Journal of Luminescence 169 (2016) 115–120

Contents lists available at ScienceDirect

Journal of Luminescence

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

Studying the interaction between trinuclear ruthenium complexes and human serum albumin by means of fluorescence quenching

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ARTICLE INFO

Article history:
Received 15 January 2015
Received in revised form
30 July 2015
Accepted 21 August 2015
Available online 7 September 2015

Keywords:
Triruthenium μ-oxo nitrosyl complexes
Human serum albumin
Fluorescence quenching
Stern–Volmer

ABSTRACT

This work reports for the first time a systematic investigation of the interaction between HSA and trinuclear ruthenium complexes, by using fluorescence spectroscopy and the Stern–Volmer model. The compounds investigated have general formula $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(L)]PF_6$, where L=NO or H_2O and 3-pic=3-methylpyridine. For both complexes it was observed that the increase of K_{sv} values (in the order of $10^4\ M^{-1}$) with increasing temperature, signaling a dynamic quenching of HSA fluorescence. However, analysis of the quenching rate constants and K_b values shows that the contribution of the static quenching is significant. Particularly in the case of the nitrosyl complex, the relatively high value of K_b observed ($178.44 \times 10^3\ M^{-1}$, 308 K) suggests that this compound can be efficiently stored and transported in the body by HSA. The interaction of the complexes with HSA is spontaneous ($\Delta G < 0$). Complex $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$ displays interaction with HSA by hydrophobic forces (ΔH=215 kJ mol^{-1} and ΔS=796 J $mol^{-1}\ K^{-1}$), likely because of the nitrosyl lipophilicity, while complex $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(H_2O)]PF_6$ is involved in the formation of hydrogen bonds with HSA (ΔH=−75.5 kJ mol^{-1} and ΔS=−231 J $mol^{-1}\ K^{-1}$), through its aquo ligand.

1. Introduction

Human serum albumin (HSA) is produced in the liver and is the most abundant protein present in blood plasma. This protein acts in several physiological processes, including the regulation of osmotic pressure, transmission, distribution and metabolism of several ligands, and it is responsible for regulating blood pH [1–6]. Binding affinity to HSA is highly related to the distribution, free concentration and metabolism of ligands or drugs, therefore it is of great importance to study these interactions [7,8]. To study the interaction between a drug and HSA protein, the fluorescence technique is widely used. It is possible to verify from which residue the observed fluorescence is derived, thus determining the number of interacting sites between the drug and the protein. It is also feasible that the determination of the binding type and the mechanism by which this binding occurs, as well as the determination of the distance that this interaction site is from the tryptophan residue [9–15].

Since the discovery of the use of ruthenium compounds as metallodrugs, many studies are being developed to synthesize new compounds that may be used in the treatment of various diseases, including cancer. The development of metallodrugs allows the replacement of currently used drugs, since the coordination to a metal center may increase the activity of a given drug, as well as reduce its side effects [16]. Such as iron, ruthenium ion has low toxicity due to their various oxidation states available in physiological environment (II, III, IV) [17]. Also, their complexes can act both as NO scavengers or releasers, increasing the interest on the development of a variety of nitrosyl ruthenium complexes [17–26]. Of interest to this work, trinuclear ruthenium complexes of general formula $[Ru_3O(CH_3COO)_6(L)_3]PF_6$, with L=N-heterocycles, have been widely studied in recent decades due to its rich mixed-valence chemistry and catalytic properties [27].

Also of interest is the nitric oxide molecule. NO is synthesized endogenously by the oxidation of L-arginine nitrogen, which is converted in L-citruline, catalyzed by NO synthase (NOS) [28,29]. An alternative for NO release from coordination compounds in physiological environment is Photodynamic Therapy, which involves the NO labilization mediated by light stimuli. Also, electrochemical stimuli of coordinated NO triggers its delivery, taking into account, in both cases, that NO^0 has low affinity for metal centers in lower oxidation states [30].

Recently we have reported the synthesis and characterization of the novel NO releaser $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$, as well as its ability to relax pre-contract rat aorta [31]. In order to extend the investigation of bioinorganic aspects of this candidate to a

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http://dx.doi.org/10.1016/j.jlumin.2015.08.066
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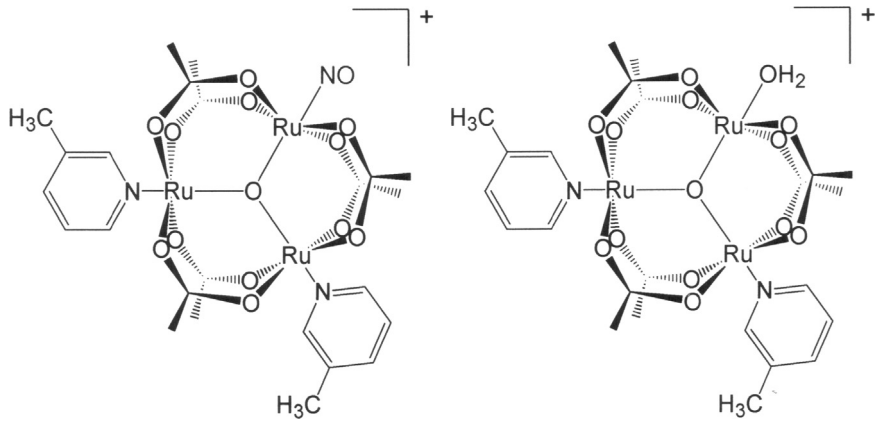

Fig. 1. Pictorial view of the structures of compounds $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$ and $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(H_2O)]PF_6$.

metallodrug, the study presented herein describes the interaction between this trinuclear ruthenium compound and the corresponding aquo complex $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(H_2O)]PF_6$ with HSA. Investigating the aquo complex is worth because it constitutes the product of NO release from $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$. To our knowledge, it is the first report on this type of investigation for the trinuclear ruthenium μ-oxo bridged class of compounds (Fig. 1).

2. Material and methods

2.1. Materials and methods

HSA was purchased from Sigma-Aldrich. The average molecular weight value of 66,500 g/mol was used in the preparation of protein solutions. Prior to each experiment, all solutions were freshly prepared in phosphate buffer (pH 7.4). Deionized water was used in the preparation of buffer solution. All chemicals were of analytical grade and were used without further purification.

The complexes were previously prepared, purified and characterized [31]. Stock solutions (2.31×10^{-3} M) were prepared by dissolving an adequate mass of each complex in acetonitrile.

2.2. Kinetic experiments

The absorption spectra were recorded in an Agilent 8453 spectrophotometer in the 190–1100 nm region, using a quartz cell with 1.0 cm optical path. A solution of the complex $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$ (2.31×10^{-3} M) was prepared in acetonitrile and an aliquot of this solution was added to a buffer solution containing albumin (1×10^{-6} M) in order to provide a concentration of 5.37×10^{-6} M. The solution was incubated at 303 K in the presence of ambient light and the absorption spectra were recorded as a function of time.

The kinetic constant and the half life time were calculated using the following equations [32]:

$$[A] = [A_0]e^{-kT} \quad (1)$$

$$t_{1/2} = \ln 2/k \quad (2)$$

where A and A_0 are the absorbance and the initial absorbance respectively, T is the temperature, $t_{1/2}$ is the half life time and k is the kinetic constant.

2.3. Fluorescence spectroscopy

Fluorescence spectra of the solution of HSA in the absence and presence of the complexes (0–18.5×10^{-6} M) were recorded in a Shimadzu fluorescence spectrophotometer model RF-5301PC, using a quartz cell with 1.0 cm optical path. During a typical fluorescence measurement, 3.0 mL of HSA solution (1.0×10^{-6} M) was firstly added to a 1.0 cm quartz cell and the fluorescence spectrum was recorded. Then, the complex solution aliquots were gradually added to the cell using a micropipette and the solution was incubated in the presence of ambient light for 5 min and for 120 min, before data acquisition. The wavelength 280 nm was used for sample excitation (tryptophan excitation) [33, 34]. The fluorescence spectrophotometer was set up with a slit width of 5 nm. The fluorescence emission spectra were measured at 298, 303, and 308 K.

The intensity of the fluorescence was corrected to eliminate the inner filter effect of HSA and complexes using the following equation [15]:

$$F_{corr} = F_{obs}e^{(A_{ex}+A_{em})/2} \quad (3)$$

where F_{corr} and F_{obs} are the corrected and observed fluorescence intensities, respectively. A_{ex} and A_{em} are the absorbance values of the drugs at the excitation and emission wavelengths, respectively.

3. Results and discussion

3.1. Fluorescence-quenching of HSA by $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$ and $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(H_2O)]PF_6$

The fluorescence of the HSA molecule is largely due to the presence of the tryptophan residue and, in a smaller extent due to tyrosine, depending on the excitation wavelength [35,36]. The fluorescence intensity decreases when certain substrates are added to a HSA solution. Therefore, fluorescence spectroscopy is widely used to investigate the interaction between the protein and other molecules [12–15].

The photochemical behavior of trinuclear ruthenium clusters with a NO ligand such as $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$, has been described in the literature [31,37]. Although the nitrosyls are thermally stable, in solution and in presence of light, NO substitution by a solvent molecule may occur [20,37]. Because of this known reactivity, a control experiment was performed. The complex $[Ru_3O(CH_3COO)_6(3\text{-pic})_2(NO)]PF_6$ was incubated with a HSA in buffer solution in order to verify, for a given time interval and in the presence of ambient light and of albumin, whether is the