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Ruthenium (II) complexes binding to human serum albumin and inducing apoptosis of tumor cells

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ABSTRACT

The binding properties of two Ru(II) complexes $[Ru(bpy)_2(ox)]^{2+}$ (RBO) and $[Ru(bpy)_2(suc)]^{2+}$ (RBS) (bpy = 2, 2-bipyridine, ox = oxalate, and suc = succinate) to human serum albumin (HSA) have been studied by fluorescence, UV–vis absorption spectroscopy and circular dichroism (CD) spectroscopy. The fluorescence quenching mechanism was determined to be a static quenching procedure. The Stern–Volmer quenching constant K_{sv} and corresponding thermodynamic parameters ΔH, ΔS and ΔG were calculated. The number of binding site was about **1**. The result of CD showed that the secondary structure of HSA molecules was changed in the presence of the Ru(II) complexes. Furthermore, the anticancer activities of the complexes were evaluated by using the MTT assay, the results indicated that the antiproliferative activity of RBS was higher than that of RBO, and RBS showed a significant antitumor activity through induction of apoptosis in A549 cells.

In recent years, alternative metal-based cancer therapies have received widespread attention [1–3] since cisplatin was developed as an anticancer drug. The purpose is to find drugs that have lower toxicity but more activity than cisplatin. Ru complexes are considered as one alternative to Pt drugs, which exhibit low toxicity to normal cells, as well as easily absorbed by tumor tissue and rapidly excreted from the body [4,5]. A number of Ru complexes have displayed promising anticancer activity [6,7]. Up to now, there are three promising Ru anticancer agents, i.e. NAMI-A, KP1019, and IndCR, which have already entered clinical trials as anticancer agents [6–9].

As the major soluble protein constituents of the circulatory system, human serum albumin (HSA) exerts a substantial impact on transporting and disposing of numerous endogenous and exogenous ligands present in blood [10]. It has been shown that the drug–albumin interactions in the blood stream can influence the drug stability, toxicity and distribution during the chemotherapeutic process. The nature and magnitude of drug–albumin interactions significantly influence the pharmacokinetics of drugs, and the binding parameters are useful in studying protein–drug binding. Consequently, it is important to know the affinity of a drug to serum albumin. In the past few years, our group has been committed to the research of anti-tumor properties of Ru complexes including their design, synthesis, structural modification, biological activity and mechanisms [11–14]. In the present paper, we will focus on the interactions of two Ru(II) complexes RBO and RBS (Scheme 1) with HSA by fluorescence, UV–vis absorption spectroscopy and CD spectroscopy, as well as the biological properties of RBS, the more active Ru complex between the two complexes studied, by showing its apoptosis-inducing activities.

Human serum albumin consists of 585 amino acid residues organized in three domains (I, II, and III), each comprising two subdomains (A and B) [15]. Intrinsic fluorescence of HSA is mainly due to the presence of the sole tryptophan residue (Trp-214) in the hydrophobic cavity of subdomain IIA [16]. The fluorescence of Trp-214 may change when HSA interacts with other molecules, which is a common response to conformational transition, subunit association, or substrate binding of HSA [17]. The results of emission titrations for the complexes with HSA are illustrated in Fig. 1. The intensity of the characteristic broad emission band at 348 nm decreased regularly with the increasing concentration of each complex, suggesting that some interactions between Ru(II) complexes and HSA have occurred. Nevertheless, the maximum emission wavelength of HSA was virtually unchanged during the interaction, indicating that Trp-214 was not exposed to any change in polarity, and the interaction may occur via the hydrophobic region located inside HSA. Meanwhile, it is noteworthy that the trends of HSA fluorescence quenching slowed down, so it can be deduced that the bonding was saturated progressively. When the [Ru]/[HSA] molar ratio was 2:1 at 300 K, we supposed that the extent of the fluorescence attenuation was in an order: RBS > RBO. This indicated that the binding affinity of RBS was higher than that of RBO when bonded to HSA. These results demonstrated that there were strong interactions between HSA and the Ru(II) complexes.

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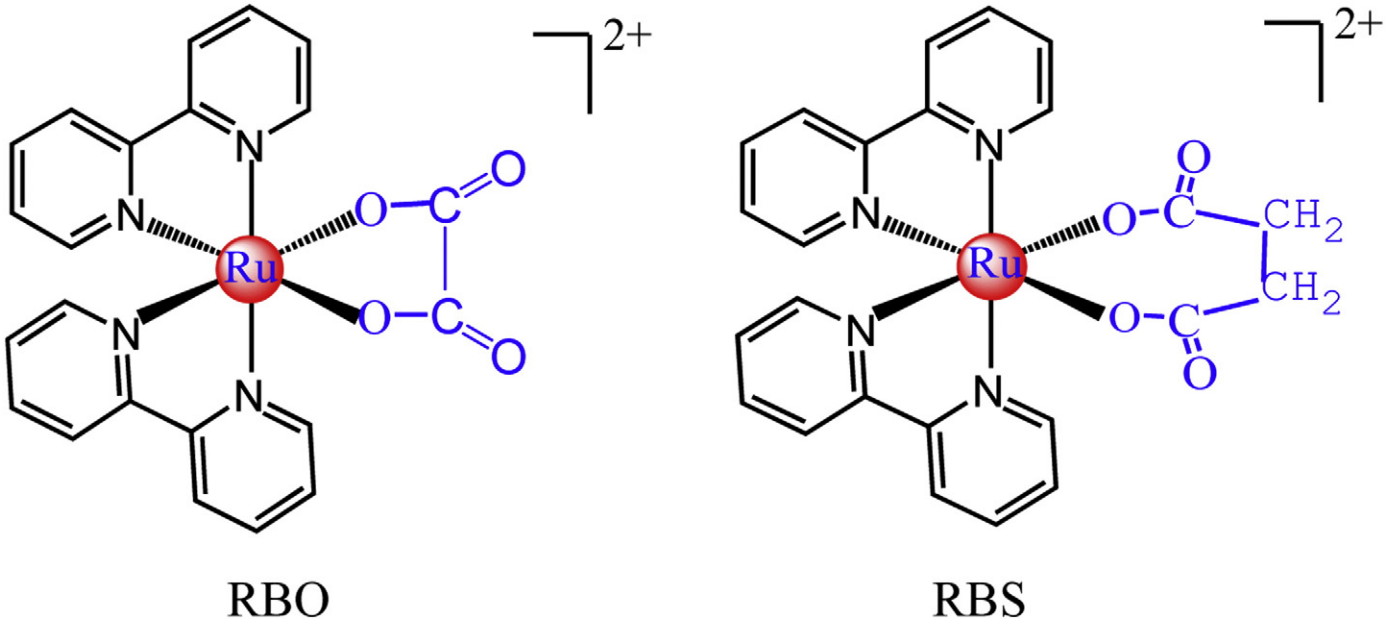

Scheme 1. Structure of the Ru(II) complexes.

Quenching can occur by different mechanisms, which is usually classified as dynamic quenching and static quenching. Dynamic quenching depends upon diffusion, and for static quenching, that is quenching by formation of a complex between quencher and fluorophore [18]. The Stern–Volmer equation could be employed to describe the fluorescence quenching [19]:

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

Where F_0 and F were the fluorescence intensity of HSA in the absence and presence of the metal complex, respectively; K_q, τ_0, K_{sv}, and [Q] were the quenching rate constant of HSA, the average lifetime of molecule without quencher, the Stern–Volmer quenching constant, and concentration of quencher, respectively. Fig. 2 showed that plots were linear and the slopes decreased with increasing temperature within the investigated concentrations range. This indicated the static quenching interaction between Ru(II) complexes and HSA [18,20]. Table 1 listed the quenching constants and quenching rate constants of the fluorescence quenching process for the two complexes binding to HSA under different temperature conditions. The slopes of the linear regression equation were for the quenching constant K_{sv}. For HSA, τ_0 is approximately 5 ns [21], values of K_q ($\sim 10^{13}$ M^{-1} S^{-1}) could be obtained from $K_{sv} = K_q\ \tau_0$, which were larger than the maximum quenching rate constant of diffusion collision, 2.0×10^{10} M^{-1} s^{-1} [20]. So here we come to the conclusion that the fluorescence quenching is caused by a specific interaction between HSA and Ru(II) complexes and the quenching mechanism mainly arise from the formation of the Ru(II)–HSA complex, while dynamic collision could be negligible in the concentration range studied [22].

A Site-binding model is a valuable method to describe the interaction between small molecular ligands and biomacromolecules. The apparent association constant (K_A) and the number of binding site (n) can be obtained from the well-known equation [23]:

$$\log[(F_0-F)/F] = n\log K_A - n\log\{1/([Q_t]-(F_0-F)[P_t]/F_0)\} \quad (2)$$

Where F_0 and F were the fluorescence intensities in the absence and presence of Ru(II) complex, respectively; $[Q_t]$ and $[P_t]$ were the total concentrations of Ru(II) complex and HSA, respectively. The plot of log $(F_0-F)/F$ versus log $\{1/([Q_t]-(F_0-F)[P_t]/F_0)\}$ gave a straight line. K_A and n were thus obtained from the intercept on the Y-axis and the slope, respectively. The values of K_A and n for each complex binding to HSA were presented in Table 2. The association constants ($\sim 10^5$ M^{-1}) relative to the strong ligand–protein complexes suggest that the affinity of Ru(II) complexes for HSA is just at a moderate level [24]. Also, the values of n were approximately

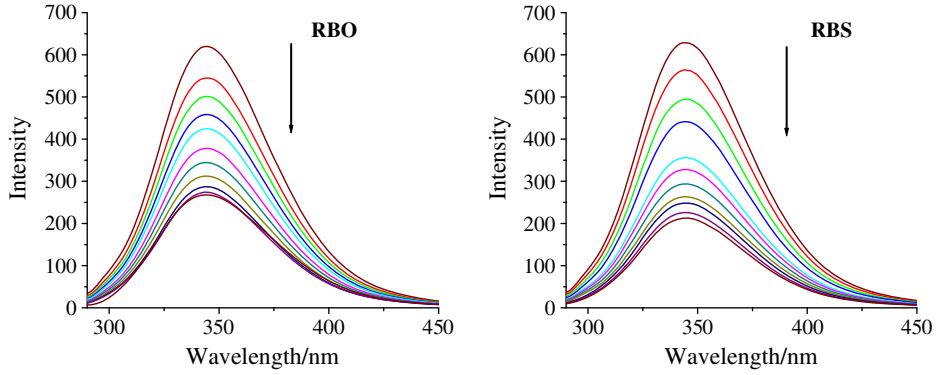

Fig. 1. Fluorescence spectra of HSA (5 μM) in the presence of RBO and RBS at different concentrations (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 μM, respectively) at 300 K. λ_{ex} = 280 nm, pH = 7.4.