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DNA as molecular target of analogous palladium and platinum anti-*Trypanosoma cruzi* compounds: A comparative study

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ABSTRACT

In the search for drugs with anti-trypanosome activity, we had previously synthesized two series of platinum and palladium analogous compounds of the formula $[M^{II}Cl_2(HL)]$, where HL were bioactive 5-nitrofuryl or 5-nitroacroleine thiosemicarbazone derivatives. In this work, we thoroughly characterized $[M^{II}Cl_2(HL)]$ complexes interaction with DNA by using different techniques: gel electrophoresis, DNA viscosity measurements, circular dichroism (CD) and atomic force microscopy (AFM). Electrophoresis results showed that all complexes induced a withdrawal of DNA superhelicity demonstrated by a decrease in electrophoretic mobility of supercoiled DNA form. This effect on migration was dependent on dose but also on the nature of both the metal and the ligand. In general, the effect produced by palladium complexes was significantly more intense than that observed for the corresponding platinum analogs. Differences between palladium and platinum complexes were also observed in CD experiments. While palladium complexes induce evident calf thymus (CT)-DNA profile changes compatible with B-DNA to Z-DNA conformational transition, no clear effect was observed for platinum ones. Additionally, AFM studies showed that changes in the shape of plasmid DNA, like supercoiling, kinks and thickness increase resulted more intense for the former. In addition, either Pd or Pt complexes increased the viscosity of CT DNA solutions in a concentration dependent manner. Although the nature of DNA interaction of both series of analogous palladium and platinum complexes seemed to be similar, an explanation for the observed differential intensity of the effect could be related to the known kinetic stability differences between palladium and platinum compounds.

1. Introduction

American Trypanosomiasis (Chagas disease) is produced by the protozoan parasite *Trypanosoma cruzi*. It is mainly transmitted to the mammalian host by reduviid bugs in a stercorarian mode. Although exhaustively described for the first time in 1909 by the Brazilian scientist Carlos Chagas, there are evidences demonstrating that this disease has been present in the American continent for more than 9000 years [1]. Nowadays it remains the major parasitic disease in the Americas, being endemic throughout Latin America [1–5]. Around 8–14 million people are currently infected and this disease causes more deaths per year in this region than any other parasitic disease. In addition, the premature disability and the effect of this disease on worker productivity lead to very significant annual losses of resources and industrial productivity. Furthermore, globalization and immigration of unknowingly infected people from Latin America has also led to several infection cases in developed countries, mainly due to lack of controls and screening in blood and organ banks and to immigrant mother to child transmission during pregnancy [6].

The chemotherapy of the disease relies on two quite unspecific nitroheterocyclic drugs, nifurtimox and benznidazole, that date back over 50 years and suffer of poor efficacy in the chronic phase of the disease, severe toxicity and increasing resistance development [3,6,7]. New drugs are urgently needed.

Currently, the development of bioactive metal complexes is a promising approach in the search for new potential drugs for the therapy of parasitic illnesses. Different attempts toward developing trypanocidal metal-based compounds have been described [2,4,5,8–10]. In particular, our group has been successfully working on the development of potential antitrypanosomal agents through different strategies [11–22]. One of these strategies involves the metal coordination of trypanocidal organic ligands. The obtained metal compounds

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could act through dual or even multiple mechanisms of action by combining the pharmacological properties of both the ligand and the metal, or could at least lead to additive effects. The development of agents that act against different parasitic targets could diminish host toxic effects by lowering therapeutic dose and/or could circumvent the development of drug resistance. Through this approach we have developed different series of bioactive metal compounds bearing antitrypanosomal activity. Among them we have exhaustively studied Pd(II), Pt(II), Ru(II) and Ru(III) coordination compounds of bioactive 5-nitrofuryl and 5-nitroacroleine containing thiosemicarbazones [11,12,15,17,18]. These ligands (Fig. 1a) had shown higher *in vitro* activity against *T. cruzi* than nifurtimox. Their main mode of action is related to the intracellular reduction of the nitro moiety followed by redox cycling, yielding reactive oxygen species (ROS) known to cause parasite damage [22]. All performed biological experiments strongly suggest that the main mechanism underlying the trypanocidal activity of their metal complexes is the production of oxidative stress as a result of their bioreduction and extensive redox cycling [11,12,15,17,18]. Many of the Pd and Pt compounds showed increased antitrypanosomal activity in respect to the free ligands and significant interaction with DNA, suggesting this biomolecule as a second molecular target. Trying to get further insight into the mechanism of action of these metal compounds and, particularly, to characterize their interaction with DNA, in this work we present a comparative study of the interaction with DNA of analogous $[MCl_2(HL)]$ compounds, where M = Pd(II) or Pt(II) and HL = 5-nitrofuryl or 5-nitroacroleine containing thiosemicarbazones, (Fig. 1b). DNA interaction has been thoroughly studied by using different techniques: gel electrophoresis, DNA viscosity measurements, circular dichroism (CD) and atomic force microscopy (AFM).

2. Experimental

2.1. Preparation of the thiosemicarbazone ligands and their metal complexes

All thiosemicarbazone ligands were synthesized and characterized using the previously reported methodology [23]. Palladium and platinum complexes of the formulae $[MCl_2(HL)]$ (Fig. 1b) were synthesized by ligand substitution on $Na_2[PdCl_4]$ or $K_2[PtCl_4]$, using a 1:1 metal to ligand molar ratio and were characterized as previously reported [12,15,17].

2.2. Electrophoresis approach

2.2.1. Preparation of plasmid DNA

Plasmid DNA (pBSK II BlueScript (Stratagene) 300 ng per reaction) was obtained and purified according to standard techniques [24]. Briefly, *Escherichia coli* XL1 cells were transformed with pBSK II. Transformation was verified by PCR (polymerase chain reaction) and plasmid DNA was purified (Qiagen Plasmid Maxi Kit). Spectrophotometric DNA quantification was carried out at 260 nm assuming an absorptivity at 260 nm of 0.02 mL/μg cm [25].

2.2.2. Analysis of DNA–metal complex interaction

1% DMSO had to be added to achieve dissolution of the Pd complexes. For the Pt compounds, being less soluble than the others, stock solutions were prepared in net DMSO. No effect on DNA due to DMSO addition was observed even for higher concentrations than the used for dissolution purposes [25]. The purified DNA was incubated in the presence of the metal complexes for 24 h at 37 °C (final volume: 20 μL, reaction buffer: Tris 10 mM, EDTA (ethylenediaminotetraacetic acid disodium salt) 0.1 mM, pH 7.4 or HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) 25 mM, pH 7.0). Various molar ratios (r_i = mol of complex:mol of DNA base pair) and different incubation times were assayed. When corresponding, distamycin (0.1 mM) was added to the incubation reaction 10 min before the addition of the metal complex. After incubation, reactions were stopped by adding of loading buffer (25% bromophenol blue, 50% glycerol, 25 mM EDTA pH 8.0). In all cases, samples were electrophoresed in 0.7% agarose buffered with TB (90 mM Tris–borate) at 70–80 V for 2 h. The gel was subsequently stained with an ethidium bromide solution (0.5 μg/mL) for 30 min and destained in water for 20 min. Relative mobility of bands visualized under UV light was quantified using OneDSCAN.

2.3. DNA viscosity measurements

Viscosity experiments were conducted at 25 °C on an automated AND viscometer model SV-10. Stock solutions of each complex were prepared by dissolution in the minimum amount of DMSO and addition of water. A 1 mM calf thymus DNA (CT DNA) solution was diluted 1:4 with Tris–EDTA (TE) buffer (pH 7.4). For each complex increasing amounts of complex stock solution were added to this DNA solution to reach complex/DNA molar ratios (r_i) in the range 0–1.0. The DMSO amount in the samples never exceeded 2%. The mixtures were incubated for 24 h at 37 °C. The viscosity of each sample was repeatedly measured at 25 °C after thermal equilibrium was achieved (10–15 min). Mean values of six measurements performed at intervals of 1 min were used to evaluate the viscosity of each sample [26]. Results were expressed as relative viscosity (η/η_0) where η_0 is the viscosity of DNA solution ($r_i = 0$).

2.4. Circular dichroism spectroscopy

CD spectra were measured in a Jasco J-815 spectropolarimeter at 20 °C and pH 7.0 (25 mM Pipes buffer) using CT-DNA (200 μM). The

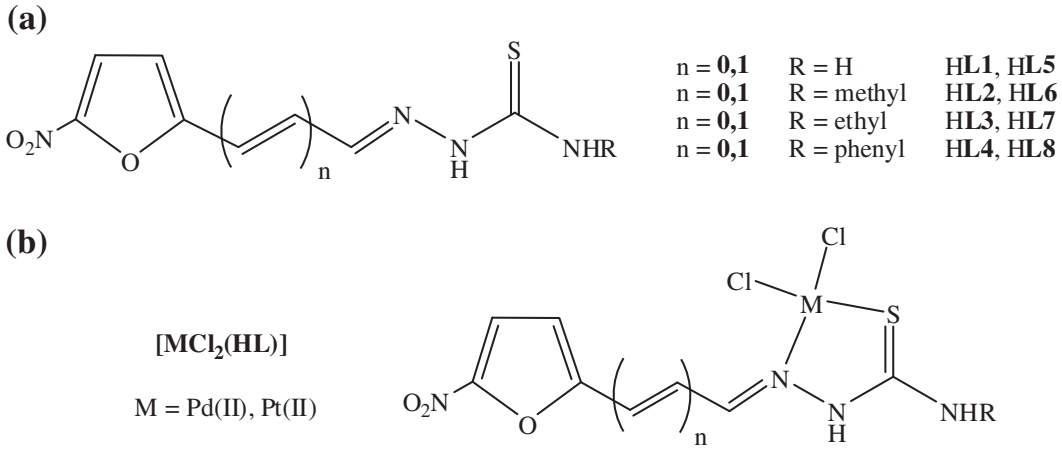

Fig. 1. (a) Bioactive 5-nitrofuryl and 5-nitroacroleine containing thiosemicarbazones (HL) and (b) their analogous metal complexes, $[MCl_2(HL)]$, where M = Pd(II) or Pt(II).