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Increasing DNA reactivity and in vitro antitumor activity of *trans* diiodido Pt(II) complexes with UVA light☆

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ABSTRACT

Trans diiodido platinum(II) complexes bearing the same as well as different aliphatic amines (mixed-amines) have interesting biological activity; cytotoxicity and interactions with some important biological models have already been demonstrated. Herein we described the interaction of such compounds with ct-DNA, supercoiled and linearized plasmid DNA and 5-GMP. Interestingly, UV irradiation of these compounds results in an increase in reactivity towards DNA and 5-GMP in such model systems. Additionally, the cytotoxicity of the *trans*-Pt(II) complexes towards human cancer cells is noticeably increased when treatment is combined for 90 min with UVA-irradiation. With this work we provide evidence that *trans* diiodido compounds can be activated by UV-light over relatively short treatment times.

1. Introduction

The design of antitumor metallodrugs has been strongly guided by the structure of cisplatin for many years [1]. However, despite the clinical successes of this drug as well as the analogues carboplatin and oxaliplatin there are still many drawbacks to their use in anticancer treatment; such as adverse side-effects, the development of resistance to the therapy, and the relatively narrow spectrum of antitumor activity [2]. The discovery of the anti-tumor potential shown by non-conventional metallodrugs, for example *trans* Pt-complexes [3], has opened up new possibilities for metallodrug development.

There are multiple advantages of using transition metal-based complexes in treating cancer. For example, many key biological structures such as DNA form strong coordination complexes with transition metal ions, which can have an anti-tumor effect. Different metal ions adopt diverse geometries for a given ligand (Ru, Pt, Au, Os, Ir are examples), thus the electronic configuration of the metals can introduce a new shape in the complex. Metal ions also give the ligands more diverse reactivity, including photochemical reactions that promote unusual reactivity, for example activating platinum anticancer complexes with visible light [4].

An important strategy in this trend is to reevaluate accepted rules, based on chemical and biological features, by using novel techniques and new approaches that were not available at the time the original rules were formulated. A reevaluation of some complexes previously discarded as inactive could provide new insights into the design of novel drug candidates. For example, pioneering work with cisplatin derivatives appeared to rule out iodido as a leaving ligand in classical structure-antitumor activity relationships [5]. Our reevaluation of the diiodido Pt(II) complexes began when we identified unexpected in vitro cytotoxicity for the complex *cis*-Pt(isopropylamine)I_2, which also showed a particular strong reactivity versus S-donors such as cytochrome c and N-acetylmethionine [6]. The studies with the *cis* configured series bearing aliphatic amines showed much lower reactivity towards sulfur donors and a clear retention of the amine ligands in the overall adducts studied [7]. On the other hand, the *trans* series appeared more affected by the size of the amine ligand, showing particular differences in cytotoxicity in cancer cell lines when the size of the amine was varied [8].

The elucidation of the mechanism of action of these complexes, possible targets and specificity versus cancer cell lines is important for the reevaluation of these complexes as potential antitumor drugs. We are particularly interested in using irradiation with UVA light to increase the reactivity of these molecules towards the biological targets in order to create a more powerful and selective metallodrug. The use of UVA radiation in photoactivation of platinum drugs has been described previously for Pt^{IV} complexes to produce photoreduction of the Pt and activation of the prodrug compounds [9,10], and in Pt^{II} complexes to promote hydrolysis and increased reactivity towards model bases

☆ To Professor Giovanni Natile on the occasion of his 70th birthday.
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[11]. The UVA irradiation of diiodido Pt(IV) complexes has been previously studied as an activation process, but these were *cis* platinum complexes bearing stabilizing chelate ligands such as ethylenediamine [12, 13].

2. Results and discussion

The synthesis and characterization of **1**–**5** were performed according to our previous work [7,8] and the information can be found in the experimental section of those publications. The complexes studied in this work were selected according to their varying reactivity towards biological model nucleophiles: a) *cis*-Pt(ipa)$_2$I$_2$ (Fig. 1, compound **1**) showed adduct formation with S-containing model compounds with only Pt–I fragments where no trace of the spectator ligand was present. Moreover the reactivity of **1** towards DNA, observed in DNA model systems (5-GMP), supercoiled pBR322 and oligonucleotides, resulted in the same binding pattern as cisplatin [14,15]. b) The parent *trans* complex (Fig. 1, compound **2**) showed a very similar pattern to cisplatin and compound **1**, but compound **2** produces noticeable changes in the mobility of supercoiled DNA at lower concentrations than cisplatin. c) the *trans* complexes series (Fig. 1, compounds **3**, **4** and **5**) showed an apparent correlation between the produced cytotoxic effects and the modification of supercoiled DNA mobility. The reactivity towards sulfur donors was similarly low or even non-reactive in some of the cases (compound **4**). DNA was obviously a potential target in this particular case, and thus chosen for more detailed study.

2.1. DNA binding performed without UV irradiation

We first investigated in detail the interactions of the compounds with double stranded (ds) DNA by using various biochemical and biophysical methods. The nature of the interaction of these complexes with DNA was studied, followed by the evaluation of their binding kinetics to ds DNA by Atomic Absorption Spectroscopy (AAS). Finally, conformational distortions of DNA induced by binding to the Pt complexes were measured. The nature of this interaction has also been investigated in cell-free media and compared with previous results.

2.1.1. Transcription mapping of DNA adducts

In order to identify the preferential binding sites of the compounds, RNA synthesis on a DNA template modified by the platinum complexes **1**–**5** was performed. It has been demonstrated that in vitro RNA synthesis on DNA templates containing several types of adducts of platinum complexes can be prematurely terminated at the site of adduct formation or in close proximity to the Pt adducts [16,17]. The experiments were carried out by using linear DNA fragment containing a T7 RNA polymerase promoter, modified at an $r_b = 0.015$ by platinum compounds **1**–**5** and for comparative purposes also by cisplatin and transplatin. Fig. 2A shows that RNA synthesis on the template modified by the platinum complexes yielded fragments of defined sizes, which indicate that

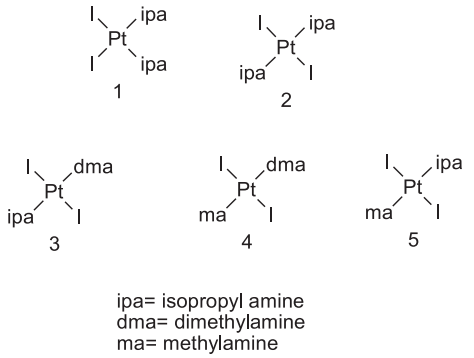

Fig. 1. Platinum complexes studied in this work.

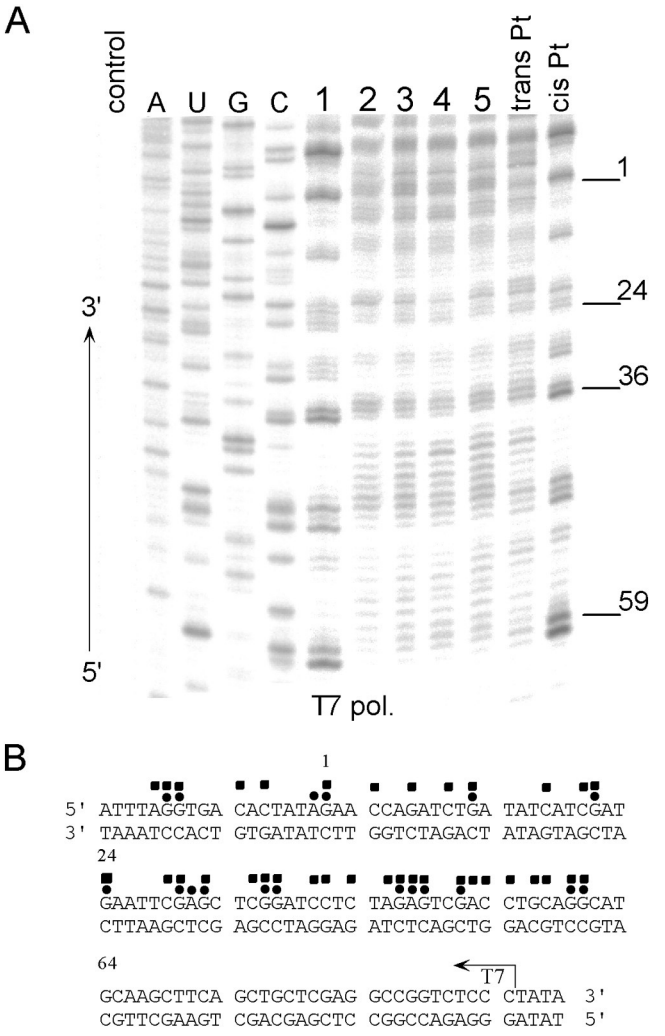

Fig. 2. 3-RNA synthesis by T7 RNA polymerase on the NdeI/HpaI fragment of pSP73KB plasmid modified by cisplatin, transplatin, **1**, **2**, **3**, **4** and **5**. A. Autoradiogram of 8% polyacrylamide/8 M urea sequencing gel. Lanes: control, non-platinated template: A, U, C, and G, chain terminated marker RNAs; **1**–**5**, transplatin, cisplatin, template modified by **1**, **2**, **3**, **4**, **5**, transplatin or cisplatin, respectively at $r_b = 0.015$. B. Sequence of the NdeI/HpaI fragment of the pSP73KB plasmid. The arrow indicates the start of the T7 RNA polymerase, which used the upper strand as template. Numbers correspond to nucleotide numbering in the sequence of the pSP73KB plasmid. Circles and squares indicate stop signals from panel A, lanes cisplatin and transplatin, respectively.

RNA synthesis on these templates was prematurely terminated. The sequence analysis revealed that the major bands resulting from termination of RNA synthesis by the adducts of the four *trans* compounds are similar to those produced by transplatin. Conversely, the bands generated by **1** are similar to those of cisplatin adducts (Fig. 2B). The results also indicate that the preferred binding sites for compounds **1**–**5** on DNA are guanine residues.

2.1.2. Interstrand cross-linking

The transcription mapping experiments (Fig. 3) suggest that the complexes **1**–**5** could form bidentate adducts on polymeric DNA, but whether these adducts were intrastrand or interstrand cross-links cannot be distinguished (Figure SM1a SM2). Therefore, we quantify the interstrand cross-linking efficiency of **1**–**5** in linearized pSP73KB plasmid (2455 bp), which was modified by Pt compounds after it had been linearized by PvuII, an enzyme that cuts only once within pSP73KB plasmid. The samples were analyzed for the interstrand cross-links by agarose gel electrophoresis under denaturing conditions (Figure SM1a and SM2). Under these conditions P^{32}-5′-end-labeled strands of linearized pSP73KB plasmid containing no interstrand cross-links migrated as a