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How half sandwich ruthenium compounds interact with DNA while not being hydrolyzed; a comparative study

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ABSTRACT

The non-coordinative interactions between the compounds [(η^6-cym)Ru(pqx)Cl]Cl, (**1**)Cl, [(η^6-cym)Ru(pbqx)Cl]Cl, (**2**)Cl (cym is 1-methyl-4-(1-methylethyl)benzene, pqx is 2-(2′-pyridyl)quinoxaline and pbqx is 2-(2′-pyridyl)benzo[*g*]quinoxaline), with the self-complementary oligonucleotide duplex d(5′-CGCG CG-3′)$_2$ in 100 mM phosphates buffer and 50 mM NaCl, were studied by means of NMR spectroscopic techniques. The compounds (**1**)Cl and (**2**)Cl were found to be stable in aqueous media without being hydrolyzed, preserving their saturated coordination sphere (η^6-arene-2N-Cl). Based on NMR techniques it was found that both (**1**) and (**2**) interact with the hexamer with the outer side of the chelating ligand L, causing elongation or disruption of the oligonucleotide Watson-Crick (W-C) hydrogen bonds. Since both compounds are highly cytotoxic, it can be concluded that their cytotoxic activity could be owed to this type of interactions, or non-coordinative interactions with other biomolecules.

1. Introduction

Ruthenium complexes have attracted a great research interest as anticancer agents due to their unique properties, such as high cytotoxicity against various cancer cell lines, light induced activated reactions, rich chemistry and stereochemistry and tunable redox properties [1–11]. Among them Ru(II)-η^6-arene half sandwich compounds have shown remarkable cytotoxicity. The discovery of the cytotoxic properties of the complex [(η^6-C_6H_6)$RuCl_2$(metronidazole)] (metronidazole is 1-β-hydroxyethyl-2-methyl-5-nitroimidazole) [12] focused the efforts of many research groups on studying the antitumor properties of arene ruthenium compounds [13–25]. At present, mainly two classes of Ru(II)-η^6-arene half sandwich complexes are of the most interest; the monofunctional compounds, represented by [(η^6-cym)Ru(en)Cl]PF_6 (cym = 1-methyl-4-(1-methylethyl)benzene, en = 1,2-ethylenediamine) [26] and the bifunctional, represented by [((η^6-cym))Ru(pta)Cl_2] (pta = 1,3,5-triaza-7-phospha-tricyclo-[3.3.1.1]decane), termed RAPTA-C [27]. Despite the fact that RAPTA-C exhibits low in vitro activity, it is active in vivo, inhibiting lung metastases in mice [26]. On the other hand, the [(η^6-cym)Ru(en)Cl](PF_6) shows significant antitumor activity, comparable to that of carboplatin, in various cancer cell lines [26]. Recently, a comparative study for the above compounds showed that [(η^6-cym)Ru(en)Cl](PF_6) targets the DNA of chromatin, while RAPTA-C preferentially forms adducts on the histone proteins [28]. For both classes of compounds aquation seems to be the crucial step in cytotoxicity, producing the ruthenium active site in order to react with biomacromolecules. In the cases of complexes with the general formula [(η^6-arene)Ru(L)Cl]$^+$ (L = aromatic diimine) the remaining moiety of the complex (arene and chelating aromatic diimine L) is related to the aquation rate, such as in the case of 2,2′-bipyridine-3,3′-diol [29].

Hydrolysis is suppressed extracellularly, due to the high chloride concentration (104 mM), but occurs inside the cell, in cytoplasm or the nucleus, where the chloride concentration is significantly lower. Thus, the monofunctional complexes hydrolyze relatively slowly, with typical values of $t_{1/2}$ being some tens of minutes [29,30]. However, the hydrolysis of such type of complexes reaches the equilibrium with an amount of the complex remaining non-hydrolyzed [30]. Taking into account that the non-hydrolyzed complex is able to interact with DNA through its ligands, it may cause significant alterations to DNA structure. In addition, inactive hydroxo species which were observed inside cells may interact with DNA non-coordinatively. Also, interaction between the ligands of the complex and DNA, may occur even during the process of hydrolysis, placing the complex in an appropriate location towards DNA helix.

In our previous work [31] we studied the interactions of the highly cytotoxic compounds [(η^6-cym)Ru(L)Cl]Cl, L is 2-(2′-pyridyl)quinoxaline (pqx) or 2-(2′-pyridyl)benzo[*g*]quinoxaline, (pbqx) with the nucleobase 9-methylogouanine by NMR spectroscopy, after the enforced subtraction

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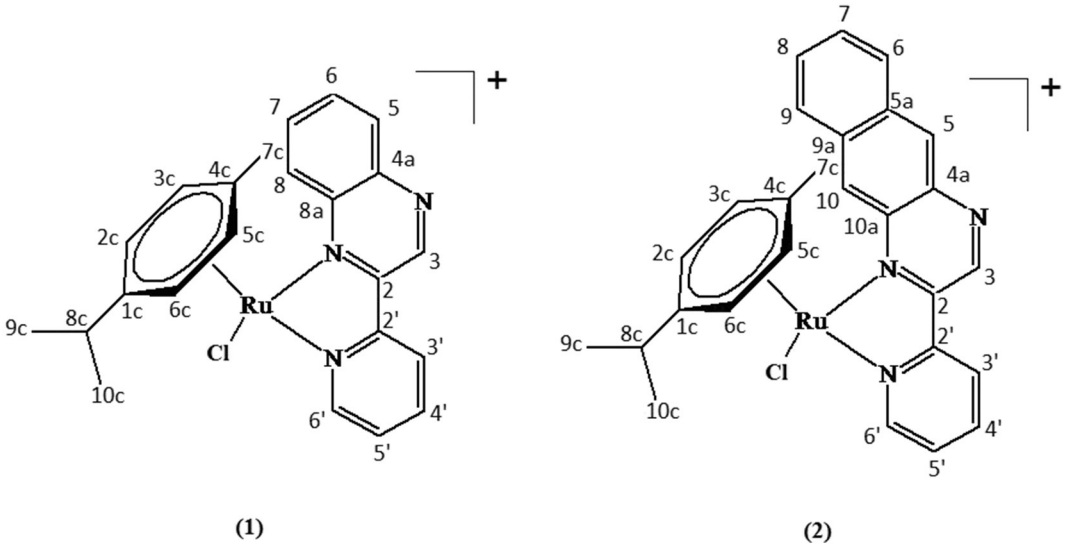

Fig. 1. The structures of the studied complexes (**1**) and (**2**), with atom numbering.

of the chloride with $AgNO_3$. Besides the expected N7 coordination of 9-methylguanine, a strong shielding effect between the aromatic ring system of the quinoxaline or benzo[*g*]quinoxaline moiety of the ligands pqx and pbqx and the H8 of the nucleobase was also observed. Intending to study further the non-coordinative interactions between the compounds [(η^6-cym)Ru(pqx)Cl]Cl, (**1**)Cl, [(η^6-cym)Ru(bpqx)Cl]Cl, (**2**)Cl (Fig. 1) and DNA, herein we report the interactions of the above compounds with the self-complementary oligonucleotide duplex d(5′-CGCGCG-3′)$_2$ as DNA model [32,33] in 100 mM phosphates buffer and 50 mM NaCl, by means of NMR spectroscopic techniques.

2. Experimental

2.1. Materials and methods

All solvents were of analytical grade and were used without further purification. The compounds (**1**)Cl and (**2**)Cl were prepared as previously described [31]. The deoxynucleotide d(5′-CGCGCG-3′) was purchased from Eurogentec and purified by chromatography on a 120 × 2.5 cm Sephadex G-25 superfine column, using distilled water as eluent. Oligonucleotide concentrations were quantified by measuring the absorbance at 260 nm, given that the extinction coefficient ε is 51,400 L mol^{-1} cm^{-1} for the single stranded hexamer. High resolution electrospray ionisation mass spectra (HR-ESI-MS) were obtained on a Thermo Scientific, LTQ Orbitrap XL™ system. Absorption spectra were measured in a Jasco V-650 spectrophotometer in a 1 cm path length cuvette for the region 900–220 nm. NMR spectra were recorded on a Bruker Avance spectrometer operating at ^{1}H frequency of 500.13 MHz and processed using Topspin 3.1 (Bruker Analytik GmbH). The spectra of (**1**)Cl and (**2**)Cl were recorded in H_2O/D_2O (9:1) in 100 mM phosphate buffer (pH 7.0) and NaCl 50 mM, at 298 K and 283 K. All spectra of the oligonucleotide titrated with the complexes were recorded at the same conditions. One-dimensional spectra were recorded for samples, with oligonucleotide concentration of approximately 20 OD_{260} units, while 2D NMR experiments were performed with more concentrated samples. Two dimensional NOESY (Nuclear Overhauser effect spectroscopy) experiments were performed with 300, 350 and 400 ms mixing time.

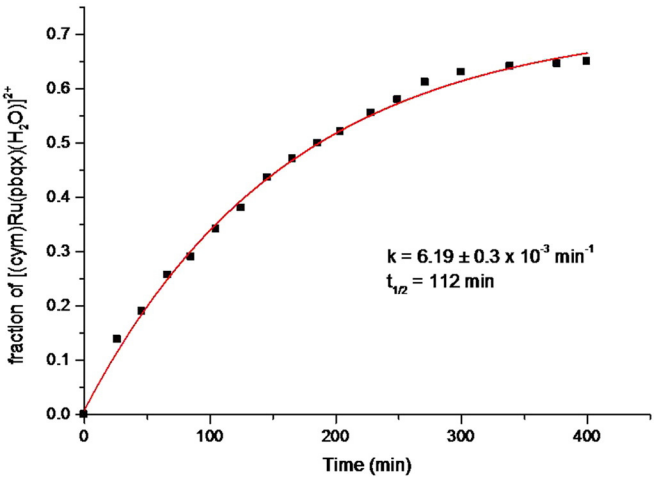

Fig. 2. Plot of fraction of [(cym)Ru(pbqx)(H_2O)]$^{2+}$ vs. time (min) during the hydrolysis of 20 mM of [(cym)Ru(pbqx)Cl]$^+$ at 298 K, fitted by the first order equation $A = A_1 - A_2e^{-kt}$, using the software Origin v. 5 (Microcal software Ltd).

2.2. Determination of hydrolysis constants

Hydrolysis of (**1**)Cl and (**2**)Cl was monitored by ^{1}H NMR spectroscopy in aqueous solutions, at concentrations of 20 mM in 298 K, after the addition of one equivalent of $AgNO_3$ in the samples and the filtration of the AgCl, at cycles of 6 min. Since the hydrolysis reaction follows slow exchange kinetics in the NMR time scale at 298 K, the proton signals of the chloro complex decrease and new signals of the hydrolyzed product arise. The fraction (F) of the aquated complex was estimated from the integral ratios (r_i) of selected signals of chloro and aquated forms, according to the following equation:

$$F = r_i/(1 + r_i). \quad (1)$$

Table 1
Kinetic parameters for (**1**) and (**2**) at 298 K, determined by ^{1}HNMR spectroscopy.

Complex	k (min^{-1}) 10^{-3}	$t_{1/2}$ (min)
(**1**)	9.29 ± 0.4	74.6 ± 3.1
(**2**)	6.19 ± 0.3	112.0 ± 2.3

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