

Intrinsically Disordered Regions in Serum Albumin: What Are They For?

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Received: 4 November 2016 / Accepted: 13 February 2017
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Abstract Serum albumin is a major plasma protein in mammalian blood. The importance of this protein lies in its roles in both bioregulation and transport phenomena. Serum albumin binds various metal ions and participates in the transport and storage of fatty acids, bilirubin, steroids amino acids, and many other ligands, usually with regions of hydrophobic surface. Although the primary role of serum albumin is to transport various ligand, its versatile binding capacities and high concentration mean that it can assume a number of additional functions. The major goal of this article is to show how intrinsic disorder is encoded in the amino acid sequence of serum albumin, and how intrinsic disorder is related to functions of this important serum protein.

Keywords Serum albumin · Ligand binding · Intrinsically disordered region

Introduction

Serum albumin (molecular mass of 66.5 kDa) is the most abundant protein of mammalian blood (35–50 g/L in human serum, i.e., 0.5–0.75 mM) [1]. It is synthesized in liver hepatocytes and released into the vascular space daily [2, 3]. Its lifetime in the blood is about 19 days [4, 5]. Termination of albumin life is typically achieved by its catabolism in skin and muscles [3, 6]. Albumin does not contain prosthetic groups or covalently linked lipids, but contains multiple hydrophobic binding pockets. It serves as a transporter and storage container of a variety of different ligands, such as fatty acids (FAs), steroids, bilirubin, and other ligands with hydrophobic surfaces, as well as different drugs [2, 7]. It binds metal ions, such as zinc, copper, calcium, and magnesium [8–13]. These unique properties enable albumin to be an universal carrier and reservoir of endogenous and exogenous substances in blood plasma and tissues throughout the mammalian body [14]. The unique binding capacities and high blood concentrations of albumin indicate that it can perform a number of other functions. Serum albumin participates in the control of osmotic pressure in blood [15] and contributes to body detoxification via trapping of poisonous metabolites [16]. Serum albumin affects the pharmacokinetic and pharmacodynamic properties of bound drugs. Since albumin possesses multiple ligand-binding sites and can trap free radicals, it is characterized by high antioxidant activity [17–19]. Bound copper and iron ions, as well as polyunsaturated FAs and sterols are involved into its antioxidant action: the binding of these transition metals affects their availability for the Fenton reaction, while lipid binding prevents their peroxidation. Furthermore, Cys34 residue of albumin acts as a free radical scavenger, trapping multiple reactive oxygen and nitrogen species [17, 20]. For instance, serum albumin

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serves as a reservoir of nitric oxide [21]. Human serum albumin (HSA) carries about 90% of β -amyloid peptide in plasma [22], thereby enabling to put forward an amyloid-targeted therapeutics in Alzheimer's disease (AD) based on the use of ligand-free serum albumin. Indeed, the replacement of endogenous albumin by 5% Human Albumin Grifols via plasma exchange showed a tendency to prevent cognitive impairment in patients with mild to moderate AD [23]. HSA is also used to treat severe hypoalbuminemia and traumatic shock [24, 25].

It is recognized now that many biologically active proteins do not have unique three-dimensional (3D) structures as a whole or in some of their parts. These are intrinsically disordered proteins and hybrid proteins containing ordered domains and intrinsically disordered regions [26–36]. Such proteins have the ability to bind to multiple partners, which enables them to function in regulation, signaling, and control, where they are commonly engaged in one-to-many and many-to-one interactions [26, 28, 33, 34, 37–43]. Disordered proteins or protein regions are often affected by posttranslational modifications (PTMs), such as phosphorylation, glycosylation, methylation, and ubiquitination [44, 45], and serve as major targets for the alternative splicing [46–48]. All these means are utilized by nature to control and regulate functions of these proteins. Importantly, these structureless, highly dynamic, promiscuously interacting proteins/regions are implicated in numerous human diseases [49–51].

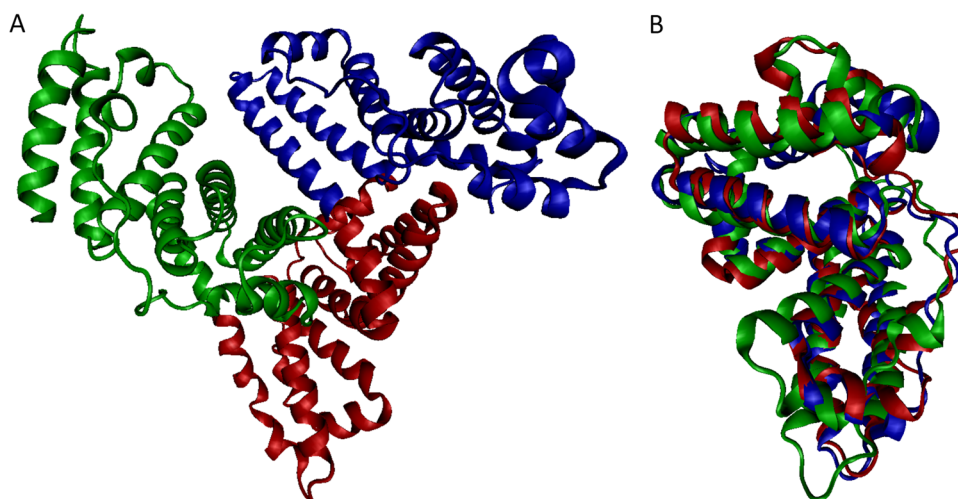
Although various aspects of serum albumin structure and function are rather well studied and were subjects of several reviews, the abundance and potential functional roles of intrinsically disordered regions in this protein have never being addressed as of yet. The major goal of this article is to fill this gap and to describe the peculiarities of the “intrinsic disorder code” of this protein.

Serum Albumin Structure

There are 332 (57%) invariant residues in the sequences of mammalian albumins. 76% of sequence identities have been noted between bovine albumins (BSA) and HSA. The determination of the first high-resolution crystal structure of HSA in 1992 [14], followed soon after by the determination of the crystal structure of equine serum albumin [52], was an important stage in the serum albumin studies. The crystal structure of BSA was published in 2012 by Majorek et al. [53]. The mature BSA/HSA is a monomeric protein that consists of 583–585 amino acids. It contains 35 cystine residues (17 internal disulfide bridges and 1 free thiol group), and has a molecular mass of 66,400 Da [2]. The numerous disulfide bridges provide the high stability of albumin. Free Cys34 (i.e., a residue that is not involved in the formation of disulfide bond) is located on the outer surface of albumin distant from the main interior drug-binding sites and is, therefore, useful for covalent conjugation of drugs [1, 54, 55]. Low tryptophan, methionine, glycine, and isoleucine content, combined with the high content of the charged amino acids, such as glutamic acid and lysine are characteristic features of serum albumin. There are two isoforms of HSA generated by alternative splicing, with isoforms #2 and #3 being different from the canonical full-length protein by missing the 43–234 and 164–376 regions, respectively (<http://www.uniprot.org/uniprot/P02768#sequences>). Both HSA and BSA are synthesized in the form of precursors and contain a signal peptide (residues 1–18) and a pro-peptide (residues 19–24) [56].

The overall 3D structure of HSA determined by X-ray crystallography is heart-shaped, with approximate dimensions of $80 \times 80 \times 30$ Å (Fig. 1a) [57]. Serum albumin molecule consists of three homologous domains: I (residues 1–195, *blue*), II (196–383, *red*), and III (384–585, *green*).

Fig. 1 **a** Crystal structure of HSA (PDB ID: 1n5u). The three domains of albumin are shown as *blue* (domain I), *red* (domain II), and *green* (domain III) ribbons. **b** Multiple structural alignment of the HSA domains I (*blue*), II (*red*), and III (*green*) from the structure shown in plot **a**. Structures were aligned with the RMSD of 1.72 Å over 99 residues. Multiple structural alignment was conducted using the MultiProt tool (<http://bioinfo3d.cs.tau.ac.il/MultiProt/>) [202]. Resulting structures are drawn using the visual molecular dynamic tool VMD [203] (color figure online)



The domains are not only topologically identical, but they also have very similar 3D structures [14, 58] (see Fig. 1b). Each domain in its turn consists of two subdomains (A and B). The A subdomain contains four α -helices, while the B subdomain contains six α -helices. The secondary structure of the protein is mainly α -helical (74%), with the remaining polypeptide chain occurring in turns and in extended or flexible regions between subdomains [53].

There are two regions showing significant differences between HSA and BSA, both found within the subdomain IB. The first region is located on the first two α -helices of the IB subdomain, comprising residues 114–135 (numbered according to the mature BSA numbering), with most of the different residues being exposed at the protein surface. BSA residues S109, D111, D37, and E38 also differ from the HSA sequence and lie in close proximity possibly extending the recognition region.

Notably, HSA is known to undergo the pH-dependent reversible conformational changes [2, 59–62]. Within pH region from 2.7 to 4.3, HSA exists in a fast (F) form, characterized by high viscosity, low solubility, and low α -helical content. Within pH region from 4.3 to 8.0 HSA exists in a neutral (N) form, which is characterized by the heart-shaped structure. In this pH region and in the presence

of drugs and long-chain FAs and at pH > 8 in the absence of ligands, HSA converts to the basic form (B), which is accompanied by a loss in α -helices and by an increase in affinity for some ligands, like warfarin.

There are at least 77 genetic variants of HSA [63]. Twenty-two genetic variants were found in domain I, 30 in domain II, and 25 in domain III. Most of the mutations are benign; 65 mutations result in bisalbuminemia [64]. No correlation was found between mutation-induced changes in the pharmacokinetic parameters and changes in α -helical content or changes in heat stability of HSA [65].

Intrinsic Disorder Predisposition of Serum Albumins

Figure 2a represents the results of the multifactorial analysis of intrinsic disorder predisposition in HSA, whereas analogous data for BSA are shown in Fig. 2b. In this analysis, we used a series of established per-residue disorder predictors, such as PONDR[®] FIT [66], PONDR[®] VLXT [67], PONDR[®] VSL2 [68], and IUPred [69]. Here, scores above 0.5 are considered to correspond to the disordered residues/regions. PONDR[®] VSL2B is one of the more accurate

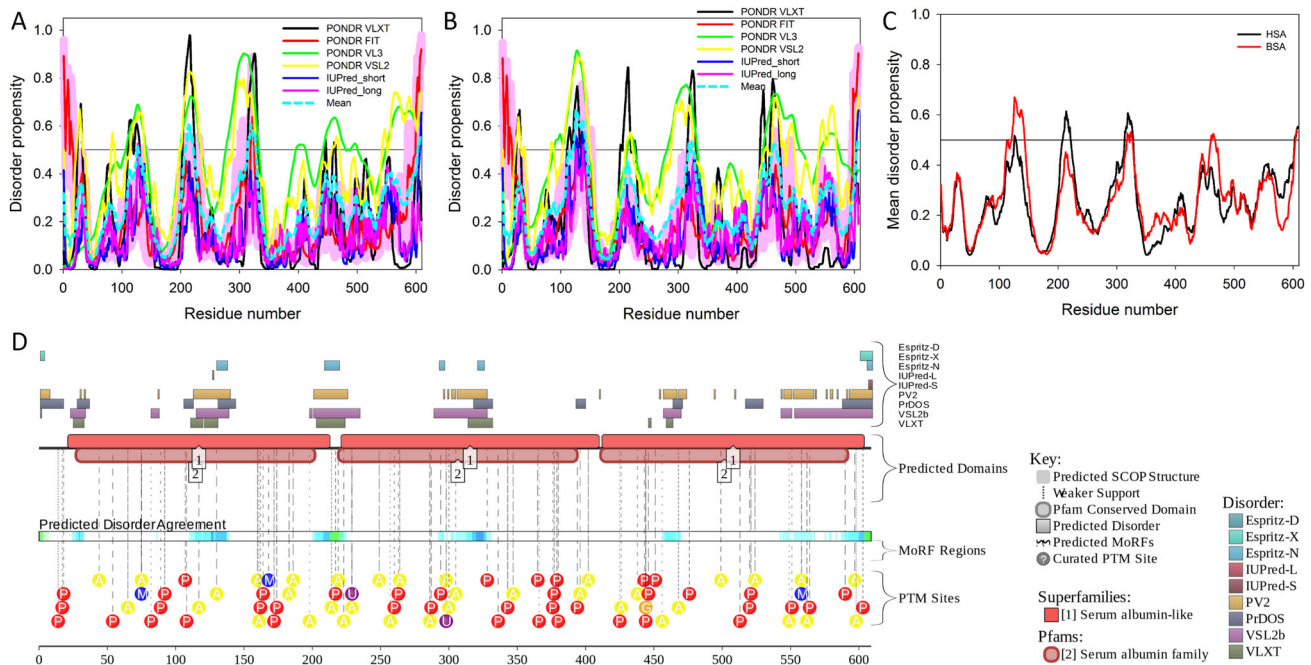


Fig. 2 Multifactorial computational disorder analysis of the serum albumins. **a** Intrinsic disorder profile of HSA (UniProt ID: P02768) generated by the superposition of the outputs of PONDR[®] VLXT, PONDR[®] FIT, PONDR[®] VL3, PONDR[®] VSL2, IUPred_{short} and IUPred_{long} and a consensus disorder profile calculated by averaging disorder profiles of individual predictors. **b** Intrinsic disorder profile of BSA (UniProt ID: P02769) generated by the superposition of the outputs of PONDR[®] VLXT, PONDR[®] FIT, PONDR[®] VL3, PONDR[®]

VSL2, IUPred_{short} and IUPred_{long} and a consensus disorder profile calculated by averaging disorder profiles of individual predictors. **c** Superposition of the consensus disorder profiles calculated for HSA (black curve) and BSA (red curve) illustrating remarkable similarity of the distribution of disorder propensities within sequences of these proteins. **d** Intrinsic disorder propensity and some important disorder-related functional information generated for HSA by the D²P² database (color figure online)

stand-alone disorder predictors [70–72], PONDR® VLXT is known to have high sensitivity to local sequence peculiarities and can be used for identifying disorder-based interaction sites [26], whereas a metapredictor PONDR-FIT is moderately more accurate than each of the component predictors [66], PONDR® VLXT [26], PONDR® VSL2 [70], PONDR® VL3 [73], FoldIndex [74], IUPred [69], TopIDP [75]. IUPred was designed to recognize IDPRs from the amino acid sequence alone based on the estimated pairwise energy content, where it was hypothesized globular proteins are composed of amino acids that have the potential to form a large number of favorable interactions, whereas IDPRs do not have unique 3D structure because their amino acid composition does not allow sufficient favorable interactions to form [69, 76]. We also analyzed mean disorder propensity calculated by averaging disorder profiles of individual predictors. Use of consensus for evaluation of intrinsic disorder is motivated by empirical observations that this approach usually increases the predictive performance compared with the use of a single predictor [72, 77, 78].

Figure 2a and b shows that although serum albumins are predicted to be mostly ordered, they have several intrinsically disordered regions. Importantly, the overall shape of disorder distribution within the amino acid sequences of HSA and BSA is well-conserved, suggesting functional importance of both ordered and disordered regions in these proteins (see Fig. 2c). Figure 2d provides further support to the idea of functionality of intrinsic disorder by representing the output of the D²P² database (<http://d2p2.pro/>) [79]. Here, complementary disorder evaluations together with some disorder-related functional information are shown. To this end, the D²P² database uses outputs of IUPred [69], PONDR® VLXT [67], PrDOS [80], PONDR® VSL2B [68, 73], PV2 [79], and ESpritz [81] and also mark locations of various curated PTMs in HSA. Figure 2d shows that HSA has multiple phosphorylation, acetylation, ubiquitination, methylation, and glycosylation sites, which are spread through the entire protein sequence. Often, sites of the enzymatically catalyzed PTMs are located within the region of intrinsic disorder, which makes them easily accessible to modifying enzymes. Discussion of functional roles of PTMs is considered below. In sections describing functionality of HSA and/or BSA, the roles of various protein regions and individual residues are also discussed. For these discussed residues/regions we provide their mean predicted disorder scores (MPDSs), which are mean disorder scores calculated by averaging outputs of individual predictors. Looking at the numbers provided below for functional regions and residues, one should keep in mind that although HSA(BSA) are known to be ordered proteins, they are predicted to be rather mobile, being characterized by the sequence-average mean disorder score of $0.26 \pm$

0.05. In these proteins, there are 76 (48), 136 (177), 168 (164), 125 (103), 42 (48), and 38 (43) residues with MPDS < 0.1, $0.1 \leq \text{MPDS} < 0.2$, $0.2 \leq \text{MPDS} < 0.3$, $0.3 \leq \text{MPDS} < 0.4$, $0.4 \leq \text{MPDS} < 0.5$, and $\text{MPDS} \geq 0.5$, respectively. According to their MPDS values, 17, 59, 61, 29, 17, and 21 residues mentioned in the following sections fall within the MPDS < 0.1, $0.1 \leq \text{MPDS} < 0.2$, $0.2 \leq \text{MPDS} < 0.3$, $0.3 \leq \text{MPDS} < 0.4$, $0.4 \leq \text{MPDS} < 0.5$, and $\text{MPDS} \geq 0.5$ categories, respectively. This clearly indicates that serum albumins in their function often rely on disordered or flexible residues characterized by $\text{MPDS} \geq 0.5$ and $0.2 \leq \text{MPDS} < 0.5$, respectively.

Multiple HSA structures (mostly frequently, in a complexed form) were solved by X-ray crystallography. In fact, there are at least 94 such structures in Protein Data Bank (PDB) with the averaged resolution of 2.6 ± 0.3 Å. Furthermore, many HSA-related PDB entries contain multiple chains (there are at least 2 HSA chains in 42 of 94 structures). This abundance of structural data provides enough information for the comparison of the predicted local intrinsic disorder or flexibility and experimentally determined structure of this protein. We started with the utilization of the PDBFlex database that “provides information on flexibility of protein structures as revealed by the analysis of variations between depositions of different structural models of the same protein in the PDB” [82]. Serum albumin structures constitute the 4l8uA cluster of this database that contains 131 chains and therefore includes almost all X-ray structures currently available for this protein. Analysis of this cluster revealed that HSA is characterized by medium structural flexibility, since the average Root Mean Square Deviation (RMSD) of C_α atoms between structures in this cluster is 2.691 Å, and these structures are characterized by the average contact map overlap (CMO) of 0.88 (here, the CMO values of 1 corresponds to the completely preserved contacts, whereas the CMO of 0 indicates that all contacts are different). On the other hand, the maximal pairwise RMSD (6.782 Å) was found for the structures with PDB IDs 4kt1A and 1o9xA. The minimal pairwise CMO, which is the proportion of inter-residue contacts conserved between a pair of similar structures, was 0.634 (<http://pdbflex.org/cluster.html#!/4l8uA/23622/4l8uA>). It is emphasized that RMSD and CMO used as measures of protein structural dynamics in the PDBFlex database are sensitive to different flexibility levels, with the high RMSD scores being indicative of the presence of hinge-like movements that reflect high global structural flexibility, whereas changes in secondary structure that are considered as high local flexibility is reflected in low CMO values [82].

Furthermore, one of the outputs of the PDBFlex database is the sequence distribution of the average local C_α RMSD between the master protein chain and other cluster members

calculated over a 10-residue sliding window. Figure 3b shows that there is some agreement between the results of this analysis and analysis of intrinsic disorder predisposition. In fact, positions of many peaks between these plots either coincide or are located in the close proximity to each other. However, the correspondence of peak position is not ideal likely due to the different size of window used for data averaging in disorder prediction and in evaluating averaged local RMSDs. Therefore, we conducted analysis of the available HSA structures for the presence of regions with missing electron density that typically correspond to the intrinsically disordered segments. This analysis revealed that the first 2–4 residues are almost invariably disordered in 131 structures of the 4l8uA cluster. Furthermore, in many structures assembled to this cluster, the C-terminal tail of this protein is also disordered, with the number of missing residues ranging from 2 to 93 (e.g., PDB IDs: 4hgm and 4hgm). Furthermore, many structures (at least 30) where characterized by missing 77–88 region or some of parts of this region. Notably, these regions of the mature HSA are predicted to be either disordered or highly flexible (see Fig. 3). The longest predicted region of intrinsic disorder (residues 182–200) corresponds to the hinge region connecting albumin domains 1 and 2, whereas the linker between the albumin domains 2 and 3 (region centered at residue 379) is predicted to be flexible. Importantly, two predicted disordered regions of HSA (residues 102–104 and 290–304) coincided or overlapped with the regions with unassigned (likely highly mobile) structures (residues 104–119 and 293–305, respectively), with the third long unassigned region (residues 491–502) being located within the C-terminal part of the protein, which is predicted to be rather disordered/flexible.

Besides numerous crystal structures NMR (nuclear magnetic resonance) solution structure was determined for the C-terminal albumin domain (PDB ID: 1ysx, residues 404–601 of the pre-protein or residues 380–577 of the mature protein with removed signal and pro-peptides) and for the 432–447 (408–423) region (PDB ID: 2n0x) which is identical to the albumin-derived EPI-X4 peptide that regulates innate immunity by acting as an effective and highly specific CXCR4 antagonist [83]. HSA serves as a precursor for the EPI-X4 peptide, which is derived from the protein by the pH-regulated proteases and is highly evolutionary conserved [83]. Within the HSA structure, this peptide corresponds to the loop linking α -helices 22 and 23 and is predicted to be rather flexible. In isolated form, the EPI-X4 peptide possesses the flexible, lasso-like structure [83].

Finally, to illustrate the role of intrinsic disorder and flexibility in functionality of this protein, Fig. 3 represents the intersection between the HSA regions predicted to be either disordered or flexible and the binding sites of the protein, and clearly shows that the flexibility is needed for ligand binding.

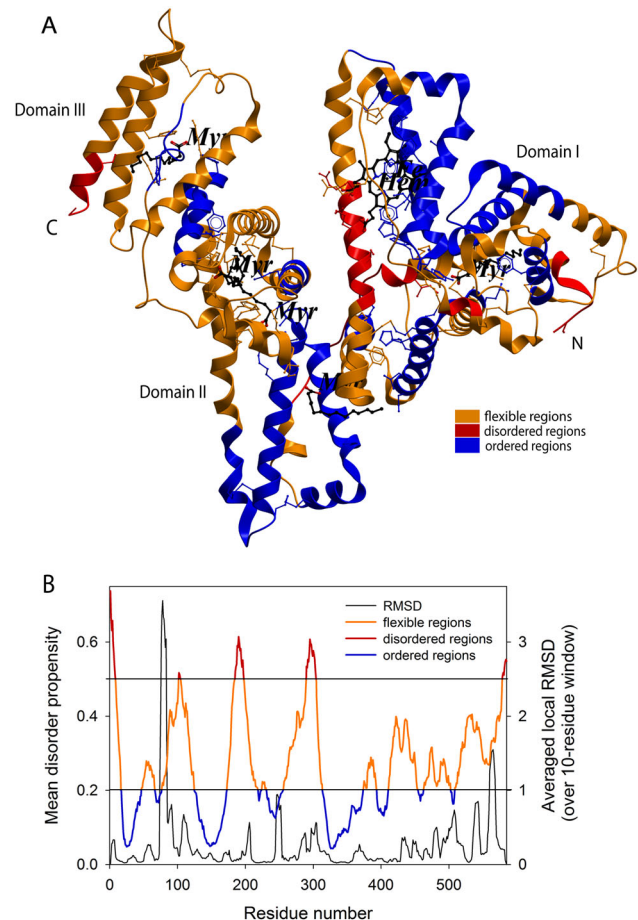


Fig. 3 The consensus per-residue disorder predictions for HSA mapped onto its tertiary (**a**; PDB entry 1n5u) and primary (**b**) structure. The regions predicted to be ordered (MPDSs < 0.2), flexible (MPDSs: 0.2–0.5), and disordered (MPDSs > 0.5) are colored *blue*, *orange*, and *red*, respectively. The RMSD values of C α atoms of HSA structures taken from PDBFlex database (see explanations in the text) are shown in *gray* (**b**) (color figure online)

Posttranslational Modifications

Serum albumin can undergo PTMs, some of which affect its ability to interact with drugs (reviewed in ref. 84). Of all the amino acids, Cys34 in domain I is the most sensitive to modifications. These modifications include glycation, cysteinylolation, S-nitrosylation, S-transnitrosation, and S-guanylation.

Protein glycation is the non-enzymatic reaction between nucleophilic groups of the biomolecules and sugars [85]. The principle sites of glycation of proteins are amino groups of lysine residues, the N-terminal amino group, and the guanidine groups of arginine residues (they all belong to the category of disorder-promoting amino acids). The initial step of glycation is the condensation reaction between a carbonyl compound and an amino group [86], leading to the formation of a Schiff's base. The second reaction step

results in accumulation of the Amadori product with an extra carbonyl group, an advanced glycation end product (AGE). AGEs are associated with the onset and complications of cardiovascular diseases, diabetes, and AD [87]. Some of AGE-binding receptors contribute to clearance of AGEs, while others are associated with propagation of inflammation and oxidative stress. They are considered as mediators of the AGEs-related diseases. The fraction of glycated albumin increases during diabetes [88].

HSA contains 24 arginine residues and 59 lysine residues and many of them can be subjected to glycation because they are solvent exposed and accessible to glycation reagents. Several tens of glycation and AGE modification sites in albumin have been identified (reviewed in ref. [89]). Arg410 (MPDS of 0.19) is found to be a principal site of modification by methylglyoxal in HSA glycated *in vivo*. Other sites of minor modification are: Arg114, Arg186, Arg218, and Arg428 [90, 91] with the MPDS values of 0.41, 0.53, 0.20, and 0.32, respectively. The other principal site of glycation on HSA is Lys525 (MPDS of 0.37), but the lysine residues in positions 199, 281, and 439 (with the MPDS values of 0.47, 0.38, and 0.36, respectively) are also susceptible to glycation. Curiously, all these arginine and lysine residues are characterized by rather high disorder scores (MPDS values are ranging from 0.19 to 0.53), which make them either flexible or disordered. AGE formation of HSA is associated with extensive damage of histidine and tryptophan side chains, main chain fragmentation, and loss of both secondary and tertiary structure as a result of oxidation [92].

In 2013, Anguizola et al. published a review article that summarized HSA glycation sites [93]. Analyses of the 59 lysine residues in HSA have confirmed that glycation occurs frequently at the following residues: Lys12 (MPDS of 0.27), Lys51 (MPDS of 0.23), Lys199 (MPDS of 0.47), Lys233 (MPDS of 0.18), Lys276 (MPDS of 0.41), Lys281 (MPDS of 0.38), Lys317 (MPDS of 0.16), Lys323 (MPDS of 0.07), Lys378 (MPDS of 0.21), Lys439 (MPDS of 0.36), Lys525 (MPDS of 0.37), and Lys545 (MPDS of 0.36). In addition to *in vitro* experiments, *in vivo* research also indicates that glycation readily occurs at Lys199, Lys439, and Lys525 [94]. However, Marashi et al. have raised questions about Lys525 being the main glycation site because of its low accessibility [95]. It was shown that glycation rates at Lys199, Lys439, Lys525 (all of which are characterized by relatively high MPDS values), and the N-terminal amino acid are faster than at other glycation sites [96].

It was found that in HSA complexes with glucose or fructose two molecules are localized in the Sudlow I binding site [97]. Glucose forms a covalent adduct with ϵ -amino group of lysine residue 195 (MPDS of 0.53) and H-bonds to nearby residues Lys199 (MPDS of 0.47),

Arg218 (MPDS of 0.20), Arg222 (MPDS of 0.20), and the backbone of Ala291 (MPDS of 0.53). The second glucose molecule is also bound to Sudlow site I, but retains its pyranose form and does not form a covalent link with any of nearby lysine residues. It is held in place by a network of H-bonds with His242 (MPDS of 0.13), Arg222 (MPDS of 0.20), Tyr150 (MPDS of 0.05), and Arg257 (MPDS of 0.22). In general, glycation “softens” the protein fold by reducing the α -helical content, increases the intrinsic flexibility of domains and secondary structure elements, and opens up additional hydrophobic-binding sites on the protein surface (reviewed in ref. [89]). At the same time, glycation can seriously affect the binding of ligands and drug molecules to albumin: for some drugs glycation enhances binding affinity, for others it is reduced.

Serum albumin can also undergo cysteinylolation, e.g., an addition of another cysteine to Cys34 (MPDS of 0.09) on HSA via a disulfide bond [98]. This modification was found in patients with liver and kidney diseases, as well as patients with diabetes.

Cysteine residues in serum albumin can also undergo S-nitrosylation or incorporation of nitric oxide [99]. Besides Cys34, Cys410 in the Arg410Cys variant of HSA has also been identified to undergo S-nitrosylation [100]. Such nitrosylating agents as sodium nitroprusside have low reactivity to the sulfhydryl groups in HSA. At the same time, S-nitrosoglutathione intermediates readily interact with the sulfhydryl groups of albumin and facilitate S-nitrosylation [101]. Ishima et al. found that S-nitrosylation of HSA affects its ability to interact with FAs, specifically with oleic acid [102].

The Cys34 residue of albumin, a free radical scavenger, is able to trap multiple reactive oxygen species (ROS) and reactive nitrogen species, such as hydrogen peroxide (H_2O_2), peroxynitrite (ONOO^-), superoxide, or hypochlorous acid [18, 20, 103, 104]. Under oxidative stress caused by ONOO^- or H_2O_2 , Cys34 thiol is oxidized with formation of sulfenic acid (HSA-SOH). Sulfenic acid may be oxidized to either sulfinic (HSA-SO₂H) or sulfonic (HSA-SO₃H) acids by usually irreversible processes leading to end products [105, 106].

Mild oxidation of HSA has no detectable effect on the binding of drugs to site I in subdomain IIA. In contrast, the ligand affinity of site II and the esterase-like activity of oxidized HSA are decreased, most probably due to changes in conformation of subdomain IIIA [107]. Bourdon et al. found that Met and Cys accounted for 40–80% of total antioxidant activity of HSA [108]. They concluded that Cys34 in domain I works as a free radical scavenger, whereas Met residues mainly act as metal chelators. In blood HSA exists in both reduced and oxidized forms, and the percentage of oxidized albumin increases in several diseases. The oxidation HSA may impair its function in a

number of pathological conditions [109, 110]. At the same time, oxidized albumin is a reliable marker of oxidative stress in hemodialysis patients [111].

In healthy adults, about 70–80% of the Cys34 in albumin contains a free sulfhydryl group; 25% of the Cys34 forms a disulfide with small sulfhydryl compounds like another cysteine, homocysteine or glutathione; and a small fraction of the Cys34 is more highly oxidized to the sulfinic or sulfonic acid form [112, 113]. Plasma concentration of albumin (up to 700 mM) is far higher than the plasma concentrations of cysteine (30–50 mM) or glutathione sulfhydryl (low micromolar range) [114, 115].

FA-Binding Sites

One of principal functions of serum albumin is transportation of FAs. The association constants for FAs increase, but not linearly, with their chain length. The number of high-affinity binding sites also increases with FAs chain length: octanoate and decanoate bind to one such site, whereas laurate and myristate most probably bind to two sites [63]. Under physiological conditions, HSA normally binds up to equivalents of unesterified FAs, although it may accommodate up to six–seven equivalents [116]. In acute hemolysis, e.g., after trauma or postischemic reperfusion, HSA binds heme that is released in the bloodstream.

In total HSA is able to bind seven equivalents of long-chain FAs at multiple binding sites with different affinities. In 1998, the publication of the crystal structure of HSA complexed with myristate provided a detailed picture of the precise locations of several FA-binding sites [117]. The crystal structure of HSA complexed with myristate [117] reveals five FA-binding sites. FA binding results in a significant conformational change in the protein. All of these sites (FA1–FA5 in Fig. 4) were recognized on the base of strong electron density for the ligand and reasonable chemistry at the FA-binding site. A sixth site (FA6) was tentatively assigned at the interface between subdomains IIA and IIB [118]. FA6-binding site is relatively open and consists of a shallow depression on the surface of domain II. The bound FA is “belted” in place by a salt bridge from Arg209 (MPDS of 0.26) to both Asp324 (MPDS of 0.06) and Glu354 (MPDS of 0.12) across the top of the hydrophobic tail of the ligand. Significant portions of the bound in FA6 fatty acid are exposed to solvent. One binding site is located in subdomain IB, one at the interface between IA and IIA (with contributions from IB), two sites within IIIA, one in IIIB, and one located at the junction of subdomains IIA and IIB (Fig. 4). All the binding sites are different in detail, nevertheless each site contains a hydrophobic pocket capped at one end with basic or polar side chains (with the

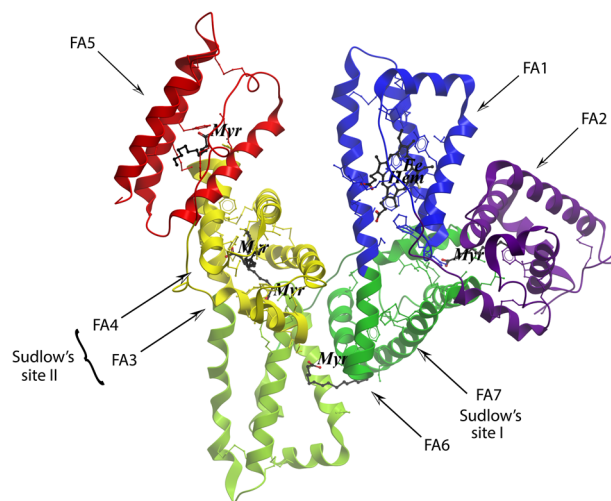


Fig. 4 The six subdomains of HSA are colored as follows: subdomain IA: violet; subdomain IB: blue; subdomain IIA: green; subdomain IIB: light green; subdomain IIIA: yellow; subdomain IIIB: red. The heme fits the primary cleft in subdomain IB. Sudlow's site I (in subdomain IIA) is empty. Sudlow's site II (in subdomain IIIA) is occupied by two myristate anions. Except for FA1 and FA7, all other FA sites are occupied by myristate. Atomic coordinates were taken from PDB entry 1n5u (color figure online)

possible exception of site 6, which is relatively open). The binding sites FA1, FA4, and FA5 in IB, IIIA, and IIIB, respectively, are essentially wholly contained within a single subdomain, while in the other three sites the pockets may be composite: binding pockets FA2 and FA3 incorporate residues from different domains [117–119].

FA-binding sites 1–5 interact with the carboxylate moiety of FAs due to electrostatic/polar interactions. In particular, the FA in FA1 forms a salt bridge with Arg117 (MPDS of 0.34) in the IB-IIA polypeptide linker, whereas the FA in FA2 is hydrogen bonded with Tyr150 from subdomain IB and with Arg257 (MPDS of 0.22) and Ser287 (MPDS of 0.43) from subdomain IIA. FAs in FA3 and FA4 are located in a large cavity in subdomain IIIA that constitutes the Sudlow's site II (see below). The FA in FA3 forms salt bridges with Arg348 (MPDS of 0.09) and Arg485 (MPDS of 0.23), and a hydrogen bond with Ser342 (MPDS of 0.08). The FA in FA4 forms a salt bridge with Arg410 (MPDS of 0.19) and hydrogen bonds with Tyr411 (MPDS of 0.21) and Ser489 (MPDS of 0.27). The FA in FA5 is located within subdomain IIIB and establishes both salt bridges and hydrogen bond with Tyr401 (MPDS of 0.14) and Lys525 (MPDS of 0.37). On the contrary, sites FA6 and FA7 have no polar interactions and therefore they are low-affinity FA-binding sites. The cavity of the FA7 site represents the Sudlow's site I (see below), whereas Sudlow's site II overlaps with FA3 and FA4 sites (see below) [2, 117, 119–128].

FA binding induces relative movements of the three domains of the protein: these are almost rigid-body rotations with very modest distortion of the individual domains [118]. FA binding displaces domains I and III to the left, opening up the central crevice, and increasing the width of the molecule from 80 to 90 Å. The rotation of domain I relative to domain II is stabilized by the binding of FA2 fatty acid to the interface between subdomains IA and IIA. Upon FA binding, Tyr150 (MPDS of 0.05) from subdomain IB moves to interact with the carboxylate moiety of the lipid bound to a site that straddles domains I and II (FA site 2). This interaction results in a relative rotation of domains I and II accompanied by extensive rearrangement of the H-bond network, involving Tyr150, Glu153 (MPDS of 0.06), Gln196 (MPDS of 0.55), His242 (MPDS of 0.13), Arg257 (MPDS of 0.22), and His288 (MPDS of 0.44). These movements open a solvent channel between Tyr150 and Gln196 (MPDS of 0.55), thus increasing the binding pocket volume and altering the distribution of polarity in it.

Petitpas et al. have reported the X-ray crystal structure of HSA complexed with the unsaturated FAs, oleic acid, and arachidonic acid [125]. These FAs bind to seven sites, primarily sites 1 and 2, and it has been shown that the ϵ -amino groups on Lys525 (MPDS of 0.37) and Lys199 (MPDS of 0.47) take part in this binding. These results are supported by NMR data, which showed that the binding of FAs is greatly reduced by alanine substitution of Lys525 [129].

Mutations in domains I and II practically do not change FAs binding [130]. The most pronounced changes are caused by mutations in domain III [131]. Thirteen recombinant isoforms mutated in four different subdomains were synthesized to study the binding of octanoate, decanoate, laurate, and myristate [132]. It turned out that octanoate and decanoate bind to a single site in subdomain IIIA; laurate binds to sites in subdomains IIIA and IIIB, whereas myristate binds to sites in subdomains IB and IIIB. It was also found that primary FA binding is sensitive to amino acid substitutions in other parts of the protein.

According to the data of differential scanning calorimetry, FA-free albumins are generally less stable, more susceptible to aggregation, and their unfolding endothermic transition is less cooperative and less reversible in comparison with the FA-bound protein [133].

Drug-Binding Sites

A fundamental characteristic of serum albumin is its useful capacity to bind a large variety of drugs [2, 134]. Many endogenous and exogenous compounds (drugs, hormones, xenobiotics, and FAs) form complexes with serum albumin. Mostly anionic drugs interact with unmodified HSA; few

cationic drugs have detectable affinity for serum albumin. Interactions of HSA with FA chains increase its affinity for cationic drugs (reviewed in ref. 84). Most drugs have association constants in the range of 10^3 – 10^6 M⁻¹ but constants over a wide range of 10^2 up to 10^{11} M⁻¹ have been recorded [2].

Sudlow's sites I and II are two main drug-binding sites [123]. Sudlow site I, located in subdomain IIA, reversibly binds bulky heterocyclic anions, e.g., anticoagulant drug warfarin [135, 136]. Sudlow site II is located in the subdomain IIIA and it binds with high-affinity aromatic carboxylates with an extended conformation, e.g., ibuprofen, a non-steroidal anti-inflammatory agent, benzodiazepine and diazepam, which is used in the treatment of anxiety [136]. The sites I and II are the primary drug-binding sites, but albumin can possess some other low-affinity binding sites for drugs [123, 137, 138]. Drugs and drug metabolites can also bind covalently to serum albumin.

Bos et al. [139] proposed that the sites I and II are located in domains II and III, respectively. Current crystallographic studies have proved that the majority of drugs bind to the Sudlow's sites I and II [119, 134, 140, 141]. In the presence of bound FAs, drugs bind preferentially to IB and IIA subdomain because IIIA subdomain contains the strong binding site for FAs and FAs occupy this site inhibiting drugs binding [129, 142]. In contrast, FAs weakly bind to IIA subdomain, and are usually replaced by drugs.

Site I is a large, flexible, multi-chamber cavity. The binding pocket of the site I is located in the core of subdomain IIA. It comprises six helices of the subdomain and a loop-helix feature (residues 148–154) from subdomain IB (reviewed in ref. 134). The interior of the pocket is formed by hydrophobic residues Trp214 (MPDS of 0.29), Leu219 (MPDS of 0.19), Phe223 (MPDS of 0.20), Leu238 (MPDS of 0.14), His242 (MPDS of 0.13), Leu260 (MPDS of 0.23), Ile264 (MPDS of 0.30), Ser287 (MPDS of 0.43), Ile290 (MPDS of 0.54), and Ala291 (MPDS of 0.53). The pocket contains also two clusters of polar residues: an inner cluster Tyr150 (MPDS of 0.05), His242 (MPDS of 0.13), and Arg257 (MPDS of 0.22), and an outer cluster composed of Lys195 (MPDS of 0.58), Lys199 (MPDS of 0.47), Arg218 (MPDS of 0.20), and Arg222 (MPDS of 0.20). The distal end of the pocket is divided by residue Leu264 (MPDS of 0.29) into left and right hydrophobic sub-chambers, whereas a third sub-chamber is composed of Phe211 (MPDS of 0.25), Trp214 (MPDS of 0.23), Ala215 (MPDS of 0.21), Leu238 (MPDS of 0.14), and aliphatic portions of residues Lys199 (MPDS of 0.47) and Arg218 (MPDS of 0.20). Trp214 is an important structural element of site I since rotation of its indole ring enables certain ligands to be accommodated at the binding site and to form π - π stacking interactions [140, 143]. The binding pocket can be accessed

through an open entrance of ~ 10 Å in diameter between helices h1 and h2.

The large central compartment of the site I is typically occupied by planar aromatic/heterocyclic rings of the ligands. Guest molecules within the pocket form multiple hydrophobic contacts with nonpolar site residues. Acidic or electronegative peripheral groups of the ligands are oriented in the sub-chamber formed around Trp214, or toward the solvent accessible entrance of the cavity where they can form intermolecular H-bonds with the basic residues [135, 140].

Crystal structure of the complex of HSA with warfarin [135] shows that multiple hydrophobic contacts and some specific electrostatic interactions ensure stability of the complex. The benzyl moiety is located in a sub-pocket formed by Phe211 (MPDS of 0.25), Trp214 (MPDS of 0.23), Leu219 (MPDS of 0.19), and Leu238 (MPDS of 0.14) with additional aliphatic contacts from Arg218 (MPDS of 0.20) and His242 (MPDS of 0.13). The coumarin moiety fits into the main chamber far from the entrance. This chamber has an additional side pocket (delimited by Leu219 (MPDS of 0.19), Arg222 (MPDS of 0.20), Phe223 (MPDS of 0.20), Leu234 (MPDS of 0.18), Ile264 (MPDS of 0.29), Leu275 (MPDS of 0.36), and Ile290 (MPDS of 0.54)) that is not occupied by warfarin but may accommodate hydrophobic portions of other site I drugs. The coumarin group makes primarily hydrophobic contacts with the surrounding side chains. “Ceiling” and “floor” of the pocket are formed by Ala291 (MPDS of 0.53) and Leu219/238 (MPDS of 0.19 and 0.15), respectively (reviewed in ref. 144); the back end of the coumarin ring contacts Ile260 (MPDS of 0.23), Ile264 (MPDS of 0.29), Ile290 (MPDS of 0.54), and the aliphatic portions of Arg257 (MPDS of 0.22) and Ser287 (MPDS of 0.43) and lies close, but does not directly contact the FA in the adjacent binding site FA2.

Artali et al. studied dynamical behavior of the structured domains of the HSA using unrestrained molecular dynamics simulations [145]. The simulation has been extended up to 2.0 ns. They revealed collective movements of different HSA domains. Relevant overall flexibility of IA, IB, IIB, and IIIB confirms the mobility of these HSA subdomains. The residual motions at the IIA binding site entrance, namely, Lys195 (MPDS of 0.53) and Lys199 (MPDS of 0.47) together with that of Glu450 (MPDS of 0.28), Asp451 (MPDS of 0.26), and Tyr452 (MPDS of 0.22) of the IIIA subdomain cause an enlargement of site entrance. This result demonstrates the central role of Glu450 (MPDS of 0.28) in the binding to subdomain IIIA, in agreement with the crystallographic data. The movement of the subdomain IB toward subdomain IIA, with a decreasing of the distance of more than 3 Å, is in agreement with the value of 4 Å obtained experimentally.

Heme allosterically influences drug binding to Sudlow's site I and vice versa. HSA has one strong heme binding site with $K_a = 2 \times 10^8 \text{ M}^{-1}$ and several weaker sites [146]. Crystal structure of the complex of HSA with heme shows that heme iron is penta-coordinated and the fifth ligand is the hydroxyl oxygen of Tyr161 (MPDS of 0.09) [147]. The binding of warfarin decreases the affinity of HSA for heme by about one order of magnitude; likewise, heme binding to HSA decreases the warfarin affinity by the same extent [60].

FAs are also effective in the allosteric regulation of ligand binding to Sudlow's site I and to the heme cleft [124, 125, 128, 147–150]. The binding of FAs results in movements of domains I and III relative to domain II. Such regulation is not observed for short FAs (e.g., octanoate) that preferably bind to Sudlow's site II displacing the specific ligands (e.g., ibuprofen) without inducing HSA allosteric rearrangements. Hydrophobic interactions between polymethylenic tail of long FA and HSA initiate allosteric rearrangements. Sudlow's site I ligands (e.g., warfarin) compete with FA binding to FA7, and Sudlow's site II ligands (e.g., ibuprofen) compete with FA binding to FA3 and FA4.

Metal Ions Binding Sites

Albumin has binding sites for a number of metal cations, including copper, nickel, calcium, magnesium, zinc, cadmium, mercury, aluminum, manganese, and cobalt [2]. At least four different metal sites have been described (reviewed in ref. 9): (i) the so-called ATCUN motif at the N-terminus, the primary site for Cu^{2+} and Ni^{2+} [151]; (ii) the so-called site A, the primary Zn^{2+} -binding site that can also bind other bivalent metal ions [152, 153]; (iii) site B, which has a high affinity toward Cd^{2+} , but the location of which is unknown [154–156]; and (iv) the reduced thiol of Cys34, which binds gold [157] and platinum [158] compounds.

The ATCUN motif is particularly suitable for metal ions with a prevalence for tetragonal or square-planar co-ordination. The high-affinity site for Cu^{2+} ions in HSA is composed of the first four amino acids Asp–Ala–His–Lys (DAHK) (MPDS values of 0.31, 0.31, 0.32, and 0.36) [2, 159–161]. It provides four nitrogen ligands: an imidazole nitrogen of His3, the N-terminal amino group, and two deprotonated backbone amide nitrogens. In addition, the carboxylate side chain of Asp1 is also implicated in metal binding, giving an overall penta-coordinate site [151], although this residue appears to be not crucial for the high-affinity Cu^{2+} and Ni^{2+} binding.

The binding constants for copper, nickel, and cobalt are quite high ($1.5 \times 10^{11} \text{ M}^{-1}$ for Cu^{2+} ; ref. 162). During ischemia/reperfusion, the structure of N-terminus of

albumin is changed in such a way that it loses its Cu^{2+} binding capacity [163].

Serum albumins bind Zn^{2+} with rather high affinity ($1.9 \times 10^7 \text{ M}^{-1}$ for BSA; ref. 162). Equilibrium dialysis experiments by Goumakos et al. revealed that, in total, 3 mol equiv. of Zn^{2+} and Cd^{2+} can be bound to albumin (one high-affinity site for Zn^{2+} and two for Cd^{2+} on both HSA and dog serum albumin) [155]. The logarithm of apparent stability constant for the Zn^{2+} site in HSA is 6.4 ± 0.8 , and that for the two Cd^{2+} sites it equals to 5.3 ± 0.6 . Competition experiments showed pronounced preference for Zn^{2+} of site A: 1 mol equivalent of Zn^{2+} is sufficient to replace Cd^{2+} which is reflected in complete suppress of the corresponding ^{113}Cd or ^{111}Cd resonance in either BSA [154] or HSA [156, 164].

Data of circular dichroism (CD) and electron paramagnetic resonance (EPR) spectroscopy and metal competition experiments [153] demonstrated that the second mol. equivalent of Cu^{2+} added to BSA or HSA binds to the high-affinity Zn^{2+} site. The site binds Zn^{2+} more strongly than Cu^{2+} , Ni^{2+} , or Cd^{2+} .

In order to identify and characterize the high-affinity zinc site on HSA, Stewart et al. used a combination of site-directed mutagenesis, molecular modeling, and NMR spectroscopy [164]. They have found that the site is located at the interface between domains I and II, and each domain provides two ligands: His67 and Asn99 from domain I, and His247 (MPDS of 0.15) and Asp249 (MPDS of 0.17) from domain II. This site is present in all published X-ray crystal structures of FA-free albumin. It was found that the binding of long-chain FAs to the FA2 site and zinc binding to site A might be mutually exclusive. Lu et al. concluded that under physiological conditions, only Zn^{2+} will be bound to the high-affinity site, as Cu^{2+} binds much more avidly to the ATCUN motif, and other metal ions, including the toxic Cd^{2+} , have a lower affinity for the zinc site [9].

The interaction of serum albumin with Ca^{2+} is relatively weak ($K_a = 1.5 \times 10^3 \text{ M}^{-1}$ for HSA [165]), but due to high concentration of albumin in the serum about 45% of the 2.4 mM of circulating Ca^{2+} is bound by albumin [2]. Majorek et al. revealed three calcium ions in the crystal structure of BSA (PDB entry 3v03) [53]. All Ca^{2+} -binding sites are located in domain I. One of the sites is composed of residues Glu6 (MPDS of 0.36), Glu243 (MPDS of 0.13), Asp248 (MPDS of 0.17), and Glu251 (MPDS of 0.19), with the electron density for the Ca^{2+} in chain A more disordered than in chain B. In chain B, Ca^{2+} is coordinated by residues Asp248 (MPDS of 0.17), Glu251 (MPDS of 0.19), and Glu243 (MPDS of 0.13). In chain A, Asp248 and Glu251 also coordinate the Ca^{2+} , but the ion is shifted closer to residue Glu6 and is coordinated by it directly, while the distance to Glu243 is long enough to require water-mediated interactions with the ion. The first Ca^{2+} -binding site is

located in close proximity to the Zn^{2+} -binding site [164], with residue Asp248 being shared between the two sites.

The second Ca^{2+} site is composed of residues Asp13 (MPDS of 0.25), Asp254 (MPDS of 0.20), and Asp258 (MPDS of 0.23) [53]. It is close in space to the first site. In the first and in the second sites all of the contacts with Ca^{2+} ion are achieved by side chain interactions. The third Ca^{2+} -binding site utilizes side chain of Asp111 (MPDS of 0.40), the side chain and main chain oxygen of Ser109 (MPDS of 0.42), and a water-mediated interactions with Asp107 (MPDS of 0.42) [53]. In this site Asp107 is conserved, Asp11 is replaced by Asn in HSA and equine SA and Ser109 is not conserved at all. In each case the coordination is completed by water molecules.

It is thought that the binding of Mg^{2+} to serum albumins occurs to sites, which are different from the Ca^{2+} -binding sites [2]. Mg^{2+} is also transported by albumin but binds with slightly lower affinity than Ca^{2+} ($K_a = 1 \times 10^2 \text{ M}^{-1}$; ref. 166). The concentration of Mg^{2+} in blood plasma is around 1.2 mM and like Ca^{2+} , approximately 45% of it is complexed with albumin [2].

The binding of free transition metals to proteins can control their reactivity and limit their availability for the Fenton reaction [19, 160]. HSA might then be able to limit damage caused by hydroxyl radicals produced from Fenton reaction between iron/copper and H_2O_2 [161].

Metal Complexes Binding Sites

Serum albumin can bind a large variety of mononuclear and polynuclear Cu^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} , Pt^{2+} complexes with aromatic ligands (some of them bearing known pharmacologically active moieties) [167–169]. The interactions between serum albumin and metal complexes can result in disrupting the disulfide bonds, which can lead to a partial loss of α -helix conformation with the subsequent unfolding of the protein [170].

Cisplatin is a platinum(II) complex, which is extensively used as an anticancer drug. The binding of cisplatin and other platinum complexes to albumin has been investigated since the 1980s [171, 172]. Up to ten molecules of cisplatin were found to be bound by one molecule of albumin [173]. Cys34 was reported to take part in these interactions [171, 172].

Epitopes and Antigenic Activity

The antigenic activity of serum albumins of different animals has been extensively studied [53, 174]. BSA has been identified as one of the most important allergens in bovine meat, whereas equine and rabbit albumins are mostly

considered as inhaled allergens. Several regions in BSA have been identified as epitopes [175–180]. In most cases the sequences identified as containing epitopes are relatively long. To narrow the size of the possible epitope region, Majorek et al. compared differences between HSA and BSA and studied the surface exposure of these residues [53].

Residues 126–144 (collectively known as the ABBOS peptide and characterized by the regions-averaged MDPS value of 0.15 ± 0.03) have been described as a reactive epitope [175, 176]. The second region that could be a cross-reactive epitope is located on the third and fourth α -helices of the IB domain, comprising residues 155–189 (with the regions-averaged MDPS value of 0.19 ± 0.02). Specifically, the BSA residues in this region that both differ from those of HSA and are highly solvent-exposed are Tyr155 (MPDS of 0.05), Asn158 (MPDS of 0.05), Lys159 (MPDS of 0.05), Asn161 (MPDS of 0.06), Gly162 (MPDS of 0.06), Val163 (MPDS of 0.06), Gln165 (MPDS of 0.08) (in the third α -helix) and Glu182 (MPDS of 0.31), Thr183 (MPDS of 0.32), Glu186 (MPDS of 0.37), Lys187 (MPDS of 0.42) (in the fourth α -helix). BSA residues Lys294 (MPDS of 0.53), Ala296 (MPDS of 0.52), Ile297 (MPDS of 0.50), Glu299 (MPDS of 0.52), and Asn300 (MPDS of 0.53) constitute a continuous patch on the protein's surface and differ from those of HSA. The residues are located on the long loop connecting subdomains IIA and IIB and could potentially be recognized as either a conformational or linear epitope.

The BSA residues that differ from those of HSA form two parallel patches on the third and fourth α -helix of subdomain IIB. Specifically residues Glu351 (MPDS of 0.17), Ala354 (MPDS of 0.19), Glu358 (MPDS of 0.20), Lys362 (MPDS of 0.18), and Asp363 (MPDS of 0.19) form the first patch and Ala367 (MPDS of 0.16), Ser370 (MPDS of 0.15), Thr371 (MPDS of 0.17), Lys375 (MPDS of 0.18), His378 (MPDS of 0.16), and Asp381 (MPDS of 0.20) form the second. Of residues 336–345 (MRPS values of 0.15, 0.16, 0.16, 0.16, 0.18, 0.19, 0.21, 0.19, 0.21, and 0.19), which have been shown to be a B-cell epitope [179], the only residue exposed to the surface that differs between HSA and BSA is Glu338.

The C-terminal subdomain of BSA (IIIB) has also been shown to contain important epitopes. Region 500–574 (with the region-average disorder score of 0.25 ± 0.08) has been suggested as an epitope-containing region for human species, with 500–518 (with the region average disorder score of 0.21 ± 0.05) being the most critical [177]. Residues 537–554 (with the region-average disorder score of 0.26 ± 0.07) have been shown to be recognized by AB3 monoclonal antibody [180], and residues 520–542 (with the region average disorder score of 0.34 ± 0.02) have been suggested to be important too [178]. Within this region of BSA, four patches of residues are located at the protein surface and differ from HSA [53]. Residues Ala500 (MPDS

of 0.21), Asp502 (MPDS of 0.19), Glu503 (MPDS of 0.17), Lys504 (MPDS of 0.15), and Leu505 (MPDS of 0.15) constitute the first patch on one side of the subdomain, whereas residues Pro516 (MPDS of 0.27), Asp517 (MPDS of 0.31), Thr518 (MPDS of 0.31), and Lys520 (MPDS of 0.32) constitute the second patch on the opposite site of the subdomain. The last two patches are located on the last two helices of BSA and are long and parallel; specifically Thr545 (MPDS of 0.28), Glu548 (MPDS of 0.21), Asn549 (MPDS of 0.20), Val551 (MPDS of 0.17), Asp555 (MPDS of 0.15), and Ala559 (MPDS of 0.15) (third patch) and Ala565 (MPDS of 0.19), Val569 (MPDS of 0.25), Pro572 (MPDS of 0.29), Val576 (MPDS of 0.34), Ser577 (MPDS of 0.39), Thr580 (MPDS of 0.51), and Ala583 (MPDS of 0.53) (fourth patch). Residues Val551 (MPDS of 0.17), Asp555 (MPDS of 0.15), and Ala559 (MPDS of 0.16) are separated from Ala565 (MPDS of 0.19), Val569 (MPDS of 0.25), and Pro572 (MPDS of 0.29) by residues that are identical between BSA and HSA. Residues Thr545 (MPDS of 0.28), Glu548 (MPDS of 0.21), and Asn549 (MPDS of 0.20) from one helix and Val576 (MPDS of 0.34), Ser577 (MPDS of 0.39), and Thr580 (MPDS of 0.51) from the other helix are spatially close, and thus could be recognized as a single epitope.

Interactions with Cellular Receptors and Other Proteins

Albumin's recycling and cellular transcytosis are achieved due to its interaction with cellular receptors. Receptors include glycoproteins Gp60, Gp30, and Gp18, a secreted protein, which is acidic and rich in cysteine (SPARC), the Megalin/Cubilin complex, and the neonatal Fc receptor (FcRn) [181–187].

Gp60 (molecular mass 60 kDa) is thought to facilitate cellular transcytosis of albumin and redirect albumin from lysosomal degradation [188]. The Gp18 and Gp30 are cell surface glycoproteins with molecular masses of 18 and 30 kDa, respectively. Gp18 and Gp30 bind to modified albumin and then direct it to lysosomal degradation [189–191]. It is possibly a safety mechanism to remove old, damaged, or altered albumin. Gp18 and Gp30 are expressed in endothelium cell membranes, in particular in the liver and peritoneal macrophages [2].

SPARC, also known as osteonectin, is an albumin-binding protein located in the extracellular matrix [192, 193]. It is expressed by a variety of cells including fibroblasts and endothelial cells and associated with tissue growth and cell movement and/or proliferation.

The albumin-binding receptors responsible for mediating the reabsorption of filtered proteins in the kidney are Cubilin and Megalin [184, 185]. It was suggested that the

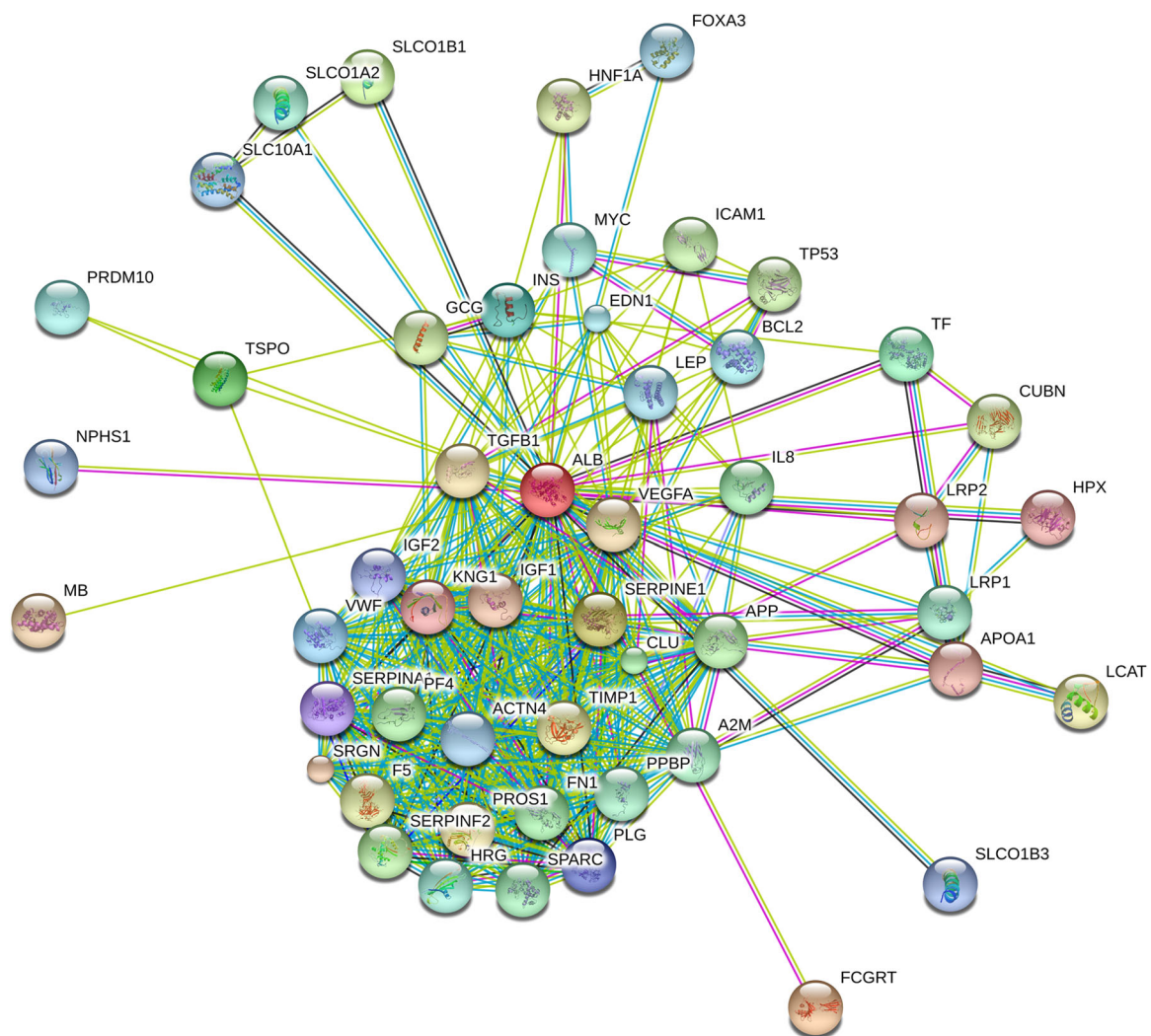


Fig. 5 Analysis of the interactivity of HSA by STRING computational platform that produces the network of predicted associations for a particular group of proteins [201]. In this plot, the nodes correspond to proteins, whereas the edges show predicted or known functional associations. Seven types of evidence are used to build the corresponding network, where they are indicated by the differently colored

lines: a *green line* represents neighborhood evidence; a *red line*—the presence of fusion evidence; a *purple line*—experimental evidence; a *blue line*—co-occurrence evidence; a *light blue line*—database evidence; a *yellow line*—text mining evidence; and a *black line*—co-expression evidence [201] (color figure online)

Megalin/Cubilin complex is responsible for the receptor-mediated endocytosis and rescuing of albumin from renal excretion.

A principal role of the neonatal Fc receptor (FcRn) is transport of IgG from mother to fetus in placenta and proximal small intestine [194]. Serum albumin binds to FcRn [183]. It was found that FcRn is involved in albumin recycling [183]. Crystal structure of human FcRn in complex with HSA shows participation of both domain III and domain I in FcRn binding [195, 196].

Glycated serum albumin is able to interact with the receptor for advanced glycation end products (RAGE) [197]. The interaction between AGE and RAGE receptor activates release of proinflammatory cytokines and causes a

production of ROSs [87]. RAGE mediates HMGB1-induced albumin transcytosis [198]. Interaction between HSA and RAGE seems to contribute to pathogenesis of diabetes and AD [199].

The natural transport function of albumin, the presence of multiple ligand-binding sites, and ability of albumin to recognize cellular receptors favor its application for drug delivery as a drug reservoir (reviewed in ref. 200).

Figure 5 represents evaluation of the HSA interactome and shows that this protein has multiple binding partners. This information was retrieved using Search Tool for the Retrieval of Interacting Genes; STRING, <http://string-db.org/>, which generates a network of predicted associations based on predicted and experimentally validated

information on the interaction partners of a protein of interest [201]. In our analysis, the most stringent criteria were used for selection of interacting proteins by choosing the highest cut-off of 0.9 as the minimal required confidence level.

Concluding Remarks

Our analysis revealed that intrinsic disorder and high structural flexibility have several implementations for functionality of serum albumin. Disordered or flexible regions frequently serve as “display sites” containing residues amenable to PTMs. The vast majority of known binding sites of serum albumin are characterized by “dual personality”, possessing both ordered and flexible/disordered residues. In these binding sites, the roles of the less ordered residues are to form lids for regulation of binding site accessibility, to control amenability of binding sites needed for fine adjustment of their environment to various ligands, and, therefore, to define and control binding promiscuity of serum albumin and the ability of this protein to interact with a wide range of ligands of very different physico-chemical nature.

Acknowledgements This work was supported by a Grant No 16-34-60229 from the Russian Foundation for Basic Research (E.A.L.).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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