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Synthesis, DNA-binding, DNA-photocleavage and antioxidant activity of ruthenium(II) complex containing triazine ring ligand: $[Ru(dmb)_2(pdta)](ClO_4)_2$

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ABSTRACT

A new Ru(II) complex $[Ru(dmb)_2(pdta)](ClO_4)_2$ (**1**) (dmb = 4,4′-dimethyl-2,2′-bipyridine, pdta = 3-(pyridine-2-yl)-as-triazino [5,6-f]-acenaphthylene) was synthesized and characterized by elemental analysis (EA), ES–MS, ^1H NMR, UV–Vis and CV. The DNA-binding properties of complex **1** were investigated by absorption titration, thermal denaturation, viscosity measurement and photoactivated cleavage. The DNA-binding constant was determined to be $2.37 \pm 0.14 \times 10^5\ M^{-1}$ ($s = 1.90 \pm 0.04$) by electronic absorption titration, indicating that complex **1** interacts with DNA through intercalative mode. The redox peak potentials of this complex in CV were rationally assigned with the frontier molecular orbital stereographs resulting from the density functional theory (DFT) calculation. The photoactivated cleavage and cleavage mechanism of plasmid pBR322 DNA in the presence of complex **1** and various inhibitors were studied. The experiment for detecting the formation of singlet oxygen photo-sensitized by complex **1** was carried out, and the antioxidant activity of the Ru-complex and the ligand pdta against hydroxyl radical was also investigated.

1. Introduction

Over the past decades, quite a large amount researches on the non-covalent interaction of transition metal coordination complex with DNA have been accumulated [1–3]. Ru(II) polypyridine chelates take very important position in such research field due to their abundant chemical, physical and optical spectroscopy properties [2,4–7]. More and more ruthenium complexes have been synthesized and explored for DNA structure probes [8–10], DNA cleavage mediators [11–13], footprinting and chemotherapeutic agents [14–17]. As well documented, the intercalation is popular in the DNA-binding modes [2,12,13,17]. In an attempt to obtain more insights on the DNA-binding properties of ruthenium complex, we selected the triazine ring containing compound pdta as DNA-intercalating ligand and dmb as ancillary one to construct the complex **1** (Scheme 1). The pdta has energetically low-lying LUMO steming from the triazine ring, as the case in structurally analogue compounds $[Ru(bpy)_2(pdta)](ClO_4)_2$ [18] and $[Co(bpy)_2(pdta)]^{3+}$[19], and this feature facilitates it to receive the electron charge from DNA base pairs, when it inserts into DNA's double-helix. On the other hand, the auxiliary ligand dmb is more hydrophobic than that of bpy for having two more methyls, being favorable to dispose along the major groove of DNA, as the main ligand of the complex intercalates to DNA. This can be easily understood from the hydrophilic coat/hydrophobic core structure of the DNA molecule [20]. This design idea may be offer us to obtain ruthenium complex with strong DNA affinity.

Considering the combination of the strong DNA-binding affinity and ubiquitous photo-chemical, photo-physical properties of the Ru(II) complex, we had investigated here the photo-induced cleavage and cleavage mechanism of supercoiled plasmid DNA (pBR322 DNA) in the presence of complex **1** and various inhibitors following the systematic studies of CT-DNA-binding properties using the spectroscopic methods, viscosity measurements and thermal denaturation. The DNA-binding and mediating cleavage behaviors were found to be similar with those of the analogues $[Ru(dmb)_2(ITAP)](ClO_4)_2$ (ITAP = satino [1,2-b]-1,4,8,9-tetraazatriphenylene) [21], and $[Co(bpy)_2(pdta)](ClO_4)_3$ (bpy = 2,2′-bipyridine) [19], and, as our expected, has a relative greater DNA-binding constant K_b ($2.37 \times 10^5\ M^{-1}$) than that for $[Ru(bpy)_2(pdta)](ClO_4)_2$ ($3.1 \times 10^4\ M^{-1}$) [18]. The cleavage mechanism experiments indicate that singlet oxygen ($^1O_2$) is likely to be the reactive species responsible for the cleavage reaction. In order to confirm this result, the photo-sensitized generation of $^1O_2$ in presence of complex **1** under the same irradiation conditions as in the cleavage experiment was studied with illumination methods. Meanwhile, we noted that a series of ruthenium coordination compounds with ligands 2-(3-aminophenyl)-imizado[4,5-f][1,10]-phenanthroline (maip) and 2-(4-aminophenyl)-imidazo[4,5-f][1,10]-phenanthroline (paip) had been found to have hydroxyl radical ($OH^{\cdot}$) scavenging ability [12,22]. In this paper, the experiment for exploring $OH^{\cdot}$-scavenging ability of

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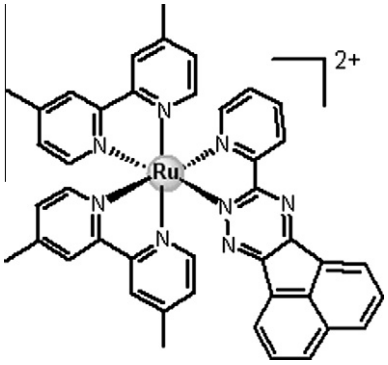

Scheme 1. Structural diagram of the complex cation $[Ru(dmb)_2(pdta)]^{2+}$.

pdta and $[Ru(dmb)_2(pdta)](ClO_4)_2$ was carried out, the result demonstrates that the complex **1** has more effective antioxidant against hydroxyl radical than the corresponding ligand. This may be offer us the alternative method to obtain the potential antioxidants, in hope of developing therapeutic reagents for some diseases as some antioxidants reported in literature [23].

2. Experimental

2.1. Materials

Calf thymus DNA (CT-DNA) was obtained from the Sino-American Biotechnology Company. pBR322 DNA was purchased from Shanghai Sangon Biological Engineering & Services Co., Ltd., 9,10-Dimethylanthracene (DMA) was from Aladdin Chemistry Co., Ltd. All the other reagents were commercially available and of analytical reagent grade. Doubly distilled water was used in all experiments. A solution of CT-DNA in the buffer (5 mM Tris(hydroxymethylaminomethane)–HCl, 50 mM NaCl, pH = 7.2) gave a ratio of UV absorbance at 260 and 280 nm of ca. 1.8–1.9: 1, indicating that the DNA was sufficiently free of protein [24]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [25].

2.2. Physical measurements

Microanalysis (C, H and N) was performed with a Perkin–Elmer 240Q elemental analyzer. Electronic spectra were recorded on a Shimadzu MPS-2000 spectrophotometer. ^{1}H NMR spectrum was measured on a Varian-500 NMR spectrometer with $(CD_3)_2SO$ as solvent at ambient temperature and TMS as the internal standard. Electrospray mass spectrum (ES–MS) was recorded on a LCQ system (Finnigan MAT, USA) using methanol as the mobile phase. Cyclic voltammetric experiment was carried out with a BAS 100-W instrument and standard three-electrode system.

2.3. Synthesis

The ligand pdta [26] and precursor cis-$[Ru(dmb)_2Cl_2]$ [27] were synthesized according to literature methods.

2.3.1. $[Ru(dmb)_2(pdta)](ClO_4)_2$ (**1**)

A mixture of $Ru(dmb)_2Cl_2{\cdot}2H_2O$ (0.144 g, 0.25 mmol) and pdta (0.071 g, 0.25 mmol) in ethanol–water (v/v, 3:1) 20 cm^3 was refluxed under argon for 8 h, while vigorous stirring was maintained. After being cooled to ambient temperature, the ethanol was removed under reduced pressure. A brown precipitate was obtained by addition of a saturated aqueous $NaClO_4$ solution, and purified on a neutral alumina column with acetonitrile–toluene (v/v, 2:1) as eluant. Yield: 72%. ^{1}H NMR [$(CD_3)_2SO$]: δ8.92 (d, 1H, J = 6.5), 8.77 (s, 1H), 8.73 (d, 1H, J = 7.5), 8.67 (s,1H), 8.64 (s,1H), 8.55 (d, 1H, J = 8.0), 8.48 (dd, 1H, J_1 = 7.5, J_2 = 8), 8.28 (m, 2H,), 8.11 (t, 1H), 8.04 (d, 1H, J = 5.5), 7.98 (m, 2H), 7.74 (m, 1H), 7.69 (d, 1H, J = 6), 7.64 (d, 1H, J = 5.5), 7.56 (d, 1H, J = 6), 7.46 (d, 1H, J = 5.5), 7.41 (d, 1H, J = 4.5), 7.37 (d, 1H, J = 5), 7.24 (d, 1H, J = 6.5), 2.61 (s, 3H), 2.57 (s, 3H), 2.51 (s, 3H), 2.47 (s, 3H). ES–MS (CH_3CN): m/z 851.00 ($[M\text{-}ClO_4]^+$) and 376.27 ($[M\text{-}2ClO_4]^{2+}$). Anal. calc. for $C_{42}H_{34}N_8Cl_2O_8Ru$: C 53.1, H 3.6, N 11.8%; Found: C 53.8, H 3.7, N 11.9%.

2.4. DNA-binding and photoactivated cleavage

Absorption titration of ruthenium(II) complex in Tris buffer was carried out by using a fixed Ru(II) concentration to which increments of the DNA stock solution were added. Ruthenium-solution employed was 20 μM and CT-DNA was added to a ratio of 8:1 of [DNA]:[Ru]. Ruthenium-DNA solutions were allowed to incubate for 5 min before the absorption spectra were recorded. The intrinsic binding constant K_b of Ru(II) complex to DNA was calculated based on Eq. (1) [28]:

$$(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f) = (b - (b^2 - 2K^2C_t[\mathrm{DNA}]/s)^{1/2})/2KC_t \quad (1a)$$

$$(b = 1 + KC_t + K[\mathrm{DNA}]/2s) \quad (1b)$$

where the apparent absorption coefficients ε_a, ε_f, and ε_b correspond to A_{obsd}/[Ru], the absorbance for the free ruthenium complex and the absorbance for the ruthenium complex in fully bound form, respectively. K_b is the equilibrium binding constant, C_t is the total metal complex concentration, and s is the binding site size (in base pairs).

Thermal denaturation experiment was performed with a Perkin–Elmer Lambda 35 spectrophotometer equipped with a Peltier temperature-controlling programmer (±0.1 °C). The melting curves were obtained by measuring the absorbance at 260 nm for solutions of CT-DNA (100 μM) in the absence and presence of the Ru(II) complex (10 μM) as a function of the temperature. The temperature of the solution was increased by 1 °C min^{-1} and ramped from 50 to 90 °C. The solutions were prepared with the buffer containing 15 mM trisodium citrate and 150 mM NaCl (pH = 7.0). The data were presented as $(A - A_0)/(A_f - A_0)$ versus temperature, where A, A_0, and A_f were the observed, the initial, and the final absorbance at 260 nm, respectively.

Viscosity measurements were performed with an Ubbelodhe viscometer maintained at 30.0 ± 0.1 °C in a thermostatic bath. DNA samples with an approximate average length of 200 base pairs were prepared by sonication in order to minimize complexities arising from DNA flexibility [29]. Flow time of each sample was measured three times, and an average value was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio ([Ru]/[DNA]), where η was the viscosity of DNA in the presence of complex and η_0 the viscosity of DNA alone [30].

For the gel electrophoresis experiment, supercoiled pBR322 DNA (0.1 μg) was treated with the Ru(II) complex solution in Tris–acetate buffer (50 mM Tris, 18 mM NaCl, pH = 7.2), then irradiated at room temperature with a UV lamp (365 nm, 10 W). Samples were analyzed by electrophoresis for 1 h at 100 V on a 0.8% agarose gel in Tris–acetate buffer. The gel was stained with 1 μg mL^{-1} ethidium bromide (EB) and photographed under UV light.

2.5. Detecting the formation of singlet oxygen photo-induced by complex **1**

A 3 ml N,N-dimethylformamide (DMF) solution containing 10 μM complex **1** and 100 μM DMA was prepared in a quartz