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Study on the interaction between a luminescent metal-ligand probe and bovine serum albumin by fluorescence spectroscopy

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ABSTRACT

The interaction between a luminescent metal-ligand probe, $[Ru(dpp)_3]^{2+}$ (dpp = 4,7-diphenyl-1,10- phenanthroline disulphonic acid) with bovine serum albumin(BSA) was investigated by means of absorption and fluorescence spectroscopy. The fluorescence of BSA was quenched by the ruthenium (II) complexes and the quenching mechanism was considered as static by forming a complex. The association constant K_b and the number of binding sites, n were calculated using the modified Stern Volmer equation. The fluorescence of the metal complex is highly enhanced by the addition of BSA. In addition, the results of synchronous fluorescence spectra showed that binding of the metal probe with the BSA induce conformational changes in BSA. The fluorescent enhancement of the system is found to be originated from the hydrophobic microenvironment provided by BSA.

Introduction

The interest as biophysical probes was stimulated by the observation that the metal-ligand complexes display strongly polarized emission. Small organic fluorophores have extinction coefficients ranging from 10,000 to 1,00,000 □-1cm-1, decay times 1 to 20 ns and display photobleaching. The timescales are useful for many biophysical measurements, but there are numerous instances where intermediate decay times are desirable. To measure rotational motions of large proteins or membrane-bound proteins, the overall rotational correlation times can be nearly 200 ns and can exceed 1 □s for larger macromolecular assemblies. Rotational motions on this timescale are not measurable using fluorophores that display ns lifetimes. Hence, there is a need for probes that display microsecond lifetimes. One such probe is a metal-ligand probe, rutheniumbathophenanthroline complex, which displays lifetime in the range of microseconds [1,2]. The ruthenium complex containing three diphenylphenanthrolines, [Ru(dpp)3]2+ has a long lifetime of nearly 5 □s and found widespread use as an oxygen sensor.

Ruthenium (II)-polypyridyl complexes, [Ru (NN)3]2+ (NN = 2,2'-bipyridine and 1,10-phenanthroline and their derivatives) are characterized by strong metal-to-ligand charge transfer (MLCT) absorption in the visible region and are highly coloured and luminescent [3-7]. These complexes have been widely used as photosensitizers because of their simple synthetic procedure and unique photophysical properties such as long-lived 3MLCT states, appreciable luminescence quantum yield, large excited state lifetime and above all their chemical and photostability. These metal complexes find extensive applications as important materials for the construction of solar energy conversion devices, light emitting diodes, dye sensitized photoelectrochemical cells, molecular probes, sensors, NLO materials and as photocatalysts [8-12].

Serum albumin is the major protein constituent of blood plasma which facilitates the disposition and transport of various exogeneous and endogenous ligands to the specific targets [13-15]. There are two major and structurally selective binding sites, namely site I and site II, which are located I three homologous domains that form a heart-shaped protein. The binding affinity offered by site I is mainly through hydrophobic interactions, whereas site II involves a combination of hydrophobic, hydrogen bonding and possessing higher affinity for serum albumin and showing preferential binding site II are found to exhibit photodynamic therapeutical applications (PDT). Therefore, detection and understanding of the nature and selective binding interactions of probes with serum albumin is important for the design of efficient drugs and sensitizers for use in PDT. Herein, we report the spectroscopic studies on the interaction of the metal-ligand probe, [Ru(dpp)3]2+ with bovine serum albumin (BSA).

Experimental Procedure

Bovine serum albumin (BSA) fraction V was purchased from Merck and is essentially fatty acid free used without further purification; the molecular weight of 67,000 Da was used to calculate the molar concentrations. All reagents were of analytical grade and distilled water is used throughout the experiment. Phosphate buffer solution is used and the pH of the medium is maintained as 7.0 throughout the measurements. Ruthenium(II)bathophenanthroline disulfonate chelate was prepared as reported in the literature [15-20]. Freshly prepared solutions of BSA and ruthenium complex were mixed and subjected for each measurement. All the measurements were performed at room temperature.

Fluorescence spectra and synchronous fluorescence spectra were measured with a F-6300 JASCO Spectrofluorometer equipped with xenon lamp source using 5 nm/5 nm slit widths. Absorption spectra were recorded on a SPECORD S100 Diode-array spectrophotometer with 1.0cm quartz cells.

Results and Discussion

UV-Vis absorption spectra titration

UV-Vis absorption measurement is a very simple method and applicable to explore the structural change and to study the complex formation.

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The molecular structure of the ruthenium metal complex is given in Chart 1. The optical absorption of the metal ligand probe, [Ru(dpp)3]2+ in aqueous medium at pH 7.4 shows a strong high energy absorption in the 285-300 nm region corresponds to the π-π* (LC) transition and low energy absorption in the region 420-470 nm is assigned to the dπ-π* (MLCT) transition. The absorption spectrum of Ruthenium bathophenanthroline homoleptic complex shows a maximum at 273 and 460 nm. The characteristic peak at 273 nm corresponds to the ligand centered peak and the peak at 460 nm is assigned to the MLCT transition. The absorption spectrum of BSA has maximum peaks at 218 nm and 279 nm. The absorption of the protein in 279 nm is mainly due to the presence of the chromophore tryptophan and tyrosine residues present in BSA. Absorption measurement is a simple but effective method in confirming the probable quenching mechanism. For dynamic quenching the absorption of the fluorophore are not changed, only excited-state fluorescence molecule is influenced by the quencher, whereas for the static quenching, a new compound is formed between ground–state of flurophore and a quencher, the absorption of fluorophore would be influenced [21].

Fig.1a shows the absorption spectrum of BSA with the addition of metal complex. BSA has two characteristic absorption bands in the region of 200 to 300 nm with absorption maxima at 218 nm and 279 nm.

Chart 1 Molecular structure of $[Ru(dpp)_3]^{2+}$

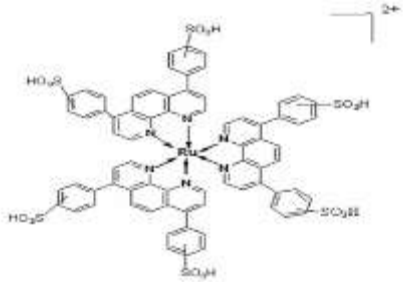
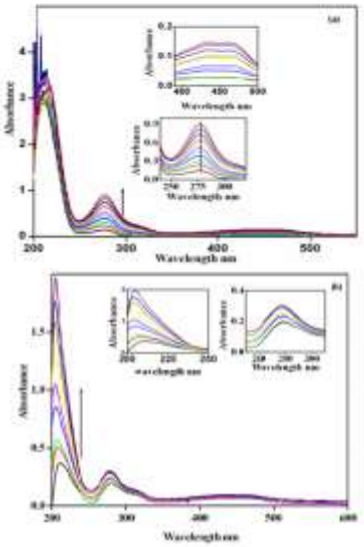


Fig 1Absorption spectra of (a) BSA in presence of different $[Ru(dpp)_3]^{2+}$ and (b) $Ru(dpp)_3^{2+}$ in presence of different [BSA]in aqueous medium at pH 7.4.

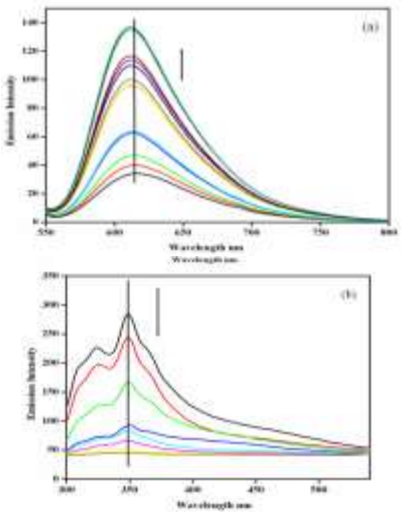


The increasing concentration of ruthenium increases the absorption at maximum 279 nm as well as at 460 nm (inset in fig.1a). These results suggest that there is binding interaction occur between the metal complex and BSA. Further in order to confirm the interaction of BSA with the metal complex, absorption spectrum was monitored by gradually increasing the concentration of BSA keeping constant amount of ruthenium in the reaction mixture (fig 1b). Thus, by the use of these two methods, it is evident from the absorption spectral studies that the metal complex and BSA interact to form an association complex. The absorption of [Ru(dpp)3]2+ increases on adding BSA and forms an association complex (fig 1b), which indicates that there is an interaction between [Ru(dpp)3]2+ and BSA. This results revealed that static quenching is occurred during the interaction process. The association constant of the BSA-ruthenium metal complex is found to be 5.2 ×10 6 M-1.

Fluorescence titration of [Ru(dpp)3]2+ (dpp=diphenylphenathroline) against BSA

The fluorescence titration of Ruthenium complexes with BSA was carried out in aqueous medium at pH 7.0 and is shown in fig 2a. Ruthenium complexes are highly luminescent and emit in the range of 600-700 nm. On incremental addition of BSA, the fluorescence intensity increases with a blue shift of 5 nm from 615 to 610 nm. The fluorescence enhancement with spectral shift indicates that the polarity of the microenvironment of ruthenium complexes decreased, which meant that the ruthenium complex entered the hydrophobic microenvironment of BSA.

Fig 2Change in emission intensity of (a) $Ru(dpp)_3^{2+}$ in presence of different [BSA] and (b) BSA in presence of different $[Ru(dpp)_3]^{2+}$ in aqueous medium

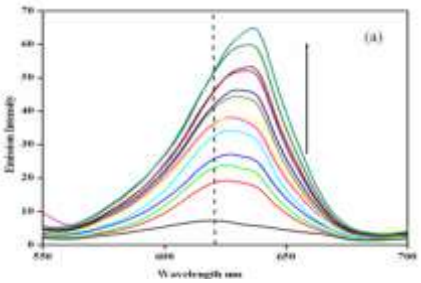


Fluorescence titration of BSA against [Ru(dpp)3]2+ (dpp=diphenylphenathroline)

Among the three intrinsic aromatic fluorophores in BSA molecules, tryptophan fluorescence is the most frequently examined to obtain information about conformational changes [22]. The interaction of ruthenium complexes to BSA and the conformational changes in BSA were evaluated by measuring the intrinsic fluorescence intensity of protein before and after the addition of ruthenium complex. Fig. 2b shows the change in emission intensity upon addition of ruthenium complexes. BSA has a strong fluorescence emission with a peak at 340 nm on excitation at 280 nm. With gradual increase in ruthenium complex concentrations, we observe slightly dual fluorescence behavior. The fluorescence intensity of protein decreased in the presence of ruthenium complexes and the emission wavelengths were shifted from 348 to 351 nm, suggesting that the microenvironment around BSA was changed in presence of ruthenium complexes. A significant decrease in the fluorescence intensity of BSA and red shift of the maximum wavelength were observed on addition of ruthenium complexes. This result suggests that the interaction between ruthenium complex and

BSA occurred. Mean while, the red shift of the maximum emission of BSA indicates that the polarity of the protein environment increased.

Fig 3 Synchronous fluorescence spectra of (a) $Ru(dpp)_3^{2+}$ in presence of different [BSA] and (b) BSA in presence of different $[Ru(dpp)_3]^{2+}$ in aqueous medium.



Fluorescence quenching mechanism

Protein could emit intrinsic fluorescence at 340nm when excited at 280 nm because three intrinsic fluorophore presented in it, i.e., tryptophan, tyrosine and phenylalanine. So the interaction of ruthenium complexes with BSA was studied by measuring the intrinsic fluorescence intensity of protein before and after addition of ruthenium complexes, Fig 2b shows the fluorescence spectrum of BSA upon addition of ruthenium complexes.

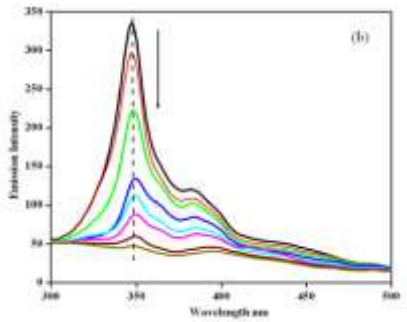


Fig 4 Stern Volmer plot for the quenching of BSA by the metal complex at 303 K

The different mechanisms of quenching are usually classified as dynamic quenching and static quenching. Dynamic and static quenching can be distinguished by their differing dependence on temperature and viscosity. Since higher temperature result in larger diffusion coefficients, the dynamic quenching constants are expected to increase with increasing temperature. In contrast, increased temperature is likely to result in decreased stability of complexes, and thus lower values of the static quenching constants [23].

The fluorescence quenching of BSA induced by the ruthenium complexes is dynamic and the quenching can be described by Stern Volmer equation (1)

$$F0/F=1 + Ksv[Q] =1+kq\tau0[Q] \quad (1)$$

where F0 and F represent the steady-state fluorescence intensities in the absence and presence of quencher (ruthenium complexes), respectively. Ksv is the Stern-Volmer quenching constant, [Q] is the concentration of ruthenium complexes and kq is the quenching rate constant of the biological macromolecule. Accordingly, Stern-Volmer equation was applied to determine Ksv by linear regression of a plot of F0/F against [Q]. τ0 is the average lifetime of the molecule in absence of quencher and the fluorescence lifetime of the biopolymer is 10-8s .Stern Volmer curves of F0/F versus [Q] is shown in Fig 4. The Stern- Volmer quenching constant is found to be 1.1x105.

Analysis of binding constant and binding sites

When small molecules bind independently to a set of equivalent sites on a macromolecule, Eq. (2) can be used to describe the relationship between fluorescence intensity and the concentration of the quencher

$$\log F0\text{-}F/F = \log Kb + n \log[Q] \quad (2)$$

where Kb is the binding constant and n is the number of binding sites per BSA . Fig 5 showed the double-logarithm curve log(F0-F)/F vs log[Q] . The double-logarithm curve yields a straight line and from the slope and the intercept, the binding constant, Kb is evaluated and found to be 5.9×106 M-1 and the binding site, n is approximately equaled to1. The very high value indicates the strong interaction between BSA and ruthenium complex.

Conformational investigation

Synchronous fluorescence spectroscopy can give information about the molecular environment in the vicinity of the chromosphere molecules in low concentration under the physiological condition. When Δλ between excitation and emission wavelength is stabilized at 15 nm or 60nm, the synchronous fluorescence gives the characteristics information of tyrosine or tryptophan residues of BSA [25]. The maximum emission wavelengths of the residues are related to the polarity of the surrounding environment, a lower hydrophobicity and a more loose structure of BSA and vice versa. The value of Δλ was set as 15nm and the environmental change was studied. It is evident (from fig 3a) that when Δλ was 15 nm, it shows a shift of 16nm (from 620 to 636 nm). The red shift indicated that the microenvironments around tryptophan and tyrosine residues were disturbed and the hydrophilicity of both the residues increased in the presence of ruthenium complex, and the spread of peptide strand increased [26]. The changes at the BSA center is also observed and a gradual decrease of the fluorescence intensity of BSA together with a red-shift is observed (fig 3b) when Δλ = 15 nm, which indicates that the polarity around the tryptophan residues is increased and the hydrophobicity is decreased. Thus the addition of the metal-ligand probe, ruthenium complex to the protein molecule induces the conformational changes of BSA. Similar results are observed when Δλ = 60 nm.

Conclusion

In this paper, the interaction between ruthenium complex and BSA was studied using fluorescence and UV-visible spectroscopy at 303K. The binding constant determined using UV-Vis and fluorescence titrations show similar affinity between the metal complex and the biomacromolecule, BSA. The results revealed that the BSA fluorescence was quenched by ruthenium through dynamic quenching. From the synchronous fluorescence spectroscopy it is understood that the tyrosine residues are placed in a more hydrophobic environment and the polarity around tryptophan residues is increased and the hydrophobicity is decreased when ruthenium interacts with BSA.

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