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Photo-induced DNA cleavage and cytotoxicity of a ruthenium(II) arene anticancer complex

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ABSTRACT

We report DNA cleavage by ruthenium(II) arene anticancer complex $[(\eta^6\text{-}p\text{-terp})Ru^{II}(en)Cl]^+$ (*p*-terp = para-terphenyl, en = 1,2-diaminoethane, complex **1**) after its photoactivation by UVA and visible light, and the toxic effects of photoactivated **1** in cancer cells. It was shown in our previous work (T. Bugarcic et al., J. Med. Chem. 51 (2008) 5310–5319) that this complex exhibits promising toxic effects in several human tumor cell lines and concomitantly its DNA binding mode involves combined intercalative and monofunctional (coordination) binding modes. We demonstrate in the present work that when photoactivated by UVA or visible light, **1** efficiently photocleaves DNA, also in hypoxic media. Studies of the mechanism underlying DNA cleavage by photoactivated **1** reveal that the photocleavage reaction does not involve generation of reactive oxygen species (ROS), although contribution of singlet oxygen ($^1O_2$) to the DNA photocleavage process cannot be entirely excluded. Notably, the mechanism of DNA photocleavage by **1** appears to involve a direct modification of mainly those guanine residues to which **1** is coordinatively bound. As some tumors are oxygen-deficient and cytotoxic effects of photoactivated ruthenium compounds containing $\{Ru(\eta^6\text{-arene})\}^{2+}$ do not require the presence of oxygen, this class of ruthenium complexes may be considered potential candidate agents for improved photodynamic anticancer chemotherapy.

1. Introduction

Metal complexes offer extensive potential in medicinal chemistry and drug design. Particularly, ruthenium complexes are attracting current interest as potential anticancer, antimetastatic, antibacterial and antifungal drugs [1–5]. During the last decade, organometallic ruthenium compounds containing $\{Ru(\eta^6\text{-arene})\}^{2+}$ have been extensively investigated, owing to their promising activity in vitro and in vivo toward cancer cells, including those resistant to conventional cisplatin [6–8]. These complexes appear to be novel anticancer agents with a mechanism of action different from that of platinum and other ruthenium complexes that have been so far tested for antitumor activity. Previously, the new complex $[(\eta^6\text{-}p\text{-terp})Ru^{II}(en)Cl]^+$ (*p*-terp = para-terphenyl, en = 1,2-diaminoethane) (complex **1**) (Scheme 1) was synthesized and investigated for its toxicity in tumor cells and DNA binding properties [7]. It was shown to exhibit promising toxic effects in several human tumor cell lines, including those resistant to conventional cisplatin. The results of the DNA binding study performed in cell-free media revealed that the DNA-binding mode involves combined intercalative and monofunctional (coordinative) binding [7]. This unusual DNA binding mode has been suggested to be an important factor contributing to the high biological potency of this complex, as it has been also shown for other monofunctional Ru^{II} complexes containing multi-ring arenes [9,10]. Moreover, there were significant differences in cellular responses to treatment with this complex compared to cisplatin, suggesting a different mode of action compared to platinum antitumor drugs in clinical use.

Interestingly, ruthenium complexes have been shown to be attractive also as potential photochemotherapeutic anticancer agents [6,11–15]. Effective photoactivation of ruthenium complexes by irradiation may increase toxicity selectively in cancer cells and avoid the damage of normal, noncancerous tissues due to unwanted side-effects. Therefore, it was of interest to examine whether the activity of **1** can also be affected by irradiation with light to the extent that its biological, including cytotoxic, properties are altered. We demonstrate here that **1** induces formation of single-strand breaks in double-helical DNA under irradiation by UVA or visible (VIS) light even in anaerobic conditions and concomitantly the irradiation of this Ru^{II} complex markedly enhances its toxicity in cancer cells.

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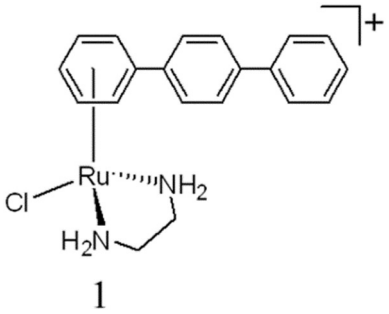

1

Scheme 1. Schematic representation of the structure of Ru^{II} arene complex [(η^6-*p*-terp)Ru(en)Cl]$^+$ (complex **1**) used in this work.

2. Experimental

2.1. Starting materials and reagents

The half-sandwich ruthenium arene complex **1** (Scheme 1) as a PF_6 salt was prepared as described previously [7]. A stock solution of the complex was prepared by dissolving the solid compound in water (Milli Q) to the final concentration of 5×10^{-4} M. The concentration was checked by flameless atomic absorption spectrometry (FAAS). Complex **1** exhibits absorption maximum at 285 nm and the absorption tail extends up to about 500 nm (Fig. 1).

Plasmids pUC19 [2686 base pairs (bp)] and pSP73 (2464 bp) were isolated according to standard procedures. *Nde*I and *Hind*III restriction endonucleases were purchased from New England Biolabs (Beverly, MA). [α-^{32}P]-dATP was obtained from MP Biomedicals, LLC (Irvine, CA). The Klenow fragment from DNA polymerase I (exonuclease minus, mutated to remove the 3′-5′ proofreading domain), KF^-, was purchased from New England Biolabs (Beverly, MA). Acrylamide and bis-acrylamide were obtained from Merck KGaA (Darmstadt, Germany), and agarose from FMC BioProducts (Rockland, ME, USA). The Wizard SV and PCR Clean-Up System used to extract and purify the 158 bp DNA fragment (vide infra) was purchased from Promega. Ethidium bromide (EtBr) and deuterium oxide (D_2O) were purchased from Merck KGaA. Superoxide dismutase from bovine erythrocytes (SOD), calf thymus (CT) DNA, mannitol, sodium azide, and dimethylsulfoxid (DMSO) were obtained from Sigma-Aldrich (Prague, Czech Republic).

2.2. Cell lines

The A2780 human ovarian carcinoma cell line and HaCaT human adult low calcium high temperature keratinocytes were kindly supplied by B. Keppler (University of Vienna, Austria) and A. Rajnochová Svobodová (Department of Medical Chemistry and Biochemistry, Faculty of Medicine, Palacky University), respectively. A2780 cells were grown in RPMI 1640 medium (GIBCO, Carlsbad, CA) supplemented with gentamycin (50 μg mL^{-1}; Serva, Heidelberg, Germany) and 10% heat inactivated fetal bovine serum (FBS) (GIBCO, Carlsbad, CA). HaCaT cells were maintained in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% FBS, 1% nonessential amino acids and gentamycin (50 μg mL^{-1}). The cells were cultured in a humidified atmosphere (5% CO_2) in incubator at 37 °C and subcultured 2–3 times a week at an appropriate plating density.

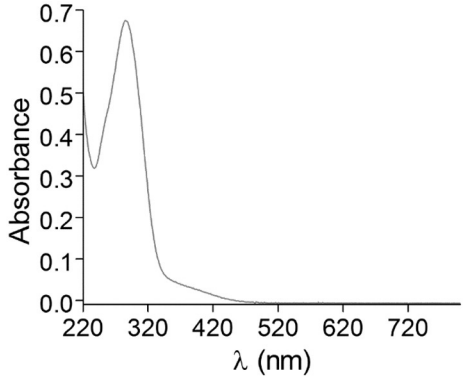

Fig. 1. The electronic absorption spectra of 3.5×10^{-5} M aqueous solution of complex **1**.

2.3. Photocleavage experiments

2.3.1. Instrumentation

The light source used in DNA photocleavage experiments was a Photoreactor LZC-ICH2 from Luzchem (Canada) fitted with UVA lamps (4.3 mW cm^{-2}, λ_{max} 365 nm) or with Vis lamps (cool white fluorescent tubes, 400–700 nm with a maximum of ca. 580 nm, 2.8 mW cm^{-2}). The temperature in the light chamber during irradiation was 37 °C.

2.3.2. Photocleavage of plasmid pUC19

Reaction mixtures containing plasmid DNA pUC19 and **1** in various molar ratios were incubated in 0.01 M $NaClO_4$ at 37 °C for 24 h in the dark to allow the ruthenium complex to bind to DNA [7]. After that, the samples were precipitated by ethanol to remove free, unbound ruthenium complex and dissolved in 0.01 M Tris·HCl, pH 7.4. Aliquots of these samples were used to determine r_b values (the number of molecules of the ruthenium complex bound per nucleotide residue) by FAAS. Samples were then irradiated by UVA or visible light for indicated time intervals. All the samples were then mixed with loading buffer (2.5% Ficoll®-400, 11 mM EDTA, 3.3 mM Tris · HCl, 0.015% bromophenol blue) and directly loaded onto a 1% agarose gel running at 25 °C in the dark with Tris-acetate-EDTA (TAE) buffer and the voltage set at 25 V. The gels were then stained with EtBr, followed by photography with a transilluminator. Intensity of fluorescence associated with bands was quantitated with the AIDA image analyzer software (Raytest, Germany).

2.3.3. Photocleavage of 158 bp DNA fragment

The 158 bp DNA fragment was prepared by digesting supercoiled pSP73 plasmid with *Nde*I restriction endonuclease and 3′-end-labeled by treatment with KF^- and [α-^{32}P]-dATP. After radioactive labeling, the linear DNA was digested with *Hind*III. The cleavage resulted in 158 and 2306 bp fragments. The 158 bp fragment was purified by electrophoresis on 1% agarose gel and isolated from the gel by Promega Wizard SV Gel cleanup system. The reaction mixtures were prepared with 150 mM CT DNA (0.048 mg mL^{-1}, 150 mM related to the phosphorus content) containing 3′-end-labeled restriction fragment modified by [(η^6-*p*-terp)Ru(en)Cl]$^+$ at $r_b = 0.05$ in 10 mM Tris·HCl (pH 7.4). The samples were irradiated by UVA or visible light for 30 or 120 min, respectively, and then analyzed on 13% polyacrylamide (PAA) gel under denaturing conditions.

2.4. Phototoxicity in cells

Human HaCaT (human adult low calcium high temperature) keratinocytes were maintained in DMEM with 10% FBS in the absence of antibiotics. A2780 human ovarian carcinoma cells were grown in RPMI supplemented with 10% FBS and gentamycin (50 μg mL^{-1}, Serva). Cells were grown in a humidified atmosphere of 5% CO_2/95% air at 37 °C. For analysis, cells were seeded into 96-well plates in 100 μL medium at a density of 10^4 cells/well and placed in the incubator for 24 h. For experiments, monolayers were washed in phosphate-buffered saline (PBS) and then incubated for 1 h at 37 °C in the Earle's Balanced Salt Solution (EBSS) containing the test compound. Test compounds were prepared in EBSS immediately before use and filtered. After this time, the cells were irradiated with UVA light (λ = 365 nm) or kept in the dark — HaCaT for 50 min irradiation (5 J cm^{-2}) and A2780 for 20 min irradiation (2.5 J cm^{-2}). Under these conditions, cell

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