Journal of Photochemistry and Photobiology A: Chemistry 227 (2012) 38–44

Contents lists available at SciVerse ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

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

Rhenium(I)-based fluorescence resonance energy transfer probe for conformational changes of bovine serum albumin

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ARTICLE INFO

Article history:
Received 13 April 2011
Received in revised form 5 October 2011
Accepted 27 October 2011
Available online 4 November 2011

Keywords:
Rhenium(I) tricarbonyl complex
Bovine serum albumin
Energy transfer
Protein binding

ABSTRACT

Protein binding properties of *fac*-rhenium(I) complexes with general structure $[Re(CO)_3(N\text{-}N)L]PF_6$, where N-N = 4,4′-dinanoyl-2,2-bipyridine and L = py-3-COOH (**1a**) and py-3-$CONH_2$ (**1b**) with bovine serum albumin (BSA) were investigated at physiological pH (7.4) using UV–visible absorption and fluorescence spectral study, excited state lifetime measurement and circular dichroism (CD). The results observed from fluorescence spectra reveal the energy transfer from BSA to Re(I) complex, and the distance r between donor (BSA) and acceptor (Re(I) complex) is 3.05 nm and 2.16 nm for **1a** and **1b** respectively according to Forster's non-radiative energy transfer theory. CD results show that the binding of Re(I) complex could induce the conformational change with the loss of α-helicity.

1. Introduction

The use of Re(I)-based luminophores has attracted significant attention due to the favorable photophysical properties associated with complexes of the generic form *fac*-$[Re(CO)_3(N\text{-}N)(L)]^+$ (where L = monodendate and N-N = bidendate ligands) [1–7]. Since the excited state is formally localized on the single diimine ligand (Metal to Ligand Charge Transfer (MLCT), Re → N-N), these complexes often serve as more sensitive probes than related tris-chelated $[Ru(diimine)_3]^{2+}$) complexes [8]. This advantage leads to the development of a variety of Re(I) derived systems exploited in cellular imaging using fluorescence microscopy [9]. Schanze et al. [10] and Yam et al. [11] reported the luminescent properties of Re(I)-anthacene system and its application as a luminescent probe for DNA binding. Coogan et al. [12] used Re(I) complex for cell imaging.

The development of transition metal complexes that target and interact non-covalently with proteins is an emerging field that links bioinorganic chemistry with chemical and synthetic biology [3,13–15]. The metal complexes are very much suited to the optimization of non-covalent interactions, since they combine flexibility in ligand design with access to a variety of coordination geometries, oxidation states and electronic configurations [16]. Once protein is bound to a metal ion, metal ion plays a structural role, particularly on protein folding, but can also determine the reactivity of a protein as part of redox or catalytic center site [17]. Shahabadi and Maghsudi studied the interaction between Cu(II) complexes and BSA using absorption, fluorescence and circular dichroism spectroscopy [18]. Palaniandavar et al. reported the cleavage of BSA using Cu(II) phenolate complex [19]. Recently Lo et al. extensively studied the protein binding properties of Re(I) complexes with BSA [20,21]. In this perspective, we report our results obtained through the study of interaction of Re(I)-complexes carrying hydrophobic ligand with protein *via* non-covalent interactions to design spectroscopic probes.

BSA belongs to a class of proteins most widely studied category and functions as a carrier for many fatty acid anions and other simple amphiphiles in a blood stream [22]. It has a molecular weight of 6.8×10^4 g mol^{-1} and contains 583 amino acids in a single polypeptide chain. The protein contains 17 disulfide bridges and one free-SH group, which can cause it to form a covalently linked dimer. The interior of the protein is almost hydrophobic, while both the charged amino acid residues and apolar patches cover the interface. The tertiary structure of BSA is composed of three domains I–III and each domain consist two subdomains A and B. Because of the common interface between domains II and III, binding of a probe to domain III is associated with the conformational changes of domain II and hence its binding affinities. BSA contains two tryptophan residues, Trp-134 and Trp-212, of which the former is located in hydrophilic subdomain 1B, and it is proposed to be located near the surface of albumin molecule in the second helix of the first domain. Trp 212 is located within a hydrophobic binding pocket of the protein. BSA is known to exhibit a very high conformational adaptability to a large variety of ligands [22,23]. In general BSA has been used extensively in the past years, partly because of its structural homology with HSA [24,25]. In this work, we report

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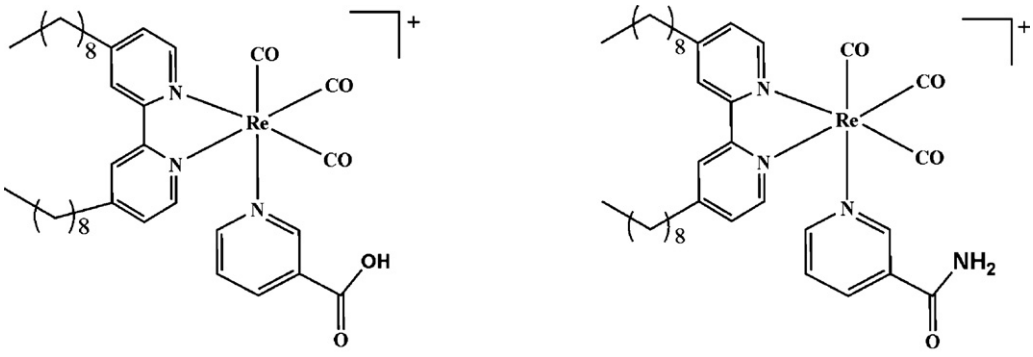
doi:10.1016/j.jphotochem.2011.10.022



[Re (CO)$_3$(dnbpy)(py-3-COOH)]PF$_6$ (1a) **[Re (CO)$_3$(dnbpy)(py-3-CONH$_2$)]PF$_6$ (1b)**

Chart 1. Structure of the rhenium(I) complexes.

the protein (BSA) binding properties of Re(I) complexes carrying surfactant ligands. The interaction of proteins with surfactants has received a great deal of interest for many years due to its application in a great variety of industrial, biological and cosmetics system [26,27].

2. Experimental

2.1. Materials

The chemicals used in this study $Re_2(CO)_{10}$ and ligands 4,4′-dinanoyl-2,2′-bipyridine (dnbpy), nicotinic acid (py-3-COOH) and nicotinamide (py-3-CONH$_2$) were obtained from Aldrich and Merck and used as such. BSA was purchased from Genei. $Re(CO)_5Br$ was obtained by the oxidative addition of Br_2 to Re_2CO_{10} [28].

2.2. Synthesis of Re(I) complexes

The complexes $[Re(N\text{-}N)(CO)_3(L)]PF_6$ (N-N = dnbpy and L = py-3-COOH or py-3-CONH$_2$) were prepared from $Re(CO)_5Br$ by the modification of the method reported by Lo et a1. [29] (see Supporting Information).

2.3. Instrumentation and methods

The stock solution of BSA (1×10^{-3} M) based on its molecular weight of 68,000 was prepared in buffer solution at physiological pH (7.4). The stock solution for complexes **1a** and **1b** (1×10^{-3} M) were prepared in acetonitrile (HPLC). Double distilled water was used throughout the experiment. The sample solutions were prepared freshly for each measurement.

The Re(I) complexes were characterized using mass, NMR and IR techniques. The mass spectra were recorded using a Quattro LC triple-quadrupole mass spectrometer (Micromass, Manchester, UK) interfaced to an ESI (Electro Spray Ionization) source; data acquisition was done under the control of Masslynx software (version 3.2), the ESI capillary voltage was maintained between 4.0 and 4.2 kV and the cone voltage kept at 25 V. Nitrogen was used as desolvation and nebulization gas. NMR spectra were recorded on Bruker (300 MHz) spectrometer and IR spectra using 8400S Shimadzu-FT-IR spectraphotometer.

Absorption spectra were recorded using Analytik Jena Specord S100 spectrometer. The emission was recorded using Varian Cary Eclipse spectrofluorometer. All the sample solutions used for the emission measurements were deaerated for about 30 min, by dry N_2 purging by keeping solution in cold water to ensure that there is no change in the volume of the solution. All the measurements were done at room temperature. Both the absorption and emission titrations were carried out by keeping the concentration of BSA as constant (1×10^{-5} M) while varying the concentration of complexes (**1a** and **1b**) from 0 μM to 24 μM.

The fluorescence measurements were performed at different concentrations of Re(I) complexes and the quenching rate constant, k_q, values were determined from the modified Stern-Volmer plot using the Eq. (1) [30].

$$\frac{F_0}{F_0 - F} = \frac{1}{fK[Q]} + \frac{1}{f} \quad (1)$$

where F_0 is the initial fluorescence intensity and F is the fluorescence intensity in the presence of quenching agent or interacting molecule (Re(I) complex). K is the Stern-Volmer quenching constant, [Q] is the molar concentration of quencher (Re(I) complex), and f is the fraction of accessible fluorophore–quencher complex, which indicates the fractional fluorescence contribution of the total emission for an interaction with a hydrophobic quencher [31]. The plot of $F_0/(F_0 - F)$ vs 1/[Q] yields f^{-1} as the intercept on the y axis and $(fK)^{-1}$ as the slope. k_q, the quenching rate constant is calculated from obtained K value.

Time resolved fluorescence measurements were carried out using a diode laser-based time correlated single photon counting (TCSPC) spectrometer from IBH, UK. In the present study, a 295 nm diode laser (50 kHz) was used as the excitation source and Hamamatsu photomultiplier tube was used for the fluorescence detection. The instrument response function for this system is ≈1.2 ns and the fluorescence decay was analyzed using the software provided by IBH (DAS-6) and PTI global analysis software.

Circular dichroism (CD) measurements were performed on a JASCO J810 spectropolarimeter at RT over the wavelength 200–260 nm. Parameters were set as follows: path length, 50 mm; resolution, 0.5 nm; scan speed, 50 nm min^{-1}; band width, 1 nm; response 1 s. Every spectrum was averaged two times. The concentration of BSA is 3×10^{-6} M and the concentration of Re(I) complex is 1×10^{-5} M.

3. Results and discussion

The structure of the Re(I) complexes synthesized for the present study are shown in Chart 1. The absorption and emission spectra of **1a** in acetonitrile are shown in Fig. 1 and the spectral data of Re(I) complexes are collected in Table 1. The electronic absorption spectra of Re(I)-tricarbonyl complexes **1a** and **1b** reveal strong absorption bands at 225–315 nm and less intense absorption shoulders at 345–360 nm. With reference to previous studies on related Re(I) complexes [32–34], these strong high energy absorption bands at 225–315 nm are assigned to ligand centered (LC) π–π^* transition and low energy absorption shoulders at 345–360 nm

Table 1
Photophysical properties of the Re(I)-complexes in CH_3CN and IR spectral data.

Complex	λ_{abs}, nm (ε_{max}, $dm^3\ mol^{-1}\ cm^{-1}$)	λ_{em} (nm)	τ (ns)	ν(CO) (cm^{-1})
Re-dnbpy-Br	216 (29,236), 245 (19,547), 288 (14,308), 369 (3461)	587	46	2019, 1915, 1890
1a	222 (31,995), 246 (26,964), 274 (23,059), 310 (16,230), 349 (5930)	549	138	2029, 1911
1b	219 (32,757), 239 (27,398), 269 (25,343), 305 (17,769), 360 (9854)	538	123	2027, 1905
BSA	278 (45,110)	348	–	–

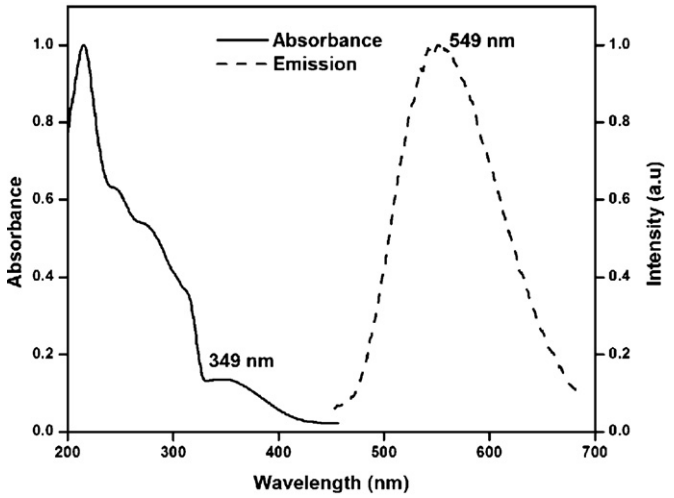


Fig. 1. Normalized absorption and emission spectra of complex **1a** in acetonitrile.

to spin allowed metal-to-ligand charge transfer (^{1}MLCT) transition from the Re dπ-orbital to the π^* orbital of the ligand dπ Re $\rightarrow \pi^*$ (diimine). Complexes **1a** and **1b** show luminescence in the region 545–555 nm in acetonitrile at RT. Variation in the axial ligand (replacement of Br with py-3-COOH and py-3-$CONH_2$) shifts the MLCT emission to higher energies (shorter wavelengths) due to the stabilization of the Re dπ-orbitals.

3.1. Steady state absorption and emission spectra

Fig. 2 shows the absorption spectrum of BSA in the absence and in the presence of complex **1b**. The absorption spectrum of BSA shows a maximum at 280 nm, which is characteristic absorption of

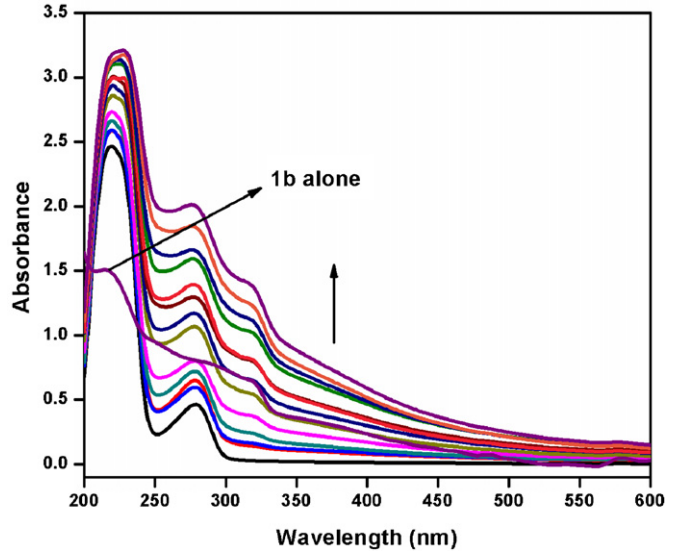


Fig. 2. Absorption spectra of BSA (1×10^{-5} M) in the absence and presence of different concentration of complex **1b** ([**1b**] = 0 → 24 μM).

tryptophan (Trp) and tyrosine (Tyr) residues in proteins [35]. The interesting point to be noted here is that in the absence of BSA the absorption intensity of **1b** ([**1b**] = 24 μM) at 318 and 360 nm are 0.64 and 0.33, respectively. But on the addition of BSA, the intensity of these bands increased up to 1.41 and 0.81. This is the first spectral evidence towards the occurrence of interaction between BSA and Re(I) complex.

Generally, the fluorescence of protein is caused by three intrinsic flours present in protein, i.e., Trp, Tyr and phenylalanine (Phe) residues [18]. Actually, the intrinsic fluorescence of many proteins is mainly contributed by tryptophan alone because of very low quantum yield of Phe, and the fluorescence of Tyr is almost quenched *via* energy transfer to Trp if Tyr is ionized, near an amino or carboxyl group. In order to confirm the interaction between Re(I) complexes and BSA, fluorescence titration is also carried out for the same sample solutions used for the absorption measurements. The result of interaction of Re(I) complex with BSA is found to produce dramatic modifications on the emission profile of both BSA (Fig. 3A) and Re(I) complex (Fig. 3B). The fluorescence of BSA is quenched in the presence of Re(I) complex with a blue shift (≈10 nm) in the emission maximum. The variation in the tryptophan emission is the consequence of the protein conformational changes. The mixing of BSA with complex **1b** induces an enhancement along with blue shift (≈540 → 525 nm) (Fig. 3B) in the luminescence of Re(I) complex (the same trend is observed for complex **1a**, blue shift from ≈550 → 535 nm, see Supporting Information). We propose that the major force for these spectral changes is the strong hydrophobic interaction between Re(I) complex and BSA. Tajmir-Riahi et al. [30] and Guchhait et al. [36] observed the similar results for polyphenols and 5-(4-dimethylamino-phenyl)-penta-2,4-dienoic acid methyl ester (DPDAME) in the presence of BSA. The fluorescence quenching of BSA in addition to blue shift in the emission maximum suggests that binding of Re(I) complex is associated with the change in the environment of at least one of the two Trp moieties in BSA. The binding constant (K_b) of BSA with Re(I)-complexes is determined using Eq. (2) [30]

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

where F_0 and F are the fluorescence intensities of BSA before and after the addition of Re(I) complex, n is the number of binding sites and [Q] is the concentration of Re(I) complex. The obtained binding constant values are collected in Table 2. The binding constants are $7.3 \times 10^5\ M^{-1}$ and $5.6 \times 10^6\ M^{-1}$ for **1a** and **1b**, respectively. This large binding constants indicate that there is a strong interaction between BSA and Re(I)-complex.

Fig. 4 shows the modified Stern-Volmer plot for the quenching of BSA with complex **1b** and the K_{SV} values are $1.5 \times 10^4\ M^{-1}$ and $2.0 \times 10^4\ M^{-1}$ for complexes **1a** and **1b**, respectively. The calculated k_q values are $2.6 \times 10^{12}\ dm^3\ mol^{-1}\ s^{-1}$ and $3.5 \times 10^{12}\ dm^3\ mol^{-1}\ s^{-1}$ for complexes **1a** and **1b**, respectively (Table 2). In general, the maximum collision quenching constant (k_q) of various kinds of quenchers to biopolymers is $2.0 \times 10^{10}\ L\ mol^{-1}\ s^{-1}$ [37]. Obviously, the quenching rate constant of BSA–Re(I) system is very high compared to the collisional quenching constant of biopolymer. This shows that the major component of quenching of BSA by Re(I) complex is *via* the formation of complex between BSA and Re(I) ion i.e., the major contribution

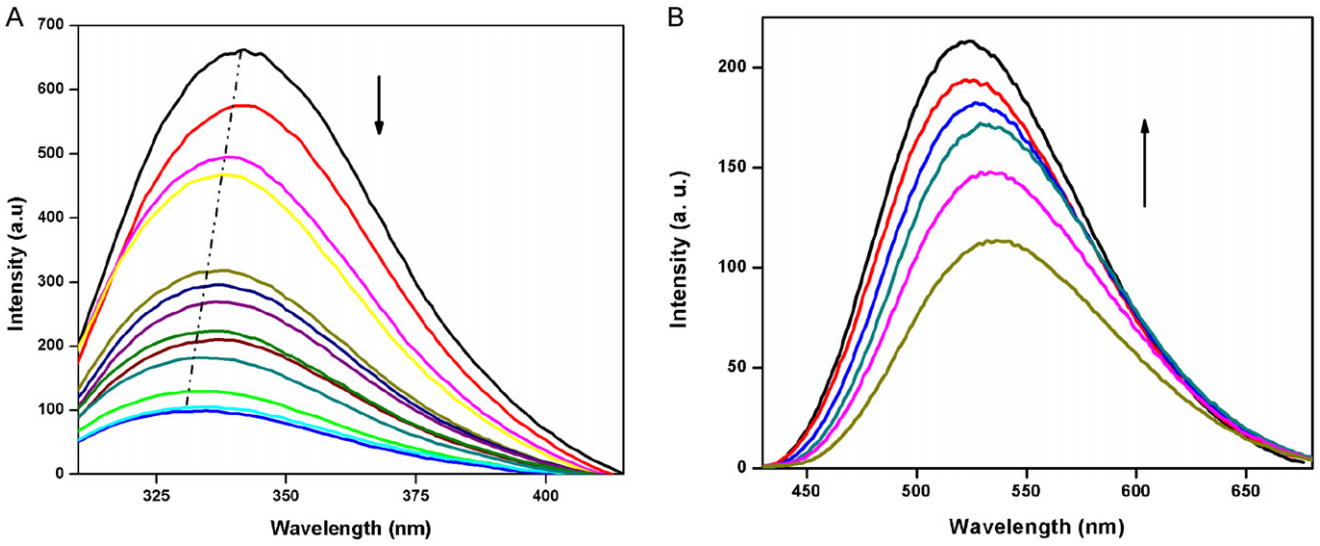


Fig. 3. (A) Fluorescence quenching of BSA in the presence of various [**1b**] (0 → 24 μM), [BSA] = 1×10^{-5} M excitation maximum – 290 nm; (B) fluorescence enhancement of complex **1b** in the presence of various [BSA] (0 → 100 μM), [**1b**] = 1×10^{-5} M excitation maximum – 360 nm.

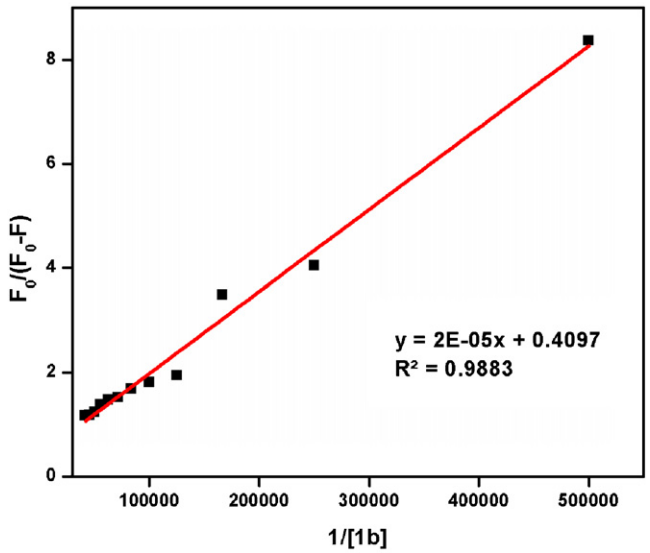


Fig. 4. Stern-Volmer plot for the quenching of BSA with complex **1b**.

for the BSA fluorescence quenching with Re(I)-complex is the static quenching process.

3.2. Fluorescence resonance energy transfer (FRET)

The past few years, in the field of photochemistry and photobiology, have witnessed an awesome progression of research related to the photophysical studies on metal complexes bound to biomolecules particularly on fluorescence resonance energy transfer in the donor (D)–acceptor (A) prototype [38–41]. During the FRET process, the donor emission intensity is found to be decreased with concominent increase in the acceptor emission intensity as the acceptor concentration increases. It is interesting to note that the quenching of fluorescence of BSA accompanies simultaneous increase in the intensity of a new band at ≈535 nm (Fig. 5A) for both complexes **1a** and **1b** which is the CT emission of Re(I) complex (blue shifted from 555 → 532 nm for complex **1a** and 540 → 535 nm for complex **1b** with [BSA] = 1×10^{-5} M). Interestingly, we have observed the luminescence enhancement of Re(I) complex on the addition of BSA at constant [Re(I) complex] (Fig. 3B). The blue shift in the fluorescence maximum of BSA in the presence of Re(I) complex and luminescence enhancement of Re(I) complex in the presence of BSA (Fig. 3) can be rationalized on the basis of the modification of the local environments of the Trp residue as well as Re(I) complex during the interaction between BSA and Re(I) complex. In addition, the clear isoemissive point at 415 nm (Fig. 5A) indicates that the quenching of the fluorescence of BSA depends on the formation of BSA–Re(I) complex [42]. Taking into account an overlap between the emission spectrum of BSA and the absorption spectrum of Re(I) complex (Fig. 5B), an excited state energy transfer mechanism may be proposed.

The importance of FRET in biochemistry is that the efficiency of transfer can be used to evaluate the distance, *r*, between the probe (Re(I)-complex) and Trp residue in the protein. According to the Forster's non-radiative energy transfer theory [43], the rate of energy transfer depends on (i) the relative orientation of the donor (BSA) and the acceptor (Re(I) complexes) dipoles, (ii) the extent of

Table 2
Binding constant (K_b), quenching constant (k_q) and lifetime (τ) data for BSA in the presence of Re(I) complexes.

Complex	K_b (M^{-1})	n	k_q ($dm^3\ mol^{-1}\ s^{-1}$)	τ_1, ns (%)	τ_2, ns (%)	χ^2
1a	$7.3 \times 10^5 \pm 0.1$	1.2	2.6×10^{12}	–	–	–
1b	$5.6 \times 10^6 \pm 0.2$	1.3	3.5×10^{12}	–	–	–
BSA[a]	–	–	–	1.32 (8.32)	5.85 (91.68)	1.20
BSA + 10 μM **1a**	–	–	–	0.96 (8.57)	5.44 (91.43)	0.99
BSA + 20 μM **1a**	–	–	–	0.24 (13.41)	5.21 (86.59)	1.05
BSA + 10 μM **1b**	–	–	–	0.60 (12.52)	5.16 (87.48)	1.07
BSA + 20 μM **1b**	–	–	–	0.11 (17.52)	4.94 (82.96)	1.02

[a] [BSA] = 1×10^{-5} M.

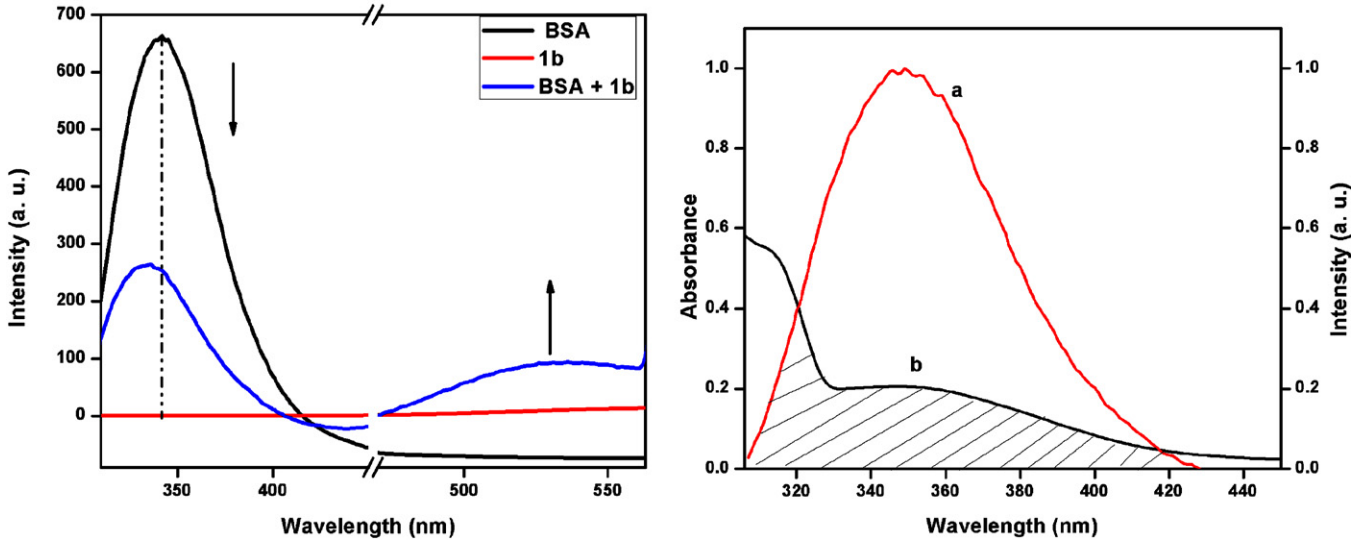


Fig. 5. Overlap between the emission spectrum of BSA (a) and the absorption spectrum of Re(I) complex (b) (right). Fluorescence resonance energy transfer (FRET) from BSA to bound Re(I) complex ([BSA] = 10 μM, [**1b**] = 10 μM) (left), excitation maximum – 290 nm.

overlap of emission spectrum of the donor with absorption spectrum of the acceptor and (iii) the distance between the donor and the acceptor. The efficiency of energy transfer, E, is calculated using Eq. (3).

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \tag{3}$$

where F and F_0 are the fluorescence intensities of BSA in the presence and absence of Re(I) complex (24 μM), r is the distance between the acceptor and the donor and R_0 is the critical distance when the transfer efficiency is 50% and the value is given by Eq. (4).

$$R_0^6 = 8.79 \times 10^{-25} \chi^2 n^{-4} \phi J \tag{4}$$

$$J = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda}{\sum F(\lambda)\Delta\lambda} \tag{5}$$

Here $F(\lambda)$ is the fluorescence intensity of the fluorescent donor at the wavelength λ and $\varepsilon(\lambda)$ the molar absorption coefficient of the acceptor at λ. The value of J is 6.6×10^{-14} cm^{-1} M^{-1} and 1.4×10^{-14} cm^{-1} for **1a** and **1b**, respectively, which is calculated by integrating the spectra collected in Fig. 5B. It has been reported for BSA that $K^2 = 2/3$, $\phi = 0.15$, and $N = 1.336$ [44]. Based on these data, we estimated R_0 and r values and the data are collected in Table 3. The donor to acceptor distance, $r < 8$ nm indicates that the energy transfer from BSA to Re(I)-complex occurs with high possibility [45]. That is the donor (tryptophan of BSA) and acceptor (Re(I)) can approach very close for very high energy transfer efficiency. This also indicates that the probe can measure the proteinous microenvironment at a distance of 3.05 and 2.16 nm. As far as we know, it seems to be the first documentation of energy transfer from BSA to Re(I) complex. That the ratio r/R_0 amounts to 0.87 and 0.80 for complex **1a** and **1b**, respectively, i.e., within the range 0.5–2.0, rationalizes the practical reliability of the FRET process to measure the distance between donor and acceptor chromophore in the present case [31].

Table 3
Fluorescence resonance energy transfer (FRET) data for complexes **1a** and **1b** with Trp of BSA.

Complex	J value/10^{-14} (cm^3 mol/L)	R_0 (nm)	E	r (nm)
1a	6.56	3.49	0.75	3.05
1b	1.39	2.69	0.85	2.16

3.3. Lifetime measurement

Time-resolved fluorescence study is also carried out to investigate the quenching mechanism of BSA fluorescence by Re(I) complexes **1a** and **1b**. Fluorescence lifetime serves as a sensitive parameter for exploring the local environment around a fluorophore, and it is sensitive to excited-state interactions [46]. It also contributes to the understanding of the interactions between the probes and the proteins [47]. The fluorescence lifetime of the tryptophan residues in proteins is a complex function of its interactions with the local environment and the solvent. The fluorescence decay curves were fitted to biexponential function with acceptable χ^2 values (Table 2) for BSA in the absence and presence of probe. The literature data show that native BSA has two lifetime components: one ($\tau_1 = 5.85 \pm 0.2$ ns) contributing 75% of the total fluorescence, and the other ($\tau_2 = 1.2 \pm 0.2$ ns) contributing the rest of the fluorescence [48]. The one with a longer lifetime is assigned to Trp-134 (hydrophilic in nature), and the other to Trp-212 (hydrophobic in nature) [30,36]. The relative contribution of each component is dependent on the folding/unfolding conditions due to the protein's multiple local configurations and changes in the extent of solvent accessibility [49]. Fig. 6 shows the decrease in the τ value of BSA with increasing the probe concentration. The lifetime of BSA alone in buffer is 5.85 ns and 1.32 ns, which are in good agreement with the reported values [49]. A glance at Table 2 and Fig. 6 reflect that two lifetime values observed, τ_1 and τ_2, decrease in the presence of the Re(I) complexes than those measured for native BSA in an aqueous buffer solution. Thus, the decrease in fluorescence lifetime is a clear consequence of the significant interaction between Re(I) complexes and BSA, with both the Trp 134 and Trp 212. The efficiency of lifetime quenching is found to be higher at Trp 212 (hydrophobic) which shows the stronger hydrophobic interaction between Re(I) complex and Trp 212 of BSA.

3.4. Conformational change of BSA in the presence of Re(I)-complex

The absorption and emission titration experiments confirm the interaction between Re(I) complexes and BSA. It is important to

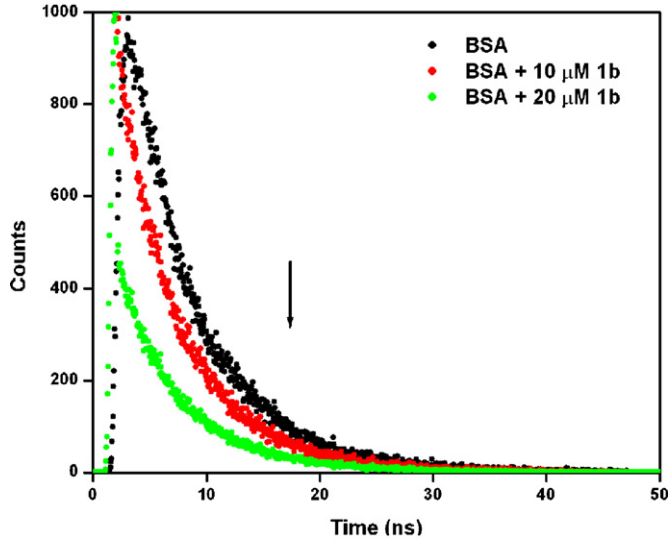


Fig. 6. Fluorescence decay curve of BSA in the presence of complex **1b**, [BSA] = 1×10^{-5} M.

examine how the structure of BSA is affected in the presence of Re(I) complexes. When probes bind to a globular protein, the intramolecular forces responsible for maintaining the secondary and tertiary structures can be altered, resulting in a conformational change of the protein [50]. Circular dichroism is a sensitive technique to monitor conformational changes of protein upon interaction with small molecules. BSA has a high percentage of α-helical structure which shows characteristic strong double minima signals at 208 and 222 nm (Fig. 7). The reasonable explanation is that the negative peaks at 208 and 222 nm are both contributed to n–π* transition of the carbonyl group of peptide. The CD result is expressed as MRE (Mean Residue Ellipticity) in° cm^2 $dmol^{-1}$, which is defined as Eq. (6).

$$\text{MRE} = \frac{\theta_{\text{obs}}}{10nlC_{\text{p}}} \tag{6}$$

Here θ_{obs} is the CD in milli-degree, n is the number of amino acid residues (583), l is the path length of the cell (5 mm) and C_{p} is

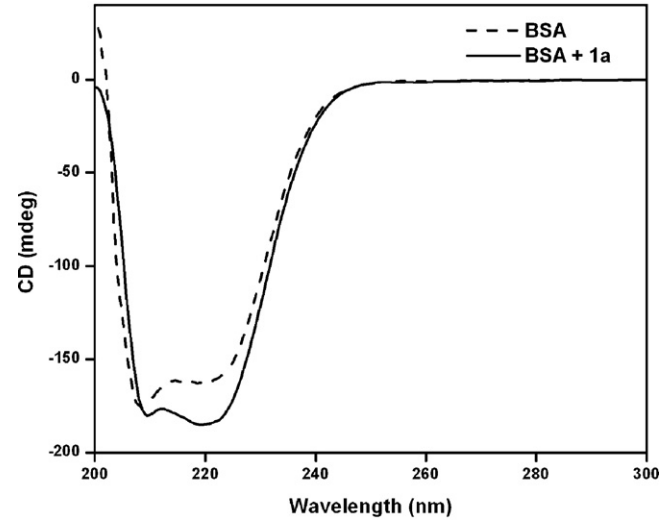


Fig. 7. CD spectra of BSA in the presence and absence of the complex **1a**, [BSA] = 3×10^{-6} M and [**1a**] = 1×10^{-5} M.

the mole fraction. The helical content is calculated from the MRE values at 208 nm using the Eq. (7) [51].

$$\alpha\text{-helix} = \left[\frac{(-\text{MRE}_{208\,\text{nm}}) - 4000}{33,000 - 4000}\right] \times 100\% \tag{7}$$

In Eq. (7) $\text{MRE}_{208\,\text{nm}}$ is the observed MRE value at 208 nm, 4000 is the MRE of the β-form and random coil conformation cross at 208 nm, and 33,000 is the MRE value of a pure α-helical at 208 nm. The quantitative analysis of the CD data using Eq. (7) provides the α-helix content in the secondary structure of serum albumin. The θ value decreased on the addition of Re(I) complex with slight red shift, which is indicative of the loss of α-helicity. Upon addition of Re(I) complexes to BSA the extent of α-helicity of the protein decreases from 62% to 54% and 52% for complexes **1a** and **1b**, respectively. It is apparent that the Re(I) complexes (**1a** and **1b**) bind to the amino acid residues of the main polypeptide chain of BSA and destroy the hydrogen bonding networks present in the amino acid residues of protein which may decrease the percentage of α-helicity of the protein [52]. Therefore, we can conclude that the effect of Re(I) complex on BSA causes a conformational change of the protein with the loss of α-helix stability.

4. Conclusions

The binding of Re(I)-complexes (**1a** and **1b**) with BSA is investigated using UV–visible, steady-state fluorescence, CD and fluorescence lifetime measurements. The BSA fluorescence exhibits an appreciable blue shift along with fluorescence intensity quenching and a decrease in the fluorescence lifetime upon binding with the Re(I) complexes. The decrease in both τ_1 and τ_2 values of BSA in the presence of Re(I) complexes show the interaction of probe with both Trp 134 and Trp 212 residues but stronger with the more hydrophobic Trp 212 residue. Quenching of BSA fluorescence and an enhancement in the Re(I) luminescence is found to be a function of the extent of spectral overlap between the donor's fluorescence and acceptor's absorption spectrum. This observation indicates that the energy transfer from the Trp residues of BSA to the probe, Re(I) complex occurs efficiently.

Acknowledgements

Financial support for this work from Rajiv Gandhi National Fellowship (UGC) is greatly acknowledged. SR thanks DST, New Delhi and Madurai Kamaraj University (UGC-UPE) for funding.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jphotochem.2011.10.022.

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