

INSIGHT INTO PROPERTIES OF HUMAN SERUM ALBUMIN FOR NOVEL DRUG DISCOVERIES

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ABSTRACT: Human serum albumin (HSA) is an opulent, non- glycosylated, most versatile carrier protein in plasma possessing multiple functions. It comprehends of multiple binding sites located in different subdomains and are responsible for binding of ligands. While antecedent research have discovered various functional and structural properties of HSA, the objective of this review paper is to shed light on some of the important properties of HSA and how binding pattern of different ligands can sustain the development of new drugs. Some significant properties include transportation, ligand- binding, distribution and metabolism of a compound. It also undergoes glycation and acts as an antioxidant. Another important feature is an esterase- like activity possessed by HSA, which is also crucial in converting the prodrugs into active therapeutics. Therefore, HSA is one of the most suitable molecule for future research in drug discovery in pharmaceutical industry because of its numerous features and binding pattern that also governs the metabolism and drug dosage.

Keywords: Human serum albumin, ligand- binding, esterase –like activity

1. INTRODUCTION

Human serum albumin (HSA), among other plasma proteins is most copious protein found in blood. The synthesis and secretion of the protein is in the liver. The molecular weight of protein 66 kDa. A single gene is responsible for translating it into prealbumin and afterwards, cleavage occurs in endoplasmic reticulum. Thereafter, it moves to Golgi before eventually being secreted out of cell [1]. Previous discoveries have observed high binding affinity to multiple endogenous and exogenous compounds with moderate to high affinity [2]. There are ample transport proteins which exist in blood plasma but only HSA associate with wide diversity of ligands reversibly with high affinity. A wide literature is available for HSA background. It was precipitated from urine in 1500 A.D. [3]. In 1940, I.S. Ravdin initiated clinical use of HSA by giving purified HSA to 7 wounded patients and successfully all of them get through [4]. Thereafter, three dimensional atomic structure was discovered using X- ray crystallography at 2.8 angstrom in 1992 [5]. Thus, the aim of current review paper is to illustrate the critical functions of HSA for their implementation in pharmaceutical industry for more innovative invention of drugs.

2. STRUCTURE OF HSA

The structure depicts several physical properties and understanding of pharmacokinetic and therapeutic applications of HSA. It is a spiral protein consisting of turns and extended loops and 585 amino acids forming a single polypeptide of known sequence. It has alpha helices and is segregated into domains. HSA has profusion charged residues like aspartic acid and lysine and not many methionine and tryptophan residues [6]. The structure has 17 pairs of disulphide bridges formed out of 35 cysteine residues, putting up to overall tertiary structure. More than half of the tertiary structure is composed of alpha – helices [Figure1]. The albumin molecule has three homologous domains that possess common structural motifs. Each domain further consists of two subdomains and have distinct feature [Figure 2]. Experimental data suggests that there are seven – long chain fatty acid binding sites spreaded across three domains [7]. Every site has different affinity for fatty acids but there are two high affinity binding sites for small heterocyclic or aromatic compounds (located on subdomains IIA and IIIA), two to three long-chain fatty acid binding sites (located on subdomains IB and IIIB). [Sudlow et. Al 1975]. X – ray crystallography has also proven that HSA has a heart shaped tertiary structure but it is ellipsoid in solution [8]. It has a negative charge (pI 4.8) which extend to high water solubility. This protein is highly flexible and alters the shape based on conditions and binding of the ligand. Some ligands which contain a free thiol group binds covalently to HSA. There is also presence of one free – cysteine derived, thiol (-SH) group (Cys 34) in plasma. The Cys 34 is reactive and capable of thiolation [9]. Also, there is irreversible binding between drugs and protein which can form acyl glucuronids. Reversible binding is mainly stereoselective in HSA [10]. It do not have any prosthetic groups and covalently bound lipids and carbohydrates.

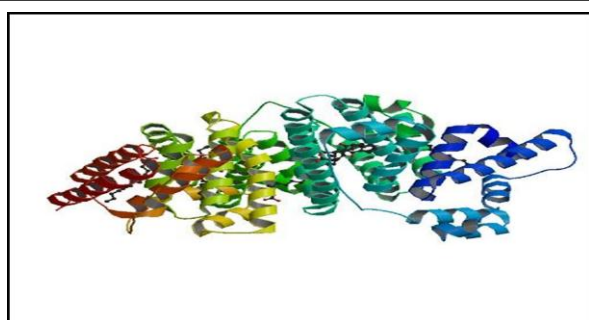


Figure 1: Three dimensional structure of Human Serum Albumin complexed with Warfarin having PDB ID: 1H9Z

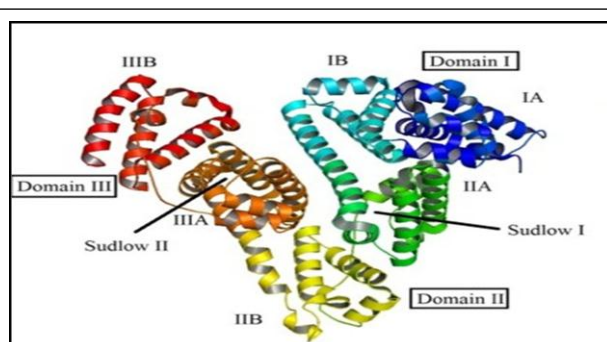


Figure 2: HSA structure showing different domains along with location of sudlow site I and II

3. PROPERTIES OF HUMAN SERUM ALBUMIN

The physiological and pharmacological properties have been extensively studied over several decades. Many studies reveal that HSA has a high empathy to a variety of components including metals, fatty acids, amino acids, metabolites such as bilirubin and for many drug compounds. The N- terminal position of HSA binds Cu, Ni and Co ions with great affinity and some other ions such as Au, Ag and Hg binds to Cysteine 34. Also, it is utmost Zn⁺ binding protein in plasma. The molecular size and high concentration in plasma facilitates the maintenance of osmotic pressure [11]. Also, it serves in transportation of various hormones, fatty acids and other compounds through blood stream. HSA is of great interest due to its carrier properties for drug delivery. It links to variety of ligands at several sites spreaded across the three domains and also acts as a carrier to deliver various drugs to their specific target. The elements which determines the metabolism and distribution of a substance is its binding to HSA. Binding with HSA assists tiny molecules to be present at a higher concentration in blood plasma. This protein also serves as depot and detoxification protein. In few cases, enzymatic activity has also been reported. It also experience glycation and acts as an antioxidant also. HSA possesses an intrinsic enolase activity which converts 3- keto form of dihydrotestosterone to 3- enol form. The enolase activity is of clinical importance as albumin get accumulated and it is seen in various tumors and cytosol of benign [12].

3.1. Ligand – binding property

Ligand binding is a significant property of this protein. HSA binding affects pharmacokinetic and distribution of the molecule. Moreover, the drug- protein interaction is also crucial in study of bioavailability, efficacy, transportation and designing of the drugs. Binding of HSA also leads to an increased solubility, and decreased toxicity [13]. Therefore, drug binding ability with HSA is chief reason considered in drug research. In addition, pharmacodynamics and pharmacokinetics of drug- protein binding are foremost in determining drug availability to and removal of drug from body and also, has consequences upon dose response relationship. Several low and high- affinity ligand binding sites have been identified on HSA spreaded across three domains [14] [Figure 3]. Among all of them, two sites have high affinity for multiple substances and they are commonly known as Sudlow site 1 and sudlow site 2 and are responsible for the binding of most pharmaceuticals that interact with the protein [15]. Both the sites are located in separate domains and have variant ligand binding affinities. Hydrophobic and electrostatic interactions play a crucial role in controlling the affinity towards drug binding for Site 1 and site 2.

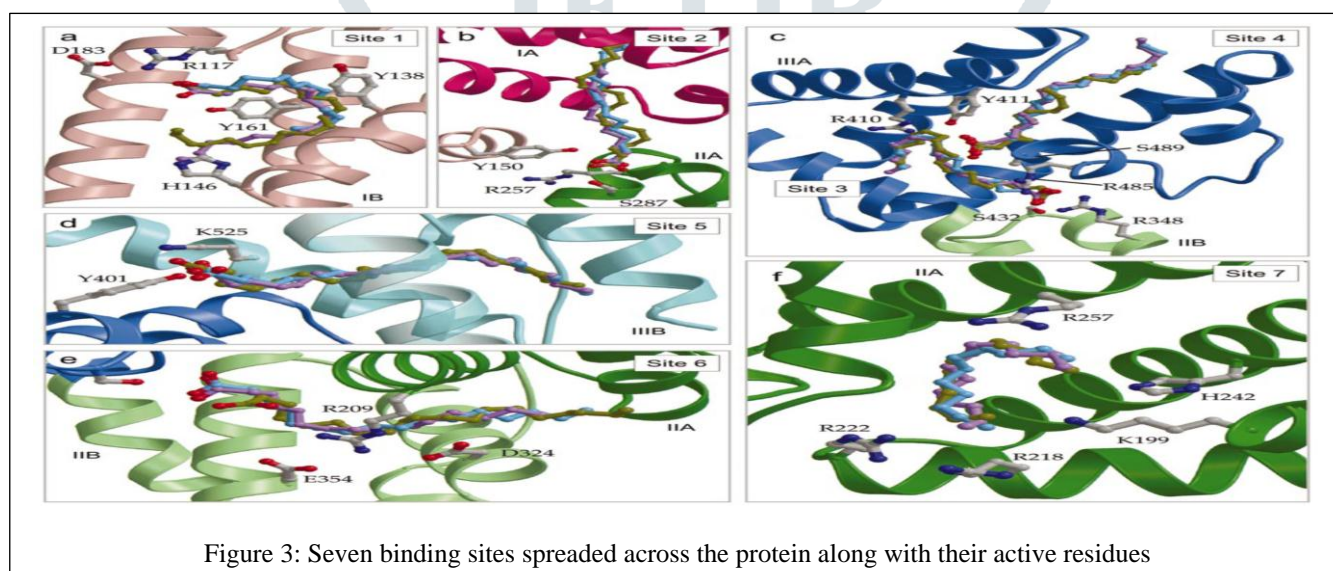


Figure 3: Seven binding sites spreaded across the protein along with their active residues

3.1.1 Sudlow site 1

Sudlow site 1, also known as Warfarin binding site is a huge, flexible and multi chamber cavity located in II A subdomain and it contains six helices and more residues from I B, II B and III B [16]. The ligands which bind at sudlow site 1 are often large, negatively charged and heterocyclic. The inside wall of the pocket is formed by hydrophobic side chains while entrance is mainly enclosed by positively charged residues [17]. Thus, site 1 appears to be versatile and capacious to have huge individual ligand binding sites that in some cases are independent while in others influence each other mutually. The individual residues of this site includes F 211, W 214, R 218, R 222, L 238, H242, R 257, I 264 and A 291 [18] [Figure 4].

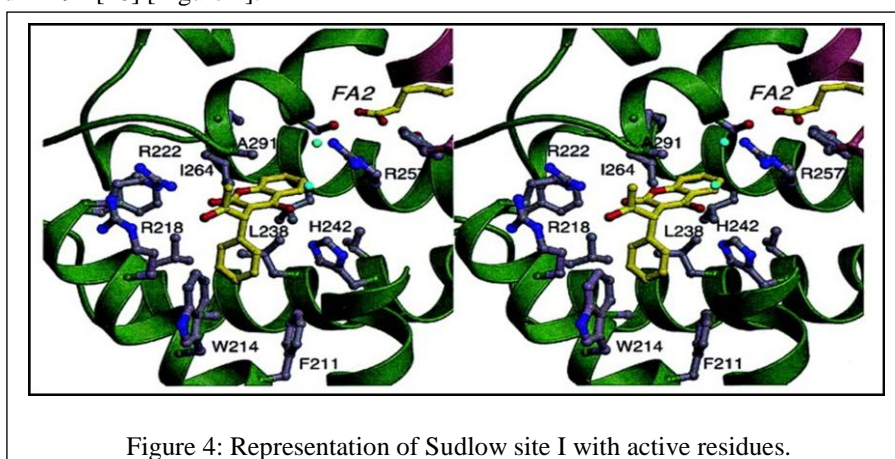


Figure 4: Representation of Sudlow site I with active residues.

3.1.2 Sudlow site 2

Site 2 involves in electrostatic, hydrophobic and hydrogen- bonding with the ligands which are small, aromatic carboxylic acids having negatively charged acidic group at one end of the molecule [19]. Site 2 seems to be smaller, or more narrow as compared to site 1 because no huge ligands associate to it and it has also been suggested that it is less flexible. It is located in subdomain III A [20]. Among individual amino acid residues of this subdomain, Arg 410 and Tyr 411 are most predominant residues. Site 2 is also composed of six helices of sub domains which forms polar cavities and the inner cavity is smaller and rigid [21] [Figure 5].

The drug- protein interaction also helps in optimization of ADME properties. Moreover, Hydrophobic interactions in HSA play major role in drug- binding. Hydrophobic and hydrophilic residues are distributed asymmetrical in the binding crevice [22]. The main non- polar residues are sequenced in the hydrophobic cavity inside the protein core and polar residues on the surface. Several lines of evidence have discovered notable association between fatty acid and drug binding sites on HSA but still there is lack of high- resolution structural information.

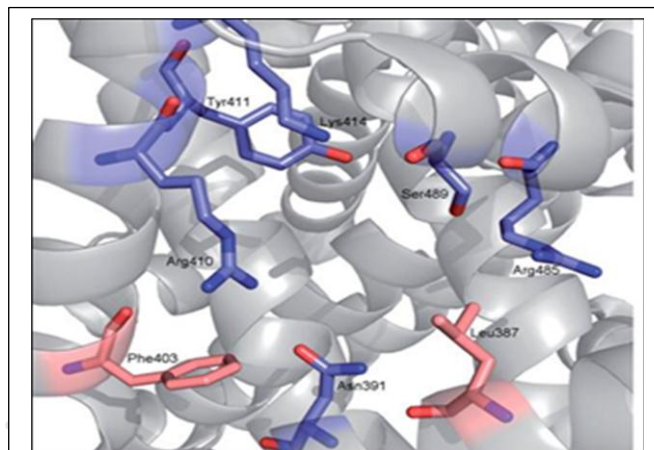


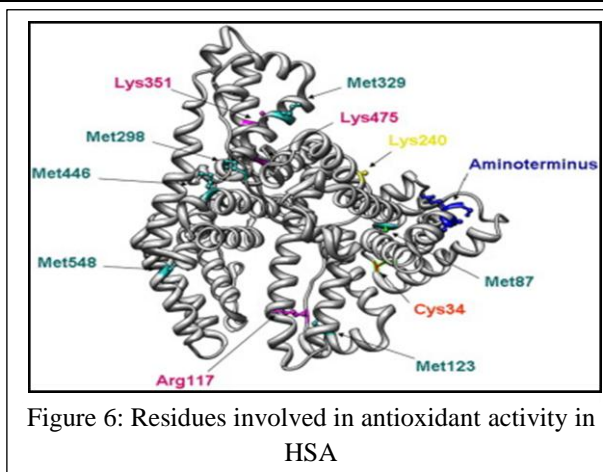
Figure 5: Representation of Sudlow site II with active residues

3.2. Enzymatic properties

The interaction of an albumin with small molecule results in enzymatic activity. An indispensable stereospecific property of HSA is an esterase- like activity that hydrolyzes drugs having an ester group and has already been studied and reported with respect to various substrate [23]. This catalytic property transfers the acetyl group from acetylsalicylic acid to lysine 199 which is pivotal for trinitrophenylation of HSA [24]. Lys 199 is essential for esterase activity and it is because Lys 199 is present in vicinity to Lys 195 and attacks by nucleophilic substitution and additionally, due to Lys 195 it forms Hydrogen bond which makes proton- transferring process stable at Lys 199 [25]. It has been found that this activity has been measured by cleavage of p- nitrophenyl esters by subdomain III A [26]. The residues existing in III A also detoxifies cyanide with elemental sulfur to form thiocyanates [27]. Apart from this, site- directed mutagenesis has proven that Arg 410 and Tyr 411 are requisite for esterase activity due to their close proximity and are present in Sudlow site II. The studies indicate that hydroxy group at position 411 and the surrounding environment are critical for this activity and thus, forms primary reactive site (R site) for esterase activity [28 and 29]. In addition, secondary site (T site) makes unpresumable benefaction due to Lys 199 and Lys 195 [30]. The free sulfhydryl group at Cys 34 in albumin is able to act as thioesterase and thus, Cys 34 is also vital for esterase activity. Therefore, further research on this property of HSA can reveal information about the drugs cleaved by this activity which could have pharmacokinetic association for adjusting dosage of drugs. Also, this esterase- like activity is crucial from clinical prospective as it converts prodrugs to active drugs in plasma. [31]

3.3. HSA as an antioxidant

The antioxidant property of HSA in plasma seems to be of paramount importance. An antioxidant is a compound which if present at low levels significantly slacken off or prevents the oxidation of an oxidizable substrate [32] The antioxidant activity is due to potential to trap oxygen radicals [33]. HSA exhibits this property to it's structure and multiple ligand binding capacity and interacting either directly or with free radical trapping property [34]. Among different residues contributing to this activity in HSA, Cys 34 is essential depending upon it's position. [35]. In addition, Methionine also contributes to this activity but it is less important than Cys 34 which plays more vital role. It has also been studied that collectively Cys 34 and 6 methionine residues contributes for more than 90% of antioxidant activity of HSA against O_2^- and H_2O_2 . Apart from this, long chain fatty acids also interact with HSA and results in antioxidant activity. An indirect antioxidant activity of this protein comes from binding with Lys 240 and it's ability to transport bilirubin [Figure 6]. Thus, due to presence of antioxidant activity HSA can act as a biomarker for numerous oxidative stress in future discoveries [36].



In addition to above properties, HSA may undergo some chemical modifications which includes non-enzymatic glycation and in hyperglycaemic condition it is more frequent. It is a process of addition of a sugar molecule to free amide group of protein without assistance of an enzyme and leads to formation of early and advanced glycation end products (AGEs) [37]. Among different proteins of body, glycation of HSA is of profound research due to its high sensitivity due to which there are variation in both structure and function of HSA due to AGEs [38] and it also leads to increase in total molecular weight of the protein [39]. Several techniques like radiolabeling colorimetric assays, fluorescence, spectroscopy, infrared spectroscopy, circular dichroism, and NMR spectroscopy have been used to study the changes in structure of HSA. This topic is of keen interest because amendments in the structure may effect the ability of binding to various molecules [40]. Also, few experiments have derived that changes in the protein structure can be on the residues that are at or near Sudlow site I or II [41]. Accumulation of numerous research studies have identified that because of high nucleophilic properties, lysine, arginine and cysteine are the main residues susceptible to glycation. L- 525 and Arg 410 are principle glycation site where Arg 410 is also involved in esterase – like activity of HSA [42]. Many studies reveals that inclined glycation of HSA is related to high blood glucose levels in diabetic patients. Also, glycated HSA remains in blood for 2- 3 weeks, so for longer periods it is useful for diagnostic purposes [43]. Therefore, many researches are related to implement glycated HSA as a biomarker to detect and control blood sugar level [44].

4. CLINICAL SIGNIFICANCE OF HSA

HSA also holds clinical significance by removal of endogenous toxins. In many cases such as renal failure, liver failure, jaundice, there is amassing of numerous endogenous toxins. This accumulation leads to necrosis and apoptosis conditions. These toxins mainly includes uremic toxins, bile acids and bilirubin and they also cause cardiovascular, kidney and cerebral impairment. HSA ardently binds toxins, including bilirubin, copper ions and breakdown these products. Recently, an approach has been developed known as molecular adsorbent recirculating system (MARS) [24]. that uses large pore size and thinner dialysis membrane and by enumerating albumin to the dialysate which consequently, leads to removal of toxins normally cleared by kidney and removed by liver.

However, the former study elaborates the binding of HSA with particular type of ligand, new research focuses on interaction with variety of components and their binding patterns to active site. It also aims to burgeon new drugs and enhance the delivery of various pharmacological drugs.

5. CONCLUSION

HSA has many physiological and biochemical properties. The future research aims to take up HSA to enhance drug delivery of pharmacological approaches to treat various diseases in human beings. The above mentioned properties can gather up to add information to the formation of drugs added in the market everyday and also, to decide the drug dosage according to their binding to active sites. Moreover, albumin can also be beneficial in specific clinical circumstances.

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