Investigation on the Interaction of Sulfonyl Derivatives with BSA and DNA Base by Spectral and Molecular Docking Analysis

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ABSTRACT

Interaction behavior of 4,4'-sulfonyldibenzoic acid (SDBA), 4,4'-sulfonyldiphenol (SDP), 4,4'-sulfonylbis(2-methylphenol) (SBMP) have been studied in BSA and adenine by UV-visible, fluorescence spectroscopy and docking methods. Upon increasing the drug concentrations the absorption and fluorescence intensities of the BSA and adenine changed at the same wavelength suggests that intermolecular interactions present between the drugs and the BSA/ adenine molecules. Hydrogen bond interactions, van der Waals and hydrophobic interactions are participate a main responsibility in stabilizing the complex. The fluorescence quenching constant (K_{sv}) results for the drug:BSA and drug:adenine was determined by using the Stern-Volmer equation. Docking method specifies that the drug molecule is interrelated with the protein molecule which is in good accordance with the experiment results.

Keywords: Sulfonyl dibenzoic acid, Sulfonyl diphenol, Sulfonylbis(2-methylphenol), BSA quenching, Molecular docking

INTRODUCTION

Albumin binds and transports numerous ligands including cations, anions, amino acids, fatty acids, variety of pharmaceuticals and hormones. It is recommended that the most important regions of ligand binding to HSA are located in hydrophobic cavities in the sub domains IIA and IIIA, which are consistent with sites I and II, respectively and single tryptophan residue of HSA is in sub domain IIA [1,2]. BSA was preferred as our protein model because of its unusual ligand-binding properties, ready availability, low cost and the results of the studies are consistent with the fact that BSA and HSA are similar proteins [1-4]. Protein-drug binding greatly influences metabolism, distribution, absorption and excretion properties of typical drugs [5,6]. Thus, it is important and fundamental to study the interaction of drug with serum albumins at molecular level.

Adenine is one of the purine nucleases and it is an essential molecule of life and evolution. Adenine has tremendous biological significance since it is one of the nitrogenous bases found on deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) to make up genetic information. It is a component of adenosine triphosphate (ATP) which is major energy releasing molecule in cells. Adenine is also a part of coenzymes and being a part of nucleic acid, it has a central role in protein synthesis [7].

In middle of the 1990's, we have been studying the solvatochromic and prototropic effects of diphenyl sulfone [8,9] diphenyl [10,11], diphenyl ether [12,13] and with one or two electron-donating groups such as OH or NH_2 and recently, we have published the dual florescence effects of sulfonyl drugs with cyclodextrins [14]. In continuation of our work, herein, we have studied the interactions of 4,4′-sulfonyldiphenol, 3,3'-dimethyl 4,4′-sulfonyldiphenol, 4,4′-sulfonyldibenzoic acid (Figure 1) with BSA and adenine. The major plan of the present study are: (i) to compare the effect of protein interactions of SDP, SBMP and SDBA molecules and (ii) the quenching properties of fluorescence emission and (iii) to determine the binding site. The results are obtained by the Spectral and computational (docking) methods.

EXPERIMENTAL SECTION

Materials

4,4'-Sulfonyldibenzoic acid, 4,4'-sulfonyldiphenol, 4,4'-sulfonylbis(2-methylphenol) (Figure 1), adenine and bovine serum albumin (BSA) were obtained from Sigma-Aldrich chemical company and used without further purification. All other reagents were of analytical grade. The purity of the compound was checked by similar fluorescence spectra when excited with different wavelengths. Triply distilled water was used for the preparation of aqueous solutions. BSA solutions were prepared in 2×10^{-5} M Tris HCl buffered at pH 7.4 and the buffer ion strengths were kept at 0.1 M. The concentration of the sulfa drug solutions was varied from 2 10^{-3} to 1×10^{-2} M. 0.2 mL of the drug in methanol solution was used for the binding experiments and percentage of methanol (dissolved with drugs) in adenine and BSA solution was under 1% in order to ensure that methanol has no effect on structure of BSA. All solutions were stored in a refrigerator at 4°C in the dark.

Apparatus

Absorption spectral measurements were carried out with a UV-visible spectrophotometer (model-UV-2600 Shimadzu, Japan) and fluorescence measurements were performed on a spectrofluorophotometer (model-RF-5301PC, Shimadzu, Japan) equipped with 1.0 cm quartz cells. The excitation wavelength for all the sulfa drugs is 270 nm. The pH values in the range 2.5-11.5 were measured on Elico pH meter model LI-120.

Molecular docking

Molecular docking data were analyzed by using online molecular docking server (https://www.dockingserver.com) [15-19]. The MMFF94 force field was used for energy minimization of ligand molecule (SDBA, SDP and SBMP) using Docking Server [20]. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on (*4F5S*) protein model [16-19]. The initial geometries of the drug molecules were constructed with Spartan 08 and then optimized by the PM3 method. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools [20]. Affinity (grid) maps of $20 \times 20 \times 20$ Å grid points and 0.375 Å spacing were generated using the Auto grid program [20].

RESULTS AND DISCUSSION

Absorption spectroscopic studies

Absorption and fluorescence spectral data of BSA and adenine in varying concentration of SDBA, SDP and SBMP are summarized in Supplementary Table S1. The absorption and emission spectra of the above compounds are displayed in Supplementary Figure S1. In aqueous solution (water), the absorption maxima of SDBA appears at ~251 and 227 nm, SDP shows at ~258 and 234 nm and SBMP shows at ~260, 235 nm (Figure 2). In aqueous solution, BSA and adenine, have an absorption maximum at ~280 nm and 260 nm, respectively.

With an addition of SDBA, SBMP and SDP, the absorption intensity of BSA gradually increased at the same wavelength. Similarly, intensity of absorption spectra of adenine gradually increases at ~260 nm with increasing concentration of the sulpha drug molecules. The above results indicate that SDBA, SBMP and SDP drugs form ground state complex with BSA and adenine molecules [21]. The inset in Supplementary Figure S1 depicts the changes in the absorbance with different sulfonyl drug concentration, indicating that the drug molecules could bind to BSA/adenine molecules. In addition to that, a clear isosbestic point was observed in the absorption spectra which designate the formation of well-defined 1:1 interaction between the drugs and BSA or adenine. These results suggest that there is one binding site in BSA and adenine for these drugs during their interaction. Further, these results were also confirmed by the following fluorescence spectroscopy and its mechanism of quenching.

Fluorescence studies

Figure 2 show emission spectrum of BSA and adenine with different sulfonyl drugs. In aqueous solution, the emission maxima of SDP appears at ~291, 348, 446 nm, SBMP appears at 293, 346, 423 nm and SDBA shows at ~289, 348, 422 nm. On progressive addition of different concentrations of SDBA, SDP and SBMP to BSA, the emission intensities decrease (Figure 2). When the drug concentration increased, the emission intensity of BSA is decreased.
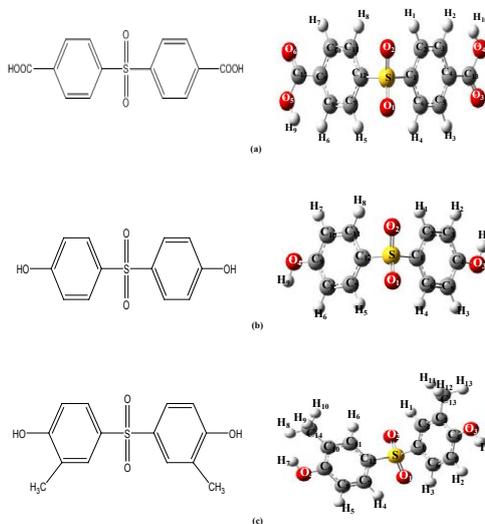
Figure 1: Chemical and optimized structures of (a) SDBA, (b) SDP and (c) SBMP
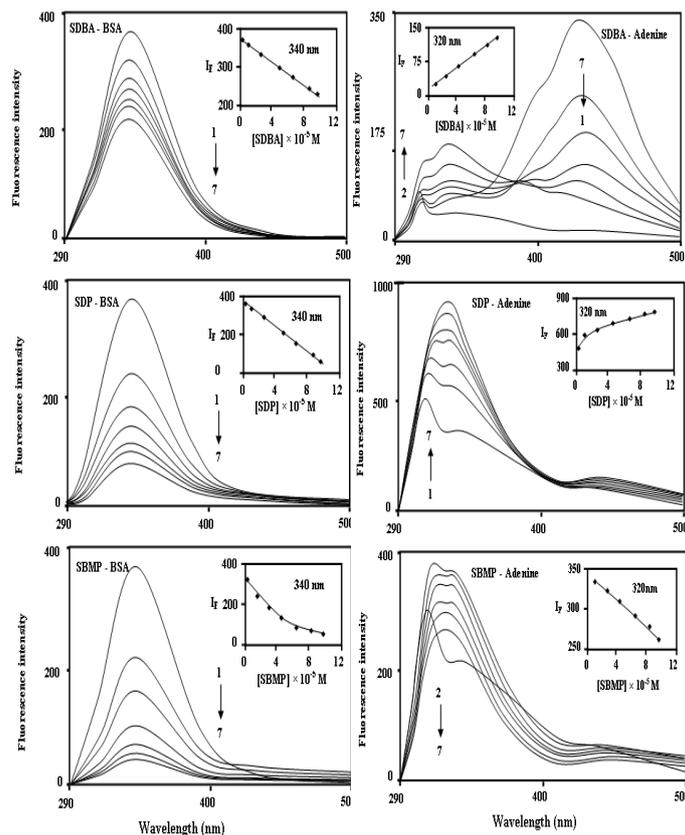

Figure 2: Emission spectra of BSA and adenine in different SDBA, SDP and SBMP drug concentrations (10^{-3} M): (1) 0, (2) 1, (3) 3, (4) 5, (5) 7, (6) 9 and (7) 10. Inset figure: Emission intensity vs. drugs concentration

The quenching mechanism is further analyzed by using the Stern-Volmer analysis (equations S1-S3). The quenching of BSA fluorescence intensity implies that all these drugs could bind and close to the tryptophan and tyrosine residues.

The absence and presence of SDBA, SDP and SBMP, the fluorescence spectra of adenine consists of three distinct bands, the maxima at around 290 nm (fluorescence maximum, denoted as F_1) 330 nm (fluorescence maximum, denoted as F_2) 430 nm (fluorescence maximum, denoted as F_3) with SDBA, SDP and SBMP. Figure 2 shows that as the drugs concentration increases in the adenine solution F_1, F_2 and F_3 band intensity gradually increased at the same wavelength.

We have also followed the quenching and noticed that it obeyed the Stern-Volmer equation very well yielding a straight line when $[(F_0-F)/F]$ was plotted against the drug concentration.

Fluorescence quenching mechanism

The Stern-Volmer (method) equation S1 and S2 is used for analyzing fluorescence quenching [22,23]. Figure 3 and Supplementary Figure S2 shown Stern-Volmer plots for the BSA and adenine by the sulfonyl drugs and the corresponding data's are listed in Table 1 (i.e., Static one) [24]. However, Supplementary Figure S2b, the Stern-Volmer plot showed a deviation indicates the presence of both static and dynamic quenching is present in adenine system.
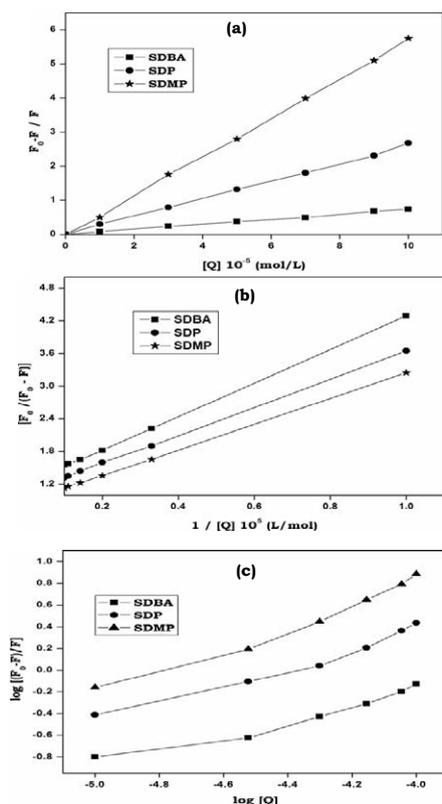
Figure 3: Stern-Volmer (a, b) and modified Stern-Volmer (c) plots for fluorescence quenching of BSA by SDBA, SDP and SDMP

Table 1: Stern-Volmer quenching constant (K_{sv}), bimolecular quenching rate constant (K_q) and modified Stern-Volmer association constant (K_a) of the BSA and adenine with SDBA, SDP and SBDP systems

		Dyes	K_{sv} (10^5 M^{-1})	K_q (10^{13} M^{-1}s^{-1})	**R^a**	**SD**
S-V quenching	BSA	SDBA	0.74	0.74	0.990	0.514
		SDP	2.62	2.62	0.997	0.391
		SBMP	5.41	5.41	0.997	0.591
	Adenine	SDBA	-6.61	-6.61	0.999	0.761
		SDP	-6.63	-6.63	0.998	0.592
		SBMP	4.97	4.97	0.998	0.761
		Drugs	K_a (10^4 M^{-1})	ΔG^0 (kJ/mol)	**n**	**R^a**
Modified S-V quenching	BSA	SDBA	0.96	-20.16	1.01	0.997
		SDP	1.84	-21.28	0.86	0.999
		SBMP	2.04	-21.53	0.82	0.995
	Adenine	SDBA	2	-17.46	-	0.996
		SDP	2	-17.46	-	0.998
		SBMP	2	-19.22	-	0.999

The K_a and n can be obtained by using modified Stern-Volmer equation (S3). The values of K_a and n were obtained from the intercept and slope of the plot of $\log[(F_0\text{-}F)/F]$ vs. $\log[Q]$ (Figure 3c). The values of K_a were found to be (0.96 × 10^5, 1.57 × 10^5 and 2.04 × 10^5 L mol^{-1}) and those of n were noticed to be 1.01, 0.86 and 0.82, respectively (Table 1). The values of binding sites close to unity indicated that there was only one independent class of binding site on BSA for sulfonyl drugs.

Free energy change and nature of the binding forces

The free energy change was calculated from the binding constant (Ka) by using the following equation:

$$\Delta G^\circ = -RT \ln K a \qquad (1)$$

where K_a is the binding constant; T is the absolute temperature and R is the gas constant. The negative value of ΔG° suggests that the interaction process between the biomolecule (BSA/adenine) and biologically active drug (sulfonyl) molecules is spontaneous.

Fluorescence resonance energy transfer (FRET)

On the basis of equations (S3)-(S5), the following parameters are obtained for BSA – with SDBA, SDP and SBMP respectively (Supplementary Table S1) J=1.83, 1.66 and 1.58 × 10^{-14} cm^3 L mol^{-1}, R$_0$=1.17, 1.10 and 1.13 nm, E=3.20, 2.82 and 2.63, r=1.57, 1.41 and 1.03 nm. Supplementary Table S1 shows, energy transfer efficiency (E) for sulfonyl drugs with BSA/adenine and SDBA drug molecule has more efficiency to bind active site of BSA than that of other two drug molecule, its supports the above implication.

Molecular docking studies

Molecular docking methods have been working to understand the different binding modes of BSA-drugs interaction [16-19]. The 3D structure of BSA was obtained from Protein Data Bank (4F5S). The possible conformations of the BSA with sulfonyl drug complexes were calculated using molecular docking server program. Out of 10 conformers, the conformer with the lowest binding free energy was used for further analysis.

The results in Table 2 and Figure 4 revealed the most possible binding sites and poses for the sulfonyl drug compounds in protein. The best energy ranked model (Figure 4) revealed that the sulfonyl drugs molecules were bound at the interface between two sub domains IIA and IIIA, which is located just above the entrance of the binding pocket of IIA. In the figures, sulfonyl drug molecules were found to be surrounded by 11-13 amino acid residues within a distance of 5 Å (Supplementary Tables S2 and S3). Hydrogen bonding and hydrophobic interactions plot was used to explore the interaction between BSA and sulfonyl drugs as shown in Figure 5 and its corresponding two-dimensional schematic diagram are shown in Figure 6.

Table 2: Estimated free energy, inhibition constant, electrostatic energy and total intermolecular energy of the BSA–SDBA, SDP and SBMP

| Drug	Estimated Free Energy of Binding (kcal/mol)	Estimated Inhibition Constant, Ki (µM)	vdW + H bond + desolve Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Total Intermolecular Energy (kcal/mol)	Frequency (%)	Interact Surface
SDBA	**-6.32**	**23.31**	**-7.65**	+0.14	-7.52	100	705.4
SDP	-5.45	100.72	-6.69	+0.01	-6.68	60	559.8
SBMP	-5.88	48.98	-6.93	-0.04	-6.96	20	674.8

In SDBA, the two oxygen atoms of acid functional groups form H-bonds with the OH of THR190 (-O$_5$…OH, 3.30 Å), of SER192 (-O$_4$…OH, 3.05 Å), respectively (Figure 6a). Similarly, the phenol oxygen atom (O$_2$) in SDP, act as hydrogen acceptor to form H–bond with the group of THR578 (-O$^{…}$HO, 2.99Å) (Figure 6b). On the contrary, in SBMP the highest affinity of ligands are actually by hydrophobic interactions instead of hydrogen bonding (Figure 6c).

From the docking analysis (Figure 6 and Supplementary Tables S2-S4), we could presume that the electrostatic, hydrogen bonding, hydrophobic interactions and the polar contacts collectively constituted the primary force for the binding of the molecule. Supplementary Table S3 shows estimation free energy, van der Waals and hydrogen bonding interactions of BSA with sulfonyl drugs. The interaction of BSA with SDBA is more negative than other two drug molecules and the above results suggest SDBA strongly interacted with BSA than other molecules, which is in well

agreement with the results of experimental binding mode study.
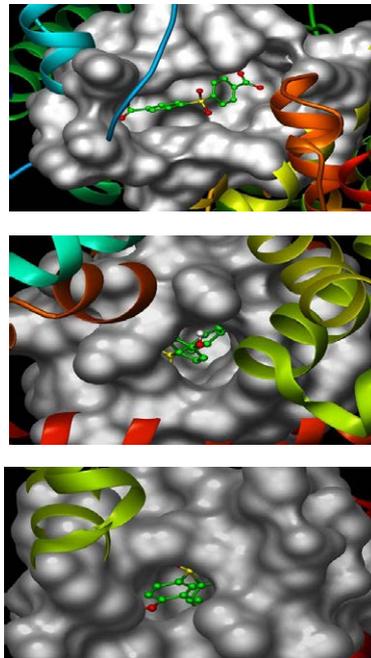
Figure 4: Binding mode between different (a) SDBA (b) SDP and (c) SDMP with BSA. The important active amino acids of SA are represented using surface format and the sulfonyl drug structure is correspond to using a "Ball and Stick" format
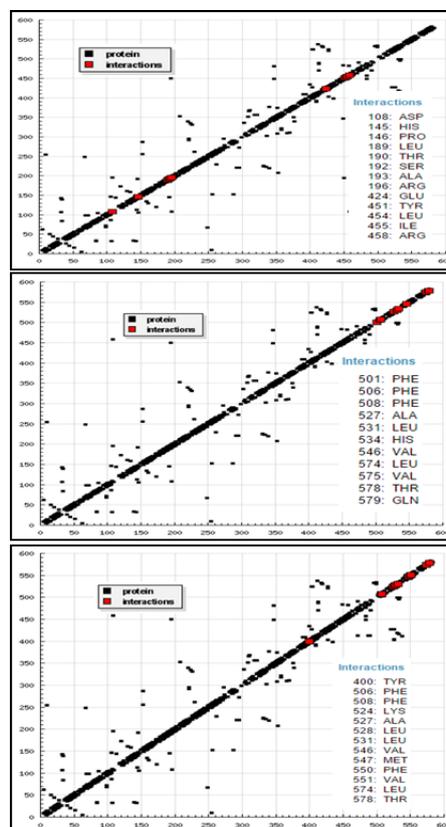
Figure 5: The Hydrogen bonding plots between different (a) SDBA, (b) SDP and (c) SDMP with BSA. The BSA residues are represented using black dots and the hydrogen bonding interactions are represented using red dots
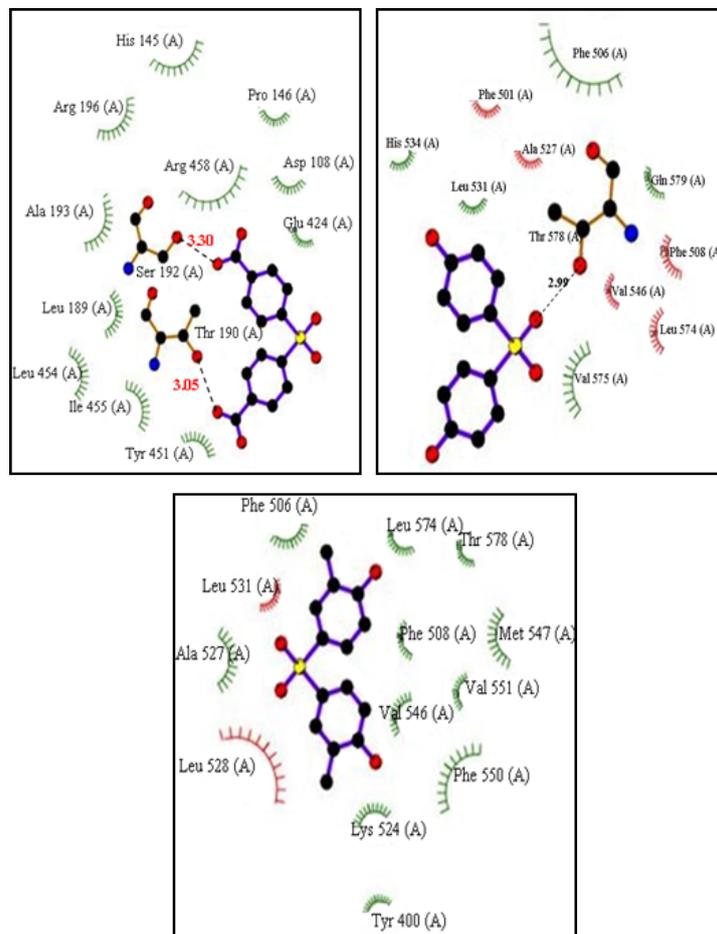
Figure 6: Two-dimensional schematic representation of hydrogen bonding and hydrophobic interactions of BSA with SDBA, SDP and SDMP. Hydrogen bonding depicted as dashed line. Active residues are represents in green colour sweeps. Colour of the atoms: blue- nitrogen, red- oxygen, yellow- sulphur, black- carbon

CONCLUSION

Interaction between BSA and adenine with three different sulfonyl drugs (SDBA, SDP and SBMP) has been examined by using different techniques. BSA fluorescence can be quenched by these drugs, which means that these drug molecules could bind to BSA. The above reaction stoichiometric ratio is 1:1 and the protein-drug complexes are stabilized mainly by van der Waals interaction, hydrophobic and hydrogen bonding. Compared to adenine, BSA contributes substantially higher binding efficiency with the drugs. The K_{sv} value of BSA/adenine with SBMP is higher than that of other drugs and BSA is more quenched than adenine. With the addition of the sulfonyl drugs the tautomeric equilibrium structure of the adenine is changed, Molecular docking studies shows, SDBA drug molecule has high affinity to bind BSA molecules than other drugs.

ACKNOWLEDGEMENT

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