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Tissopi, Rengka
Department of Chemistry, National Institute of Technology Nagaland

Bhattacharyya, Jhimli
Department of Chemistry, National Institute of Technology Nagaland

https://doi.org/10.5109/7158001

出版情報：Proceedings of International Exchange and Innovation Conference on Engineering & Sciences (IEICES). 9, pp.355-360, 2023-10-19. 九州大学大学院総合理工学府
バージョン：

Biophysical investigation of the binding mechanism for industrial dye molecular association with serum protein

Rengka Tissopi, Jhimli Bhattacharyya*

Department of Chemistry, National Institute of Technology Nagaland, Dimapur, Nagaland-797103, India.

*Corresponding author email: jhimli@nitnagaland.ac.in, jhimli.bhattacharyya@gmail.com

Abstract: *The dynamics of biomolecules are very different from that of atoms or molecules. Biomolecules are often highly damped due to solvent and exhibit either few or no oscillations because of their massive nature. In the given study we used dye as a ligand to see the characteristic changes of the serum albumin through spectroscopic and theoretical (molecular docking) techniques. Dye molecules are one of the most used toxic materials which significantly affects the quality of water bodies. Dyes are usually carcinogenic; mutagens and they interact with living organisms at the molecular level. There are many unanswered questions that remain about the connections of protein structure to protein function. The nature of the binding interaction of ethidium bromide with serum albumin, binding constant, and stoichiometry was investigated using various biophysical methods like UV-vis and fluorescence spectroscopies. Molecular docking tools were utilized to explore detailed structural information and energetics of the ligand-protein interactions.*

Keywords: Bovine serum albumin; Industrial dyes; Biophysical studies; Molecular docking; Binding mechanism

1. INTRODUCTION

Dye use is one of the most hazardous substances that adversely impacts water quality since it raises BOD & COD. It is one of the most dangerous compounds that has a negative influence on water quality. During the dyeing process, around 10 to 15 percent of the dyes seep into the water system; they wind up in rivers and sewers and accumulate in the environment [1]. They are widely used within the waterbody as a coloring and tracing agent to determine the direction of flow and transit. It serves as a fluorescent dye that stains Common uses for dyes include coloring and printing on textiles, paper, paint, leather, and other materials as well as numerous industrial applications. It is essential to understand protein interactions in order to fully appreciate a variety of biological events, processes, and mechanisms. They offer a database for theoretical and experimental drug research and design. Biomacromolecules (protein, DNA, RNA, etc.) and dye molecules ligate to form stable complexes [2]. Therefore, dyes are progressively turning into pollutants. According to recent studies, dyes may be harmful to both human and animal health. It can irritate the skin and eyes and cause serious biological and environmental problems [3]. Polluted water bodies see a fall in the aquatic population in addition to the deterioration of the soil and contaminated drinking water. Protein molecules like serum albumin are strongly attracted to fluorophores, often known as dye molecules. The most prevalent protein in the blood of animals is serum albumin. The source of bovine serum albumin is the cow. In laboratories, it is regarded as a starting protein concentration. Bovine serum albumin has been utilized extensively as a pharmaceutical due to its close structural similarity to serum albumin derived from humans, constancy, low cost, and ease of supply [4]. Bovine serum albumin, sometimes known as BSA, is frequently given as a model protein in studies on how proteins interact with ligands. The link between dyes and bovine serum albumin (BSA) was investigated using a range of spectroscopic techniques in order to analyze the binding/interaction of dyes at the molecular level [5]. It is particularly beneficial to integrate theoretical and experimental approaches since it clarifies the nature of the interaction between serum albumin and dyes at the molecular level. In the study, a molecular docking probe is used to look at the many ways that proteins engage with ligands [6]. The discipline of biophysical chemistry allows for communication between chemists and biologists or pharmacists. The activity of medications or ligands and the structural alterations they undergo provide a wealth of information regarding interactions and binding events. Signaling, immune reactions, and gene regulation all depend on interactions between proteins and their ligands. Studies of protein-ligand interactions are crucial for comprehending how biological regulation works because they offer a theoretical basis for the development and discovery of novel therapeutic targets [7]. As the binding constant increases, the binding affinity will increase and become more harmful to the biomolecules. Research on protein interactions is frequently essential for understanding a variety of biological behaviors, processes, and mechanisms. They offer theoretical and experimental databases for developing novel pharmacological targets and finding new medications [8]. To fully comprehend the negative effects of such coloring chemicals, it is crucial to understand how dye molecules interact with proteins. In this work, we're using ethidium bromide, a fluorescent marker that's often employed in molecular biology labs [9]. The goal of the current research is to provide a comprehensive analysis of the dyes' binding mechanism and interactions with serum albumin (BSA). This study enables us to analyze a binding site or region of contact between a dye and a protein molecule and aids in our understanding of the precise interactions predicted by various docking approaches. Information regarding likely biochemical interactions is provided by a BSA-dye absorption study [10]. The absorption spectra will change as a result of the binding, either increasing or decreasing.

To determine the stoichiometry of the binding and binding constant of the BSA-ethidium bromide complex, fluorescence photo spectroscopy was performed on a dye molecule [11].

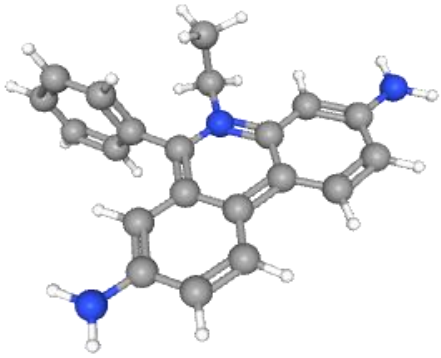

Fig. 1a. 3D conformers of Ethidium Bromide (EB) molecule

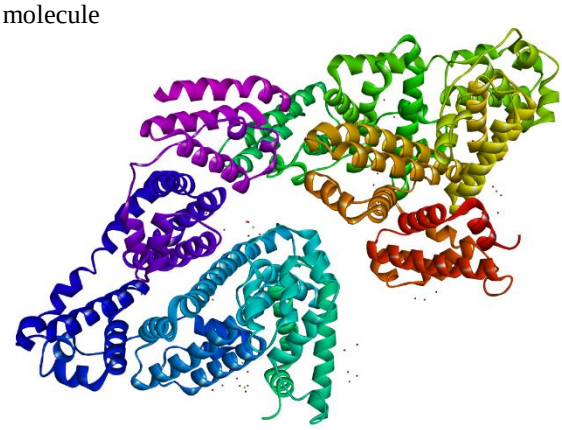

Fig. 1b. 3D structure of bovine serum albumin (BSA) molecule.

A challenging and fascinating research area at the interface of chemistry, biochemistry, and materials science is the study of the binding mechanism between industrial dyes and serum proteins. While some studies on the interaction of dyes with serum proteins may already exist, there may still be questions regarding the specific molecular interactions and processes behind this connection. There may be a research void in investigating these relationships at a more fundamental level. A deeper comprehension of the mechanisms underlying dye-protein interactions may be attained by characterizing the binding kinetics (rate of association and dissociation) and thermodynamics (enthalpy, entropy, and free energy changes) of dye-protein interactions. Designing and enhancing dye-protein interactions for particular purposes may need this information.
Computing-based modeling Computational modeling may be quite useful in predicting and explaining binding processes, even though experimental methods are vital. A more complete picture of the interactions may be obtained by combining experimental data with computer simulations, perhaps revealing fresh insights that would not be obvious from experiments alone.

2. MATERIALS AND METHODS

2.1 Materials

Ethidium bromide and bovine serum albumin (BSA) were acquired from Sigma-Aldrich. Analytical-grade chemicals were used in all assays; further purification was not done [12].

2.2 Sample preparation

The BSA stock solution was made using the citrate-phosphate buffer (10 mM, p^H 7), and it was then diluted as necessary. The samples were maintained in a refrigerator while buffer and dye solutions were being produced, and the experiment used Millipore (deionized) water. All experiments were conducted at three different temperatures i.e., 298.15, 303.15, and 308.15 K [13, 14].

2.3 Absorption Spectroscopy

The UV-Vis spectral data were recorded using an Agilent Cary 100 series UV-Visible spectrophotometer. BSA was maintained at a constant concentration of 5 µM while dye concentrations were increased from 0 to 15 µM. Between 200 and 800 nm were used to capture the UV spectra [15].

2.4 Fluorescence Spectroscopy

The fluorescence spectra were recorded with the Agilent Cary Eclipse Spectro fluorophotometer. BSA was maintained at a constant concentration of 5 µM while dye concentrations were increased from 0 to 15 µM. BSA was excited at the 280 nm wavelength [16].

2.5 Molecular Docking

Swiss Dock, a docking web server, was used for the molecular docking assessments, which were then carried out using Discovery Studio Visualizer 2021, Chimera, and PyMOL. Further research was done on the energy value with the lowest value. The protein databank with the ID: 3V03 [17] was used to construct the 3D crystal structure of BSA for docking preparations, and the structures of the dye were obtained from PubChem [18].

3. RESULTS AND DISCUSSIONS

3.1 Absorption Spectroscopy

The tryptophan, tyrosine, and phenylalanine residues' phenyl groups give tryptophan, tyrosine, and phenylalanine residues their distinctive peak at 278 nm in the absorption spectra of Bovine serum albumin. To further understand the processes governing interaction, various spectra of BSA were taken in both the presence and absence of ethidium bromide (Fig. 2).

Although there was no appreciable peak shift after the addition of both dyes, it is clear that the absorbance of BSA increased. This suggests that the ligand and protein molecules were effectively bound by the dyes. Ethidium bromide followed the same trends in absorption spectra with no specific peak shift or appearance of isosbestic points. Because of this, the data point to potential biochemical interactions between dyes and BSA. The absorbance data was not further processed since

equilibrium calculations were not feasible in the absence of isosbestic points.

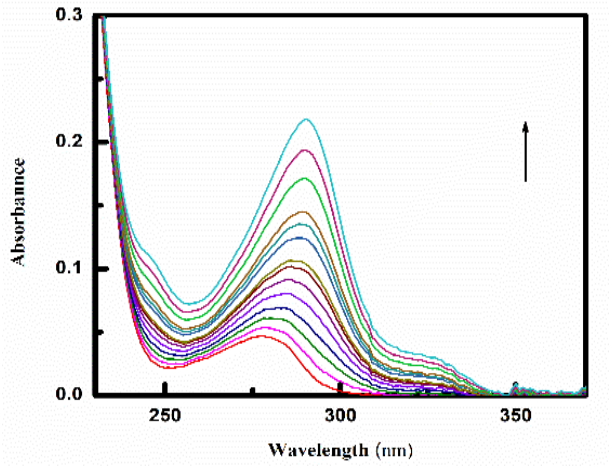

Fig. 2. BSA UV absorption spectra with various Ethidium Bromide alignment conditions.

3.2 Temperature-dependent Fluorescence spectroscopy

Amino acid residues *i.e., Tyr, Trp, Phe, etc* are present in proteins, they exhibit fluorescence characteristics. Similarly, dyes are well-known fluorophores that exhibit excellent binding affinity. Fluorescence spectroscopy was used in both the absence and presence of Ethidium Bromide molecules to identify the mechanism of interaction between Ethidium Bromide (EB) and BSA. At an excitation wavelength of 280 nm, BSA exhibits a λ_{max} peak at 334 nm (Fig. 3). It demonstrates a reduction in the quenching pattern with an increase in dye concentration from 0 to 15 µM. The complexes' binding constants and binding stoichiometry are determined using Scatchard plot analysis. The total binding ratio can be viewed as a 1:1 ratio. Complex temperature-dependent quenching analyses were performed at three distinct temperatures to better understand the quenching mechanism (Table 1).

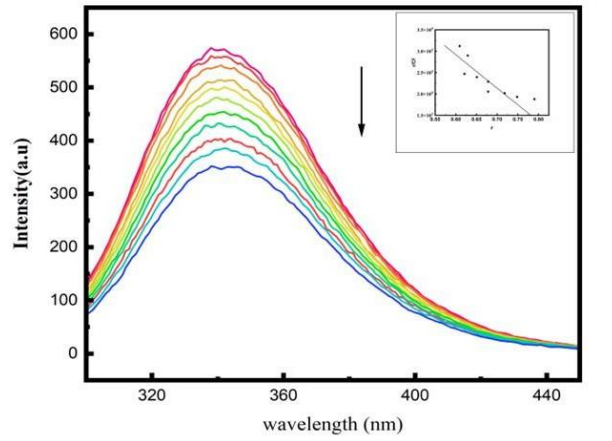

Fig. 3. Fluorescence spectra of Bovine serum albumin (5 µM) at various ethidium bromide concentrations [Onset: Scatchard plot].

Table 1. Temperature-dependent binding constant values (*K*) and the total number of binding sites (n) for BSA-EB interactions.

Molecule	Temp. (T)	Binding stoichiometry (n)	Binding constant (*K*)
Ethidium Bromide	298.15	0.9	0.9×10^5
	303.15	0.8	0.84×10^5
	308.15	0.9	0.8×10^5

The data demonstrate a reduction in binding constant value as temperature rises, indicating the stability of the BSA-EB complex and suggesting that static quenching may be the binding process.

3.3 Thermodynamics of binding

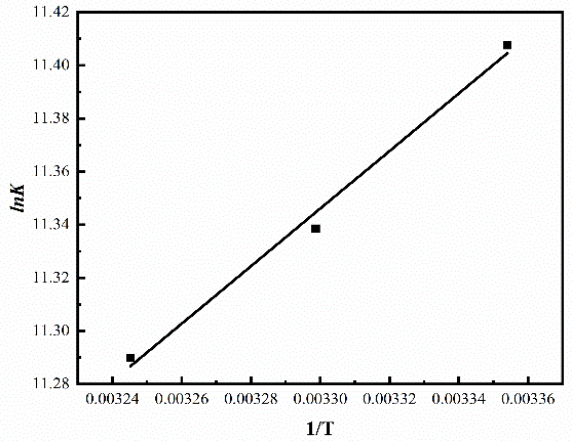

Fig. 4. *ln*(*K*) vs 1/T of EB with BSA van 't Hoff plot. The buffer solution of 10 mM citrate phosphate buffer was used for all studies.

Thermodynamic variables Enthalpy, entropy, and Gibb's free energy were studied by analyzing the results of temperature-dependent fluorescence spectroscopy. The values were obtained by employing the Van't Hoff equation [3]:

$$lnK = -\frac{\Delta H^\circ}{RT} + \Delta S/R \qquad (1)$$

The binding constant is *K*. R is the gas constant, and T is the temperature (298.15, 303.15, and 308.15K). ΔH and ΔS were computed in accordance with the table below: 2. ΔG was determined with the formula [3]:

$$\Delta G = \Delta H^\circ - T\Delta S^\circ = -RTlnK \qquad (2)$$

Table 2. Fluorescence quenching at various temperatures was used to extract data for BSA-EB's thermodynamic parameters. All experiments were carried out in 10 mM citrate-phosphate (CP) buffer.

Temp (K)	ΔG° (Kcal /mol)	ΔH° (Kcal/ mol)	ΔS (Kcal /mol)	$T\Delta S^\circ$ (Kcal/ mol)
298.15	-6.758	-2.152	0.0154	4.605
303.15	-6.830			4.682
308.15	-6.913			4.759

According to the thermodynamic characteristics, exothermic binding was encouraged by a negative change in enthalpy. Both the reactions were spontaneous as evident from the negative Δ*G* values.

3.4 Salt-dependent fluorescence analysis

Salt-dependent fluorescence tests were examined to determine the type of molecular forces used during the binding process. By splitting the Gibbs free energy into its polyelectrolytic and non-polyelectrolytic components, it is feasible to calculate the polyelectrolytic contribution. An experiment was carried out using varied salt concentrations and fluorescence spectroscopy to perform the van 't Hoff analysis.

The following equation [3] relates the relationship of protein-drug binding to sodium ion concentration:

$$(\partial lnK/\partial ln[Na^+])T,P = -z\Psi \qquad (3)$$

Where zΨ is the net ion liberation after the formation of a protein and ligand complex and z is the probable charge of the bound ligand. Plotting the *lnK* values against the *ln*[Na^+], which reflected the number of ions released during complexation, yielded a straight line and a slope value of 0.0713.

[Na^+]	$K\times10^5$	n	ΔG°	ΔG_t°	ΔG_{pe}°	zψ
10	0.91	1.01	-6.74	-6.66	-0.09	-0.0713
20	0.85	0.9	-6.72	-6.59	-0.12	
50	0.81	0.9	-6.69	-6.53	-0.16	

Table 3 portrays Δ*G*°, ΔG_t° and ΔG_{pe}° values for binding of ethidium bromide and BSA shows that ΔG_t° part contribute more to Δ*G*° than ΔG_{pe}° at every salt concentration. Thus, it may be concluded that non-polyelectrolytic forces are crucial in maintaining the stability of the BSA-EB complex. Salt-dependent fluorescence revealed that non-polyelectrolytic forces, such as Van der Waals contacts, hydrogen bonds, pi-pi stacking, etc., drove the interaction between the ligand and protein.

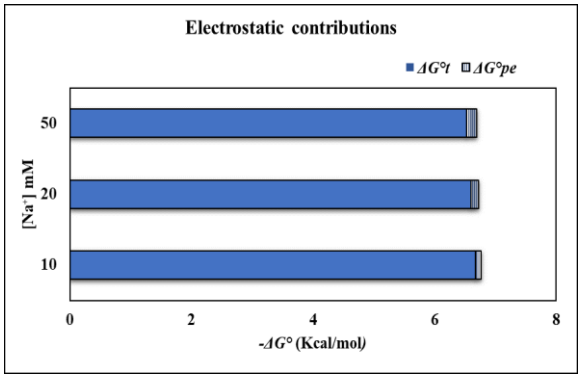

Fig. 6. At the temperature of 298.15 K, the standard molar Gibbs free energy at concentrations of 10, 20, and 50 mM [Na^+] in the BSA-EB complex was affected by

Table 3. Study on fluorescence depending on salt, the importance of electrostatic forces discovered for BSA-EB at a temperature of 298.15K.

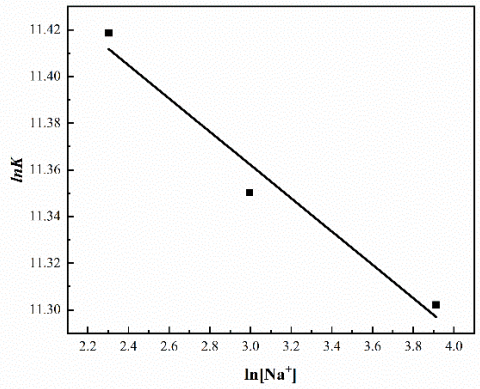

Fig. 5. Van't Hoff plot shows the relationship between the *lnK* and *ln*[Na+] of EB and BSA at various ionic strength concentrations.

The equation [3] may be used to calculate the resulting Gibbs free energy:

$$\Delta G^\circ = -RTlnK = (\Delta G_t^\circ) + (\Delta G_{pe}^\circ) \qquad (4)$$

both polyelectrolytic and non-polyelectrolytic contributions.

3.5 Molecular Docking Probe

The 3D structure was acquired from the RSCB Protein data bank ahead of the molecular docking investigation. To confirm the precise binding site and residues involved in the binding of ethidium bromide molecules to BSA molecules, molecular docking experiments for the same were conducted. Fig. 4. shows the closest dock with the least amount of energy residues. HSE129 forms conventional Hydrogen bonding and TyR108 of BSA forms a Pi-Pi T-shaped bond with Ethidium Bromide.

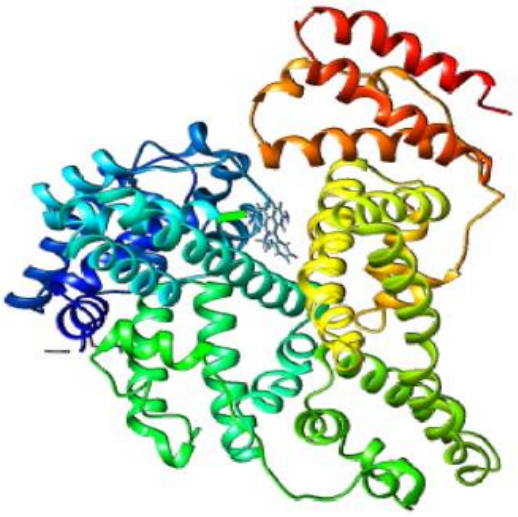

Fig. 7a. Lowest energy docked possible for BSA-EB (ΔG = -6.845Kcal/mol).

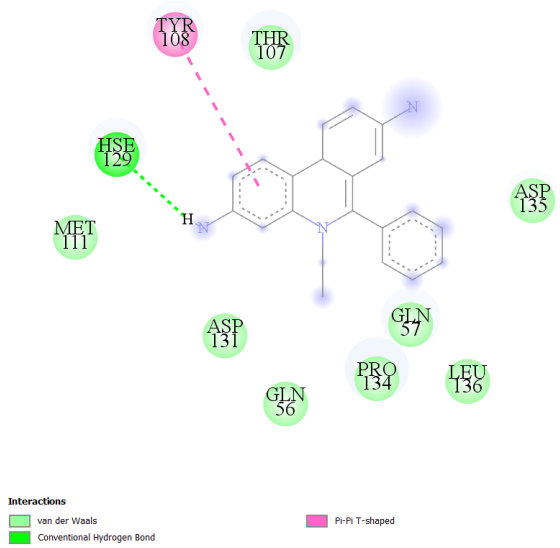

Fig. 7b. Various types of non-covalent interactions for binding of BSA-EB complex.

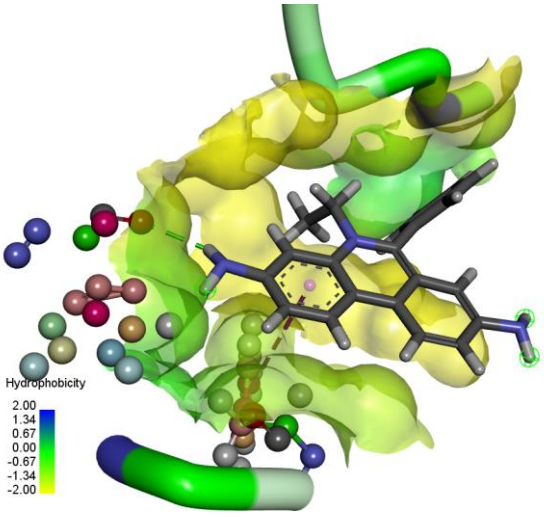

Fig. 8. Surface illustration of the interactions between different amino groups and the ethidium bromide molecule. (This picture was produced using Discovery Studio Visualizer 2021.)

4. CONCLUSION

Dye-related environmental contaminants are on the rise. In this work, successful interactions between BSA and dyes were deduced using UV-visible absorption spectroscopy, different fluorescence emission spectroscopic experiments, and molecular docking data. When dyes were bound to the BSA solution, the fluorescence spectrum was quenched. The system's 1:1 binding stoichiometry is found to indicate static quenching, and the interaction's spontaneously occurring binding affinity is demonstrated by the system's negative Gibbs free energy value. All of the experimental evidence points to successful protein-dye binding. Van Der Waals forces, Pi-Pi interactions, hydrogen bonds, and hydrophobic interactions were discovered to be the key components in dyes and BSA. Combining experimental data with molecular docking data has led to the creation of a more comprehensive picture of the interactions. The result depicts the possible binding constants, stoichiometry, and mechanism of dye-protein complexation.

This study possesses the thread at the molecular level, for the dye molecules to compete with other essential ligands in biological systems; hence can provide important insights for further risk assessment of such chemical moieties.

Conflict of interest

The authors state that they do not have any conflicts of interest.

Acknowledgment

JB acknowledges the financial support received from DRDO, Govt. of India (Project Order No. DFTM/07/3603/NESTC/EWM/P-04).

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