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ORIGINAL PAPER

Association studies to transporting proteins of fac-$Re^{I}(CO)_3$(pterin)(H_2O) complex

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Abstract A new synthetic route to acquire the water soluble complex *fac*-$Re^{I}(CO)_3$(pterin)(H_2O) was carried out in aqueous solution. The complex has been obtained with success via the *fac*-[$Re^{I}(CO)_3(H_2O)_3$]Cl precursor complex. $Re^{I}(CO)_3$(pterin)(H_2O) has been found to bind strongly with bovine and human serum albumins (BSA and HSA) with intrinsic-binding constants, K_b, of 6.5×10^5 M^{-1} and 5.6×10^5 M^{-1} at 310 K, respectively. The interactions of serum albumins with $Re^{I}(CO)_3$(pterin)(H_2O) were evaluated employing UV–vis fluorescence and absorption spectroscopy and circular dichroism. The results suggest that the serum albumins-$Re^{I}(CO)_3$(pterin)(H_2O) interactions occurred in the domain IIA-binding pocket without loss of helical stability of the proteins. The comparison of the fluorescence quenching of BSA and HSA due to the binding to the Re(I) complex suggested that local interaction around the Trp 214 residue had taken place. The analysis of the thermodynamic parameters ΔG^0, ΔH^0, and ΔS^0 indicated that the hydrophobic interactions played a major role in both HSA-Re(I) and BSA-Re(I) association processes. All these experimental results suggest that these proteins can be considered as good carriers for transportation of $Re^{I}(CO)_3$(pterin)(H_2O) complex. This is of significant importance in relation to the use of this Re(I) complex in several biomedical fields, such as photodynamic therapy and radiopharmacy.

Keywords Rhenium(I) complex · Serum albumins · Binding study · Fluorescence quenching · Circular dichroism · Thermodynamic parameters

Introduction

Re(I) tricarbonyl complexes have been proposed as novel therapeutic agents of relevance in photodynamic therapy, chemo and radiotherapy (anti-cancer drugs) [1], and antimicrobial activity [2]. The ability to rationally design the nature of the ligand allows us to obtain complexes with a large structural variety and stereochemistry that define their physicochemical properties (e.g., solubility, catalytic activity, and photoactivity) [3–6].

In this sense, the photoluminescence of these complexes can be modulated to apply them as intracellular fluorescent probes [7, 8] for diagnostic purposes. Moreover, "hot" analogues of the Re organometallic complexes (using ^{188}Re and ^{186}Re isotopes) can be prepared for be applied for radio imaging as well as for therapy [9, 10]. In order for these properties can be fully exploited, the complexes must interact efficiently with transport proteins in the bloodstream and have a good solubility in water at physiological pH (pH = 7.4).

Serum albumins are the major protein constituents in the circulatory system of mammalians. These macromolecules have important physiological role as they contribute to the osmotic blood pressure [11] as well as they help to control

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the blood pH and increase the solubility of fatty acids. However, the most important physiological function of albumins is the transport of numerous ligands in the blood stream [12]. The delivery and pharmacokinetics of these pharmacologic compounds depend on their binding ability with albumins. Therefore, it is important to study the interactions of drugs with these proteins. These kinds of studies are usually carried out using a model protein to Human Serum Albumin (HSA), such as Bovine Serum Albumin (BSA).

Albumins fluorescence is originated by three amino-acid residues with intrinsic fluorophore groups present in the protein, i.e., tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe). In fact, the fluorescence of albumins comes almost completely from Trp and Tyr, since the fluorescence from phenylalanine has a very low quantum yield [13]. The emission spectra of HSA and BSA at $\lambda_{exc} = 280$ nm arise from both tryptophan and tyrosine residues, because the incident radiation can be absorbed efficiently by both. Tryptophan emission comes not only from tryptophans excited by the external radiation, but also from tryptophans excited by radiationless energy transfer from excited tyrosine. Whereas at $\lambda_{exc} = 295$ nm, HSA and BSA fluorescence is only due to tryptophan residues, because tyrosine is not electronically excited with such wavelength and thus it neither emits nor transfers energy [14, 15]. When a small molecule binds to albumins, it may prompt changes in the intrinsic fluorescence intensity due to alterations in the micro environment of the emitter residues. Albumin consists of a single polypeptide chain with three homologous domains (I, II, and III). Each domain is composed of two subdomains (A and B), which are predominantly helical and extensively cross-linked by several disulphide bridges. BSA contains two tryptophans located in two different subdomains, i.e., Trp214 deeply buried in the hydrophobic loop in the IIA subdomain, and Trp 135 exposed to a hydrophilic environment in the IB subdomain [16], while HSA contains only one tryptophan located in the IIA subdomain (Trp212). The crystal structure analyses indicate that the principal regions of ligand-binding sites in albumins are located in hydrophobic cavities in subdomains IIA and IIIA.

To date, there are only a few reports in the literature about association studies of Rhenium complexes with BSA [17–19]. In addition, these Re complexes studied had not high water solubility. However, as far as we know, there are not reports about interactions studies between these kinds of complexes and HSA.

In this study, we evaluated the association with both HSA and BSA of the complex *fac*-$Re^{I}(CO)_3$(pterin)(H_2O), where pterin = 2-amino-4-oxo-pterinate. We report a new synthetic route to obtain this Re(I)-pterin complex which has interesting features such as both its relatively high solubility in water and its remarkable stability in a broad pH range [20]. Our results show that *fac*-$Re^{I}(CO)_3$(pterin)(H_2O) complex is able to bind to both HSA and BSA. The study of the binding properties between Re(I)-pterin complex with these transport proteins was performed using steady-state and time-resolved luminescence. Fluorescence measurements can offer information of the binding of small molecules to biopolymers as DNA and proteins at the molecular level, such as about the binding mechanism and mode of the interaction, binding constant, intermolecular distances, etc. [21, 22].

The nature of the binding forces was established by studying the interaction at different temperatures, in the 298–310 K range. The thermodynamic parameters of the association process ΔG^0, ΔH^0, and ΔS^0 were calculated.

The results obtained regarding the association constants, binding forces, and the binding distances show that there is strong-binding affinity between Re(I) complex with BSA and HSA. Moreover, the analysis of the circular dichroism spectra shows that no significant conformation changes are induced after the interaction between this Re(I) complex and the albumins.

Materials and methods

Materials

$ClRe(CO)_5$ and 2-amino-4-oxo-3H-pteridine (pterin) were purchased from Sigma–Aldrich. The solutions of *fac*-$Re^{I}(CO)_3$(pterin)(H_2O) (RePtr) was prepared in Tris buffer (0.1 M Tris, 0.1 M NaCl, pH 7.4 ± 0.1) and the concentrations were calculated using the molar absorption coefficient $\varepsilon_{366nm} = 4200\ M^{-1}\ cm^{-1}$ [20]. HSA (albumin human ~99%, fatty acids free (~0.005%), essentially globulin free A3782) and BSA (albumin from bovine serum, minimum 98% electrophoresis A7906) were purchased from Sigma Chemical Co. and used without further purification. Ultrapure water was obtained with a Milli-Q water purification system (Millipore, USA). All other chemical reagents used throughout the experiments were analytical grade.

Equipment and spectroscopic measurements

The UV–visible spectra were recorded on an Agilent 8453 diode array detector spectrophotometer and a Shimadzu UV-1800 spectrophotometer. FTIR spectra were recorded with a Nicolet 8700 Thermo Scientific instrument. NMR spectra were recorded at 300 K with a Bruker AM-500 spectrometer operating at 500 MHz. [D_6]DMSO was used as a solvent and the chemical shifts were referenced relative to the $(CH_3)_2SO$ in [D_6]DMSO ($\delta = 2.50$ ppm).

Emission spectra were recorded at several temperatures on a Fluoromax 3 spectrometer from *Horiba Jobin Ivon* equipped with a Hamamatsu R928 PMT in a photon counting detector using a Peltier F-3004. The excitation wavelengths were 280 and 295 nm, the excitation and the emission slit widths were set at 3.0 nm, and appropriate blanks corresponding to the buffer were subtracted to correct background fluorescence. Fluorescence lifetimes were measured using a Fluoromax 3 spectrometer equipped with NanoLED source (λ_{exc} = 295 nm). The emitted photons, after passing through the monochromator, were detected by a TBX-04 detector and counted by a FluoroHub-B module. Fluorescence decay data were analysed by the DAS6 decay analysis software. The circular dichroism (CD) spectra were recorded on a JASCO J-810 spectropolarimeter. All the spectrometric measurements were carried out in quartz cuvettes of 1 cm path length.

Procedures

An albumin solution was prepared in Tris buffer, pH 7.4 (BSA and HSA). The emission spectrum of each protein was recorded using 2 mL of the solution. Subsequently, aliquots of albumin solution with the same concentration containing Re(I) complex were added to the above solution. Final concentrations of RePtr were in the range 0–28 μM, while the protein concentration was fixed at 1.55 and 1.95 μM for BSA and HSA, respectively. After each addition, the sample solution was incubated during 5 min prior to record the spectrum. The spectra of the solution with increasing concentrations of Re(I) complex were recorded.

Emission spectra were corrected for internal filter effect according to Eq. (1) [23].

$$I_{cor} = I_{obs} 10^{\left(\frac{\sum \varepsilon_i^{exc} c_i l}{2}\right)} 10^{\left(\frac{\sum \varepsilon_i^{em} c_i l}{2}\right)} \quad (1)$$

where I_{cor} and I_{obs} are the corrected fluorescence intensities and observed fluorescence intensities at 344 nm, respectively, whereas ε_i^{exc}, ε_i^{em},c_i, and l are the molar absorption coefficients of the absorbent species at excitation and emission wavelengths, their concentrations, and the optical path, respectively.

The emission spectrum of proteins was recorded at four temperatures using a Peltier heat pump (298–310 K).

Time-resolved fluorescence data were fitted to biexponential functions after deconvolution of the instrumental response function by an iterative reconvolution approach utilizing reduced χ^2 and weighted residuals as parameters for goodness of fit. Average fluorescence lifetime (τ) for biexponential iterative fittings was calculated from the decay times and the normalized pre-exponential factors (B_i) using the following equation:

$$\langle \tau \rangle = \frac{\sum B_i \tau^2}{\sum B_i \tau}. \quad (2)$$

Fluorescence study

For a simple 1:1 ligand binding, the intrinsic-binding constants between RePtr with albumins BSA and HSA were determined by decrease of the fluorescence intensity of albumins at 344 nm, with increasing concentrations of the complex, by the Ryan–Weber equation (Eq. (3) [24, 25].

$$\frac{F_0 - F}{F_0 - F_\infty} = \frac{[PQ]}{[P]_t} = \frac{[P]_t + [Q]_t + 1/K_b - \sqrt{([P]_t + [Q]_t + 1/K_b)^2 + 4[P]_t[Q]_t}}{2[P]_t} \quad (3)$$

where F is the measured fluorescence, while F_0 and F_∞ are the fluorescence intensities when the protein is completely free or complexed; $[Q]_t$ is the total concentration of quencher (RePtr); $[P]_t$ is the concentration of albumin; [PQ] is the concentration of associated quencher-protein at equilibrium; and K_b is the binding constant.

Nature of binding forces

The acting forces between a Re(I) complex and albumin may include van der Waals, hydrophobic, electrostatic, and hydrogen-bond interaction forces, within others [26, 27]. Enthalpy change of the interaction can be regarded as a constant if the temperature range is not too wide. Temperature effect on thermodynamic interaction parameters has been followed in the 298–310 K range.

Assuming that the standard enthalpy change (ΔH^0) does not vary significantly over the temperature range studied, then its value and that of entropy change (ΔS^0) can be determined from the Van't Hoff equation (Eq. 4) combined with the relationship of Gibb's free energy (ΔG^0) (Eq. 5).

$$\frac{d(\ln K_b)}{dT} = \frac{\Delta H^0}{RT^2} \quad (4)$$

$$\ln K_b = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R}. \quad (5)$$

The term R has its usual meaning. The change in Gibb's free energy was calculated from the relation shown in the following:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0. \quad (6)$$

Circular dichroism study

The secondary structure of proteins is composed of α-helical, β-sheet, random coil, and β-turn. The decrease

in α-helical content in the secondary structure can reflect the unfolding extent of proteins [28, 29]. To investigate the effect of RePtr complex on the secondary structure of albumins, CD spectra of albumins ([protein] = 1×10^{-4} M in phosphate buffer) and the albumins-Re(I) complex adduct, at a molar ratio (p_i) of Re(I) to protein, were recorded in the wavelength region between 200 and 250 nm. The corresponding absorbance contributions of buffer and free Re(I) complex solutions were recorded and subtracted with the same instrumental parameters. The CD results were expressed as MRE (mean residue ellipticity) in deg cm^2 $dmol^{-1}$ according to the following equation:

$$\mathrm{MRE} = \frac{[\theta]_{\mathrm{obs}}}{(10 C_{\mathrm{p}} n l)} \quad (7)$$

where $[\theta]_{obs}$ is the observed molar ellipticity in millidegree, C_p is the molar concentration of the protein, n the number of amino-acid residues (582 and 585 for BSA and HSA, respectively [11]), and l is the path length (0.2 cm) [28, 30].

Results and discussion

Synthesis

fac-$Re^{I}(CO)_3$(pterin)(H_2O) complex was prepared by a modification of procedures reported recently in the literature [20]. In a first step, we obtained the precursor complex *fac*-$[Re^{I}(CO)_3(H_2O)_3]Cl$ (**1**). To this end, a suspension of $Re(CO)_5Cl$ in water was refluxed for 20 hs (Eq. (8)) in nitrogen atmosphere. Upon boiling, the mixture became a clear solution. After cooling to room temperature, the solvent was evaporated under vacuum until dryness and the resulting light green powder was dried under vacuum at 60 °C for 24 h.

$$[Re(CO)_5]Cl \xrightarrow{H_2O,20hs,N_2} [Re(CO)_3(H_2O)_3]Cl + 2CO. \quad (8)$$

The FTIR spectrum of **1** in aqueous solution shows strong absorptions at 2028 (with shoulder at 2036) and 1918 cm^{-1}, assigned to ν(C≡O), Fig. S1. These infrared absorptions are consistent with the facial configuration of the carbonyl ligands in tricarbonyl complexes of Re(I) and are in agreement with those observed for $[Re(CO)_3(H_2O)_3]$Br [31]. In the second step, we proceeded to obtain the RePtr complex as described below. A 100 mL round-bottomed flask was loaded with an aqueous solution of pterin (163 mg, 1.0 mmol) (75 mL) and was heated at ca. 60 °C. Then, 75 mL of an aqueous suspension of **1** (360 mg, 1.0 mmol) was slowly added to the hot aqueous solution. The mixture refluxed for 6 h under a N_2 atmosphere. The solution, initially colourless, turned orange during reaction (Eq. (9)). The progress of the reaction was monitored continuously by UV–vis spectrometry. The reaction mixture was then cooled to room temperature, concentrated under reduced pressure, and purified by chromatography in column of silica gel 60 (eluent: deionised water). Finally, the solvent was evaporated to dryness under vacuum.

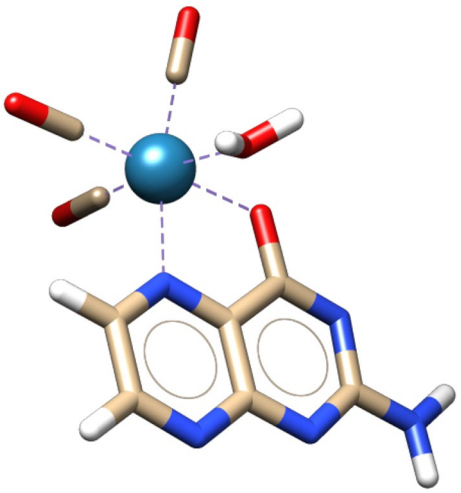

Fig. 1 View of *fac*-$Re(CO)_3$(pterin)(H_2O) complex [20]

$$[Re(CO)_3(H_2O)_3]Cl + Ptr \xrightarrow{H_2O,6hs,N_2} Re(CO)_3(H_2O)(Ptr) + 2H_2O + HCl. \quad (9)$$

The Re(I) complex was obtained in good yield (75%) and it was characterized by elemental analysis, FTIR, ^{1}H and ^{13}C-NMR, MS–ESI, and UV–vis spectroscopy.

IR (KBr, ν [cm^{-1}]): 2028 (s) and 1901 (s). ^{13}C-NMR ([D_6]DMSO): δ = 197.9, 196.9, 192.6, and 190.4 [*fac*-$Re(CO)_3$], 177.5, 157.8, 152.2, 141.9, and 126.9 [pterin]. MS (ESI): *m/z* = 452.0 $[M + H]^+$, 434.0 $[M–H_2O + H]^+$, Fig. S2. UV–vis spectrum was recorded in water at pH 7 and shows two absorption bands. FTIR, ^{1}H-NMR, and UV–vis spectroscopic features are in good agreement with those previously published for this complex [20].

The structure of the Re(I)-pterin complex is shown in Fig. 1.

Association study

Figures 2 and 3 show the effect of RePtr concentration on the fluorescence intensity of HSA and BSA, respectively, in physiological condition (pH = 7.4, buffer Tris), λ_{exc} = 280 nm and λ_{exc} = 295 nm.

The fluorescent emission spectra of HSA exciting at 280 and 295 nm show maximum at 348 nm, while the spectrum

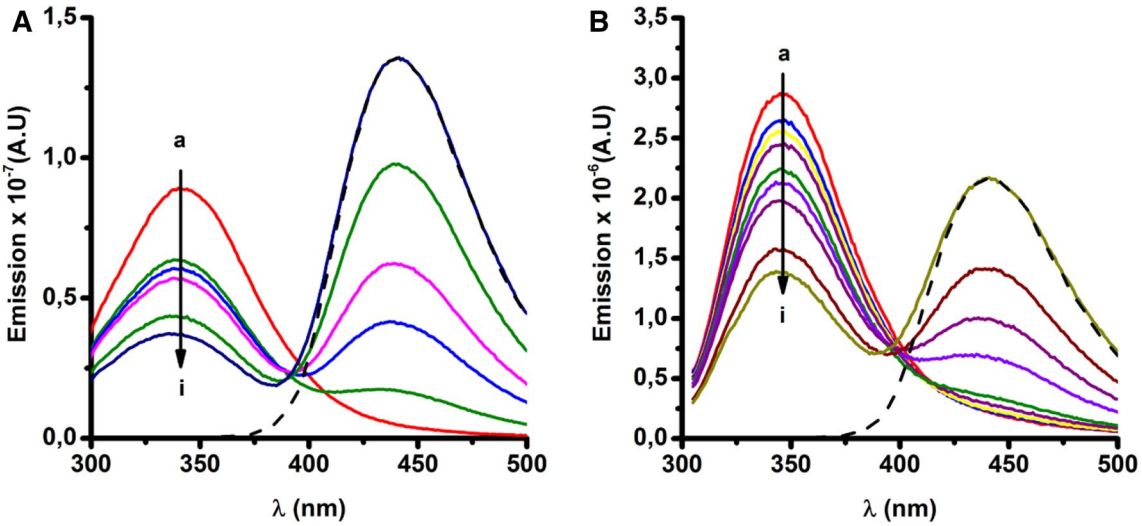

Fig. 2 Fluorescence quenching of HSA by RePtr (concentration from $a = 0$ to $i = 24$ μM) at [HSA] = 1.95 μM and $T = 310$ K, **a** $\lambda_{exc} = 280$ nm, **b** $\lambda_{exc} = 295$ nm. Fluorescence of RePtr in *dash line*, $\lambda_{exc} = 280$ nm

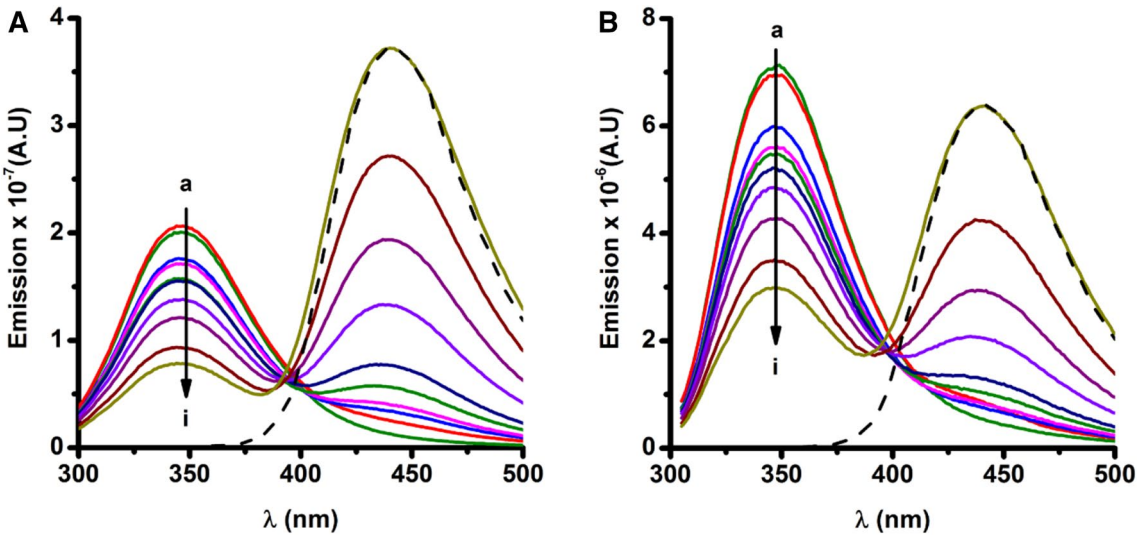

Fig. 3 Fluorescence quenching of BSA by RePtr (concentration from $a = 0$ to $k = 27$ μM) at [BSA] = 1.55 μM and $T = 310$ K, **a** $\lambda_{exc} = 280$ nm, **b** $\lambda_{exc} = 295$ nm. Fluorescence of RePtr in *dash line*, $\lambda_{exc} = 280$ nm

of BSA exciting at 280 and 295 nm shows a maximum at 344 nm. In all spectra, the emission band of the Re(I) complex centred at 440 nm can be observed [32].

We can see from Figs. 2 and 3 that the intensities of fluorescence emission of BSA and HSA decreased gradually with the increase of RePtr concentration. F/F_0 values at 344 nm, for both excitation wavelengths used, decrease when the ratio p = [RePtr]/[albumin] increases (Fig. 4).

To determine which fluorophores are involved in the interaction with the Re(I) complex, the relative fluorescence of albumins excited at 280 and 295 nm in the presence of RePtr was compared. As shown in Fig. 4a, b, no significant differences can be observed between the extent of quenching for BSA and HSA exciting at $\lambda_{exc} = 280$ and 295 nm. This absence of difference between the quenching of both proteins at both excitation wavelengths suggests that tyrosine has not taken part in the molecular interactions with RePtr, since at $\lambda_{exc} = 295$ nm, only tryptophan is excited. This is consistent with results reported in the literature which shows that when quenching by interaction of tyrosine happens, the extent of quenching for albumins at $\lambda_{exc} = 280$ nm is greater than that at $\lambda_{exc} = 295$ nm, i.e., the curves of the (F/F_0) plots do not overlap due the stronger quenching effect at 280 nm by the significant contribution

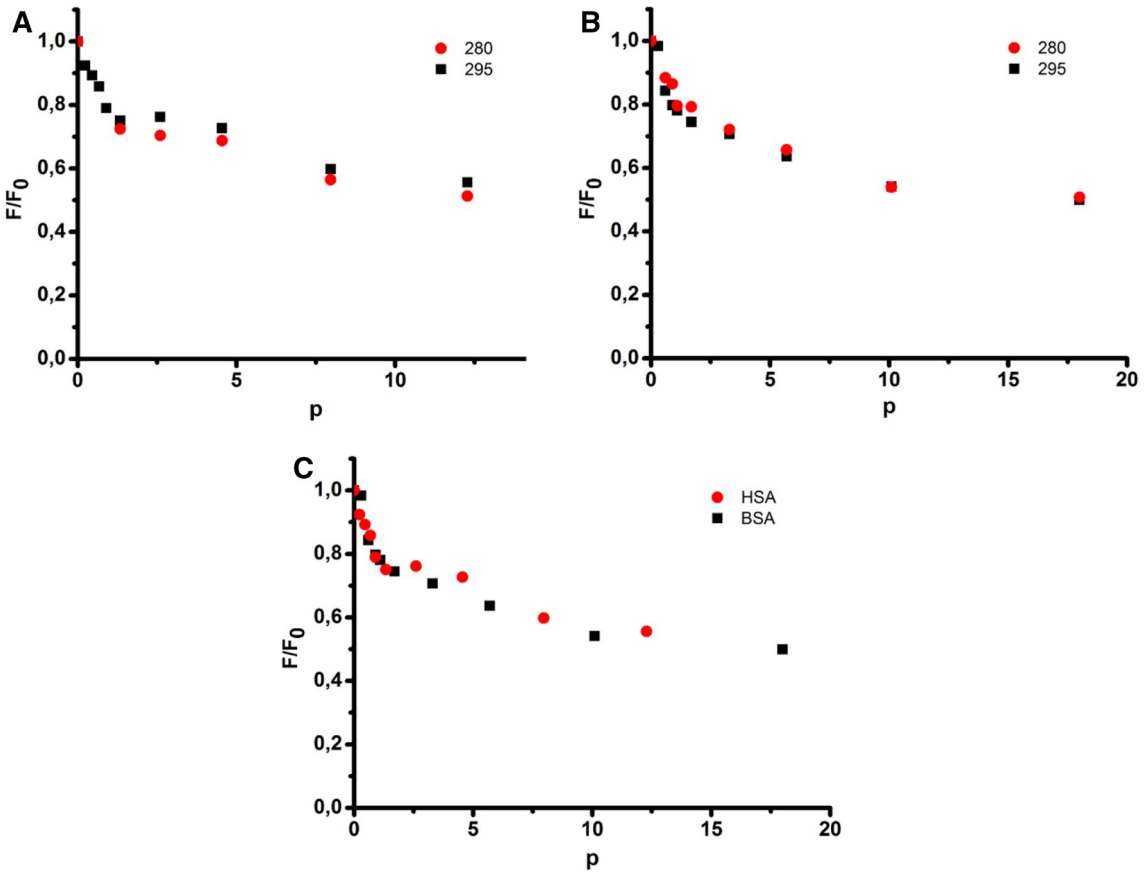

Fig. 4 Plot of relative fluorescence intensity upon titration with increasing [RePtr]/[albumin] ratio (p) for HSA at $\lambda_{exc} = 280$ and 295 nm (**a**); BSA at $\lambda_{exc} = 280$ and 295 nm (**b**); and BSA and HSA at $\lambda_{exc} = 295$ nm (**c**). $T = 310$ K; Tris buffer pH 7.4

Table 1 Lifetimes and pre-exponential factors (see Ec. 2) of HSA with RePtr

[RePtr], μM	p (RePtr/HSA)	B_1	τ_1, ns	B_2	τ_2, ns	$<\tau>$, ns	χ^2
0.00	0.0	0.032	3.75	0.026	7.48	6.07	1.14
0.59	0.4	0.031	3.63	0.027	7.41	6.05	1.18
1.76	1.2	0.031	3.63	0.027	7.43	6.07	1.13
4.58	3.0	0.030	3.54	0.027	7.36	6.03	1.27
9.83	6.5	0.031	3.63	0.026	7.45	6.04	1.12
14.62	9.7	0.033	3.71	0.025	7.50	6.01	1.13
23.04	15.3	0.033	3.80	0.023	7.61	6.00	1.17
39.15	26.0	0.031	3.53	0.025	7.37	5.96	1.14

$\lambda_{exc} = 295$ nm, $\lambda_{emi} = 344$ nm, [HSA] = 1.5 μM

of the tyrosine residues [27, 30]. For further insight into the binding process, the lifetimes of the fluorescent excited state of HSA were measured with increasing concentrations of Re(I) complex at $\lambda_{exc} = 295$ nm and $\lambda_{emi} = 344$ nm. No significant changes were observed in the average lifetime when the complex was added (Table 1).

Furthermore, the lifetimes of the fluorescent excited state of RePtr were measured with increasing concentrations of HSA and BSA albumins at $\lambda_{exc} = 341$ nm and $\lambda_{emi} = 450$ nm. No significant changes were observed in the lifetime of the complex neither in absorption nor emission spectra when the proteins were added (Table 2).

On the basis of no significant changes observed in the lifetime of the albumins, we can affirm that the mode of emission quenching is static (that only occurs at short distances). When a ligand quenches the fluorescence of HSA,

Table 2 Lifetimes and pre-exponential factors of RePtr with HSA

[HSA], μM	*r* (HSA/RePtr)	τ, ns	χ^2
0.00	0.0	8.19	1.09
1.61	0.2	8.41	1.06
3.42	0.4	8.34	0.99
4.76	0.5	8.29	1.01
5.80	0.6	8.25	1.11
6.85	0.8	8.20	1.05
7.83	0.9	8.11	1.00
8.43	0.9	8.10	0.97
14.62	1.2	8.05	0.97
15.32	2.4	8.03	1.04

$\lambda_{exc} = 341$ nm. $\lambda_{emi} = 450$ nm. [RePtr] = 0.8 μM

the process is shown to be taking place in the IIA subdomain, since in this albumin, there is only one tryptophan which is located there [16]. As found in Fig. 4c, no difference can be seen between the extension of quenching for BSA and HSA at $\lambda_{exc} = 295$ nm. Thus, it could be stated that due to the Trp 214 is located in the subdomain IIA, it is the binding site of Re(I) complex. Since BSA contains two tryptophans in 135 (IB) and 214 (IIA) positions, the extension of quenching showed in Fig. 4c would suggest that Trp 135 is not the target in the quenching mechanism for BSA and that the contribution to the emission from Trp 135 would be much lower than that from Trp 214 in this albumin. Therefore, under this considerations, the binding site in the IIA subdomain may be the major association site of Re(I) complex in both proteins. Similar results were reported previously in interactions studies between albumins and other metal complexes [30].

Thermodynamic analysis and nature of the binding forces

The binding constants for the interaction of the complex with albumins were determined from the fluorescence quenching data according to Eq. 3. The binding constants at five temperatures are shown in Table 3, see below.

According to Eqs. (4)–(6), the thermodynamic parameters (ΔH^0 and ΔS^0) were evaluated from the slope and *y*-interception of Van't Hoff's equation by plotting the values of ln K_b vs $1/T$ (Fig. 5).

The values of ΔG^0 were further calculated from the values of ΔH^0 and ΔS^0 (Table 3).

It was clear from the values of ΔG^0, ΔS^0, and ΔH^0 that binding of the Re(I) complex to both HSA and BSA occurs through endothermic processes accompanied by positive value of ΔS^0 (i.e., entropic dominance over the enthalpy factor). Thus, the binding process was in all cases spontaneous as evidenced by the negative sign of ΔG^0 values. According to an endothermic association process, a temperature increase promotes the interaction as reflected by higher K_b values. Positive values of both ΔH^0 and ΔS^0 usually allow us to assume that interactions between Re(I) complex and albumins are dominated by hydrophobic interactions [33, 34]. The positive entropy changes arise from the random rearrangement of water molecules which take place after the hydrophobic interaction between the albumin and RePtr. Therefore, hydrophobic interactions might play a major role in the binding process.

These results are in agreement with the results of association studies in which it was indicated IIA site (hydrophobic pocket) as the likely site of interaction.

Study of conformational changes of albumins induced by the RePtr binding

Circular dichroism (CD) spectra of BSA and HSA in the presence and absence of RePtr are shown in Fig. 6.

These spectra exhibit two negative bands in the UV region at 208 and 222 nm, a typical characteristic of the α-helix structure of these proteins [35].

Upon further addition of RePtr, the CD spectra do not show any significant variation indicating that there is no measurable change in the proteins conformation.

Table 3 Binding constants and thermodynamic parameters for the interaction of albumins and RePtr

Albumin	*T* [K]	$K_b \times 10^{-5}$ [M^{-1}]	ΔG^0 [KJ/mol]	ΔH^0 [KJ/mol]	ΔS^0 [J/mol K]
HSA	297.96	2.97	−31.3	51.7	278
	301.05	4.01	−32.1		
	303.95	4.09	−32.9		
	306.98	6.18	−33.8		
	309.95	6.54	−34.6		
BSA	297.96	3.0	−31.3	39.5	238
	301.05	3.8	−32.1		
	303.95	4.3	−32.8		
	306.98	5.0	−33.5		
	309.95	5.6	−34.2		

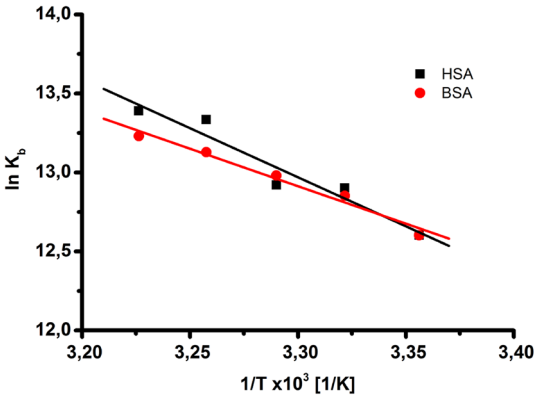

Fig. 5 Van't Hoff plot (ln K_b vs $1/T$) for HSA and BSA with RePtr complex

Summary and conclusions

We have developed a new synthetic route for obtaining the *fac*-$Re^{I}(CO)_3$(pterin)(H_2O) complex in aqueous solution via the *fac*-$[Re^{I}(CO)_3(H_2O)_3]Cl$ precursor complex.

The interactions between the Re(I) complex and albumins have been investigated by steady-state and time-resolved fluorescence, and UV–vis and CD spectroscopy. The results show that RePtr binds to both BSA and HSA with high association constants ($K_b \approx 6 \times 10^5$ M^{-1}) at physiological temperature. These association constants are of the same order of magnitude as those obtained for a large number of drugs transported through blood plasma by albumin [18, 36, 37]. There is a remarkable difference between the values of association constants found for the complex and that obtained for the free pterin ligand ($K_b = 1.3 \times 10^3$ M^{-1}) [38]. The difference may be due to the experimental conditions in which these constants were measured, since, at quite high quencher concentration, the measured constant refers to both specific and unspecific interaction. However, the neutral condition of the complex (zero net charge) could be the main cause of the stronger interaction.

The calculated thermodynamic parameters indicate that the acting forces are mainly hydrophobic. Again, these results can be rationalized in relation with the neutral state of the RePtr complex at the experimental pH conditions.

The observed saturation in emission quenching plots along the absence of changes in the lifetime is consistent with a mainly static quenching mode.

We have determined that the process is taking place in the IIA subdomain based on: the comparative analysis of the relative fluorescence quenching of BSA and HSA; the static quenching mode of the emission of Trp 214 located into IIA site; the similar values of binding constant found for both albumins; and the predominance of hydrophobic forces in the association process.

Unlike other Re(I) tricarbonyl complexes reported previously, RePtr complex does not modify the conformational structure of both HSA and BSA proteins after the interaction as shown by the results of CD. This suggests that these proteins can be considered as good carriers for transportation of RePtr complex.

These properties along with an excellent solubility in water may be important features in relation to its potential applications in several biomedical fields, such as photodynamic therapy and radiopharmacy [39].

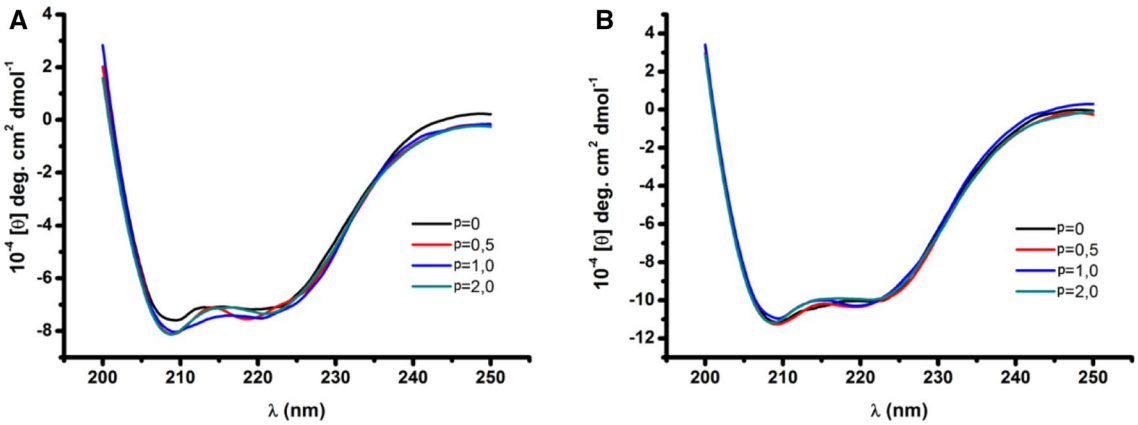

Fig. 6 CD spectra of HSA-RePtr (**a**) and BSA-RePtr (**b**). [albumins] $= 5 \times 10^{-6}$ M; [RePtr] from 0 M, $p = 0$–1×10^{-5}M, $p = 2$; $T = 298$ K; phosphate buffer pH 7.4

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