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Caffeine and sulfadiazine interact differently with human serum albumin: A combined fluorescence and molecular docking study

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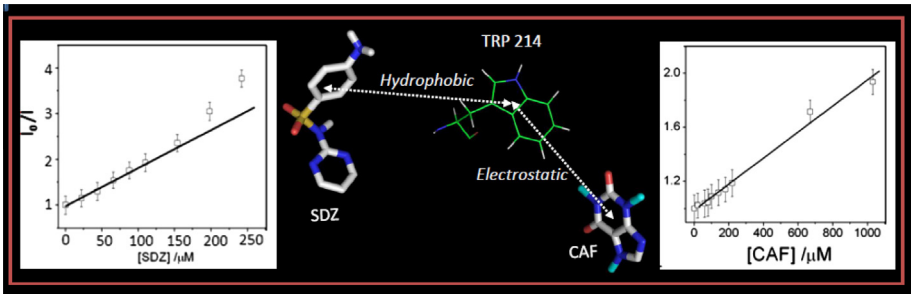
HIGHLIGHTS

- Interaction of caffeine and sulfadiazine with HSA was studied.
- Intrinsic Trp fluorescence of HSA is quenched by both the drugs.
- Both CAF and SDZ bind to similar site in HSA.
- Thermodynamics and mode of interaction for the drugs with HSA is different.
- Docking calculation supports the fluorescence spectroscopic results.

GRAPHICAL ABSTRACT

The intrinsic tryptophan fluorescence quenching of HSA in presence of sulfadiazine (SDZ) and caffeine (CAF) reveals the difference in binding of these two drugs in Sudlow's 1 site.



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ABSTRACT

The interaction and binding behavior of the well-known drug sulfadiazine (SDZ) and psychoactive stimulant caffeine (CAF) with human serum albumin (HSA) was monitored by *in vitro* fluorescence titration and molecular docking calculations under physiological condition. The quenching of protein fluorescence on addition of CAF is due to the formation of protein–drug complex in the ground state; whereas in case of SDZ, the experimental results were explained on the basis of sphere of action model. Although both these compounds bind preferentially in Sudlow's site 1 of the protein, the association constant is approximately two fold higher in case of SDZ ($\sim 4.0 \times 10^4\ M^{-1}$) in comparison with CAF ($\sim 9.3 \times 10^2\ M^{-1}$) and correlates well with physico-chemical properties like pK_a and lipophilicity of the drugs. Temperature dependent fluorescence study reveals that both SDZ and CAF bind spontaneously with HSA. However, the binding of SDZ with the protein is mainly governed by the hydrophobic forces in contrast with that of CAF; where, the interaction is best explained in terms of electrostatic mechanism. Molecular docking calculation predicts the binding of these drugs in different location of sub-domain IIA in the protein structure.

1. Introduction

Human serum albumin (HSA) is the most widely distributed protein in human blood plasma with a negatively charged surface. It binds to a series of chemically diverse exogenous and endogenous compounds with moderate to high affinity (10^4–$10^6\ M^{-1}$) and also acts as transport capacity enhancer of blood [1–3]. Analysis of protein structure shows that it consists of ~70% α-helix and ~30% random coils. Further, HSA shows higher order conformational flexibility due to the absence of any β-sheet [1]. The non-glycosylated single polypeptide chain of HSA has

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molecular weight of 66.5 kDa. It consists of 585 amino acids and there are 17 disulfide bonds which connect the individual helices that results the formation of 9 structural loops (Scheme 1). There are three 190 amino acid residue domains (labeled as I, II and III from the N terminus) which are structurally almost identical to each other and characterized by 10 helices each [1].

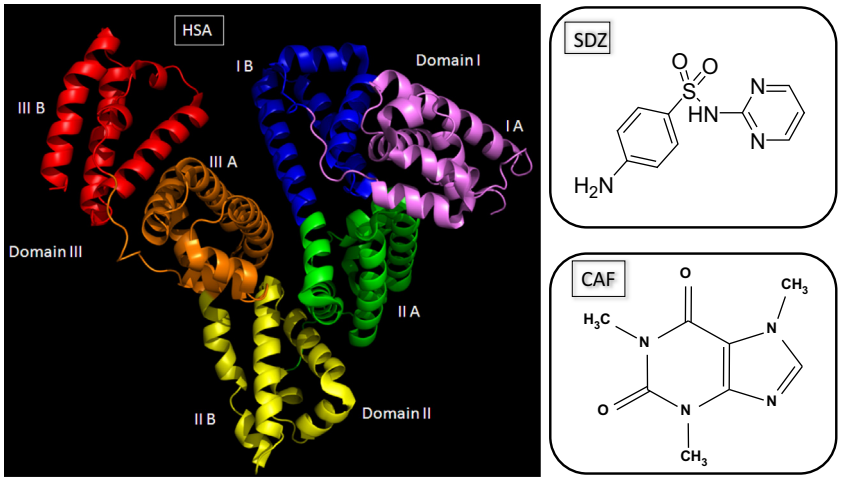
Further division of each domain into a six-helix and a four-helix sub-domain (identified as A and B, respectively) is also known and they contain a common four antiparallel α-helix core. Sudlow et al. were able to identify the two most active drug binding sites in HSA which are widely known as site 1 and site 2 (or subdomain IIA and subdomain IIIA, respectively) in the literature [4,5]. Normally, heterocyclic compounds with a diffused negative charge at the center of the molecule often act as site 1 specific drug [6–10]. On the other hand, the site 2, usually referred as the indole-benzodiazepine site, generally binds with stick-like aromatic carboxylic acids [7]. However, these structural limits are not strict prerequisites for specific drug binding sites since many ligands bind to both these sites, albeit with different affinity (e.g. L-thyroxine [11], indoxyl sulfate [7], dansyl-L-asparagine [9], or ibuprofen [7]).

Sulfadiazine (4-amino-N-pyrimidin-2-yl-benzenesulfonamide, SDZ, see Scheme 1 for structure) is a short-acting anti-bacterial drug of the parent sulfonamide class of synthetic antibiotic compounds. The therapeutic use of sulfonamide antibiotics is restricted in veterinary field because of the health risk associated with consumption of sulfonamide residue contaminated animal products [12,13]. Furthermore, hypersensitive patients carry significant allergic risk to many other drugs when they were found to be sensitive to sulfonamides [14], although the mechanism of sulfonamide-related reactions is not clear till now. Also, exposure to sulfonamide residues to the consumers for prolonged period is known to cause the development of drug-resistant bacteria [15]. The distribution of sulfonamides in the blood normally occurs in three different forms – free, conjugated (acetylated and possibly others) and protein bound. The free form is considered to be the therapeutically active. Particularly for SDZ (with LD_{50} = 1500 mg/kg), the initial clinically recommended dose of 100 mg/kg of body weight followed by 50 mg/kg four times a day (QID), the free drug level in blood serum reaches to about 7 mg/100 ml (ca. 300 μM) with a considerable fraction (~38% to 48%) bound in plasma protein [16].

On the other hand, caffeine (1,3,7-trimethyl-1H-purine-2,6(3 H,7H)-dione 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione, CAF, also shown in Scheme 1) is a bitter, white crystalline xanthene. Caffeine is the world's most widely consumed psychoactive substance, particularly in the Western countries, and believed to stimulate central nervous system (CNS). In fact, caffeine and its derivatives are known antagonists for both A_1 and A_2 – adenosine receptors in brain preparations; although with varying degree of effectiveness [17]. Caffeine influences the body water excretion mechanism when consumed in sufficient doses. It is evident that the adverse effect of caffeine ingestion (with LD_{50} = 127 mg/kg) is manifested only in the event of at least 20–30 times overdose to reach the concentration level of ca. >50 mg/L (~250 μM) than those found in normal diet [18,19], although some reports observe that caffeine concentration in human circulatory plasma rarely exceeds ca. 100 μM [20]. The peak plasma caffeine level in humans reach normally within 15–45 min after ingestion [21]; however, serum caffeine reaches to the level as low as <0.8 mg/L (~5 μM) only after 24 h of abstention [22]. Furthermore, results suggest that caffeine may be associated with enhanced reactions to some psycho-stimulants like nicotine [23] even in moderate dose. The cytotoxic and/or cytostatic effects of the DNA-IA cancer drugs doxorubicin and novantrone, in different cell lines are reported to decrease substantially in presence of CAF [24,25]. Being the principal component of coffee, CAF is also found to be partly responsible for increased homocysteine concentration in human plasma with potential risk for cardiovascular diseases [26].

Protein–ligand interactions are important in the distribution and transport of small drug molecules in living systems. Therefore, understanding the molecular basis of these interactions is indispensable toward designing of new and more efficient specific therapeutic agents for improved drug action [27,28]. Fluorescence spectroscopy is one of the most widely used techniques to achieve this goal because of its high sensitivity coupled with easy operational procedure. The modular structural domain of HSA to bind a series of exogenous drugs led to an intense research toward understanding the forces responsible as well as comparative thermodynamic parameters for drug binding etc. Also, a common trend is to note the drug binding domain by comparative experimental studies with site specific markers [29,30] in conjunction with molecular docking results [31–33].

In view of the multifarious functions as well as medicinal importance, studies on the interactions of SDZ as well as CAF drugs with HSA protein seem to be very important. In fact, binding studies of both these drugs with proteins were reported using different techniques [34–39]. However, while some of the earlier



Scheme 1. Cartoon diagram of human serum albumin (HSA) and its different binding domains. The line diagram of the drugs caffeine (CAF) and sulfadiazine (SDZ) used in the present study is also shown.