Synthesis, characterization and HSA interactions of a new piano-stool ruthenium(II) complex containing a thioamide-type ligand

Danijela Stojković[1,*], Maja Đukić[2] , Marija Ristić[2], Marina Ćendić Serafinović[2], Svetlana Belošević[3], Emina Mrkalić[1], Ivan Jakovljević[2]

[1] University of Kragujevac, Institut for Information Technologies, Department of Science, Jovana Cvijića bb, 34000 Kragujevac, Republic of Serbia; e-mail: danijela.stojkovic@kg.ac.rs, emina.mrkalic@pmf.kg.ac.rs

[2] University of Kragujevac, Faculty of Science, Radoja Domanovića 12, 34000 Kragujevac, Republic of Serbia; maja.djukic@pmf.kg.ac.rs , marija.jeremic@pmf.kg.ac.rs , marina.cendic@pmf.kg.ac.rs , ivan.jakovljevic@pmf.kg.ac.rs

[3] University of Priština in Kosovska Mitrovica, Faculty of Technical Sciences, Knjaza Miloša 7, 38220 Kosovska Mitrovica, Republic of Serbia; svetlana.belosevic@pr.ac.rs

* *Corresponding author*

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Abstract: The synthesis and characterization of a newly synthesized piano-stool [Ru-(η^6-*p*-cymene)Cl_2L] complex with a ligand 3-amino-2-cyano-*N*-phenyl-3-(4-phenyl-1-piperazinyl)-2-propenethioamide are presented. The complex was obtained in good yield as an ochre powder and was characterized by elemental analysis, ^{1}H and ^{13}C NMR, IR, conductometry, and melting point. The fluorescence binding studies showed that the interaction of the complex with albumin occurs by a static quenching mechanism and that the complex showed a very high value of the binding constant with one binding site (K_b = 1.68 × 10^7; n = 1.37). In order to identify the binding location in the HSA molecule, competitive experiments with site markers (eosin Y (site I) and ibuprofen (site II)) were performed. Obtained results showed that the examined complex binds to the site I of subdomain IIA (for eosin Y: K_b = 5.94 × 10^2; n = 0.65 vs. for ibuprofen: K_b = 1.38 × 10^7; n = 1.34).

Keywords: ruthenium(II) complex, thioamide, synthesis, HSA interactions

1. Introduction

The success of cisplatin as an antitumor agent has encouraged many research groups worldwide to direct their efforts toward the synthesis of other clinically important metal complexes [1,2]. An ideal anticancer drug must be able to destroy tumor cells while leaving adjacent healthy tissue unharmed. Great attention is paid to platinum group metals, i.e., their complexes, including ruthenium(II)-*p*-cymene complexes. Over the last few years, our research group has reported several scientific papers and described in

detail a structure-activity relationship and *in vitro* cytotoxicity of imidazole and isothiazole piano-stool Ru(II) complexes [3-5]. Since thioamides are precursors in the synthesis of isothiazoles, which were already the subject of our research and showed good antitumor activity [3,4], we have synthesized and characterized a new Ru(II) complex containing thioamide. Additionally, we have studied the binding affinity of complex toward human serum albumin (HSA).

2. Experimental

All chemicals were purchased from Sigma-Aldrich and used as received. Elemental analysis was performed on a Carlo Erba Elemental Analyser 1106. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 2000 spectrometer. IR spectra were recorded on a Perkin Elmer FT-IR spectrophotometer. Fluorescence spectra were performed using an RF-1501 PC spectrofluorometer. Molar conductivity was made using an EC-metre BASIC 30+ conductometer. The melting point was measured on the Stuart melter.

2.1 Synthesis of the complex [Ru-(η^{6}-p-cymene)Cl$_2$L]

The complex was synthesized according to the method described elsewhere [3], where L = 3-amino-2-cyano-*N*-phenyl-3-(4-phenyl-1-piperazinyl)-2-propenethioamide [6]. Yield: 73%. Anal. Calcd. for ($C_{30}H_{35}Cl_2N_5RuS$) C: 53.81; H: 5.27; N: 10.46. Found: C: 53.72; H: 5.35; N: 10.39. ^{1}HNMR (200 MHz, $CDCl_3$): δ = 7.52-7.44 (m, Ar-CH, 3H), 7.29-6.94 (m, Ar-CH, 8H), 6.82-6.70 (m, Ar-CH, 3H), 3.59 (t, -CH_2-, 4H), 3.14 (t, -CH_2-, 4H), 3.01-2.86 (sept, 1-$CH(CH_3)_2$, 1H), 1.92 (s, Ar-CH_3, 3H), 1.28 (d, CH_3, 6H). ^{13}CNMR (50 MHz, $CDCl_3$): 178.92, 162.52, 149.25, 143.86, 136.28, 131.73, 127.88, 124.19, 119.83, 113.08, 111.26, 62.32, 45.03, 43.62, 31.25, 21.83, 19.52. IR (KBr, 4000-400 cm-1): ν (cm^{-1}) 3412 (ν=CH), 3059 (νCH), 2187 (νC≡N), 1578 (νC=C). Mol. Cond. (DMSO, Ω^{-1} cm^{-1} mol^{-1}): Λ_m 12. Melting point: 221 °C.

2.3 Albumin-binding studies

Protein binding studies have been performed using human serum albumin (HSA, 2 μM) in buffer (10 mM PBS, pH 7.4). The quenching of the emission intensity of HSA tryptophan residues at 361 nm was monitored using the investigated complex as a quencher with increasing concentration (up to 2.0 x 10^{-5} M). Fluorescence spectra were recorded from 300 to 500 nm at an excitation wavelength of 295 nm. Besides, we have studied the competitive interactions of the site markers (eosin Y, as a marker for site I of subdomain IIA, and ibuprofen, as a marker for site II of subdomain IIIA) [7] and complex toward HSA using the same method as above ([HSA] : [markers] = 1:1).

3. Results and discussion

The HSA solutions exhibit strong fluorescence emission with a peak at about 350 nm, which is due to the tryptophan residues when excited at 295 nm [8]. The addition of complex to the solution of HSA results in a decrease of the intensity of the peak (Figure 1a). The Stern-Volmer constant (K_{SV}) and quenching rate constant (k_q) were calculated using the Stern-Volmer quenching equation (1):

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{sv}[Q] \quad (1)$$

where F_0 and F are the emission intensity in the absence and presence of the [Q], K_{SV} is the Stern-Volmer constant, k_q is the quenching rate constant, τ_0 is the average fluorescence lifetime of HSA without a quencher. The k_q is higher than the different quenching types for biopolymer fluorescence (10^{10} M^{-1} s^{-1}), indicating a static quenching mechanism. The binding constant (K_b) and the number of binding sites per HSA (n), were calculated using the Scatchard equation (2):

$$log \frac{F_0 - F}{F} = log K_b + n\, log[Q] \quad (2)$$

The values of K_b and n were obtained from the intercept and slope of the plots of log (F_0-F)/F versus log [Q] (Table 1). The obtained values for K_b and n indicate the ability of the complex to strongly bind to HSA occupying one binding site.

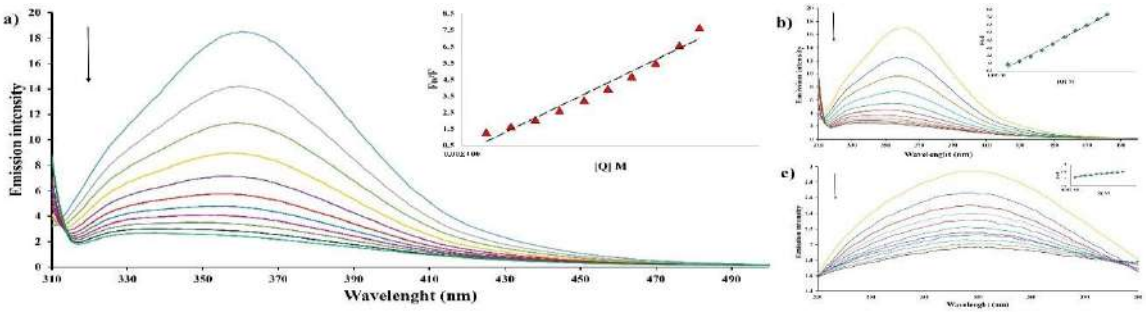

Figure 1. Emission spectra of: complex-HSA (a), complex-HSA-ibuprofen (b) and complex-HSA-eosin Y. [HSA] = [ibuprofen] = [eosin Y] = 2 μM, [Q] = 0-20 μM. Insert: plots of F_0/F vs. [Q].

Table 1. The values of K_{sv}, k_q, K_b, and the possible number of binding sites (n) for various systems.

System	K_{sv} [M^{-1}]	k_q [M^{-1} s^{-1}]	K_b [M^{-1}]	n
Complex-HSA	3.49×10^5	3.49×10^{13}	1.68×10^7	1.37
Complex-HSA-ibuprofen	3.76×10^5	3.76×10^{13}	1.38×10^7	1.34
Complex-HSA-eosin Y	2.11×10^4	2.11×10^{12}	5.94×10^2	0.65

Competitive experiments with site markers were performed to determine the preferred binding site of the complex to the HSA (Table 1 and Figure 1(b, c)). The decrease in the fluorescence intensity of the HSA solution after the addition of site markers indicates that the marker molecules bind to HSA. If the complex binds to the same site as the corresponding marker the complex must compete with the marker in order to bind to HSA, leading to a significant change in the constant value. In Table 1 it can be seen that the presence of eosin Y significantly decreased values of K_b and n, which

means that the complex binding should be mainly located in site I (subdomain IIA) of HSA, occupying a single binding site.

4. Conclusions

In this work we have synthesized and characterized a new piano-stool [Ru-(η^6-*p*-cymene)Cl_2L] complex, L = 3-amino-2-cyano-N-phenyl-3-(4-phenyl-1-piperazinyl)-2-propenethioamide. The complex was obtained in good yield as an ochre powder. The results of elemental microanalysis, molar conductivity, melting point, and spectroscopic characterization (IR, ^{1}H and ^{13}C NMR) are in agreement with the assumed structure of the complex. The obtained fluorescence results show that the quenching mechanism is static and that the complex strongly binds to one site for HSA (site I of subdomain IIA), which enables the safe transport of the complex to biological targets.

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