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Interaction of DNA with a novel photoactive platinum diimine complex

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Abstract Interaction of DNA with a novel photoactive platinum diimine compound has been studied by electronic absorption spectra, fluorescence spectra and viscosity measurements. The red light-induced DNA cleavage activity of the platinum compound has also been studied by agarose gel electrophoresis. The results suggest that the platinum compound may interact with DNA by intercalation mode. When irradiated with red light, the platinum compound can generate singlet oxygen, resulting in cleavage of DNA.

Keywords Platinum diimine complex · Photoactive · DNA binding

Introduction

The photodynamic therapy (PDT) of cancer is a fascinating method for cancer diagnosis and treatment of various types of tumors by the combined action of oxygen, light, and a sensitizer (Levy and Obochi 1996). PDT is already used in clinical applications, but is still the subject of intensive investigations in order to improve the efficiency and diminish its side effects.

The first photosensitizer in clinical PDT use, Photofin®, is a mixture of haematoporphyrin derivatives (Bonnett 1995) and most studies of singlet oxygen photosensitization involve organic molecules such as porphyrins, chlorins, bacteriochlorins, phthalocyanines, naphthalocyanines, and texaphyrins (Szacilowski et al. 2005). However, some inorganic complexes, particularly some platinum complexes, have also been shown to be efficient photosensitizers.

For example, some platinum diimine dithiolate complexes, such as Pt(bpy)(tdt), Pt(bpy)(bdt) (where bpy = 2,2′-pyridyl, bdt = 1,2-benzenedithiolate, tdt = 3,4-tolulenedithiolate), have been shown to produce singlet oxygen with moderate efficiency (Tiyabhorn and Zahir 1996; Connick and Gray 1997; Cocker and Bachman 2001; Puthraya and Srivastava 1985). Weinstein et al. (2006) have synthesized a series of red-luminescent diimine or cylometalated platinum(II) complexes, some of which can generate singlet oxygen efficiently (Adams et al. 2006; Shavaleev et al. 2006). Lu et al. (2005) found that some photoexcited platinum diimine intercalators, upon intercalation into the DNA π stack, promote reductive and oxidative damage within the DNA duplex. These findings suggest that platinum(II) diimine complexes may be promising candidates as potential photodynamic therapeutic agents.

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Bis(*o*-semiquinonediimine)platinum(II) as a photosensitizer has drawn much attention due to its unique properties (Balch and Holm 1966; Konno and Matsushita 2006; Vogler and Kunkely 1980). However, the compound has poor solubility in water. In order to improve its solubility in water, we introduced a carboxylate group on the ligand of the compound. In this paper, we report the novel platinum diimine complex, which may interact with DNA by intercalation mode and generate singlet oxygen when irradiated with red light.

Experimental

Materials

Ethidium bromide (EB) was purchased from Fluka Co., calf thymus DNA and pBR322 DNA were purchased from Sino-American Biotechnology Co. in China. *O*-phenylenediamine was commercially available from Beijing Chemical Reagents Company. Dichloro(3,4-diaminobenzoic acid)platinum(II) was prepared according to a reported method (Howell and Walles 1988). All the solvents used were analytical reagents.

Methods and instrumentation

The solution of calf thymus DNA was made by dissolving DNA in 50 mM aq. NaCl. Its purity was checked by UV spectroscopy to ensure that the ratio of A_{260}–A_{280} is 1.8–2.0, indicating that DNA was sufficiently free of protein (Marmur 1961). DNA concentration per nucleotide was determined by $\varepsilon_{260} = 6{,}600\ M^{-1}\ cm^{-1}$ (Barton et al. 1986).

All the experiments involving calf thymus DNA were carried out at pH 7.26 employing buffer (10 mM Tris, pH 7.26, 50 mM NaCl, 1% DMF).

^{1}H NMR spectra were recorded on a Bruker DRX300 NMR spectrometer. Electronic absorption spectra were recorded on a Hewlett Packard HP-8453 spectrophotometer. Fluorescence spectra were recorded on a Perkin–Elmer fluorescence spectrophotometer. The sample was excited at 520 nm and its emission spectra were scanned in the range 540–700 nm. Viscosity experiments were performed at 20 (±0.1)°C using a Ubbelodhe-type viscometer.

Synthesis of Complex **I**

To a 0.5 mM aqueous NaOH solution of dichloro(3,4-diaminobenzoic acid)platinum(II) (0.1 mM) was added an aqueous solution of *O*-phenylenediamine (0.1 mM) and the reaction mixture stirred in darkness for about 48 h. The resulting blue precipitate was filtered and washed successively with water, acetone, and diethyl ether, then further purified by Soxhlet extraction in a 5:1 acetone:methanol mixture, affording the blue solid which was collected and dried in the desiccator (Complex **I**). Elemental analysis of **I**: Calc. C 32.98%, H 2.33%, N 11.84% for $PtC_{13}H_{11}N_4O_2Na$; Found: C 32.69%, H 2.88%, N 11.26%. ^{1}H NMR (300 MHz, DMSO-d_6, 25°C) of **I**: $\delta = 7.75$ (doublet), 7.20 (multiplet), 6.71 (broad). IR of **I** (cm^{-1}): 3175 w (ν_{N-H}), 1699 s ($\nu_{C=O}$), 1348 s (ν_{Ar-N}), 1225 s (ν_{C-O}), 789 s (ν_{N-H}). Electronic absorption spectra of **I** in DMSO: $\lambda_{max} = 723$ nm, $\varepsilon_{max} = 87444\ M^{-1}\ cm^{-1}$.

Electronic absorption spectra studies

The Complex **I** (2.48×10^{-5} M) and calf thymus DNA were mixed together in different molar ratios (C_{DNA}:$C_{Complex} = 0$, 0.8, 1.6, 3.2, 4.8, 6.4, 8), then stood in darkness for 30 min at room temperature. Afterwards, electronic absorption spectra were recorded for each sample.

Inhibition effect of Complex **I** on DNA-associated EB fluorescence enhancement

Different concentrations of Complex **I** ($C_{Complex}$: $C_{DNA} = 0$, 0.08, 0.13, 0.18, 0.23, 0.33, 0.41, 0.50, 0.66, 0.83, 1.0, 2.0) were added into DNA-EB mixtures ($C_{DNA} = 1.00 \times 10^{-4}$ M, $C_{EB} = 6.35 \times 10^{-5}$ M), respectively. After the reaction mixtures were stood in darkness for 30 min at 25°C, the fluorescence spectra were recorded at 25°C.

Measurement of fluorescence Scatchard plots

Calf thymus DNA (1.27×10^{-5} M) was mixed with Complex **I** in different molar ratios ($C_{Complex}$: $C_{DNA} = 0, 0.25, 1.0$). After the reaction mixtures were stood in darkness for 30 min, spectrophotometric titrations were performed by stepwise addition of EB

(2.54×10^{-4} M) to the above reaction mixtures and the fluorescence intensities were measured at 25°C.

Viscosity measurements

Calf thymus DNA (1.00×10^{-3} M) was mixed with different concentrations of Complex **I**. After the reaction mixtures were stood in darkness for 30 min, the flow times of the reaction mixtures were recorded at 20 (±0.1)°C, respectively. Relative viscosities for DNA in the presence and absence of Complex **I** were calculated accordingly.

The red light-induced DNA cleavage activity by Complex I

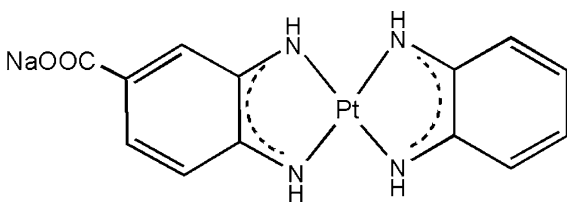
The extent of pBR322 DNA cleavage was monitored by agarose gel electrophoresis. DNA samples were treated with Complex **I** and irradiated with red light ($\lambda > 630$ nm) for a period of time, then electrophoresed for 2 h at 90 V on 0.8% agarose gel in TAE buffer. After that, the gel was stained using 1 mg ml^{-1} EB and photographed under ultraviolet light. All the experiments were performed at room temperature unless otherwise noted.

Results and discussion

Synthesis of Complex I

^{1}H NMR spectra and IR spectra of Complex **I** are all in good agreement with the structure of Complex **I** as shown in Scheme 1.

Compared to its analog bis(*o*-semiquinonediimine)platinum(II) ($\lambda_{max} = 710$ nm, $\varepsilon_{max} = 96{,}700$ M^{-1} cm^{-1} in DMSO) (Balch and Holm 1966), Complex **I** has also strong absorption band in the red light region.



Scheme 1 Complex **I**

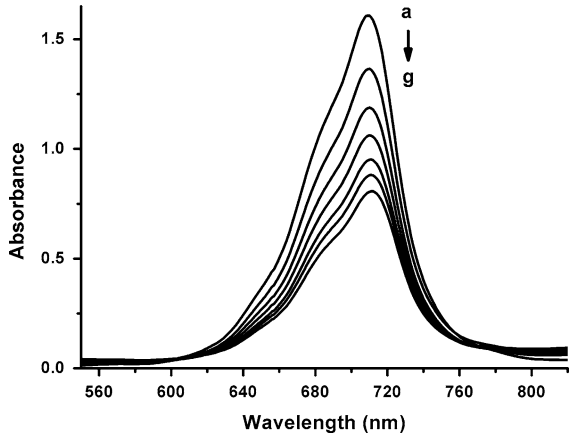


Fig. 1 Absorption spectra of Complex **I** at pH 7.26 employing buffer (10 mM Tris, pH 7.26, 50 mM NaCl, 1% DMF) in the presence of different concentrations of Calf Thymus DNA. The ratio of DNA to Complex **I** increases in the order of 0, 0.8, 1.6, 3.2, 4.8, 6.4, 8 for curves a–g, respectively

Electronic absorption spectra studies

As shown in Fig. 1, Complex **I** has a strong absorption band at around 710 nm. With the increase of DNA amounts, the band shows hypochromism and a small red shift of about 1 nm.

Interaction between an intercalator and DNA bases commonly result in hypochromism and a red shift of the transition of the intercalated chromophore (Long and Barton 1990). Interaction between Complex **I** and DNA also results in hypochromism and a small red shift. Thus, the platinum(II) diimine complex might bind to DNA by an intercalation mechanism.

Inhibition effect of Complex I on DNA-associated EB fluorescence enhancement

As shown in Fig. 2, the fluorescence intensity of DNA-EB adduct decreases dramatically with the increase of amount of Complex **I**, suggesting that EB was driven out of DNA base pairs in the presence of Complex **I**, and Complex **I** might bind to DNA by an intercalation mode.

Scatchard plots of fluorescence data

The fluorescence data were processed by Scatchard plots as described in the literature (Lepecq and Paoletti 1967). The binding of EB to DNA is expressed using the equation, $r/C = K(n - r)$, where

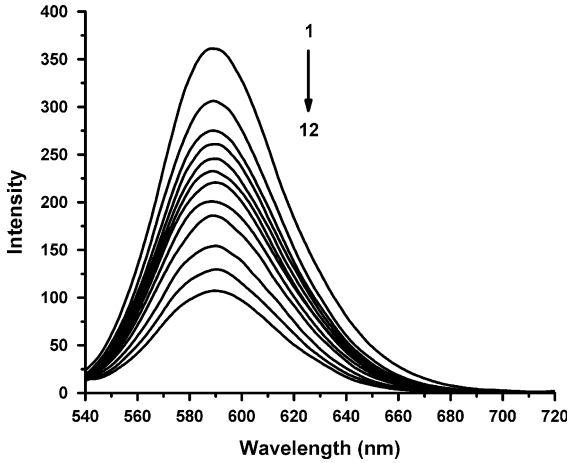


Fig. 2 Inhibition of Complex **I** on DNA-associated EB fluorescence enhancement. The ratio of Complex I to DNA increases in the order of 0, 0.08, 0.13, 0.18, 0.23, 0.33, 0.41, 0.50, 0.66, 0.83, 1.0, 2.0 for curves 1–12, respectively

r is the number of mole of EB bound to one mole of DNA phosphate, *n* the number of binding sites per DNA phosphate, *K* the intrinsic association constant to a site, and *C* the free EB concentration. Using data of fluorescence intensity to determine *r*, binding isotherms were obtained and the corresponding Scatchard plots were constructed.

As shown in Fig. 3, inhibition of Complex **I** on DNA-EB binding produces a Scatchard plot in which the intercept on the abscissa (*n*) keeps constant while the slope (*K*) decreases in the presence of increasing

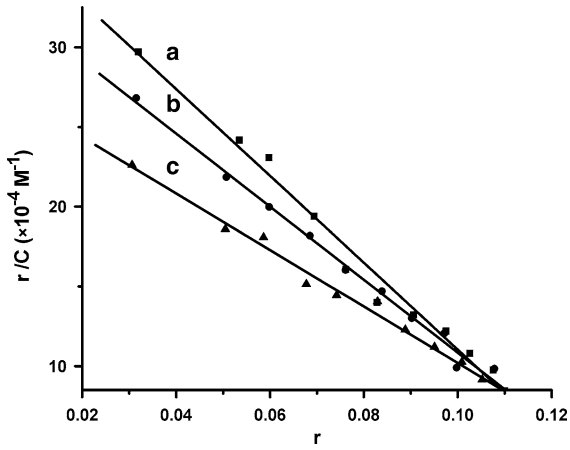


Fig. 3 Fluorescence Scatchard plots of the binding of EB to DNA in the absence and the presence of Complex **I**. The ratio of Complex **I** to DNA increases in the order of 0, 0.25, 1.0 for lines a–c, respectively

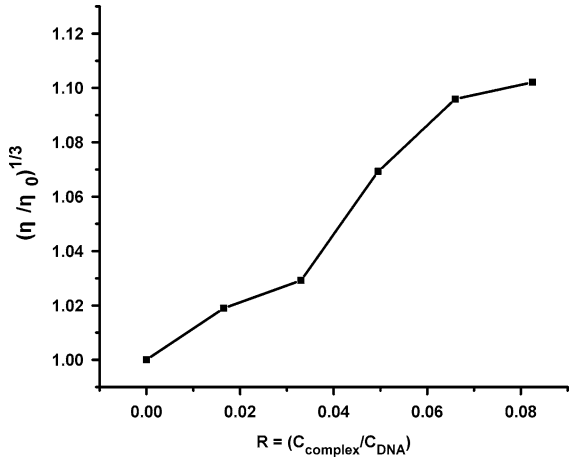


Fig. 4 Effect of increasing amounts of Complex **I** on the relative specific viscosity of Calf Thymus DNA

amounts of Complex **I**. The result suggests that Complex **I** and EB have same binding sites on DNA (Lepecq and Paoletti 1967), and Complex **I** might bind to DNA by intercalation, which is in good agreement with the studies by electronic absorption spectra.

Viscosity measurements

In order to compare with other data previously reported (Satyanarayana et al. 1992), viscosity is presented as $(\eta/\eta_0)^{1/3}$(Fig. 4).

Viscosity changes are sensitive to length increases and hydrodynamic measurements are regarded as an essential method to support an intercalation model in the absence of crystallographic structural data or NMR data (Satyanarayana et al. 1992). The intercalator ethidium was found to increase the relative specific viscosity of DNA due to the lengthening of DNA double helix resulting from intercalation; the groove-binding antibiotic Hoechst 33258 does not appreciably alter DNA viscosity; whereas Δ-tris(phenanthroline)ruthenium(II) decreases the relative specific viscosity of DNA, and the behavior was explained by a binding mode that produces bends or kinks in DNA helix (Satyanarayana et al. 1992).

Figure 4 shows that Complex **I** increases the relative specific viscosity of DNA, and it might be due to the lengthening of DNA double helix resultant from intercalation as well. The conclusion is in agreement with above-mentioned other studies.

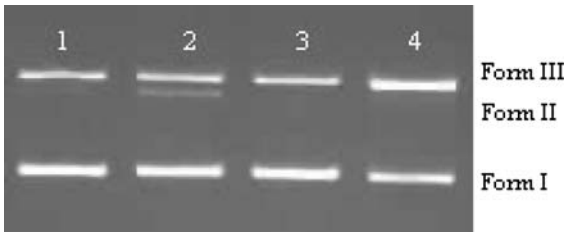


Fig. 5 Photoactivated cleavage of pBR322 DNA (0.01 μg/μl) by Complex **I** (0.50 mM) under different conditions when irradiated with red light for 2.5 h. Lane 1: DNA alone; Lane 2: DNA + Complex **I**; Lane 3: DNA + Complex **I** + histidine; Lane 4: DNA + Complex **I** + D_2O

The red light-induced DNA cleavage activity by Complex **I**

As shown in Fig. 5, when irradiated with red light, pBR322 DNA was cleaved by Complex **I**. As Complex **I** did not cleave pBR322 DNA in the control experiment without irradiation, the results suggest that the cleavage of pBR322 DNA by Complex **I** is induced by red light. The extent of pBR322 DNA cleavage by Complex **I** is enhanced in the presence of D_2O, indicating that the cleavage of DNA might be due to the generation of singlet oxygen which is produced by the platinum complex under red light since singlet oxygen has a longer lifetime in D_2O than in H_2O (Rodgers and Snowden 1982). On the contrary, the cleavage of DNA by Complex **I** when irradiated with red light is inhibited by the singlet oxygen quencher histidine. The result is in good agreement with the above-mentioned assumption that the cleavage of DNA is attributed to the production of singlet oxygen.

Conclusions

Oxygen in cells is essential for the treatment of cancers by photodynamic therapy, but there is much evidence available that solid tumors possess significant numbers of hypoxic cells (Palmer et al. 1990), and therefore these cells might limit the clinical efficacy of photodynamic therapy. Our studies reveal that the novel platinum diimine complex has a strong absorption band within the phototherapeutic window (620–850 nm). The novel platinum diimine complex can not only generate singlet oxygen when irradiated with red light, but can interact with DNA by intercalation without irradiation as well. Thus, the platinum complex might have higher selectivity against tumor cells, and might be a promising candidate for potential photodynamic therapeutic use. Further studies need to be carried out.

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