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Interaction of sulfanilamide and sulfamethoxazole with bovine serum albumin and adenine: Spectroscopic and molecular docking investigations

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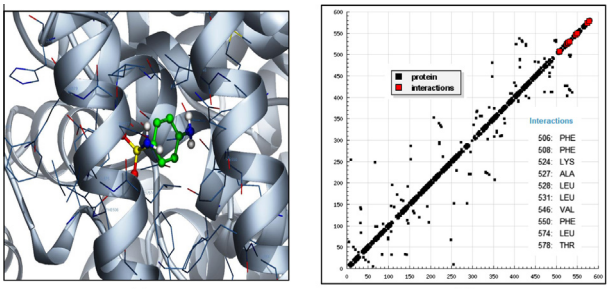
HIGHLIGHTS

- Interactions between SAM and SMO with BSA and adenine was investigated.
- Both adenine and BSA fluorescence intensities decreased as the drug concentrations increased.
- Hydrophobic forces, electrostatic interactions and hydrogen bonds played vital roles in the binding interactions.
- Docking method provided the interactions of specific chemical groups in the complex stabilization.

GRAPHICAL ABSTRACT

The best binding mode between BSA with (a) SAM. The important residues of BSA are represented using lines and the ligand structure is represented using a "Ball and Stick" format. The hydrogen bonding plots between (b) SAM with BSA. The BSA residues are represented using black dots and the hydrogen bonding interactions are represented using red dots.



(a) (b)

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ABSTRACT

Interaction between sulfanilamide (SAM) and sulfamethoxazole (SMO) with BSA and DNA base (adenine) was investigated by UV–visible, fluorescence, cyclic voltammetry and molecular docking studies. Stern–Volmer fluorescence quenching constant (K_a) suggests SMO is more quenched with BSA/adenine than that of SAM. The distance r between donor (BSA/adenine) and acceptor (SAM and SMO) was obtained according to fluorescence resonance energy transfer (FRET). The results showed that hydrophobic forces, electrostatic interactions, and hydrogen bonds played vital roles in the SAM and SMO with BSA/adenine binding interaction. During the interaction, sulfa drugs could insert into the hydrophobic pocket, where the non-radioactive energy transfer from BSA/adenine to sulfa drugs occurred with high possibility. Cyclic voltammetry results suggested that when the drug concentration is increased, the anodic electrode potential deceased. The docking method indicates aniline group is interacted with the BSA molecules.

Introduction

Serum albumin is the major protein constituent of blood plasma which facilitates the disposition and transport of various exogenous and endogenous drugs to the specific targets [1]. The specific delivery of drugs by serum albumin originates from the presence of

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two major and structurally selective binding sites, namely, site I and site II, which are located in three homologous domains that form a heart-shaped protein [2]. Bovine serum albumin (BSA) is a single-chain 582 amino acid globular non-glycoprotein cross-linked with 17 cystine residues (eight disulfide bonds and one free thiol) [3–5]. BSA has two tryptophans, Trp-134 and Trp-212, embedded in the first sub-domain IB and sub-domain IIA, respectively [6]. The binding affinity offered by site I is mainly through hydrophobic interactions, whereas site II involves a combination of hydrophobic, hydrogen bonding, and electrostatic interactions [7]. Due to its structural homology (80%) with human serum albumin, water solubility and versatile binding ability, BSA has been used as a model protein for a great variety of biophysical and physicochemical studies.

Adenine is one of the purine nucleobases 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 [8].

Among the many and different families of organic and inorganic chemicals being currently investigated because of their applications, sulfonamides and their N-derivatives are one of the outstanding groups. Sulfonamides were the first effective chemotherapeutic agents employed systematically for the prevention and cure of bacterial infections in humans. After the introduction of penicillin and other antibiotics, the popularity of sulfonamides decreased. However, they are still considered useful in certain therapeutic fields, especially in the case of ophthalmic infections as well as infections in the urinary and gastrointestinal tract. Besides, sulfa drugs are still today among the drugs of first election (together with ampicillin and gentamycin) as chemotherapeutic agents in bacterial infections by *Escherichia coli* in humans. The sulfanilamides exert their antibacterial action by the competitive inhibition of the enzyme dihydropterase synthetase towards the substrate p-aminobenzoate. Sulfonamides belong to the group of antibacterial drugs which are used for human and animal therapy, to cure infectious diseases of digestive and respiratory systems, infections of the skin (in the form of ointments) and for prevention or therapy of coccidiosis of small domestic animals [9]. Quality control of sulfonamide formulations and their quick systematic monitoring in body fluids are important analytical tasks. A number of articles have been published concerning the determination of sulfonamides by different analytical methods.

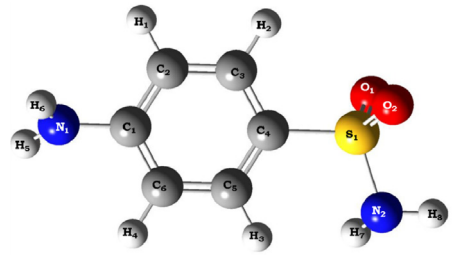
Sulfamethoxazole (SMO) is widely used as an antibacterial, mainly in combination with trimethoprim. This is a well-recognized preparation, as the combination of a sulfonamide with an inhibitor of the dihydrofolate reductase increases the bacteriostatic effect of the sulfonamide, by blocking the metabolic pathways of the microorganisms at two different points. Different methods have been described for determining the sulfonamides, but, in general, they are based on separation methods using different detection types. Its determination in urine has been made by micellar liquid chromatography and by supported liquid membrane with high pressure liquid chromatography–electrospray mass spectrometry detection [10]. The fluorescence characteristics of different sulfonamides have been studied [11] and proposed for the determination of several of these compounds. Thus, sulfanilamide can be determined by reaction with homophthaldehyde. The analysis of sulfa drugs [12,13] has been performed in foods and pharmaceuticals using the fluorescamine reaction. The reaction of 9-cloroacridine with sulfonamides produces a fluorescence quenching which allows the determination of sulfonamides [14]. Sulfa drugs were determined in milk and pharmaceutical preparations by photochemically-induced fluorometry [15]. Fluorescence has also been used as HPLC detection for determining sulfa drugs in milk and eggs [16] and recently, fluorescence after pre-column derivatization with fluorescamine has been applied as a detection technique [16].

In our previous studies, the sequences of sulfonamide derivatives with cyclodextrins were investigated through experimental [17–23] and theoretical methods [24]. In the view of increasing attention directed toward the importance of investigating interaction between proteins and drugs, herein we report the interaction behavior of sulfanilamide (SAM, 4-aminobenzene sulfonamide) and sulfamethoxazole (SMO, N1-(5-methyl-3-isoxazolyl) sulfanilamide) (Fig. 1) with BSA and adenine. We utilized absorption and fluorescence spectral techniques to determine the interaction stoichiometry, quenching mechanism, binding constant (K_a) and number of binding sites (n) of SAM/SMO-BSA/adenine complex. We report combined results of experimental and molecular docking methods on the protein – drugs complexes of SAM/SMO – BSA/adenine. Sulfa drugs are an easily available drug and have been a strong research field in life sciences, chemistry and clinical medicine. Hence, we are interested in investigating the interaction mechanism of sulfa drugs with BSA/adenine in solution and in computational methods.

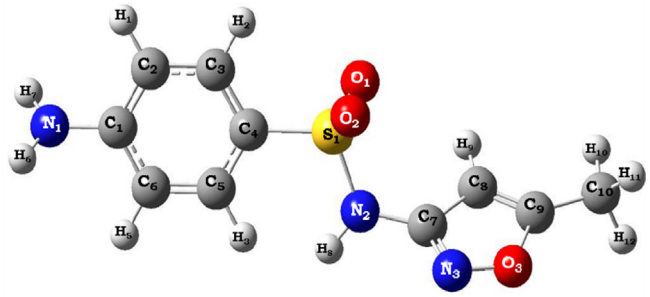
Experimental section

Materials

Sulfanilamide (SAM), sulfamethoxazole (SMO), adenine and bovine serum albumin (BSA) were obtained from Sigma–Aldrich Chemical Company, USA 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



(SAM)



(SMO)

Fig. 1. Chemical structures of sulfanilamide (SAM) and sulfamethoxazole (SMO). Color of the atoms: blue – nitrogen, red – oxygen, yellow – sulfur, gray – carbon, white – hydrogen. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)