Journal of Inorganic Biochemistry 129 (2013) 94–101

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Exploring the effect of the ligand design on the interactions between $[Ru(\eta^5\text{-}C_5H_5)(PPh_3)(N,O)][CF_3SO_3]$ complexes and human serum albumin

Tânia S. Morais, Filipa C. Santos, Leonor Corte-Real, M. Helena Garcia *

Centro de Ciências Moleculares e Materiais, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 23 April 2013
Received in revised form 10 September 2013
Accepted 10 September 2013
Available online 18 September 2013

Keywords:
Ruthenium(II)
Heteroaromatic ligand
Organometallic
Human serum albumin
Warfarin
Fluorescence

ABSTRACT

Ruthenium complexes hold a great potential in chemotherapy as an alternative to the classical platinum based drugs. The organometallic compounds studied in the present work were previously found to exhibit important anticancer activities. Here we have investigated the binding of three ruthenium compounds, namely $[Ru(\eta^5\text{-}C_5H_5)(PPh_3)(bopy)][CF_3SO_3]$ **1**, $[Ru(\eta^5\text{-}C_5H_5)(PPh_3)(2\text{-}ap)][CF_3SO_3]$ **2**, and $[Ru(\eta^5\text{-}C_5H_5)(PPh_3)(isoquinpk)][CF_3SO_3]$ **3** (bopy = 2-benzoylpyridine; 2-ap = 2-acetylpyridine; isoquinpk = 1-isoquinolinyl phenyl ketone) to fatty acid human serum albumin (HSA) and fatty acid-free human serum albumin (HSA^{faf}) at physiological pH 7.4. The influence of the substituent groups on the heteroaromatic (N,O) coordinated ligand was also studied by fluorescence spectroscopy to get information about this binding. The Stern–Volmer quenching constants (K_{SV}) were calculated at 293, 298 and 310 K, with the corresponding thermodynamic parameters ΔG, ΔH and ΔS as well. The fluorescence quenching method was used to determine the number of binding sites (n) and association constants (K_a) at the same temperatures. The binding site to HSA was confirmed by competitive studies of the ruthenium compounds with warfarin.

1. Introduction

Studies of the interaction between drugs and plasma proteins have been an object of interest in medicine. Human serum albumin (HSA) is the most abundant protein in plasma. HSA displays an extraordinary ligand binding capacity, which makes it the most important drug carrier protein [1]. The main function of albumin is to transport endogenous and exogenous compounds and fatty acids, as well as a broad range of drug molecules to its targets [2,3]. In addition, HSA often increases the solubility of hydrophobic drugs in plasma [4] and is a valuable biomarker of many diseases, like cancer, rheumatoid arthritis, ischemia, postmenopausal obesity and diseases that need monitoring of the glycemic control [1].

The well-known HSA structure consisting of a single polypeptide chain with 585 amino acid residues, presents a three-dimensional structure described in terms of three homologous chains (I, II and III), each of them formed by two subdomains (A and B). Site I (subdomain IIA) and site II (subdomain IIIA), which pockets show quite different geometries, have important physiological effects in the binding to a large number of drugs. Since HSA serves as a drug transport carrier, the knowledge of the kind of interaction between drugs and plasma proteins is of major importance to understand the drug pharmacokinetics and pharmacodynamics.

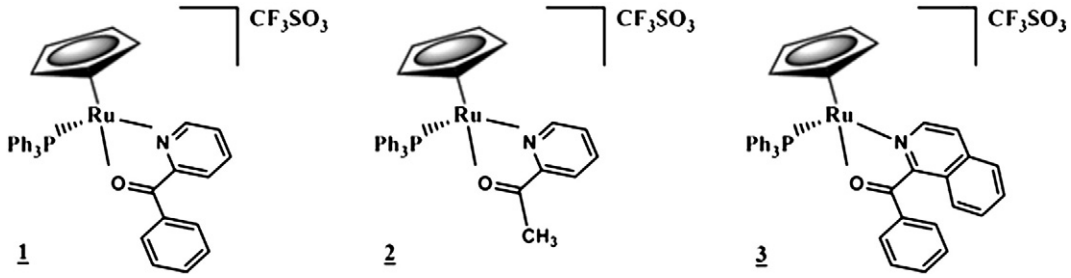
Ruthenium compounds were found to present some features such as low toxicity, good selectivity for tumors, inhibition of the antimetastic progression and antiangiogenic properties that make them very interesting for drug development [5–11].

In our approach to this field, we pioneered the report of the strong cytotoxic activity against LoVo and MiaPaca cancer cell lines of two half sandwich cationic complexes derived of "$Ru(\eta^5\text{-}C_5H_5)$" fragment [12]. The promising results displayed by these two compounds prompted us to continue the exploitation of this family of compounds that also have been revealing potent cytotoxicity against several human tumor cell lines (A2780, A2780CisR, MCF7, HT29, MDAMB231, PC3, HL60) with IC_{50} values much lower than those found for cisplatin [13–17].

The three ruthenium compounds studied here, of general formula $[Ru(\eta^5\text{-}C_5H_5)(PPh_3)(N,O)]$ $[CF_3SO_3]$ [17], belong to a sub-family of compounds where (N,O) is a heteroaromatic bidentate ligand coordinated by the N and O atoms. These compounds revealed an exceptional activity in the human cancer lines mentioned above with IC50 values in the nanomolar range. The important activity of these compounds against the highly glycolytic cell lines MCF7 and MDAMB231, the later one presenting highly metastatic properties is worthy of mention [17]. Curiously, it was also found that small differences on the (N,O) heteroaromatic ligand considerably influence the cytotoxic activity of these compounds.

* Corresponding author. Tel.: +351 217500972; fax: +351 217500088.
E-mail address: lena.garcia@fc.ul.pt (M.H. Garcia).

0162-0134/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jinorgbio.2013.09.008



Scheme 1. Chemical structure of ruthenium complexes: $[Ru(\eta^5\text{-}C_5H_5)(PPh_3)(bopy)][CF_3SO_3]$ **1**, $[Ru(\eta^5\text{-}C_5H_5)(PPh_3)(2\text{-}ap)][CF_3SO_3]$ **2**, $[Ru(\eta^5\text{-}C_5H_5)(PPh_3)(isoquinpk)][CF_3SO_3]$ **3.**

The important potentialities of these compounds as cytotoxic agents led us to pursue our studies with three compounds of this family, in order to investigate their ability to be transported by human serum albumin in blood, and how small differences on the structure of the heteroaromatic ligand (see structures depicted on Scheme 1) could affect the binding capacity of each compound to albumin. Thus, compounds $[Ru(\eta^5\text{-}C_5H_5)(PPh_3)(bopy)]$ $[CF_3SO_3]$ **1**, $[Ru(\eta^5\text{-}C_5H_5)(PPh_3)(2\text{-}ap)][CF_3SO_3]$ **2**, $[Ru(\eta^5\text{-}C_5H_5)(PPh_3)(isoquinpk)]$ $[CF_3SO_3]$ **3** (where bopy = 2-benzoylpyridine; 2-ap = 2-acetylpyri- dine; isoquinpk = 1-isoquinolinyl phenyl ketone) were prepared and the interaction studies with two variants of albumin (fatty acid HSA and fatty acid-free HSA) in aqueous solutions were performed at three temperatures under physiological conditions using fluorescence spectroscopy.

2. Materials and methods

2.1. Materials

All chemicals were analytical or reagent grade and were used as received from chemical suppliers, unless otherwise stated. The ruthenium complexes (Scheme 1) were synthesized as we previously described [17] under dinitrogen atmosphere using current Schlenk techniques. The doubly purified water used in all experiments was from a Millipore® system. Albumin and warfarin samples were purchased from Sigma Aldrich; fatty acid HSA (≥96% lyophilized powder, A1653), fatty acid-free HSA (approx. 99%, lyophilized powder, A3782) and warfarin (A2250) were used as received.

2.2. Preparation of stock solutions

Human serum albumin was dissolved in 10 mM Hepes buffer (pH 7.4). The protein concentration was determined spectrophotometrically using the molar absorption coefficient of 36,500 M^{-1} cm^{-1} at 280 nm [18]. Ruthenium solutions were first prepared in 1 mM concentration in DMSO. A series of drug-protein solutions were prepared by mixing protein solutions with different concentrations of drug solutions. After addition of the drug solution to the protein solution, the concentration of DMSO was 1%. For fluorescence determination, the final protein concentration was 2.5 μM, and the drug concentrations were 0, 0.6, 1.2, 1.8, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, 42.5 and 45 μM. The mixtures were stirred to ensure the formation of a homogeneous solution and then stood in an incubator at 37 ºC (310 K) for 24 h. The reference solutions were also prepared according to the above procedures and without protein.

2.3. Fluorescence spectroscopic measurements

Steady state fluorescence was executed with a Fluorolog-3 spectrofluorimeter (Horiba Jobin Yvon) equipped with a 1.0 cm quartz cell. The excitation and emission slit widths were fixed at 4.0 nm, the excitation wavelength was set at 295 nm to excite the tryptophan 214 residue selectively, the emission spectra were recorded from 305 to 550 nm. Buffer solutions of ruthenium complex in corresponding concentrations were used as reference when measuring the fluorescence spectra of protein-complex mixtures. The fluorescence intensities were corrected for the absorption of the exciting light and reabsorption of the emitted light to decrease the inner filter effect [19,20] using UV–visible absorption data recorded for each sample on a Jasco V-660 spectrophotometer in the range of 250/300 to 800/900 nm with 1 cm path quartz cells.

For time-resolved measurements by the single photon counting technique a nanoLED N-289 (Horiba Jobin Yvon) was used for the excitation of HSA, and the emission wavelength was 340 nm with a 15 nm bandwidth. Ludox® (from Sigma-Aldrich) was used as the scatterer to obtain the instrumental response function. The program TRFA data processor version 1.4 (Minsk, Belarus) was used for the analysis of the experimental fluorescence decays. The fluorescence intensity decays were analyzed by fitting a sum of exponentials according to

$$I(t) = \sum_{i=1}^{n} \alpha_i \exp\left(-\frac{t}{\tau_i}\right) \quad (1)$$

where α_i and τ_i are the normalized amplitude and lifetime of component i, respectively. The mean fluorescence lifetime was obtained through Eq. (2):

$$\langle\tau\rangle = \sum \alpha_i \tau_i^2 / \sum \alpha_i \tau_i. \quad (2)$$

The changes in quantum yield by processes affecting fluorescence life-time were evaluated from the amplitude-weighted mean fluorescence lifetime, which is given by

$$\bar{\tau} = \sum_{i=1}^{n} \alpha_i \tau_i. \quad (3)$$

2.4. Site marker competitive studies

Binding location studies between HSA and ruthenium complexes in the presence of one classical site marker – warfarin – were performed using fluorescence. The concentrations of HSA and warfarin were kept in equimolar, then Ru complex was added to {HSA-warfarin} adduct. The excitation wavelength of 305 nm was chosen and the emission spectra were recorded from 315 to 550 nm.

3. Results and discussion

3.1. Fluorescence quenching of HSA by ruthenium complexes

Fluorescence spectroscopy is an effective technique used to explore the interaction between small molecules and biomolecules such as proteins. Proteins contain three aromatic amino acid residues – tryptophan, tyrosine and phenylalanine – which may contribute to their intrinsic fluorescence [21]. In fact, the intrinsic fluorescence of HSA is essentially contributed by tryptophan residue alone, because of the very low quantum yield of phenylalanine and tyrosine [21,22]. When the excitation wavelength of albumin was selected at 280 nm both tryptophan and tyrosine amino acid residues give fluorescence emissions. However,