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Research paper

The reduction of ruthenium(III) complexes with triazolopyrimidine ligands by ascorbic acid and mechanistic insight into their action in anticancer therapy

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A B S T R A C T

Kinetic studies of the reduction of two ruthenium(III) complexes, *mer*-[$RuCl_3$(dmso)(H_2O)(tmtp)]·2H_2O (**1**) and *mer,cis*-[$RuCl_3$(dbtp)$_2$(dmso)] (**2**) (where dmso – dimethylsulfoxide, tmtp – 5,6,7-trimethyl-1,2,4-triazolo[1,5-*a*] pyrimidine and dbtp – 5,7-ditertbutyl-1,2,4-triazolo[1,5-*a*]pyrimidine), by ascorbic acid were performed as a function of antioxidant concentration in acetate buffer within the pH range of 2.9–5. The rapid reduction of the ruthenium(III) complexes (**1**) and (**2**) resulted in the formation of the *mer*-[$RuCl_3$(dmso)(H_2O)(tmtp)]$^-$ and *mer,cis*-[$RuCl_3$(dbtp)$_2$(dmso)]$^-$ ions and was followed by successive dissociation of the chloride ligands. The second-order rate constant (k_1) for the reduction of the *mer*-[$RuCl_3$(dmso)(H_2O)(tmtp)]·2H_2O complex and the first-order rate constant for the hydrolysis of its reduced form were found to be 134 ± 2 $M^{-1}s^{-1}$ and (3.8 ± 0.9) × 10^{-2} s^{-1} at 25 °C and pH = 2.9, respectively. Similarly, the fast process assigned to the reduction of the *mer,cis*-[$RuCl_3$(dbtp)$_2$(dmso)] complex and the subsequent, slower process attributed to the hydrolysis of the *cis*-[$RuCl_3$(dbtp)$_2$(dmso)]$^-$ ion were characterized by rate constants of 145.5 ± 0.8 $M^{-1}s^{-1}$ and (9 ± 2) × 10^{-3} s^{-1} at 25 °C and pH = 2.9, respectively. Obtained data indicated that the reduction of the ruthenium(III) complexes strongly depends on pH and accelerates with increasing pH. The kinetic data indicates that the redox process followed an inner-sphere electron-transfer mechanism at pH higher than 3.

1. Introduction

Compounds of almost all metals of the periodic table have been investigated for *in vitro* anticancer activity against cancer cell lines [1–11]. Among them, the ruthenium compounds are the potent chemotherapeutics that are the most promising to be competitive against platinum-based inorganic drugs [12–22]. A possible explanation for these effects of ruthenium compounds may be derived from their kinetic properties. Similar to platinum, ruthenium is a relatively inert metal, and its ligand exchange kinetics are typically within the same timescale as cellular division processes [20,23]. Ruthenium compounds exhibit a wide spectrum of redox properties and can exist at various oxidation states under biologically relevant conditions. The crucial points are to check whether ruthenium is transformed reductively and to discover the possible pathways of these transformations *in vivo*. Moreover, ruthenium complexes have shown significant ability to bind many biological molecules, including serum proteins (e.g., transferrin and albumin). In the human body, ruthenium compounds circulate in the bloodstream and are then transported to the cell through the cellular membrane, mostly by transferrin-mediated uptake. It is important to determine whether ruthenium(III) is reduced to ruthenium(II) in the cytoplasm or extracellular fluids by endogenic reducing agents, which essentially modifies its reactivity with biomolecules or cell components and modulates its transformation into the active form of drugs. It is necessary to check whether the levels of antioxidant concentrations (vitamin C and glutathione) and the rate of redox processes are relevant and effective for such a process to actually occur in the cell environment. The answer to this question and those above will allow us to better understand the mechanism of action of potential ruthenium pharmaceuticals and to better plan the synthesis of new compounds

Abbreviations: k_s, rate constant for slow process; k_f, rate constant for fast process; k_{1obs}, observed first-order rate constant for process 1 derived from two-exponential function; k_{2obs}, observed first-order rate constant for process 2 derived from two-exponential function; k_1, second-order rate constant for reduction by H_2Asc; k_{-1}, second-order rate constant for reverse reaction; k_2, second-order rate constant for fast reduction by ascorbyl radical (not directly observed); k_{1aq}, first-order rate constant for aquation of ruthenium(III); k_{2aq}, first-order rate constant for aquation of ruthenium(II) in the second step of reaction; k_{1b}, second-order rate constant for reduction by $HAsc^-$

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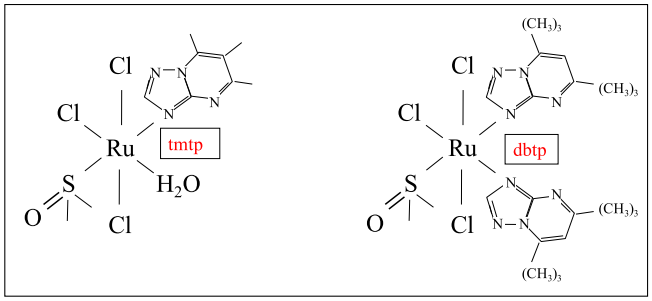

Scheme 1. Structural formulas of the *mer*-$[RuCl_3(dmso)(H_2O)(tmtp)]\cdot 2H_2O$ (**1**) and *mer,cis*-$[RuCl_3(dbtp)_2(dmso)]$ (**2**) complexes.

with potential anticancer activity in future.

Recently, the hypothesis that the antitumor activity of ruthenium (III) complexes involves activation by reduction *in vivo* prior to metal coordination to nucleic acids or other biological molecules has been proposed [19,24–26]. According to this hypothesis, ruthenium complexes should possess biologically accessible reduction potential (in the range from −0.4 V to +0.8 V vs NHE) in their *in vivo* activity to act as a prodrugs [27,28]. Nevertheless, the biological target and mechanism of action of ruthenium compounds are largely unknown. Particularly, it is still unknown whether ruthenium(II) binds to nucleic acid or other biological molecules in a cancer cell more effectively than ruthenium (III) as a result of much higher lability of ruthenium(II) in relevance to ruthenium(III).

Recently, we have reported the synthesis, structures and spectroscopic properties of new ruthenium(III) complexes with triazolopyrimidine ligands, *mer*-$[RuCl_3(dmso)(H_2O)(tmtp)]\cdot 2H_2O$ (**1**) [29], *mer,cis*-$[RuCl_3(dbtp)_2(dmso)]$ (**2**) [30] (Scheme 1), *trans*-$[RuCl_3(dbtp)_2(H_2O)]$ and *mer*-$[RuCl_3(dbtp)_3]$ [31]. The last two compounds were determined to be significant relative to cisplatin, as they are inhibitors of tumour cell proliferation with IC_{50} values at micromolar concentrations against 4 cancer cell lines: a human lung adenocarcinoma epithelial cell line (A549), a cisplatin-resistant human ductal breast epithelial tumour cell line (T47D), a human breast adenocarcinoma and grade III-causing bone metastases line (MDA-MB-231), and a human breast adenocarcinoma line (MCF-7) [31]. Although complexes (**1**) and (**2**) were less toxic against the cancer cell lines mentioned above, they were also determined to be less toxic against normal murine embryonic fibroblast cells (BALB/3T3) and a non-tumourigenic human epithelial cell line (MCF-10A) than cisplatin [29,30]. More attention should be given to kinetic and mechanistic studies of any transformations of these potential ruthenium(III) pharmaceutics in aqueous solution, which functions as an adequate model of the cell environment, with a special emphasis on reduction processes. This aspect has now been studied in additional detail for these newly synthesized complexes. This report is the first example of mechanistic insight into the reduction processes of these ruthenium(III) complexes with triazolopyrimidine derivatives. Introduction of spectator chelates, i.e., innocent, non-innocent and labilizing ligands, may have a drastic effect on the reaction rate and the underlying reaction mechanism.

2. Experimental

2.1. Materials

Ascorbic acid (Sigma-Aldrich), acetic acid (70%, Sigma-Aldrich), sodium acetate (Sigma-Aldrich) and all other chemicals were analytical grade reagents. The *mer*-$[RuCl_3(dmso)(H_2O)(tmtp)]\cdot 2H_2O$ (**1**), *mer,cis*-$[RuCl_3(dbtp)_2(dmso)]$ (**2**) complexes were prepared as described elsewhere [29,30]. Solutions of (**1**) and (**2**) were prepared freshly in methanol just before being mixed with aqueous acetate buffer (1:9) and used immediately in measurements. The 0.05 M ascorbic acid solution was prepared in water due to its instability at high pH [32] and stored for no longer than one day. Ascorbic acid solutions of desired concentrations (0.002, 0.0025, 0.005, 0.0075, 0.01, 0.0125 and 0.02 M) were prepared freshly by dilution of 1, 1.25, 2.5, 3.75, 5, 6.25 and 10 mL of ascorbic acid stock solution and 5 mL of 0.5 M acetate buffer in 25 mL volumetric flasks. Ultrapure water was obtained from a Milli-Q system (Millipore/Waters, Milford, MA, USA) and used to prepare all the solutions.

2.2. Instrumentation

Cyclic voltammetry measurements were performed using an Eco Chemie Autolab PGSTAT30 Potentiostat under an inert atmosphere (Ar) in a three-electrode cell at 25 °C. The working electrode was a Pt-wire, the auxiliary electrode was a platinum–carbon electrode, and the reference electrode was the saturated Ag/AgCl electrode. The potentials were measured in a 0.1 M $[nBu_4N][PF_6]$/acetonitrile (ACN) versus a Ag/AgCl electrode. All measurements were performed at laboratory temperature and at 100 V s^{-1} scan rate.

2.3. Kinetic measurements

Time-resolved spectra and kinetic measurements were recorded on an SX 18 MV Applied Photophysics apparatus equipped with a J&M TIDAS diode-array detector. The data were acquired and analysed with Applied Photophysics software. In the experiments, the concentration of ruthenium(III) was fixed at $(1–2) \times 10^{-4}$ M. The concentration of ascorbic acid used was in excess and varied over the range of $(1–10) \times 10^{-3}$ M for complexes (**1**) and (**2**). The other experimental conditions were as follows: pH = 4.5, 0.1 M acetate buffer, $I \neq$ constant (H^+, Na^+, CH_3COO^-), T = 298 K, l = 1 cm. In another series of experiments, the rate was measured at a lower pH value, where ascorbic acid (H_2A) is present in its fully protonated form. The other experimental conditions were as follows: pH = 2.9, 0.1 M acetic acid, I = constant (H^+, CH_3COO^-), T = 298 K, l = 1 cm. The rate was also analysed at different H^+ concentrations: pH = 2.9–5.0, 0.1 M acetate buffer, $I \neq$ constant (H^+, Na^+, CH_3COO^-), T = 298 K, l = 1 cm. The progress of the reaction was followed by monitoring the absorbance at 370 nm (electronic transitions in the ruthenium(III) complex region). Reactions were studied under pseudo-first-order conditions, i.e., ascorbic acid was in excess over the ruthenium(III) complex. In the reactions of (**1**) and (**2**), two steps were observed that could not be separated kinetically. Therefore, the data were analysed by a Gauss-Newton nonlinear least-squares fit of a two-exponential function of absorbance vs time. The reported rate constants are the mean values of at least four determinations. In the reaction of (**2**), due to some fluctuations in absorbance after mixing the reactant solutions, the analysis was performed in an adequate time scale, with the first 0.1 s of the overall process omitted. The relative standard errors of the pseudo-first-order rate constants for a single kinetic trace were *ca.* 0.1–0.2%, and relative standard errors of the mean value were usually *ca.* 0.1–0.5%.

3. Results and discussion

The stopped-flow rapid-scan spectra revealed characteristic spectral changes in the visible range attributed to rapid reduction of the ruthenium(III) complexes to ruthenium(II) complexes and the subsequent solvolysis. The former process is accompanied by a subsequent decrease in absorbance from the electron-transition band at 366 nm assigned to complex (**1**) ($[Ru^{III}] = 2 \times 10^{-4}$ M, $[H_2Asc] = 1 \times 10^{-3}$ M, pH = 2.9, 0.1 M acetic acid, T = 298 K), which appears simultaneously with a decrease in the absorbance of its low-energy transition band at 430 nm. The spectral changes characteristic for the degradation of the *mer*-$[RuCl_3(dmso)(H_2O)(tmtp)]^-$ ion are consistent with a further decrease in the intensity of the electron-transition bands at 366 and 430 nm and with a blueshift of the high-energy transition band from 366 nm to