Available online at www.sciencedirect.com
SCIENCE DIRECT
Inorganica Chimica Acta 359 (2006) 2888–2895
Inorganica Chimica Acta
www.elsevier.com/locate/ica

Binding of cysteine and glutathione to Ru(II) and Ru(III) centers: Formation and products reactivities

José Ribamar O. Filho [a,b], Welter C. Silva [a,c], José Clayston M. Pereira [a], Douglas W. Franco [a,*]

[a] *Instituto de Química de São Carlos, Departamento de Química e Física Molecular, Universidade de São Paulo, Caixa Postal 780, CEP 13560-970, São Carlos-SP, Brazil*
[b] *Departamento de Química, Centro Tecnológico, Universidade Federal do Maranhão, CEP 65085-580, São Luís-MA, Brazil*
[c] *Departamento de Química, Centro de Ciências da Natureza, Universidade Federal do Piauí, CEP 64049-550, Teresina-PI, Brazil*

Received 27 October 2005; accepted 20 November 2005
Available online 17 February 2006

Dedicated to Brian James.

Abstract

The interactions of L-cysteine (CysSH) and the tripeptide glutathione (γ-L-glutamate-L-cysteine–glycine, GSH) molecules with the *trans*-$[Ru(NH_3)_4(4\text{-pic})(H_2O)]^{2+}$ ion (4-pic = 4-picoline) have been investigated by cyclic voltammetry, UV–Vis, ^1H NMR, and EPR spectroscopies. Experimental data strongly suggest that the sulfur atom of the SH group, present in CysSH and in GSH molecule, is the binding site of these ligands to the *trans*-$[Ru(NH_3)_4(4\text{-pic})(H_2O)]^{2+}$ species. The *trans*-$[Ru^{II}(NH_3)_4(4\text{-pic})(CysSH)]^{+3}$ and *trans*-$[Ru^{II}(NH_3)_4(4\text{-pic})(GSH)]^{3+}$ ions showed a maximum absorption band at 346 nm ($\varepsilon = 5.0\times10^3$ and 5.4×10^3 M^{-1} cm^{-1}, respectively) at pH 1.0 attributed to the transition $4d_\pi\ Ru^{II} \rightarrow \pi^*(4\text{-pic})$. Solutions containing the CysSH and GSH complexes exhibited a reversible electrochemical behavior at pH 7.2 and 7.8 with values of $E_{1/2}$ equal to −0.378 V and −0.400 V (SCE), respectively, attributed to the Ru(III)/Ru(II) redox couple. The pK_a values measured from changes in the electronic and voltammetric spectra of *trans*-$[Ru^{II}(NH_3)_4(4\text{-pic})(L)]^{3+/+2}$ ions as a function of changes in the hydrogen ion concentration of the solution are, respectively, 5.6 ± 0.1 and 6.6 ± 0.2 for L = CysSH and GSH. The second-order specific rate constants (k_1 and k_{-1}) and equilibrium constants (K_{eq}) values for the reaction (1) (LH = CysSH or GSH) calculated from spectrophotometric data (25 ± 0.2 °C, $\mu = 0.20$ M $NaCF_3COO/CF_3COOH$) are $k_1 = (4.7\pm0.2)\times10^{-2}$ M^{-1} s^{-1}; $k_{-1} = (4.4\pm1.0)\times10^{-4}$ s^{-1} and $K_{eq(II)} = (1.2\pm0.2)\times10^2$ $M^{-1}$to CysSH system and $k_1 = (5.6\pm0.2)\times10^{-2}$ M^{-1} s^{-1}, $k_{-1} = (5.3\pm1.4)\times10^{-4}$ s^{-1} and $K_{eq(II)} = (1.1\pm0.4)\times10^2$ M^{-1} to GSH system.

$$trans\text{-}[Ru^{II}(NH_3)_4(4\text{-pic})(OH_2)]^{2+} + LH \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} trans\text{-}[Ru^{II}(NH_3)_4(4\text{-pic})(LH)]^{n+} + H_2O \quad (1)$$

The K_{eq} value for reaction (1) at pH 4.0 calculated from ^1H NMR data are $1.2\pm0.1\times10^2$ M^{-1} for both CysSH and GSH and is shown to be in good agreement with the K_{eq} calculated from the UV–Vis measurements (pH 4.0). Combining the $K_{eq(II)}$ and $E^{0'}_{Ru(III)/Ru(II)}$ data, the equilibrium constants $K_{eq(III)}$ values for reactions (2) and (3) were calculated to be 8.4×10^9 and 1.4×10^9 M^{-1} for the $CysS^-$ and GS^- derivatives, respectively.

$$trans\text{-}[Ru^{III}(NH_3)_4(4\text{-pic})(OH_2)]^{3+} + CysS^- \overset{K_{eq(III)}}{\rightleftharpoons} trans\text{-}[Ru^{III}(NH_3)_4(4\text{-pic})(CysS)]^{+2} + H_2O \quad (2)$$

$$trans\text{-}[Ru^{III}(NH_3)_4(4\text{-pic})(OH_2)]^{3+} + GS^- \overset{K_{eq(III)}}{\rightleftharpoons} trans\text{-}[Ru^{III}(NH_3)_4(4\text{-pic})(GS)]^{+} + H_2O \quad (3)$$

* Corresponding author. Tel.: +55 16 3373 9970; fax: +55 16 3373 9976.
E-mail address: douglas@iqsc.usp.br (D.W. Franco).

0020-1693/$ - see front matter
doi:10.1016/j.ica.2005.11.044

CysSH and GSH complexes are oxidized by H_2O_2 yielding their oxidized deprotonated analogs. The *trans*-$[Ru^{III}(NH_3)_4$-(4-pic)(GS)$]^{3+}$ ion at pH 1.0 exhibits a ligand to metal charge transfer (λ_{max} at 518 nm, $\varepsilon \sim 2.0 \times 10^3$ M^{-1} cm^{-1}) attributed to the transition $^*S \rightarrow 4d_\pi$ Ru^{III} and a well defined EPR spectrum $g_\perp = 2.27$ and $g_\parallel = 1.91$ in the 2700–3800 G region.

Keywords: Ruthenium complexes; Glutathione; Cysteine; Equilibrium constants; NMR

1. Introduction

Metabolic conditions such as hypoxia and low pH in tumor cells provide a more reductive environment than the normal tissue [1]. Ruthenium complexes have been described [2–8] as anticancer compounds due to their ability to coordinate to the DNA of tumor cells after being activated by reduction in vivo. Thus, tumor cell metabolism favors the presence of Ru(II) relative to Ru(III), thus increasing selective tumor toxicity [2,9].

In addition, ruthenium complexes exhibit promising results in the treatment of autoimmune diseases [10] and in the prevention of graft rejection in transplantation. Recently, it was reported [2] that ruthenium complexes coordinate to ammines and N-heterocyclic ligands such as $[Ru(NH_3)_5(4\text{-pic})]Cl_2$ (4-pic = 4-picoline), which shows an immunosuppressant activity very superior to that used clinically, cyclosporin, [11] thus inhibiting the proliferation of T lymphocyte with less toxicity. Since the potential redoxes of the Ru(III)/Ru(II) couples are in the range of 0.1–0.4 V, their immunosuppressant activity could be related to the reduction of Ru(III) by biomolecules such as glutathione [11,12].

The $[Ru(NH_3)_5Cl]Cl_2$, $[Ru(NH_3)_6]Cl_3$ and $[Ru(NH_3)_5$-(4-pic)$]Cl_3$ [12] complexes are already being employed as models in studying chemical and biological interactions with glutathione (GSH). According to the literature report [12], glutathione favors the binding of Ru complexes to DNA when the ratio is [GSH]/[Ru] < 1. When this ratio is higher than 1, the coordination of Ru to DNA is inhibited. Furthermore, the above ruthenium complexes can easily lose ammonia and 4-picoline when the concentration of GSH exceeds 8-fold over that of ruthenium concentration [12]. This would be a limiting factor for its potential clinical use and therefore deserves a further investigation.

Based on growing interest in the understanding of the interaction between ruthenium complexes and biological reductors [2,13–16], the scope of this paper is to contribute to the enlightenment of the reaction between *trans*-$[Ru(NH_3)_4(4\text{-pic})(OH_2)]^{2+}$ and glutathione and cysteine molecules.

The reactions between nitrosyl compounds *trans*-$[Ru(NH_3)_4(4\text{-pic})(NO)](BF_4)_3$ and CysSH and GSH are already being studied at our laboratory. Since complexes of Ru(II) and Ru(III) with CysSH and GSH can be formed during these reactions, therefore, the knowledge of their chemistry becomes relevant on its own right. This was an additional incentive undertaken in this study.

2. Experimental procedure

2.1. Chemicals and reagents

Organic solvents as ethanol, acetone, and ethyl ether were purified as described in the literature [17]. Doubly distilled water was used throughout the experiments. Ruthenium trichloride ($RuCl_3 \cdot XH_2O$ – Aldrich) was the starting material for the synthesis of the ruthenium complexes. Ionic strength was adjusted with CF_3COONa. All other chemical reagents were of analytical grade purity (Aldrich and Merck) and used without further purification.

The compounds $[Ru(NH_3)_5Cl]Cl_2$ [18], *trans*-$[Ru(NH_3)_4$-$SO_2Cl]Cl$ [19] and *trans*-$[Ru(NH_3)_4SO_4(4\text{-pic})]Cl$ [20], were prepared according to the methods published in the literature and their characteristics checked by UV–Vis, EPR and IR spectroscopies and electrochemical techniques.

2.2. Instruments

The ^1H NMR spectra were measured in D_2O solutions using tetramethylsilane (TMS) as internal standard and recorded on a Bruker AC-200 spectrophotometer.

The EPR spectra were recorded on a X-band Bruker spectrometer model ESP 300E coupled to a standard cavity. All experiments were carried out at liquid nitrogen temperature (77 K) and the samples used were both solid powders and solutions frozen in an ethylene glycol (70%) water (30%) mixture.

Cyclic voltammetry (CV) experiments were performed with a PARC system model 173 potentiostat/galvanostat, a model 175 universal programmer, and a RE 0074X-Y recorder and a model 264A polarographic analyzer. The three-electrode system consisted of a saturated calomel electrode (SCE) as the reference electrode, a working glassy carbon electrode, and a platinum wire as the auxiliary electrode under controlled argon atmosphere.

UV–Vis measurements were performed in a 1.0 cm quartz cell on a Hitachi U3501 spectrophotometer. During the kinetics experiments, the temperature was controlled within ±0.2 °C using a Tecnal TE 184 thermostat.

2.3. Measurements

All manipulations were carried out in the absence of oxygen [21]. The inert gas (argon or nitrogen with high purity 99.9%) was deoxygenated (Cr^{II}) prior to use [21]. All the complexes were stored under vacuum and protected from light. UV–Vis, NMR, and EPR spectra of air-sensitive