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Coordination capacity of cytosine, adenine and derivatives towards open-paddlewheel diruthenium compounds

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ABSTRACT

$[Ru_2Cl_2(DPhF)_3]$ (DPhF = diphenylformamidinate) links preferentially to the junctions of RNA (ribonucleic acid) structures, although the bonding mode is not known. In order to clarify this question the reactions between $[Ru_2Cl_2(DPhF)_3]$ and cytosine (Hcyto), cytidine (Hcyti), cytidine 2′,3′-cyclic monophosphate sodium salt (NacCMP), adenine (Hade), adenosine (Haden) and adenosine 3′,5′-cyclic monophosphate (HcAMP) have been carried out. In the resultant complexes, cyto (cytosinate), cyti (cytidinate), cCMP (cytidine 2′,3′-cyclic monophosphate monoanion), ade (adeninate), aden (adenosinate) and cAMP (deprotonated adenosine 3′,5′-cyclic monophosphate) are bonded to the diruthenium unit as *N,N′*-bridging ligands, as confirmed by the solution of the crystal structures of $[RuCl(DPhF)_3(cyto)]$ and $[RuCl(DPhF)_3(ade)]$ by X-ray diffraction. The axial positions of the diruthenium species are still available for additional interactions with other residues that could explain its preference towards RNA junctions.

1. Introduction

Coordination compounds with transition metals are receiving increasing interest in biochemistry and pharmacology [1–7]. That is because transition metals offer a large variety of coordination numbers, different possibilities of stereochemistry and several oxidation states. Furthermore, substitution kinetics of the coordination compounds make them a great tool to design species capable to interact with biological targets [1–8].

The use of dinuclear complexes increases the possibilities of interaction with biological systems due to their different stereochemistry [9]. From this perspective, dirhodium and diruthenium metal-metal bonded complexes with paddlewheel structure stand out for their possibilities to interact with biological molecules [9–19]. Some evidences indicate that dirhodium compounds covalently link with DNA (deoxyribonucleic acid) [12,13], tRNA (transfer ribonucleic acid) [14] and proteins [15]. Although their diruthenium counterparts have been less investigated in biological systems, they are thought to be comparable to those found for dirhodium paddlewheel compounds [16]. In fact, paddlewheel diruthenium compounds can form metal-protein adducts [17] and act as inhibitors of C6 rat glioma cell proliferation [18] or of glioma tumour growth in vivo [19].

Open-paddlewheel diruthenium species reactivity is higher than that of the corresponding paddlewheel complexes [20,21] and their interaction with RNA has also been recently proved [22]. In particular, it has been shown that the open-paddlewheel compound $[Ru_2Cl_2(\mu\text{-}DPhF)_3]$ (DPhF = *N,N′*-diphenylformamidinate) can be covalently linked to RNA, specifically to residues located at or close to the junctions of the RNA structure [22], which play crucial roles in RNA folding. Also, it has been shown that this compound has no preference for any kind of nucleotides of the RNA, either purines or pyrimidines. Although $[Ru_2Cl_2(DPhF)_3]$ provides a very useful structural information of the RNA, the chemical groups involved in the covalent bonds between RNA and the diruthenium unit remain elusive. To clarify this question, we have studied the interaction between the diruthenium complex and the pyrimidine cytosine (Hcyto) and the purine adenine (Hade). Then, we substituted cytosine and adenine for some of their derivatives, increasing their complexity. Thus, the nucleosides cytidine (Hcyti) and adenosine (Haden) and the nucleotides cytidine 3′,5′-cyclic monophosphate monosodium salt (NacCMP) and adenosine 3′,5′-cyclic monophosphate (HcAMP) have been tested to study a possible competition between the nucleobase, the monosaccharide, and the phosphate group (Figs. 1 and 2).

In the present study, the compounds $[Ru_2Cl(DPhF)_3(cyto)]$ (**1**), $[Ru_2Cl(DPhF)_3(cyti)]$ (**2**), $[Ru_2Cl(DPhF)_3(cCMP)]$ (**3**), $[Ru_2Cl(DPhF)_3(ade)]$ (**4**), $[Ru_2Cl(DPhF)_3(aden)]$ (**5**) and $[Ru_2Cl$

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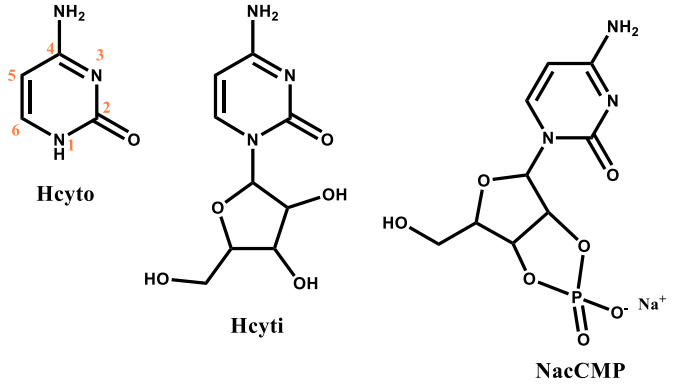

Fig. 1. Cytosine (Hcyto) and cytidine (Hcyti) molecules and cytidine 2′,3′-cyclic monophosphate sodium salt (NacCMP) employed as ligand precursors.

$(DPhF)_3(cAMP)]$ (**6**) have been synthesized and characterized using elemental analysis, infrared and electronic spectroscopy, mass spectrometry and magnetic measurements. Also, the chemical structures of **1** and **4** were determined by single-crystal X-ray diffraction.

2. Results and discussion

Compounds **1** and **4** were synthesized by microwave-assisted solvothermal synthesis and by conventional synthesis at room temperature employing $[Ru_2Cl(DPhF)_3(O_2CCH_3)]$ [23] or $[Ru_2Cl_2(DPhF)_3]$ [20], respectively, as starting materials, and at least two equivalents of nucleobase because these molecules act simultaneously as ligand precursors and as deprotonation agents. In the case of the cytosine, the reaction at room temperature takes 48 h but can be accelerated if the ratio nucleobase/complex is increased. The soft reaction conditions are more similar to those employed in the experiments with $[Ru_2Cl_2(DPhF)_3]$ and RNA performed by Lozano et al. [22]. Once the syntheses using the nucleobases were carried out successfully at room temperature, the same conditions were tried employing Hcyti, NacCMP, Haden and HcAMP, and in all the cases the results were satisfactory. The elemental analysis of compounds **1–6** are in accordance with the proposed composition. IR spectra (Figs. S1–S6) show the typical pattern of tris(*N,N′*-diphenylformamidinato)diruthenium complexes [24]. Moreover, bands due to the additional ligands are observed. Electrospray ionization (ESI) mass spectrometry confirms the stoichiometry proposed for the compounds **1–5** (Figs. S7–S11). In the case of complexes **1**, **2**, **4** and **5** very intense peaks corresponding to the $[M-Cl]^+$ fragment are observed in the positive ESI spectrogram, similarly to those observed for other paddlewheel compounds [23,24]. For **3** (Fig. S9) a very intense peak corresponding to the $[M-Cl-2H]^-$ fragment is observed in the negative ESI spectrogram. Complex **6** shows an important fragmentation and the molecular peak is not detected. Nevertheless, the most intense peak in the positive ESI can be assigned to $[Ru_2(DPhF)_3(H_2NCH_2NH)]^+$ species, which suggests a *N1,N6* coordination mode for the cAMP ligand (Fig. S12). A central question in the interaction of the diruthenium moieties with biological species is, precisely, to determine the coordination mode of the active sites of the potential ligands to the ruthenium atoms, since the diruthenium species behaves in its interaction with RNA differently than other RNA probing compounds. Open-paddlewheel species have the tendency to coordinate a bidentate ligand as a bridge to form a paddlewheel structure and gain stability [21]. The cytosinate ligand potentially may act as *N,N′*- or *N,O*-donor ligand although the only dinuclear complex with metal-metal bond bridged by a cytosinate whose crystal structure has been reported is bis(μ_2- cytosinato-*N3,N4*-tetraammine)diplatinum(II) [25]. In the cases of cytidinate and cytidine 2′,3′-cyclic monophosphate, *N1* is not available to be linked to a ruthenium atom but other additional coordination modes are possible. For example, the phosphate group of cCMP can bridge the ruthenium atoms. However, the adeninate can act as *N,N′*-donor ligand via *N3,N9* atoms or via *N1,N6* atoms (Fig. 2) and in the adenosinate and adenosine 3′,5′-monophosphate the coordination via *N1,N6* is also possible together with other coordination modes.

Electronic spectroscopy can help to determine the coordination mode of these ligands (Table S1). The electronic spectra of compounds **1–3** (Fig. 3), with two maxima around 500 and 650 nm and an intermediate absorption, are very similar to other paddlewheel diruthenium compounds containing four μ-*N,N′*-donor ligands [20,26,27]. This circumstance, together with the strong similarity of the electronic spectra of these complexes among themselves, indicate that cyto, cyti and cCMP ligands are bonded by two nitrogen atoms in compounds **1–3** leading to a $[Ru_2(N\text{-}N')_4]$ core. This coordination mode is also supported by the crystal structure of complex **1** (see below). In complexes **1–3** the absorptions can be assigned to the following transitions (from higher to lower energy): $\pi(Ru\text{-}N,Ru_2) \rightarrow \pi^*(Ru_2)$, $\pi^*(Ru_2) \rightarrow \sigma^*(Ru\text{-}N)$ and $\delta(Ru_2) \rightarrow \pi^*(Ru_2)$ in accordance with previous studies in other formamidinate compounds [23,24,26,27].

The electronic spectra of complexes **4**, **5** and **6** are different from each other (Fig. 4). In compound **4**, a coordination of ade via *N3,N9* is proved by single-crystal X-ray diffraction measurements (see below) but in aden and cAMP the position *N9* is occupied and, therefore, it cannot be used to be linked to the metal. The diruthenium unit could be bonded to the ribose group of the aden ligand or by the phosphate group of the cAMP ligand. In fact, the differences in these spectra suggest a different coordination mode with respect to the other compounds. However, aden and cAMP can still behave as *N,N′*-bridging ligands, which is particularly favorable to stabilize paddlewheel structures. If that is the case, it is expected for **5** and **6** to have dimetallic cores richer in electronic density with respect to **1–4**. This makes the Ru—Cl bond weaker and it can be broken eventually, at least in solution. Electronic spectra similar to those registered for compounds **5** and **6**, have been previously observed for diruthenium(II,III) with paddlewheel structure in which the axial position is not occupied for a strong π-donor ligand such as chloride: $[Ru_2(DPhF)_3(O_2CMe)(OH_2)]^+$ and $[Ru_2(NCS)(DPhF)_3(O_2CMe)]$ [24]. Also, the more basic the equatorial ligands are, the more likely an intermediate spin or even low spin configuration is found [20,24,28–31]. Therefore, the magnetic moment at room temperature of the compounds was measured.

Compounds **1–4** have a magnetic moment at room temperature of 4.04, 3.94, 4.19 and 4.22 μ_B (Bohr magneton), respectively. These data are in accordance with the $\sigma^2\pi^4\delta^2(\pi^*\delta^*)^3$ ground–state proposed by Norman et al. [32] that confirms a Ru_2^{5+} core with high spin configuration. However, compounds **5** and **6** present lower values for the magnetic moment: 2.63 and 3.28 μ_B, respectively. Those values are consistent with the existence of an intermediate spin configuration, that have been found in other diruthenium(II,III) compounds with paddlewheel structure [20,24,28–31], although an oxidation to diruthenium (III,III) species cannot be ruled out [33–35].

Single crystals suitable for SCXRD (single crystal X-ray diffraction) experiments of compounds **1** and **4** were obtained by diffusion of a layer of a solution of each compound in dichloromethane into a second layer of diethyl ether on top. The resulting structures are shown in Fig. 5, and structural information is collected in Tables 1 and 2 and in the Supporting information (Tables S2–S5; Figs. S13–S18).

The crystal structure of **1** (Fig. 5, left) was found to be the same for samples obtained by both microwave and room temperature synthetic procedures. Therefore, the μ-*N,N′* mode of coordination is preferred despite the strong energetic differences between the method of synthesis. As we anticipated, it is confirmed that the deprotonated