

Research paper

Interaction with calf-thymus DNA and photoinduced cleavage of pBR322 by rhodium(III) and iridium(III) complexes containing crown thioether ligands

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ABSTRACT

In this report, we present our investigation on the photoinduced cleavage of plasmid pBR322 and the binding interactions with calf-thymus (CT) DNA by a series of thioether metal complexes. The complexes of interest are rhodium and iridium complexes containing thiacycrown ligands 1,4,7-trithiacyclononane (9S3) and 1-oxa-4,7-dithiacyclononane (9S2O), and the complexes are abbreviated as [Rh(9S3)Cl₃], [Rh(9S2O)Cl₃], and [Ir(9S3)Cl₃]. In the nicking assay, pBR322 was treated with each complex and irradiated at 254 and 350 nm, respectively, in concentration- and time-dependent studies. The nicking assay revealed that, under exposure to 254-nm radiation, [Rh(9S3)Cl₃] and [Rh(9S2O)Cl₃] cleaved pBR322 efficiently forming a nicked form, while [Ir(9S3)Cl₃] was least efficient. For the 350-nm irradiation, a similar trend was observed, with [Rh(9S3)Cl₃] being the most efficient one, however with a lower efficiency than at 254 nm. An ethidium bromide displacement assay was also carried out to evaluate the binding interaction of each compound with CT-DNA by titrating the pre-equilibrated complex of CT-DNA and EB with the investigated complexes. The efficient concentration to achieve a 50% loss in fluorescent emission was found to be 24 μM for [Rh(9S3)Cl₃] and 35 μM for [Rh(9S2O)Cl₃], while [Ir(9S3)Cl₃] was an ineffective DNA binder.

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1. Introduction

Cisplatin has been a popular anticancer drug and is commonly used for treating various types of cancers such as ovarian/cervical cancer, bladder cancer, and/or melanoma [1–3]. The mechanism of its action involves several steps such as cellular delivery of cisplatin, dissociation of Cl[−] inside the cells, binding of cisplatin to nitrogen (N-7 position) in guanines, formation of intra-strand cross-linkage to the target DNA, and apoptosis triggered by DNA damage [1,3,4]. In spite of its efficiency as an anticancer agent, the usage of cisplatin comes with issues such as potential deactivation in cells, cytotoxicity, and drug resistance [1–5]. In an effort to overcome drawbacks of cisplatin, there have been great efforts in finding new anticancer inorganic compounds. Several studies spearheaded by photo-activation approaches showed promising results, focusing mainly on Ru complexes and also including Fe, Cu, Rh, and Ir complexes [2,6–28]. The idea of utilizing light as on/off switch to activate a drug for targeting DNA in cancer cells

is attractive since it is possible to design a drug which can be activated only in the presence of light, while remaining inert or less active without irradiation. Furthermore, it is possible to tune the effectiveness of a photoactivated inorganic compound by controlling a time lapse for light exposure to the localized target tissue or by choosing an effective irradiating wavelength selectively.

Whether photoactivated or not, the mechanism of DNA modifications caused by inorganic compounds can be complex, and it involves binding interactions with DNA that can be covalent, non-covalent, tight binding (via intercalation or groove binding), loose binding in a nonspecific manner, oxygen-mediated, or oxygen-independent [2,23,28,29]. The most likely results of interactions between inorganic complexes and DNA are either adduct formation or DNA strand cleavage. The adduct formation with DNA was observed for platinum containing complexes such as cisplatin, carboplatin, and satraplatin [1,3,23,30], while the DNA cleavage was observed mostly in ruthenium complexes. The core structure of the Ru complexes is [Ru(bpy)_nL_{3-n}]²⁺, where bpy = 2,2'-bipyridine and L can be ferrocene/non-ferrocene conjugated imidazole phenol ligand [6], a series of *o*-, *m*-, or *p*-(nitrophenyl)imidazo[4,5-*f*] [1,10]phenanthroline [7], 1,12-diazaperylene (*i.e.*

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DAP) [2,8], dipyrro[3,2-*a*:2',3'-*c*]phenazine (i.e. dppz) [9,10], or 4,5,9,16-tetraaza-dibenzo[*a,c*]naphthacene (i.e. dppn) [11,12].

In addition to extensively studied Pt- and Ru-containing complexes, a number of studies were focused on the biological activity of complexes with other metal centers such as Fe [19,21], Cu [15,19,31], Rh [16,20], or Ir [17,18]. Rh(III) and Ir(III) complexes did not receive as much attention until recently since these complexes were perceived to be relatively less reactive due to their chemical stability and slow solvent exchange rate [18,32,33]. The stability of Rh(III) and Ir(III) are in part due to the low spin state, with a d^6 electron configuration, and an octahedral geometry [18,32,33]. Interestingly, some studies focus on activating Rh(III) and Ir(III) complexes by adopting suitable ligands upon irradiation. Examples are $[\text{Rh}(\text{bpy})_2\text{Cl}_2]^+$ [34], $[\text{Rh}(\text{phen})_2\text{Cl}_2]^+$ (phen, 1,10-phenanthroline) [33,34], $[\text{RhCl}(\text{bpy})9\text{S3}]^{2+}$ (9S3 = 1,4,7-trithiacyclononane) [35,36], $[\text{IrL}_n]^{3+}$ where L is a terpyridyl-like ligand [17], and $[(\eta^5\text{-Cp}^*)\text{Ir}(\text{phen})\text{Cl}]$ (Cp^* = tetramethyl(phenyl)cyclopentadiene) [18]. The presence of one or more chloride ions coordinated to Rh(III) or Ir(III) metal center appears to be essential in photoactivation of the complexes in these cases. Importantly, many of the complexes above were also shown to be active as DNA cleaving agents upon irradiation, and this finding is promising in utilizing phototherapy for selective and efficient cancer treatment.

Herein, we present the results of our investigation on the biological activities of three complexes $[\text{Rh}(9\text{S3})\text{Cl}_3]$ **1**, $[\text{Rh}(9\text{S2O})\text{Cl}_3]$ **2**, and $[\text{Ir}(9\text{S3})\text{Cl}_3]$ **3** toward DNA, for both plasmid and calf-thymus (CT) DNA. As shown in Fig. 1, each investigated complex has three chloride ions bound to a metal center, and a facially coordinating thiocrown ligand to complete the octahedral structure (Fig. 1). These compounds were chosen for this study since the compounds of interest have chloride ions as ligand, which was found to be critical for photoinduced reactivity for the known Rh(III) and Ir(III) complexes. Moreover, a similar complex, $[\text{Rh}(9\text{S2N})\text{Cl}_3]$ (9S2N = 1-aza-4,7-dithiacyclononane) was shown to be active against ovarian cancer [37]. We carried out a photonuclease activity assay by treating plasmid pBR322 with compounds **1**, **2**, and **3** in a concentration- and a time-dependent manner, upon irradiation at 254 and 350 nm, respectively. For evaluating the DNA binding properties of each complex, we conducted an ethidium bromide displacement assay (EBDA) by exciting the complex of $[\text{DNA} + \text{EB} \pm \text{1, 2, or 3}]$ at 520 nm, and monitored the fluorescence emission in the 540–800 nm range. Additionally, an electronic structure theory study of these compounds was carried out and is reported here. The findings in this study will advance the knowledge of the interaction between Rh(III)/Ir(III) complexes and DNA.

2. Experimental

2.1. General methodologies and instrumentation

All chemicals were purchased from Fisher Scientific and are of reagent grade unless specified otherwise. The water used in the study was deionized water (DI- H_2O) purified by a Millipore system (Milli-Q water). pBR322 was purchased from Fermentas (SD0041),

and CT-DNA was purchased from Rockland (MB-102-0100). Gels were imaged using a Spectroline UV Transilluminator equipped with a Fotodyne Foto Analyst Apprentice system and a Panasonic DMC-FX580 digital camera. Then, the stained gel images were submitted to quantitation using ImageJ Software to compare the percent of nicked plasmid vs. supercoiled plasmid. Fluorescence analysis was carried out using a Horiba Jobin Yvon Fluorolog-3 spectrophotometer equipped with polarization accessories and a full-spectrum xenon lamp. UV-Vis spectra were obtained using a Shimadzu Biospec-1601 spectrophotometer, and data collection was obtained using UV Probe 2.3 Software by Shimadzu.

2.2. Synthesis of $[\text{Rh}(9\text{S3})\text{Cl}_3]$, $[\text{Rh}(9\text{S2O})\text{Cl}_3]$, and $[\text{Ir}(9\text{S3})\text{Cl}_3]$

The syntheses of the three complexes were carried out by following the published procedures [38,39] with minor modifications. A representative description is as follows.

- Preparation of $[\text{Rh}(9\text{S3})\text{Cl}_3]$:** A mixture of $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$ (250.0 mg, 0.949 mmol) and 9S3 (171 mg, 0.949 mmol) were placed in a 100 mL round bottom flask. To this mixture was added 34.2 mL of EtOH. The solution was refluxed for 1.5 h while stirring. As the solution was cooled to room temperature, a clear supernatant formed with a solid yellow precipitate. The product is filtered, and the gooeey precipitate was washed with ethanol (3×15 mL) followed by ether (3×15 mL) to make the product dry and clean. The product of $[\text{Rh}(9\text{S3})\text{Cl}_3]$ weighed 351 mg with a 95.0% yield.
- Preparation of $[\text{Rh}(9\text{S2O})\text{Cl}_3]$:** A mixture of $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$ (35.0 mg, 0.210 mmol) and 9S2O (55.9 mg, 0.210 mmol) were placed in a 50 mL round bottom flask. To this mixture was added 10 mL of MeOH and 5 mL of DI H_2O . The solution was refluxed for 3 h. The reagent 9S2O is a liquid at room temperature, so it was pipetted into the flask. The maroon solution turned bright orange when heated and became cloudy. The orange suspension was then filtered to recover an insoluble orange solid weighed at 55 mg, with a 69.4% yield. A fraction of the product was submitted to solubility test in various solvents or solvent mixtures (DMSO, isopropanol, ethanol, methanol, chloroform, acetonitrile, water, trifluoroacetic acid); the mixture of DMSO and H_2O appeared to be the best system for dissolving $[\text{Rh}(9\text{S2O})\text{Cl}_3]$.
- Preparation of $[\text{Ir}(9\text{S3})\text{Cl}_3]$:** The preparation of $[\text{Ir}(9\text{S3})\text{Cl}_3]$ was carried out similarly to the procedure for $[\text{Rh}(9\text{S3})\text{Cl}_3]$ with $\text{IrCl}_3 \cdot 3\text{H}_2\text{O}$, instead of $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$, following the procedure described by Timonen and coworkers [38].

2.3. Preparation of CT-DNA

The CT-DNA (30 mg) was hydrolyzed in filtered phosphate buffer (3.0 mL, 50 mM phosphate, 50 mM NaCl, pH 7.0) overnight. Then, the hydrolyzed DNA solution was submitted to dialysis ($\times 3$) over 24 h in saline phosphate buffer (i.e. SPB, 50 mM phosphate, 50 mM NaCl, pH 7.0). The solution above was diluted with SPB for assays and the DNA concentration per nucleotide was determined by A_{260} obtained at UV-Vis spectroscopic scans using the molecular extinction coefficient value of $6600 \text{ M}^{-1} \text{ cm}^{-1}$ [40]. Then, aliquots were stored at -80°C and diluted freshly prior to each ethidium bromide displacement assay (EBDA).

2.4. Preparation of stock solutions of complexes

Stock solutions of 5.0 mM **1** and **2** were prepared by adding the weighed amount of the complex to DMSO. A stock solution of 2.5 mM **2** was prepared by adding the weighed amount of the complex to the mixture of 1:1 DMSO:DI H_2O by volume. The 5.0 mM solu-

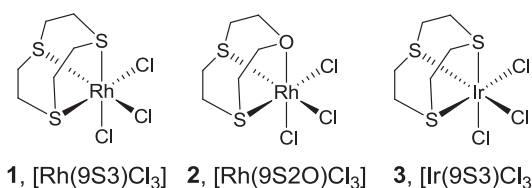


Fig. 1. Structures of compounds **1**–**3**.

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