Kinetics, mechanism, and equilibrium studies of the reactions between a ruthenium(II) complex and some nitrogen- and sulfur-donor nucleophiles

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ORIGINAL PAPER

Kinetics, mechanism, and equilibrium studies of the reactions between a ruthenium(II) complex and some nitrogen- and sulfur-donor nucleophiles

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Abstract Kinetics and mechanism of the substitution reactions between $[Ru(trpy)(bpy)Cl]^+$ with nucleophiles guanosine-5′-monophosphate, L-histidine, thiourea, and dimethylsulfoxide were studied spectrophotometrically in 0.1 M $NaClO_4$ at 310 K. The observed order of reactivity for selected ligands is: thiourea > guanosine-5′-monophosphate > L-histidine > DMSO. This order is associated with the electronic, structural, and chemical characteristics of complex and nucleophiles. The substitution reaction with thiourea was studied at three different temperatures (288, 298, and 310 K). Negative entropy of activation $\Delta S^{\neq}$ confirms the associative mode of activation. The complex formation of $[Ru(trpy)(bpy)H_2O]^{2+}$ with ligands guanosine-5′-monophosphate and L-histidine was investigated by potentiometry and spectrophotometry as well. The stoichiometry and stability constants of the species formed in these systems were determined. The concentration distribution diagram of the various complexes has been evaluated as a function of pH. Comparing the calculated values for $\log\beta$, we determined that the product with nucleotide is more stable than the product with L-histidine.

Keywords Kinetics · Equilibrium · Ru(II) · L-Histidine · Guanosine-5′-monophosphate · Dimethylsulfoxide · Thiourea

Introduction

Since the antitumor activity of cisplatin was discovered, a number of platinum complexes have been synthesized and evaluated as potential chemotherapeutic agents [1–3]. The limited range of activity of cisplatin and its analogs and their several side effects have stimulated the search for other metal-based anticancer drugs with metal ions different from platinum [4, 5]. Ruthenium complexes are today the most promising compounds for the investigation of antitumor activity of metal-containing pharmaceuticals [6–10].

The first ruthenium compounds studied for antitumor activity were the chlorido-ammine complexes *fac*-$[Ru(NH_3)_3Cl_3]$ and *cis*-$[Ru(NH_3)_4Cl_2]$ [11]. However, although active, these compounds were not soluble enough for pharmaceutical use [6, 7]. In the following years, a large number of different Ru(II) and Ru(III) compounds were studied for their cytotoxic properties, such as polypyridyl complexes *cis*-$[Ru(N,N\text{-bpy})_2Cl_2]$ and *mer*-$[Ru(N,N,N\text{-trpy})Cl_3]$ [12, 13], aminocarboxylato complexes $[Ru(N,N,O,O\text{-pdta})Cl_2]$ (pdta = 1,2- propylenediaminetetraacetato) and $[Ru(N,N,O,O,O\text{-edta})Cl]$ (edta = ethylenediaminetetraacetato) [14, 15], dimethylsulfoxide complexes *cis*- and *trans*-$[Ru(S\text{-DMSO})_4Cl_2]$ [16–18], and arylazopyrdine complexes $[Ru(N,N,N,N\text{-azpy})_2Cl_2]$ (azpy = 2-phenylazopyridine) [19, 20]. More recently, two classes of structurally similar Ru(III) complexes were synthesized and investigated for antitumor activity: [HL]*trans*-$[RuCl_4L_2]$ (L = imidazole or indazole) [21] and [HL]*trans*-$[RuCl_4(DMSO\text{-}S)L]$

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(L = heterocyclic nitrogen ligand) [22]. In particular just two compounds are undergoing clinical evaluation today, the indazole (ind) derivative cytotoxic to cancer cells [Hind]-*trans*-[$RuCl_4(ind)_2$], known as KP1019, and the imidazole (im) derivative [Him]*trans*-[$RuCl_4$(DMSO-S)(im)], known as NAMI-A, which is relatively non-toxic but has antimetastatic activity [21–25].

It is generally accepted that the antitumor activity of platinum drugs can be ascribed to interactions between complex and DNA molecules [1–5]. However, the mechanism of action of ruthenium compounds has not yet been clarified. It was proposed that they are activated by hydrolysis, mainly of the chlorido ligands. After hydrolysis, the reduction of Ru(III) to Ru(II) complex occurs because cells contain different amounts of reducing agents [23–25]. Finally, the Ru(II) complex formed reacts with the DNA molecule, binding preferentially to guanine residues via N7 coordination [26]. This activation mechanism, proposed by Clarke, has become known as the "activation by reduction" hypothesis [25]. In accordance with the "activation by reduction" hypothesis, NAMI-AR, obtained by the reduction of NAMI-A with ascorbic acid prior to administration was found to be more efficient than NAMI-A against metastasis growth [27].

Today, many different ruthenium complexes have been synthesized and investigated to elucidate the relationship between the structure of inert ligands and properties of the complexes. Complexes with polypyridyl ligands [9, 28–30] and organometallic half sandwich ligands [31–37] are studied frequently in order to gain insight into the factors that influence hydrolysis and binding to bio-molecules. In particular, activation through hydrolysis is important for the mechanism of action of this class of compounds, and their chemical behavior depends to a great extent on the acidity and chloride concentration.

Taking into account that biomedical and pharmaceutical utilizations of terpyridine-type ligands (e.g., as DNA-binding or active antitumor agents) are currently fast growing fields of research [38–41], we studied the kinetics of the substitution reactions between $[Ru(trpy)(bpy)Cl]^+$ and nucleophiles such as thiourea (Tu), dimethylsulfoxide (DMSO), L-histidine (L-His), and guanosine-5′-monophosphate (5′-GMP) by conventional UV–Vis spectrophotometry. The ligands L-His and 5′-GMP are biologically relevant molecules. Tu is commonly used as a "protective agent" for the better excretion of "soft" metal ions [3–5], while DMSO is already present in the structures of some Ru complexes, such as NAMI-A. Also, we studied the hydrolysis of $[Ru(trpy)(bpy)H_2O]^+$ as well as complex formation equilibria between $[Ru(trpy)(bpy)H_2O]^+$ and L-His or 5′-GMP by potentiometric and spectrophotometric methods. The structures of $[Ru(trpy)(bpy)Cl]^+$ and nucleophiles are shown in Fig. 1.

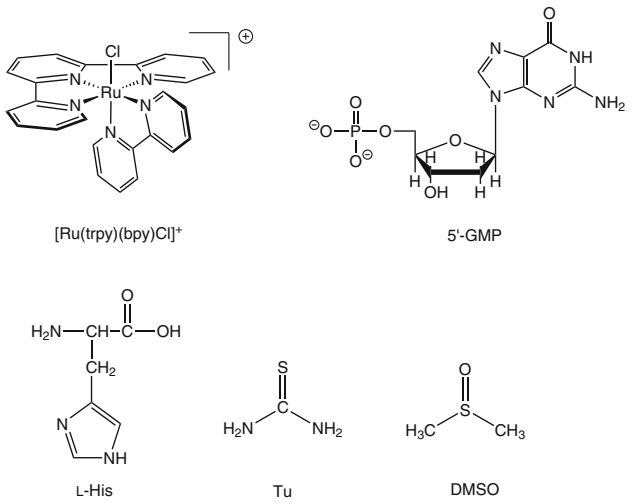

Fig. 1 The structures of complex and nucleophiles

Results and discussion

Kinetic studies

Kinetics of the substitution reactions of $[Ru(trpy)(bpy)Cl]^+$ with nucleophiles Tu, L-His, DMSO, and 5′-GMP were investigated spectrophotometrically by following the changes in absorbance at suitable wavelengths as a function of time at 310 K. The complex and ligands were dissolved in aqueous 0.1 M $NaClO_4$ with the addition of 20 mM NaCl to prevent the spontaneous hydrolysis of Ru(II) complex [42–44]. The concentration of 20 mM NaCl was chosen after recording the changes in absorbance of the complex at different chloride concentrations. The obtained results are given in the Supplementary material (Table 1S; Fig. S1). All kinetic experiments were performed under pseudo first-order conditions, where the concentration of nucleophiles was always in at least tenfold excess (Supplementary Material, Tables 2S–5S).

Substitution reaction of selected octahedral ruthenium(II) complex can be presented as shown in Scheme 1. The pseudo first-order rate constants were found to be linearly dependent upon the concentration of nucleophile (L), as presented in Eq. (1).

$$k_{obsd} = k_2[L] + k_1 \quad (1)$$

The second-order rate constant k_2 characterizing the formation of the reaction product can be evaluated from the slope of a plot k_{obsd} vs. [L]. The experimentally obtained results are summarized in Table 1 and presented in Fig. 2. The value for the rate constant of the reverse reaction k_1, which is independent on the concentration of nucleophile L, is determined from the intercept of the observed lines (Fig. 2). It is very small and contributes little to k_{obsd}.

According to the results shown in Table 1 and Fig. 2, the following order of reactivity of the selected nucleophiles was observed: Tu > 5′-GMP > L-His > DMSO. It was expected that thiourea has the highest reactivity toward the Ru(II) complex studied, because it combines the ligand properties of thiolates (σ-donor) and thioethers (σ-donor, π-acceptor) [45, 46]. However, 5′-GMP reacts slightly slower than thiourea. Taking into account the size of the molecule, we expected that 5′-GMP reacts very slowly. But, besides nitrogen donor atoms in the structure of the purine base and the well-known coordination to metal ions via N7 atoms, this nucleotide could be bound to Ru(II) via phosphate oxygen atoms as well. This kind of coordination has been already published for some Ru(II) complexes [9, 26]. After formation of an adduct where the metal ion is coordinated to oxygen from the phosphate group, very slow isomerization to the N7 atom of purine takes place. The reaction with amino acid L-His could also proceed in a similar way by coordination via oxygen atoms followed by slow isomerization to N3 from the imidazole ring. The reaction with DMSO is the slowest. This was unexpected because DMSO could coordinate via sulfur or oxygen. However, here a very rigid geometry of the nucleophile makes access and bond formation difficult.

Scheme 1

$$[Ru(trpy)(bpy)Cl]^{+} + L \underset{k_1}{\overset{k_2}{\rightleftharpoons}} [Ru(trpy)(bpy)L]^{+} + Cl^{-}$$

L = 5'-GMP, L-His, Tu, DMSO

Table 1 Rate constants for the substitution reaction of [Ru(trpy)(bpy)Cl]$^{2+}$ complex with selected nucleophiles in 0.1 M $NaClO_4$, 20 mM NaCl at 310 K

Nucleophile	$k_2/M^{-1}s^{-1}$	$10^4\ k_1/s^{-1}$
Thiourea	1.35 ± 0.03	1.4 ± 0.3
5′-GMP	1.33 ± 0.03	0.16 ± 0.2
L-His	0.41 ± 0.02	11 ± 1
DMSO	0.020 ± 0.002	1.2 ± 0.6

As mentioned above, Ru(II) complexes have a huge potential for antitumor activity. The investigation of their interactions with biomolecules could help a lot toward better understanding of some cell processes. On the basis of the chemical characteristics of such complexes and biologically relevant molecules, some interactions could be predicted. One of the most important facts is knowledge of their "hard" and "soft" behavior. The ruthenium compounds belong to the "border line" group, which means that they are somehow

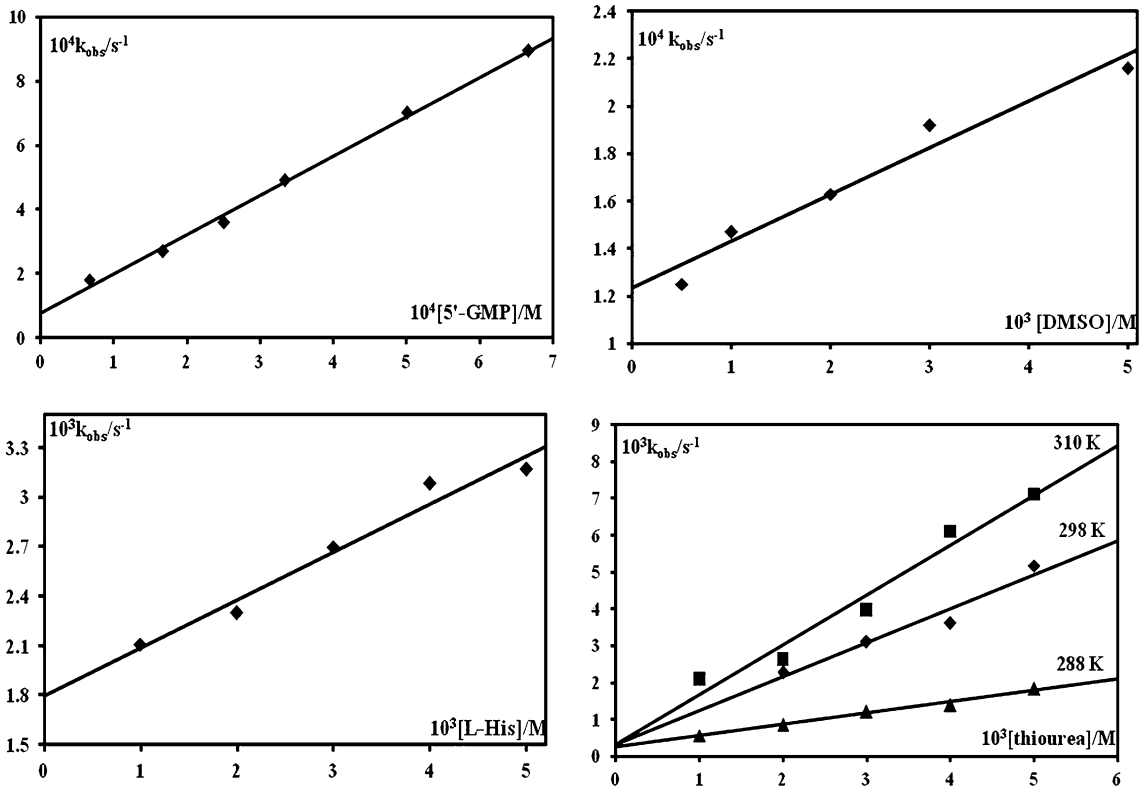

Fig. 2 Pseudo-first-order rate constants as a function of nucleophile concentrations for the substitution reactions of [Ru(trpy)(bpy)Cl]$^{+}$ in 0.1 M $NaClO_4$, 20 mM NaCl at 310 K

"harder" than platinum(II) complexes [47]. This clearly explains and supports the bond formation via oxygen atoms rather than via sulfur or nitrogen, as was the case with anti-tumor "soft" platinum complexes.

For determination of the values for thermodynamic parameters to define a mechanism of substitution, the substitution reaction between $[Ru(trpy)(bpy)Cl]^+$ and thiourea was studied at three different temperatures. Values for k_{obsd} as a function of different ligand concentrations and temperature are given in the Supplementary material (Table 4S). Calculated values for the rate constants at 288 and 298 K are $k_1^{288} = 0.31 \pm 0.02\ M^{-1}s^{-1}$ and $k_1^{298} = 0.92 \pm 0.04\ M^{-1}s^{-1}$. Finally, a negative value for the entropy of activation, $\Delta S^{\neq} = -105 \pm 5\ K^{-1}M^{-1}$, confirms the fact that the substitution reaction between $[Ru(trpy)(bpy)Cl]^+$ and thiourea undergoes an associative mechanism.

Hydrolysis of [Ru(trpy)(bpy)H_2O]$^{2+}$

Potentiometric measurements

The hydrolysis constants of the complex were determined by titration of 1.0, 1.5, and 2.0 mM solutions of $[Ru(trpy)(bpy)H_2O]^{2+}$ with NaOH. The acid-base chemistry was characterized by fitting the potentiometric data to various acid-base models. The best model, selected according to the above-mentioned method of calculation, was consistent with the deprotonation of water molecules and formation of hydroxo and μ-hydroxo complexes, as given in Eqs. (2–4). The calculated values of hydrolysis constants are given in Table 2.

$$\underset{(1,0,0)}{[Ru(trpy)(bpy)H_2O]^{2+}} \rightleftharpoons \underset{(1,-1,0)}{[Ru(trpy)(bpy)(OH)]^{+}} + H^{+} \quad (2)$$

$$\underset{(1,0,0)}{[Ru(trpy)(bpy)(OH)]^{+}} \rightleftharpoons \underset{(1,-2,0)}{[Ru(trpy)(bpy)(O)]} + H^{+} \quad (3)$$

A distribution diagram for $[Ru(trpy)(bpy)H_2O]^{2+}$ hydrolytic species is shown in Fig. 3. The complex ion $[Ru_2(trpy)_2(bpy)_2(OH)]^{3+}$ is present in the system in a pH range between 2.0 and 7.0, with the maximum in concentration at pH = 4. This ion is assumed to form through the dimerization of $[Ru(trpy)(bpy)H_2O]^{2+}$ and $[Ru(trpy)(bpy)(OH)]^+$ complexes via the hydroxo group as shown in Eq. (4). The $[Ru(trpy)(bpy)(OH)]^+$ ion begins to form at pH = 2 and reaches the maximum in concentration at pH = 8. The complex ion $[Ru(trpy)(bpy)(O)]^+$ begins to form at pH = 8, and its concentration increases with further increasing of pH. Very important is the fact that at physiological pH the aqua complex is completely converted into a hydroxo form.

Spectrophotometric titration

Spectral measurements were performed on $[Ru(trpy)(bpy)H_2O]^{2+}$ solutions in which the concentration of complex was kept constant while pH was varied by the addition of standard HCl or NaOH solutions, as appropriate. All UV–Vis spectra show evidence of an intensive band between 280 and 300 nm and another lower energy broad band between 450 and 470 nm (Fig. 4).

The spectral data were first evaluated with the aid of the computational program pHAb 2006 [49]. The calculations were carried out in the following way: the complexes found by potentiometry were included in pHAb calculations, and their stability constants were allowed to float. When the best fit of the spectra was achieved, the stability constants were varied one at a time simultaneously with variation of molar absorptivities. The accepted results of the calculation are given in Table 2. Along with the stability constants, in spectral calculations, the molar absorptivities of the complexes were calculated. Finally, the calculated spectra of different hydrolytic species are presented in Fig. 5.

$$\underset{(1,0,0)}{[Ru(trpy)(bpy)H_2O]^{2+}} + \underset{(1,-1,0)}{[Ru(trpy)(bpy)(OH)]^{+}} \overset{K}{\leftrightarrows} \underset{(2,-1,0)}{[Ru_2(trpy)_2(bpy)_2(OH)]^{3+}} + H_2O \quad (4)$$

Proton dissociation from an $[Ru(trpy)(bpy)H_2O]^{2+}$ complex proceeded in two steps ($pK_{a1} = 4.27$ and $pK_{a2} = 10.11$). The first step simply produced $[Ru(trpy)(bpy)(OH)]^+$ species, while the second one gave an unusual $[Ru(trpy)(bpy)(O)]^+$. The formation of complex with oxyl radical (O^-) has already been published [29, 48]. The equilibrium constant (K) for the dimerization reaction (Eq. 4) was determined to be $\log K = 3.45$ ($=\log\beta_{20\text{-}1} - \log\beta_{10\text{-}1}$).

The obtained values confirm a good agreement between potentiometric and spectrophotometric measurements.

Complex formation of [Ru(trpy)(bpy)H_2O]$^{2+}$ with HL (where HL = 5′-GMP or L-His)

The complex formation of $[Ru(trpy)(bpy)H_2O]^{2+}$ with ligands 5′-GMP or L-His, symbolized by HL, was studied

Table 2 Stability constants of $[Ru(trpy)(bpy)H_2O]^{2+}$–HL complexes (HL = 5′-GMP or L-His) formed in a 0.1 M $NaClO_4$ ionic medium at 298 K

Complexes	Log $\beta_{p,q,r} \pm \sigma$			
	Potentiometric	Spectrophotometric	Potentiometric	
	OH^-	OH^-	5′-GMP	L-His
$[Ru(trpy)(bpy)(OH)]^+$	−4.27(2)	−4.19(6)		
$[Ru(trpy)(bpy)(O)]^+$	−14.38(4)	−14.29(6)		
$[Ru_2(trpy)_2(bpy)_2(OH)]^{3+}$	−0.82(6)	–		
$[Ru(trpy)(bpy)(L)]^+$	–	–	8.69(9)	7.53(7)
$[Ru(trpy)(bpy)(HL)]^{2+}$	–	–	15.62(6)	14.20(6)
$[[Ru(trpy)(bpy)]_2(HL)]^{4+}$	–	–	–	18.69(13)
Statistics	$\chi^2 = 13.32$	$\chi^2 = 10.32$	$\chi^2 = 13.06$	$\chi^2 = 12.79$
	$s = 1.96$	$s = 1.73$	$s = 1.51$	$s = 2.73$

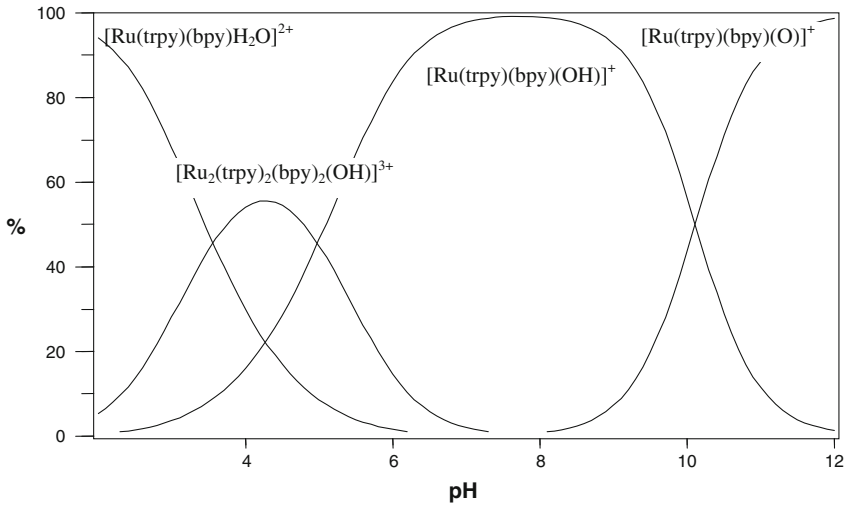

Fig. 3 Distribution diagram of $[Ru(trpy)(bpy)H_2O]^{2+}$ hydrolytic species in 0.1 M $NaClO_4$ ionic medium at 298 K ($C_{[Ru(trpy)(bpy)H_2O]^{2+}} = 2.00\,mM$)

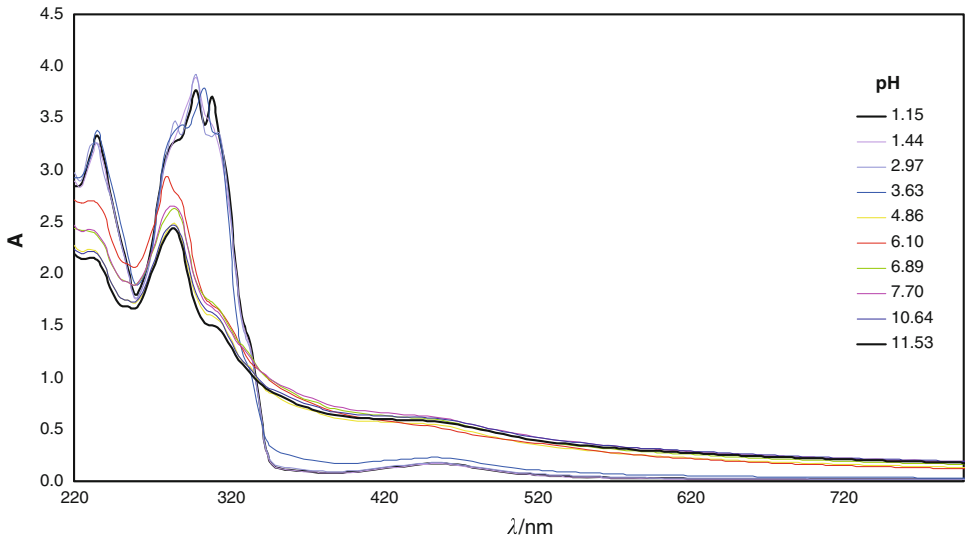

Fig. 4 The UV–Vis spectra of $[Ru(trpy)(bpy)H_2O]^{2+}$ at different pH values

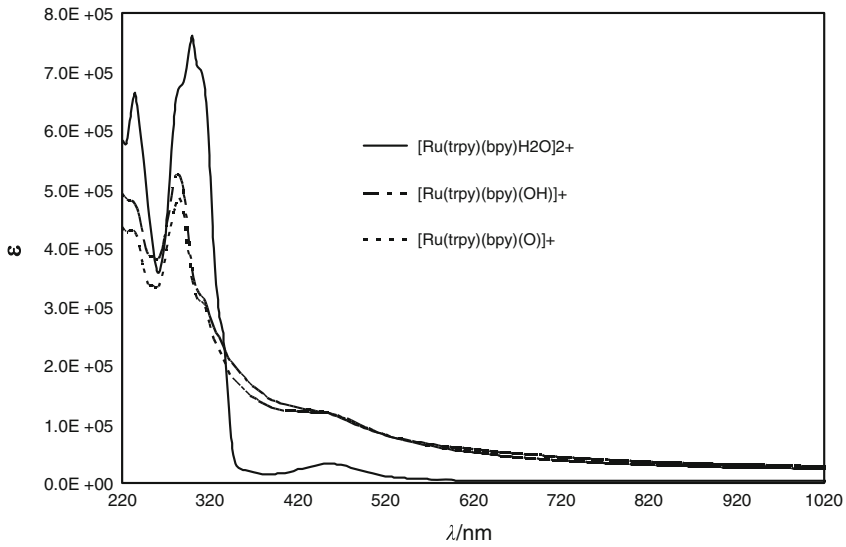

Fig. 5 The calculated spectra of $[Ru(trpy)(bpy)H_2O]^{2+}$ hydrolytic species

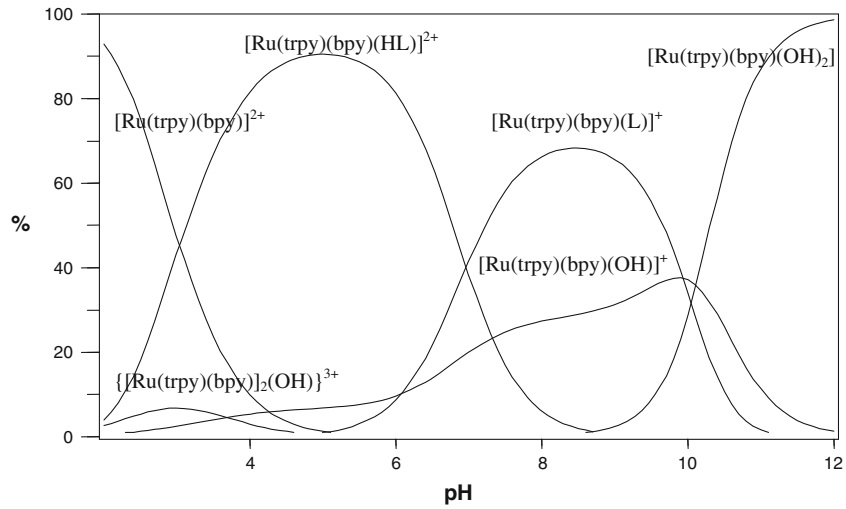

Fig. 6 Distribution diagram of $[Ru(trpy)(bpy)H_2O]^{2+}$–HL (where HL = 5′-GMP) species at a ligand-to-metal concentration ratio = 1:2 and total $[Ru(trpy)(bpy)H_2O]^{2+}$ concentration 1.0 mM

by potentiometric titrations in aqueous 0.1 M $NaClO_4$ at 298 K in concentration ratios of 1:1, 1:2, and 2:1 (metal:-ligand). The composition of the species of the general formula $M_pH_qL_r$ (where M = $[Ru(trpy)(bpy)H_2O]^{2+}$, HL = 5′-GMP or L-His) was calculated using the computer program Hyperquad 2006 [50]. The formation constants calculated for the selected systems are given in Table 2, while the distributions are shown in Figs. 6 and 7 (Supplementary Material, Figs. 3S and 4S).

It is well known that 5′-GMP can coordinate to metal ions via N1 and N7 positions, but binding through the N7 position in a neutral or weakly acidic medium has been verified. Also, depending mainly on the type of metal ion 5′-GMP has the possibility to coordinate via phosphate oxygen. However, the product formed usually undergoes isomerization to an N7 bounded form. L-His could be coordinated to metal ions via amino, imidazole, and carboxylate groups. In biological systems, there are numerous metalloproteins in which metal ions are bound to an L-histidine through N1 or N3 atoms of imidazole. However, this amino acid can coordinate some metal ions via carboxylate oxygen but the thermodynamically more stable product is always N3 or N1 bounded.

Figure 6 shows that complex formation between $[Ru(trpy)(bpy)H_2O]^{2+}$ and nucleotide 5′-GMP starts almost at the beginning of the potentiometric titration giving $[Ru(trpy)(bpy)(HL)]^{2+}$ (HL = 5′-GMP) complex with the maximum in concentration at a pH of about 5. The complex with deprotoneted nucleotide $[Ru(trpy)(bpy)(L)]^{+}$ starts to form at pH 5, and its maximum in concentration is reached at a pH of about 8.5. The pure hydrolytic complexes of $[Ru(terpy)(bipy)H_2O]^{2+}$ are present in solution in considerable amounts (Supplementary Material, Fig. 3S).

The distribution diagram of the $[Ru(trpy)(bpy)H_2O]^{2+}$ + L-His system, shown in Fig. 7, indicates that in highly acidic solution the complex [Ru(trpy)

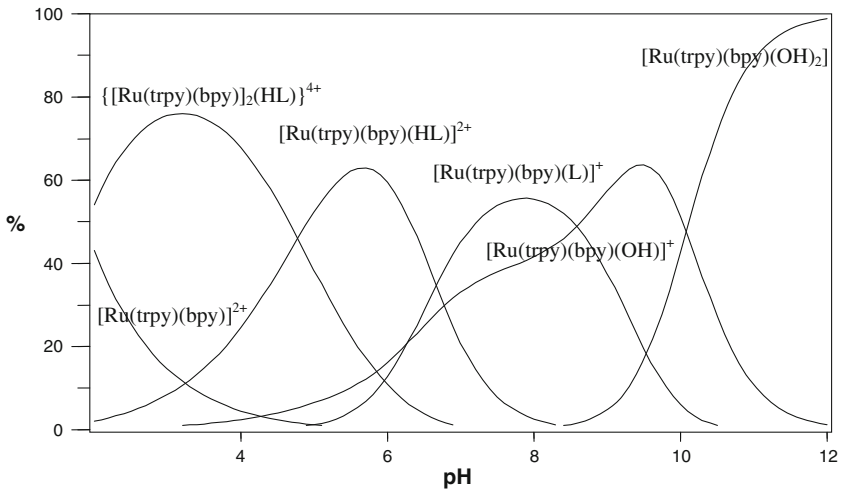

Fig. 7 Distribution diagram of $[Ru(trpy)(bpy)H_2O]^{2+}$–HL (where HL = L-His) species at a ligand-to-metal concentration ratio = 1:2 and total $[Ru(trpy)(bpy)H_2O]^{2+}$ concentration 1.0 Mm

$(bpy)]_2(HL)]^{4+}$ (HL = L-His) is dominant. Formation of the complex $[Ru(trpy)(bpy)(HL)]^{2+}$ starts at pH = 2 and reaches its maximum concentration at pH ≈ 6. The complex $[Ru(trpy)(bpy)(L)]^{+}$ starts to form at pH = 5 and reaches its maximum concentration at pH ≈ 8. The pure hydrolytic complexes $[Ru(trpy)(bpy)OH]^{+}$ and $[Ru(trpy)(bpy)H_2O]^{2+}$ are also present in considerable amounts at pH > 9 (Supplementary Material, Fig. 4S).

Calculated stability constants for the species (Table 2) show that complexes with 5′-GMP are more stable than complexes with L-His. Taking into account the voluminosity of 5′-GMP and L-His as well as the bulkiness of inert ligands in the structure of $[Ru(trpy)(bpy)H_2O]^{2+}$, it can be concluded that here intramolecular hydrogen bonds play a significant role for the stability of products. In both systems studied, mixtures of $[Ru(trpy)(bpy)(HL)]^{2+}$ and $[Ru(trpy)(bpy)(L)]^{+}$ complexes are present at physiological pH. This observation could be very important for further understanding of interactions between Ru(II) complexes and bio-molecules structurally similar to those investigated in this work.

Conclusions

We present results for the rate constants of the substitution reactions between $[Ru(trpy)(bpy)Cl]^{+}$ with the nucleophiles 5′-GMP, L-His, Tu, and DMSO obtained by conventional spectrophotometry. The best nucleophile is thiourea. The order of reactivity for selected ligands is: Tu > 5′- GMP > L-His > DMSO. This is in a good agreement with their electronic, structural, and chemical characteristics. The associative mode of substitution is confirmed for the substitution reaction with Tu. The monofunctional complex $[Ru(trpy)(bpy)Cl]^{+}$ with 5′-GMP and L-His forms very stable products, especially with 5′-GMP. Finally, knowledge of the composition and stability of the species in the studied systems, especially at physiological pH, could contribute to a better understanding of some interactions in biological systems.

Experimental

$RuCl_3 \cdot xH_2O$, a starting salt for other synthesis, was purchased from Acros Organics. The ligands thiourea (Tu), dimethylsulfoxide (DMSO), guanosine-5′-monophosphate sodium salt (5′-GMP), 2,2′-bipyridine (bpy) (Acros Organics), L-histidine (L-His) (Merck), and 2,2′:6′,2″-terpyridine (trpy) (Sigma Aldrich) were used without further purification. All other chemicals were of the highest purity commercially available.

The solutions of complex and ligands for kinetic measurements were prepared in 0.1 M $NaClO_4$. To prevent hydrolysis of the Ru(II) complex 20 mM NaCl was added to the solution. Ligand stock solutions were prepared shortly before use by dissolving the chemicals in purified, deionized water. The ionic strength of the solutions was adjusted to 0.10 M using $NaClO_4$ (Merck, p.a.). The pH of the solutions was adjusted using $HClO_4$ and NaOH. The sodium hydroxide solution was prepared from concentrated volumetric solution (Merck, p.a.) by diluting with freshly boiled double-distilled water, cooled under constant flow of purified nitrogen. The alkali concentration was checked by titration against potassium hydrogenphthalate. For the preparation of perchloric acid solution, $HClO_4$ (Merck, "Suprapure", p.a.) was used. The concentration of the resulting solution was determined by potentiometric titration against tris(hydroxymethyl)aminomethane. The concentration of $HClO_4$ solution was 0.0923 M, and the concentration of NaOH solution was 0.0982 M. Nitrogen gas, used for the stirring of solutions and providing an inert

atmosphere during the titrations, was purified by passing it through 10 % NaOH, then 10 % H_2SO_4, and finally distilled water. Ultrapure water was used for preparation of all solutions.

Preparation of the complexes

The complex [Ru(trpy)(bpy)Cl]Cl was prepared according to the published procedures [29, 30, 51]. Ruthenium salt $RuCl_3 \cdot xH_2O$ (assuming x = 3; 0.20 g, 0.89 mmol) was dissolved with stirring and heating in 30 cm^3 absolute ethanol. After dissolution, 0.21 g ligand 2,2′:6′,2″-terpyridine (0.89 mmol) was added and the mixture refluxed about 3 h. Then, an equimolar amount of 2,2′-bipyridine (0.14 g, 0.89 mmol) was injected into the flask. The system was refluxed for 4 h with the addition of an excess of LiCl (1.5 mmol) and triethylamine (0.4 mmol) as a reductant. Under this procedure, the reduction of Ru(III) occurs, and the final product is the Ru(II) complex. Finally, when the mixture was cooled to room temperature, the red-orange precipitate that formed was filtered, washed with ethanol and ether, and air-dried. The chemical analysis, ^{1}H NMR and UV–Vis spectroscopic data were in good agreement with previously published results.

The chlorido complex was converted into the aqua analog $[Ru(trpy)(bpy)H_2O]^{2+}$ by addition of two equivalents of $AgClO_4$, heating the mixture to 50–60 °C for 1 h and removing the formed precipitate AgCl by filtration through a 0.10-μm pore membrane filter. Great care was taken to ensure that the resulting solution was free of Ag^+ ions and that the chlorido complex had been converted completely into the aqua species.

Kinetic measurements

UV–Vis kinetic measurements were carried out on a Perkin-Elmer Lamda 35 double-beam spectrophotometer equipped with thermostated 1.00-cm quartz Suprasil cells. The kinetics of the substitution reactions of $[Ru(trpy)(bpy)Cl]^+$ with nucleophiles 5′-GMP, L-His, Tu, and DMSO were studied by following the changes in absorbance at a suitable wavelength as a function of time. The working wavelength for each reaction system was determined by recording the spectra of the reaction mixture over the wavelength range between 220 and 450 nm. These values are presented in the Supplementary material (Tables 1S–4S). The reactions were initiated by mixing equal volumes of complex and nuclephile solutions (1.5 cm^3) in the quartz cuvette. The concentration of ligand was always large enough (at least a tenfold excess) to provide pseudo first-order conditions. The kinetic traces gave an excellent fit to a single exponential (Supplementary Material Fig. 2S).

Potentiometric measurements

Potentiometric titrations were carried out in a double-walled glass vessel, thermostatted at 298 ± 0.1 K. Measurements were made by a Mettler Delta 350 digital pH meter (precision ± 0.01 mV or ±0.002 pH units) equipped with a combination glass electrode. This electrode was calibrated using standard buffer solutions of pH 4 and 7, obtained from Sigma. The Metrohm Dosimat model 665 automatic burette with an anti-diffusion tip was used for delivery of the titrant. The ionic strength of all test solutions was adjusted to 0.1 M with $NaClO_4$.

To reduce the concentration of the hydrogen ions, the alkali was added stepwise from an autoburette in small aliquots (0.005–0.01 cm^3). The potential was monitored after each addition of titrant. The titration protocol was chosen in such a way that the hydrolysis and complex formation reactions would proceed in conditions as close as possible to true equilibrium. The potential readings were taken every 2 min until steady values to ±0.1 mV min^{-1} were obtained. The average equilibration time for each point was 5 min at the beginning of the titration and 10 min when the complexation occurred. Stability constants were determined by titrating 1.0 and 2.0 mM solutions of complex with standard NaOH solution. The formation constants of complexes formed were determined by titrating the solution mixture of Ru(II) complex (2 mM) and ligand (5′-GMP or L-His) in concentration ratios of 1:1 and 1:2 (complex:ligand). The titration solution mixtures had a volume of 20.0 cm^3. All titrations were carried out in duplicate. The agreement between duplicate titration was better than 1 %.

Spectrophotometric titrations

Spectral measurements were made on a model Lambda 35 double-beam UV–Vis spectrophotometer (Perkin-Elmer, USA). Operational parameters were: scan speed: 2 nm/s; slit width: 0.3 nm; photometric sensitivity: 0.2 abs. units. Matching pairs of 1-cm quartz cuvettes were used for measuring the spectra. Spectral measurements were made on solutions in which the concentration of $[Ru(trpy)(bpy)H_2O]^{2+}$ complex was 0.02 mM while the pH was varied between 1.15 and 11.53. The pH of the test solutions was measured with a combined electrode, which was calibrated using standard buffer solutions of pH 4 and 7 obtained from Sigma. Spectra of the test solutions were recorded in 220–1.020-nm wavelength intervals.

Data treatment

The species formed in the studied systems were characterized by the general equilibrium:

$$pM + qH + rL = M_pH_qL_r; \quad \beta_{p,q,r}$$
$$(M = [Ru(trpy)(bpy)H_2O]^{2+}; \quad HL = 5\prime - GMPH \text{ or } L - HisH) \tag{5}$$

and the corresponding constants are given by:

$$\beta_{p,q,r} = \frac{[M_pH_qL_r]}{[M]^p[H]^q[L]^r} \tag{6}$$

where L is the deprotonated molecule of the ligand.

In this study, the convention has been adopted whereby a complex containing a metal ion, M, proton, H, and ligand, L, takes the general formula $M_pH_qL_r$, where p, q, and r are the stoichiometric indices of the components in the complex. A negative value for q refers to proton removal or hydroxide ion addition during formation of the complex. Thermodynamically, these two processes are equivalent and cannot be distinguished by potentiometry. The equilibrium constant for the formation of this complex from its components is then designated by the symbol $\beta_{p,q,r}$. For simplicity, the charges of these species are omitted.

Three kinds of equilibria should be considered in the present study: (a) protonation of the ligand anion, (b) hydrolysis of the $[Ru(trpy)(bpy)H_2O]^{2+}$ ion, and (c) general three-component equilibria, which include the case $q = 0$, i.e., the formation of pure binary complexes of $[Ru(trpy)(bpy)H_2O]^{2+}$. The overall protonation constants of 5′-GMP and L-His anion were taken from the literature [52]. The stability constants of hydrolytic complexes of $[Ru(trpy)(bpy)H_2O]^{2+}$ ion were determined in separate experiments. Thus, in evaluation of three-component equilibria (c), the binary models (a) and (b) were considered as known. The concentration stability constants of the complexes, $\beta_{p,q,r}$ were calculated with the aid of the suite of computer programs Hyperquad 2006 [50]. In Hyperquad calculations, the identity and stability of complexes that give the best fit to the experimental data were determined by minimizing the objective function U, defined by:

$$U = \Sigma_i W_i (E_{obs} - E_{calc})^2 \tag{7}$$

where E_{obs} and E_{calc} refer to the measured potential calculated from Eq. 5. The weighting factor W_i is defined as the reciprocal of the estimated variance of the measurement.

$$W_i = 1/\sigma^2 = 1/[\sigma_E^2 + (\delta E/\delta V)^2 \sigma_V^2] \tag{8}$$

where σ_E and σ_V are the estimated variances of the potential and volume readings, respectively. The quality of the fit was judged by the values of the sample standard deviation S and the goodness of fit χ^2 (Pearson's test). At $\sigma_E = 0.1$ mV (0.001 pH error) and $\sigma_V = 0.005$ cm^3, the values of S in different sets of titrations were between 1.0 and 1.8, and χ^2 was between 11.0 and 13.0. The scatter of residuals ($E_{obs} - E_{calc}$) versus pH was reasonably random, without any significant systematic trends, indicating a good fit of the experimental data. The finally accepted sets of complexes are given in Table 2. Statistical parameters, which determine the quality of the fit, are also given.

The spectrophotometric data were evaluated with the aid of the program pHAb 2006 [49] (which also belongs to the Hyperquad family but possesses some additional and improved features) and the program Hyperquad 2006, which can treat spectral data. Potentiometric and spectrometric data were made consistent by concomitantly evaluating both kinds of data with the aid of the Hyperquad 2006 suite of programs using the best model obtained in separate treatments. Distribution of species in solution was calculated by the program Hyss 2006 [53].

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