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Investigation on bindings of a binaphthoquinone derivative with serum albumin proteins by fluorescence spectroscopy

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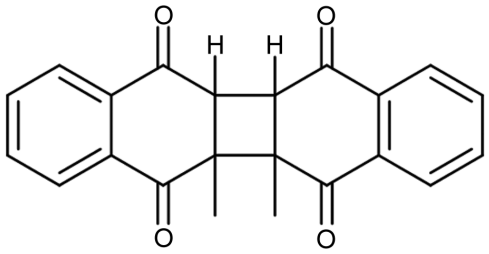
Binding of a binaphthoquinone derivative namely, 5a,5b-dimethyldibenzo[b,h]biphenylene-5,6,11,12(5aH,5bH,11aH,11bH)-tetraone (L, $C_{22}H_{16}O_4$) with bovine serum albumin (BSA) and human serum albumin (HSA) have been examined by using fluorescence spectroscopy. The fluorescence emission of the L is quenched upon addition of L to a solution of BSA or that of HSA, but the BSA has shown a higher affinity towards L over the HSA protein. A molecular docking study is also performed to suggest the sites of BSA for weak interactions to bind the L. The docking analysis, has revealed the N-H···O hydrogen bonds of L with different amino acid residues. The L is located at about 7.7Å away from the Trp-213 which is the fluorescent unit of the BSA suggesting the role of environment of the tryptophan residue to be an important to have changed the emission intensities.

Keywords: Naphthoquinone, BSA, HSA, Fluorescence, Molecular docking

The selective and specific binding of substrates with proteins have noteworthy roles in industrial, pharmaceutical and environmtantal chemistry.[1,2] The interactions of a protein and a substrate are mainly guided by the reorganised structure of the protein while interacting with the particular substrate under consideration.[3,4] Such studies also help to design drugs and other essential applications related to substrate bindings. Serum albumins are the most important proteins in the circulatory system. The effective interaction between the serum albumins with various molecules are very important to understand the distribution and absorption of a drug to search for possible delivery at specific site. From structural point of view, the tertiary structures of the BSA and HSA have similarities to an extent of 76%, hence, these proteins are homologous proteins[5-7].

In general, due to high sensitivity, selectivity and ease of execution of the fluorescence spectroscopy, it has been employed to investigate the effective binding of various substrates with these proteins. Fluorescence techniques also provide means to evaluate various binding parameters and different fluorescence paths and through space interactions.[8,9] For substrate binding study with protieins quinone and their derivatives have interest as they are biologically important probes and have relevances in electron-transfer and photochemical processes[10,11]. It is well known that the 2-methyl-1,4-naphthoquinone as the quinone part of the Vitamin K_1 and Vitamin K_2 is primarily responsible for anti-inflammatory, anticancer and toxicities.[12,13] Quinone derivatives show biological or pharmacological activity, and some of them such as lapachol, plumbagin, dichloroallyl lawsonedemonstrate anticancer activities.[14-17] So, it is essential to explore protein binding and bio-medical applications of the quinone derivatives. The limited literature on the dinaphthoquinone derivative namely 5a,5b-dimethyl dibenzo[b,h]biphenylene-5,6,11,12(5aH,5bH,11aH, 11bH)-tetraone (**L**, $C_{22}H_{16}O_4$) whose structure is shown in Fig. 1 has made us interested to explore its protein binding properties.[18-20] We report here the binding behaviour of the two serum albumin proteins, namely BSA and HSA proteins with **L** which were studied by us by montoring steady state fluorescence, measuring fluorescence anisotropy and by molecular docking.

Bovine serum albumin (BSA) is a large globular protein with molecular weight 66000 Da[21,22]. It has 582 constituent amino acids. It also has 17 disulphide bridges and a free -SH group. It is made up of three distinct homologous domains generally demarcated as



(L)

Fig. 1 — 5a, 5b-dimethyldibenzo[b,h]biphenylene-5,6,11,12 (5aH,5bH,11aH,11bH)-tetraone (**L**) used for protein (BSA and HSA) binding studies

domain I, II and III, respectively. On the other hand, Human serum albumin (HSA) is the most abundant protein in human blood plasma has molecular weight is 66.5 KDa constituted by 585 amino acid residues. Both these proteins contain tryptophan residue in distinguishable amount, namely the BSA has two tryptophan residues (TRP-131 and TRP-214), whereas, HSA has only one (TRP-214).[23] The tryptophan residues are responsible for the fluorescent behaviour of these proptiens. Hence, a substrate bin with BSA or HSA influences fluorescence emissions. Such changes in fluorescence also provide idea about the conformational changes of proteins.

Material and Methods

Materials

BSA, HSA and spectroscopic grade solvents were obtained from Sigma-Aldrich, USA and Merck, India. These were used as directly without further purification. The fluorescence spectra were obtained by using a Perkin-Elmer LS-55 spectrofluorimeter.

Preparation of solution of BSA and HSA proteins in buffer

A 10 mM phosphate buffer of pH 7 was used to prepare the protein solutions. The fluorescence titrations were carried out by taking the respective protein solution in a cuvette and adding definite aliquots of solutions of **L** (1.0 μM) by a micropipette from a stock solution of **L** in dimethylsulphoxide (DMSO). After each addition the solution is mixed well and emission spectra were recorded by excitation at 295 nm in each case, Due to low solubility of **L** in water, a solution in DMSO was used to prepare the solution of it.

Calculation of binding constants

Binding constant of BSA or HSA with the substrate was determined using the Benesi-Hildebrand equation by using the data from the fluorescence intensity change during each fluorescence titration using the following equation,[24]

$$1/\Delta F = 1/\Delta F_{max} + (1/K_a[C]^n) \times (1/\Delta F_{max}) \quad \ldots(1)$$

where, $\Delta F = (F_x - F_0)$ and $\Delta F_{max} = F_\infty - F_0$, where F_0, F_x, and F_∞ are the emission intensities of BSA or HSA in the absence of **L**, at an intermittent concentration of **L**, and at a concentration when the interaction was complete, respectively. K_a is the binding constant, C is the concentration of **L** and n is the number of **L** binding to BSA or HSA (here n = 1).

Principle of fluorescence quenching

The fluorescence quenching emissions in the two titrations with the proteins were analyzed by using Stern-Volmer equation[25] as shown in Eqn. (2),

$$F_0/F = 1 + Kq\ \tau_0\ [Q] = 1 + K_{SV}\,[Q] \quad \ldots(2)$$

where, F_0 and F respectively are the fluorescence intensities before and after the addition of the quencher **L**. Kq, K_{SV}, τ_0 and [Q] are the quenching rate constant of the bimolecular interaction, the Stern-Volmer dynamic quenching constant, the average lifetime of the bimolecular interaction without quencher ($\tau_0 = 10^{-8}$ s) and the concentration of the quencher, respectively. Obviously, $K_q\tau_0 = K_{SV}$, hence, later portion of the Eqn. (2) was applied to determine K_{SV} by linear regression of a plot of F_0/F versus [Q].

Protein sequence and ligand preparation

The protein sequence of BSA was retrieved from the Protein Data Bank (PDB id: 4f5s). The file was opened in Discovery Studio Visualizer where Hetero atoms and chain B was removed and protein file was saved in pdb format. The **L** was drawn in the Chemdraw (Chemoffice-17). The cdx file of **L** was opened in Chem3D and subjected to energy minimized using the force field MMFF94 with maximum number of iteration 500 and 0.1 of RMS gradient. The energy-minimized structure was saved in pdb format and further used for the docking purpose.

Molecular docking study

The docking study on HSA with **L** was performed by using Autodock 4.2.5 program. Autodock generally predicts the bound conformations of a supramolecular substrate to a bio-macromolecular target using Lamarckian genetic algorithm. It was done according to the literature report[26,27]. In brief, the PDBQT files were generated for protein and **L** molecule by adding polar hydrogen and Kollman charges in the case of BSA, whereas, in the **L**,

non-polar bonds were merged and Gasteiger charges were applied. The dimension of grid box was fixed to 74, 68, 66 (*x*, *y*, *z* coordinates) with default of 0.375 Å grid point spacing. For the docking simulation and conformational search Lamarckian Genetic Algorithm was used. The numbers of evaluation were set as 20 and rest of the parameters was kept as default. The molecular docking result was evaluated in PyMOL (PyMOL Molecular System, Version 2.0 Schrodinger, LLC as well as in Discovery Studio Visualiser.

Results and Discussion

Steady state fluorescence emission interactions between of BSA and HSA with L

The fluorescence emission spectra of the respective solution BSA or HSA was measured at pH 7 in the presence and absence of **L**. A solution upon excitation at 295 nm of BSA had showed fluorescence emission at 349 nm (Fig. 2a). On the other hand a solution of HSA under similar conditions showed fluorescence emission maxima at 332 nm Fig. 2b. Upon gradual addition of a DMSO solution of **L** to the solution of BSA, the intensity of the fluorescence emission at 349 nm was decreased with each aliquot, that was reflecting a fluorescence emission quenching process (Fig. 3a). The emission quenching had occurred due to the hydrophobic interactions and/or the hydrogen bonds of **L** with the protein.[28,30] The quenching took place through a blue shift 14 nm as illustrated in the shift from 349 nm to 335 nm in the Fig. 3a. This shift was indicative of the binding of the **L** to BSA, furthermore there was formation of a new emission at longer wavelength through an iso-emissive point, which was suggestive of a transformation from one

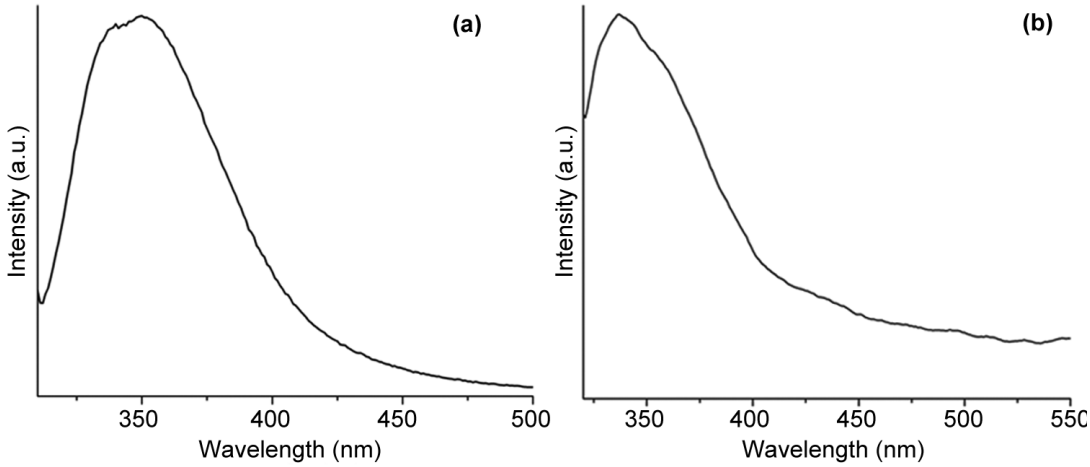


Fig. 2 — Fluorescence spectra of (a) BSA (2 μM in 3 mL) and (b) HSA (2 μM in 3 mL, in phosphate buffer, pH 7)

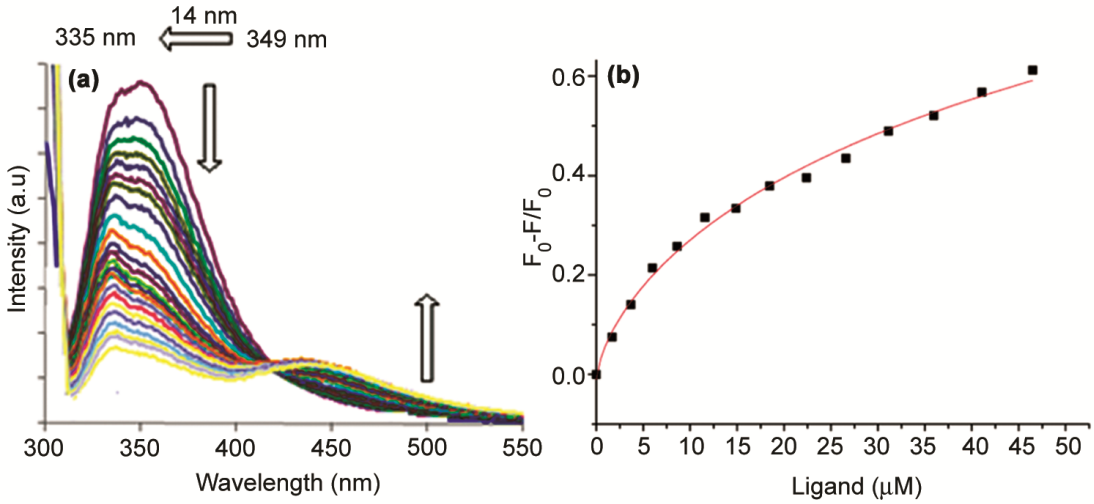


Fig. 3 — (a) Fluorescence spectra of BSA (2.0 μM in phosphate buffer, pH 7, 3 mL) in the presence of **L**, (1 μL in each aliquiot, 1.0 μM **L** in DMSO) and (b) Stern-Volmer plot of BSA with **L**

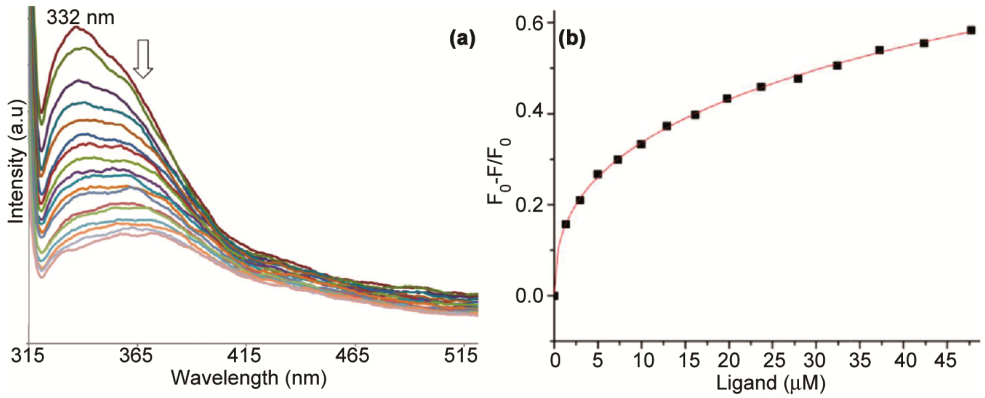


Fig. 4 — (a) Fluorescence spectra of HSA (2 μM in phosphate buffer, pH 7, 3 mL) and upon addition of different aliqots of **L** (1 μL in each aliquot, 1.0 μM of **L** in DMSO) and (b) Stern-Volmer plot of HSA with **L**

Table 1 — Binding constant (K_D), K_{SV}, n, and anisotropy of BSA and HSA with L

Sample	$K_D \times 10^3$ (M^{-1})	K_{SV}	Sample	Anisotropy value
BSA+ L	5.30	6.78	BSA	0.0369
HSA+ L	4.23	5.28	BSA+ compound	0.1455
			HSA	0.322
			HSA+ compound	0.1350

BSA (2 μL, 3 mL and HSA 2 μL, 3 mL) with compound 70 μL (in phosphate buffer, pH 7)

state to another without involving an intermediate state and likely to have occurred due to a conformational change through substrate binding. Hence, the quenching could be due to a change in the polarity of the original environment in the native sate of the protein. The compound **L** did not have an overlapping fluorescence emission with the proteins.

The gradual addition of **L** to BSA, fluorescence emission was quenched. The quenching is due to hydrophobic interactions and/or hydrogen bonding interactions[28,30]. The fluorescence emission reduced with increasing the concentration of L with a blue shifts as shown in Fig. 2a. The quenching with blue shifts is indicative of the binding of L to BSA. The quenching is due to decrease in polarity of the environment. The compound (L) chosen in this study do not have overlapping fluorescence emission with the proteins. Thus, the emission spectroscopy has been proved to be a suitable method for this study.

Similarly, a gradual addition of of a solution **L** to a solution of HSA also displayed decrease in the fluorescence emission that was orginally observed for HSA solution. In this case also a blue shift was observed while decrease in fluorescence intensities were taking place, but overlapoing nature of the broad peak. A peak maxima slanting towards lower wavelenghth could be seen (Fig. 4a). Decrease in the fluorescence intensities with increasing the concentration of L, with the blue shift was due to the change in tertiary structure of the native protein structure.[31] From the plots obtained in the Stern-Volmer plot of $(F_0\text{-}F)/F_0$ vs concentration of **L** (Fig. 3b and 4b), the plots correlated to static binding processes and the fluorescence quenching of emissions were due to the binding of **L** inside the hydrophobic pocket of the proteins.[32] The values of Stern-Volmer constant (K_{SV}) were calculated by using Eqn. (2) and listed in the Table 1. The nature of the plots implied that the static quenching were dominant during these interactions. From the plots shown in the Fig. 3b and 4b, the binding constants for **L** with the two proteins were dtermined and listed in the Table 1. It was found that **L** had a higher binding with BSA over HSA. The binding contant of **L** with BSA was 1.26 times higher than HSA, which clearly depicted a higher selectivity of **L** to bind with BSA over HSA.

Fluoresence anisotrpy between of BSA and HSA with L

Steady-state fluorescence anisotropy of bovine proteins provided the idea of deviation from globular nature of them in the absence or the presence of a binding substrate. The degree of anisotropy (r) in the tryptophan fluorescence of the proteins was calculated using following formula[33,35],

$$r = \frac{I_{VV} - G.I_{VH}}{I_{VV} + 2G.I_{VH}} \quad \ldots(3)$$

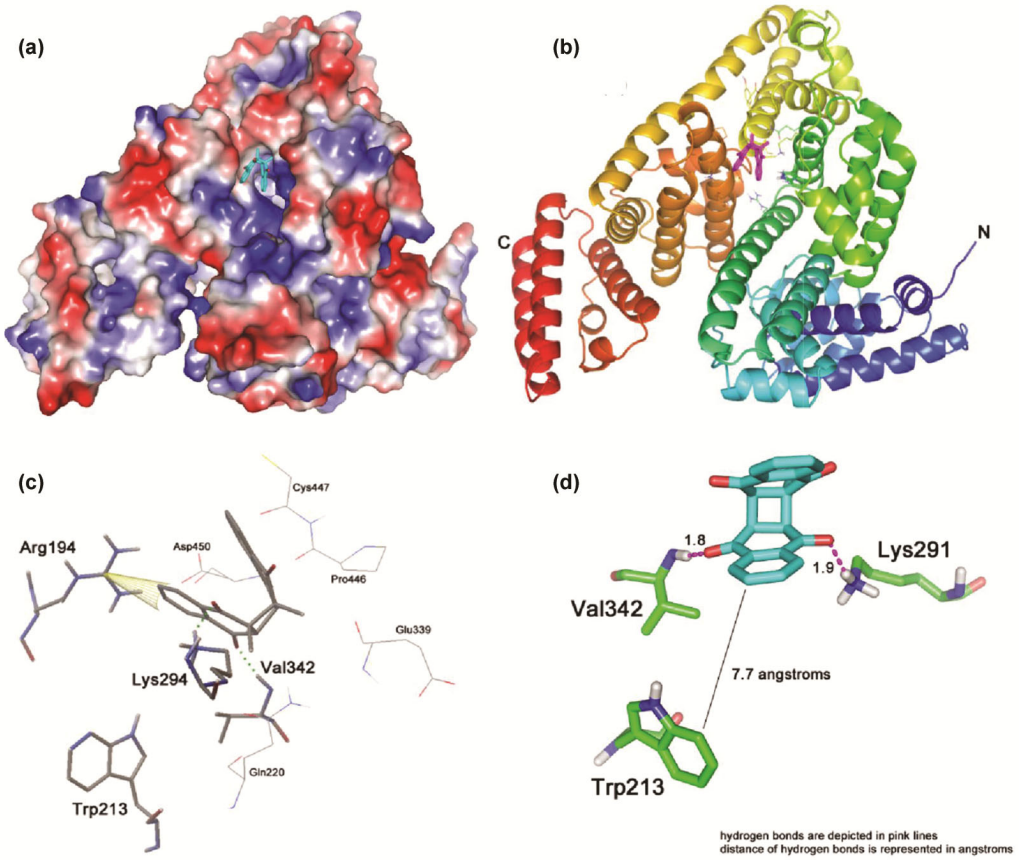


Fig. 5 — Molecular docking analysis results (a) L entered inside the hydrophobic pocket of BSA, (b) hydrogen bonding interaction, (c) electrostatic interactions of L with BSA and (d) cartoon represation rainbow of BSA

where, G = I_{VH}/I_{HH} is called the instrumental grating factor, calculated by using Eqn. (3), where I_{VV} and I_{VH} were the fluorescence intensities of the emitted light in parallel and perpendicular directions to the direction of the light used in excitation, respectively. The increase in anisotropy values from the native form of BSA and HSA in the presence of **L** had supported the binding of the substrate (**L**) (Table 1) with the proteins. It was also clear from these values that a symmetric shaped sturcture of BSA having an anisotrpoy value close to zero had changed upon binding with **L**. The observed values for the anisotropies for HSA as well as BSA were between 0.13-0.14, which were comparable to each other. These indicated that the bindings of **L** with the proteins independently induced changes in the shape and symmetry of the native structures of BSA and HSA.

Molecular docking studies of L with BSA

The molecular docking experiment was carried out to depict the interactions of BSA with **L**. The shape of the native state of BSA is shown in Fig. 5a, whereas the molecular docking had showed a similar shape after encapulating the **L** in BSA as shown in the Fig. 5b. The optimised positions of the **L** in the BSA and the different interations from the docking study are shown in the Fig. 5c and 5d. The Arg-194 exhibited a C-H···π interaction with the **L**. The Trp-213 was found to be present at a distance of 7.7Å from the **L** (Fig. 5c). It was also found that the **L** formed N-H···O hydrogen bonds with different amino acid residues, namely, Val-342 (N-H···O; $d_{H..A}$, 1.8 Å) and Lys-291 (N-H···O; $d_{H..A}$, 1.9 Å) as shown in Fig. 5d. This observation was intrguing as the tryptophan being the fluorescent site of the BSA, its fluorescence emission was quenced by the **L** which was located far away from the fluorophoric site. The **L** had no prominent interactions with tryptophan residue. Thus, the entire changes in the emissions of the proteins that were caused by the **L** were attributed to the changes in the local environment of the trptophan fluorophore. In fact, this was also true with other quinonic compounds we had studied earlier, where a

similar fluorescence emission quencings were cuaused by different naphthoquinone derivatives.[34] Docking experimental calulation with free energy of binding information regarding docking analysis, fluorescence emission spectra of L are given in Suplementary Data, Fig. S1.

Conclusions

In summary, the interactions between **L** with BSA and HSA were reflected in the changes in the fluorescence emission intensities and fluorescence anisotropy of the native states. The molecular docking analysis had revealed encapsulation of **L** in the cavity of BSA where the charge-assisted hydrogen bonds, N-H···O and C-H···π interaction had held it. The **L** had a better ability to bind with BSA over HSA.

Supplementary Data

Supplementary data associated with this article are available in the electronic form at http://nopr.niscair.res.in/jinfo/ijca/IJCA_60A(06)824-829_SupplData.pdf.

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