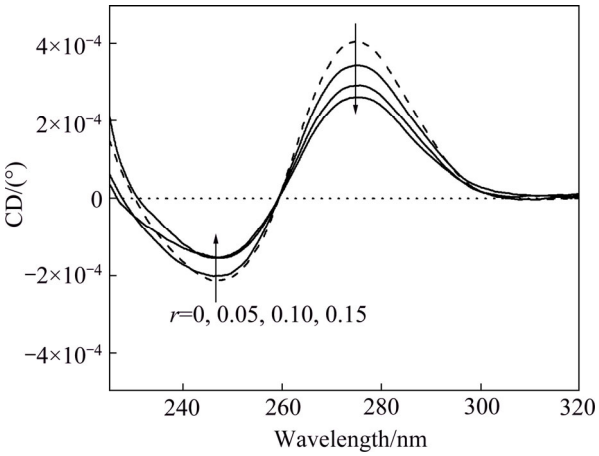

Fig. 4 CD spectra of 1.0×10^{-4} mol/L CT-DNA in the absence (dashed line) and presence (solid line) of increasing amounts of [Cu(PDP$_H$)Cl] at ratio r = 0, 0.05, 0.10, 0.15

The DNA binding behavior of [Cu(PDP$_H$)Cl] was further investigated using the EB-DNA system. EB is one of the most sensitive fluorescent probes that can bind with DNA. It is weakly fluorescent due to the quenching by the solvent molecules and its fluorescence intensity is greatly enhanced when inserting into the base pair of DNA, but the increased fluorescence intensity can be quenched or partly quenched by addition of a second complex that can replace the EB or break the secondary structure of DNA [41]. In this work, the emission spectra of EB bound to DNA in the absence and presence of [Cu(PDP$_H$)Cl] are shown in Fig. 5. The fluorescence intensity of CT-DNA-EB system at 620 nm shows a decreasing trend with the increase of complex, indicating that the complex can replace EB from the DNA. The results suggest that groove binding interactions would occur between the copper complex and DNA [42−43]. The electronic absorption spectra and CD spectral analysis also exclude intercalative binding mode.

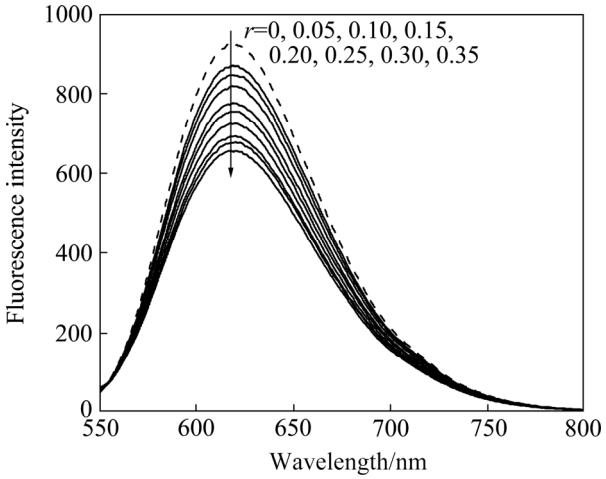

Fig. 5 Fluorescence emission spectra (excited at 530 nm) of CT-DNA-EB system (1.0×10^{-5} mol/L EB, 1.0×10^{-4} mol/L CT-DNA) in the absence (dashed line) and presence (solid line) of 6.2×10^{-4} mol/L [Cu(PDP$_H$)Cl] (10 μL per scan).

3.3 DNA cleavage activity

The DNA cleavage activity of [Cu(PDP$_H$)Cl] was studied using supercoiled pBR322 DNA by agarose gel electrophoresis. Figure 6 shows the results of DNA cleavage at different concentrations of the complex for 60 min reaction time (pH 7.4, 310 K). Control experiments using only H_2A or [Cu(PDP$_H$)Cl] did not show apparent cleavage of DNA (Lanes 2 and 3). However, the complex showed efficient DNA cleavage activity in the presence of H_2A. With the increasing of complex concentrations, the supercoiled DNA (Form I) decreased rapidly and was finally converted completely to nicked (Form II) and linear DNA (Form III). The

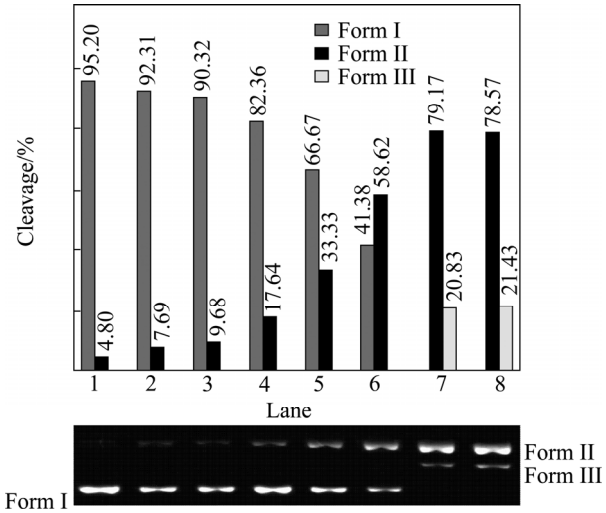

Fig. 6 Agarose gel electrophoresis patterns for cleavage of supercoiled pBR322 DNA (0.2 μg) by [Cu(PDP$_H$)Cl] in the presence of H_2A (Lane 1−DNA control; Lane 2−DNA + H_2A; Lane 3−DNA + complex (26 μmol/L); Lanes 4−8−DNA cleavage status at 8, 12, 18, 26, and 34 μmol/L of [Cu(PDP$_H$)Cl] in the presence of 50-fold excess of H_2A, respectively)

cleavage efficiency of the complex reached 100% at a concentration of 26 μmol/L (Lane 7) in converting Form I to Form II and Form III, and the amount of From III reached 20.83%. This revealed that [Cu(PDP$_H$)Cl] was a potent DNA cleavage agent in the presence of H_2A as a reductant under the experimental conditions, and the cleavage reaction mediated by the complex may be oxidative.

In order to identify the preliminary mechanism of DNA cleavage by [Cu(PDP$_H$)Cl], a new electrophoretic study was carried out with inhibiting reagents such as hydroxyl radical scavenger (DMSO) and singlet oxygen scavenger (tryptohan and histidine), and the results are shown in Fig. 7. It is evident that DMSO diminished the DNA cleavage activity significantly (Lane 8), but tryptohan and histidine (Lanes 6 and 7) have no inhibition effect. These results suggested that hydroxyl radical may be reactive species involved in the DNA cleavage process and non-involvement of singlet oxygen in the cleavage reactions. [Cu(PDP$_H$)Cl] may react with DNA to form Cu(II)-DNA species at first, and then was reduced to Cu(I)-DNA intermediate by reductant (H_2A) with the generation of hydroxyl radicals. The hydroxyl radicals attack DNA and thus lead to DNA strand scission.

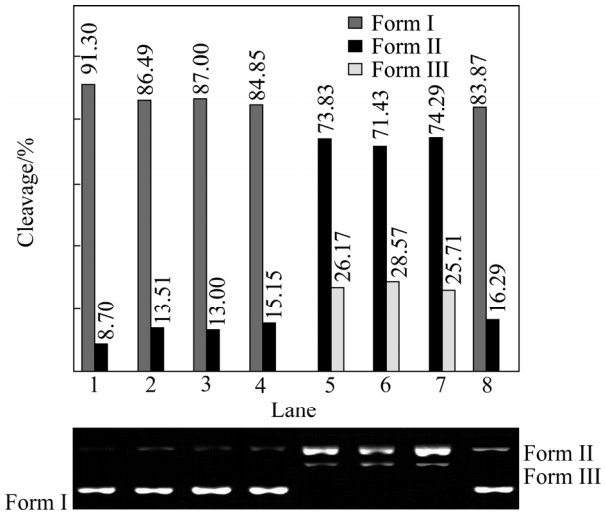

Fig. 7 Agarose gel electrophoresis patterns for cleavage of supercoiled pBR322 DNA (0.2 μg) by [Cu(PDP$_H$)Cl] in the presence of H_2A and inhibiting reagents (Lane 1−DNA control; Lane 2−DNA + DMSO; Lane 3−DNA + histidine; Lane 4−DNA + tryptohan; Lane 5−DNA + H_2A + complex (26 μmol/L); Lane 6−DNA + H_2A + complex (26 μmol/L)+ tryptohan; Lane 7−DNA + H_2A + complex (26 μmol/L) + histidine; Lane 8−DNA+ H_2A + complex (26 μmol/L) + DMSO)

4 Conclusions

Copper(II) complex of 2,5-bis(2′-pyridyl) pyrrole,

[Cu(PDP_H)Cl], has been synthesized and characterized. This copper(II) complex displays DNA groove binding and shows efficient oxidative DNA cleavage activity with hydroxyl radicals as the reactive species in the DNA cleavage process, which suggests that this copper(II) complex might be a choice as potent artificial nuclease.

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(Edited by YANG Hua)