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Review

Binding, unfolding and refolding dynamics of serum albumins ☆,☆☆

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ABSTRACT

Background: The serum albumins (human and bovine serum albumin) occupy a seminal position among all proteins investigated until today as they are the most abundant circulatory proteins. They play the major role of a transporter of many bio-active substances which include various fatty acids, drug molecules, and amino acids to the target cells. Hence, studying the interaction of these serum albumins with different binding agents has attracted enormous research interests from decades. The nature and magnitude of these bindings have direct consequence on drug delivery, pharmacokinetics, therapeutic efficacy and drug design and control.
Scope of the review: In the present review, we summarize the binding characteristics of both the serum albumins with surfactants, lipids and vesicles, polymers and dendrimers, nanomaterials and drugs. Finally we have reviewed the effect of various chemical and physical denaturants on these albumins with a special emphasis on protein unfolding and refolding dynamics.
Major conclusions: The topic of binding and dynamics of protein unfolding and refolding spans across all areas of inter-disciplinary sciences and will benefit clinical toxicology and medicines. The extensive data from several contemporary research based on albumins will help us to understand protein dynamics in a more illustrious manner.
General significance: These data have immense significance in understanding and unravelling the mechanisms of protein unfolding/refolding and thus can pave the way to prevent protein mis-folding/aggregation which sometimes leads to severe consequences like Parkinson's and Alzheimer's diseases. This article is a part of a Special Issue entitled Serum Albumin. This article is part of a Special Issue entitled Serum Albumin.

1. Introduction

Serum albumins are the most abundant soluble circulatory proteins in the blood comprising nearly 52–60% of plasma and play the pivotal role as a transporter of various fatty acids, amino acids, hemin, bilirubin and drug molecules to the target sites [1,2]. Their presence in the circulatory system helps in the metabolic modifications of some ligands, reduces the activity of toxins, affects the pharmacokinetics of many drugs, controls the anti-oxidant properties of the plasma and sometimes displays pseudo-enzymatic properties [2–8]. The serum albumins are synthesized by the parenchymal cells of the liver and eventually enter the blood stream as the non-glycosylated form of the proteins having a half-life of 19 days [9]. Human serum albumin (HSA) is a single poly-peptide chain having 585 amino acid residues, characterized by low tryptophan and high cysteine content [10]. The secondary structure of the protein consists of about 67% α-helix having six turns and seventeen disulphide bridges [11]. Under physiological conditions, HSA adopts a heart-shaped three dimensional structure having three homologous domains I–III [10]. Each of these three domains are further subdivided into two sub-domains A and B that consist of 4 and 6-helices, respectively [1,10]. Using X-ray crystallography, He and Carter showed that the two halves of the albumin molecule form a 10 Å wide and 12 Å deep crevice [10]. The other globular protein, bovine serum albumin (BSA) shares approximately 76% sequence homology with HSA [12–16]. It is a single polypeptide chain having a molecular weight of ~66 kDa and consists of 583 amino acid residues. Like HSA, BSA also has seventeen disulphide bridges and one free −SH group, which can cause it to form a covalently linked dimer. While both charged amino acid residues and apolar patches cover the interface, the interior of the protein is almost hydrophobic in nature. BSA has a net charge of −18 and an iso-electric point of 4.7 [1,2,17,18] and HSA has a net charge of −15, at neutral pH and an iso-electric point of about 5 [1,2,19]. BSA is also characterized by three domains, each consisting of a large and a small double loop and a short and a long (hinge) connecting segment, whereas, the next domain houses another large double loop and a connecting segment. The fundamental difference between HSA and BSA is that the former contains only one tryptophan amino acid residue (W214), while the latter has two, W212 and W135 [1,2]. The spectral properties of HSA and BSA in their native states do not overlap with each other due to the presence of the additional Trp residue, W135. Punyiczki and Rosenberg [20] have proposed that W135 of BSA resides on the surface of the protein molecule.

☆ Dedicated to Professor Theodore Peters, Jr.
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Trp is susceptible to ultra-violet irradiation damage and the extent of photo-degradation is dependent on the location of Trp in the amino acid sequence of the protein [21,22]. Using ultra-violet irradiation, Takeda and co-workers [23] followed the ratio of the fluorescence intensities of both HSA and BSA at an arbitrary time (FI_t) to that at a time 0 (FI_0). They concluded that the decay profiles for both the albumin proteins were the same signifying that W212 may not be more buried than W135. The chemical microenvironment of W212 of BSA is almost similar to that of W214 of HSA and in spite of having rather complicated secondary structures, both the serum albumins are well studied making use of the inherent intrinsic fluorescence rendered by the tryptophan amino acid residues [13–16,24–30].

In this review, we will focus our attention to the various binding aspects of serum albumins with surfactants, drug molecules, polymers and dendrimers, lipids and vesicles, and nanomaterials. We will also highlight how these binding studies are used to monitor the various conformational dynamics the proteins undergo which are studied by several physico-chemical characterizations. In the second half of the review, we will shift our focus to the mechanistic pathways of protein unfolding (brought in by several denaturants like surfactants, urea, guanidine hydrochloride, pressure, temperature, etc.) and then unravel the pathways of subsequent refolding of the denatured protein (when subjected to many-fold dilution or treatment of sugars, specific surfactants and osmolytes).

2. Binding studies of serum albumins

2.1. Binding of surfactants

Interaction of proteins with surfactants has been a topic of burgeoning interest [31–38]. The three dimensional structure of the protein is supposedly destroyed at high concentrations of the surfactants and the protein is said to be denatured. Contrary to this concept, way back in 1948 Duggan and Luck [39] showed that the urea induced denaturation of proteins may be prevented by the addition of small amount of the anionic surfactant, sodium dodecyl sulphate (SDS). Moriyama and Takeda [31] also proposed that the secondary structure of HSA remains more or less unchanged up to the addition of 0.2 mM of SDS. Using Circular Dichroism (CD) spectroscopy, they have studied the effect of interaction of the double-tailed surfactant, sodium bis(2-ethylhexyl) sulphosuccinate (AOT) on the structure of HSA [32]. They have shown that although the helicity of HSA decreased from 66% to 44% at 65 °C in the absence of AOT, low concentrations of AOT however, prevent such decrement in the helicity of HSA thereby establishing the protective nature of the surfactant [32].

In order to study protein–surfactant interactions, fluorescence quenching is vastly used. The quenching of fluorescence is known to occur by two processes, namely collisional (dynamic) quenching and/or formation of a complex between the quencher and fluorophore (static quenching) [24,40]. The fluorescence quenching data were analyzed according to the Stern–Volmer equation:

$$F_0/F = 1 + K_{SV}[Q]. \quad (1)$$

In the above equation, F_0 and F are the fluorescence peak intensities of the fluorophore (W214 in HSA) in the absence and presence of quencher. [Q] is the quencher concentration and K_{SV} is the Stern–Volmer quenching constant. Besides estimating the magnitude of quenching using Eq. (1), one can further estimate the binding constant (K) and the binding affinity (n). For this purpose, a modified version of the Stern–Volmer equation is used which is given by:

$$\log[(F_0 - F)/F] = \log K + n \log[Q]. \quad (2)$$

The thermodynamics of binding can be estimated by measuring the Free Energy of Binding ($\Delta G^0_{Binding}$) given by:

$$\Delta G^0_{Binding} = -2.303 RT \log K. \quad (3)$$

Very recently, Anand et al. thoroughly investigated the interaction of SDS with HSA and proved that the addition of the surfactant takes place in a sequential manner, depending upon the molar ratio of the protein:surfactant [24]. Using the intrinsic fluorescence, they monitored the fluorescence quenching of the sole W214 amino acid residue as a function of [SDS]. The added surfactant molecules serve as quenchers of the Trp fluorescence and the fluorescence intensity of the protein decreases with a rise in SDS concentration. Steady-state and time-resolved experiments suggested that the addition of SDS to HSA takes place in three different stages which was followed by a saturation stage (Figs. 1 and 2). The three binding stages, as studied by using Eq. (2) ("specific" for region I, "non-cooperative" for region II and "cooperative" for region III) have been characterized by different magnitudes of fluorescence quenching and thermodynamic parameters as listed in Table 1. Although the high magnitudes of binding parameters suggest that the interaction of SDS with HSA is indeed rather strong as evidenced from Table 1, yet the concept of SDS denaturating the entire protein so as to make it behave like an extended polymer seems to be unreal [24,37]. CD spectroscopy which probes the secondary and tertiary structures of a protein, reveal that even at high SDS concentrations, HSA loses only about 27% of its native helical structure; the structural integrity of HSA as rendered by the seventeen disulphide bonds has thus been established [24]. Bhattacharyya and co-workers have studied the interaction of HSA with very low concentration of SDS (80 μM) and have shown that when SDS binds to HSA, the solvation dynamics becomes faster which is attributed to the displacement of the bound water molecules by SDS in the immediate vicinity of the fluorescent probe, TNS [34]. In another work they have shown that the solvation dynamics of the probe CPM (7-dimethylamino-3-(4-maleimidophenyl)-4-methyl-coumarin) covalently attached to HSA becomes faster by 1.3 times in the presence of SDS due to the binding of SDS to HSA [35].

Haertlé and co-workers [41] studied the interaction of HSA with the cationic surfactant cetylpyridinium chloride (CPC) using Isothermal Titration Calorimetry (ITC) at close to physiological conditions and concluded that at low concentrations of CPC (less than 0.022 mM) the binding of the surfactant to HSA is exothermic and temperature independent. However, at higher concentrations the interaction becomes

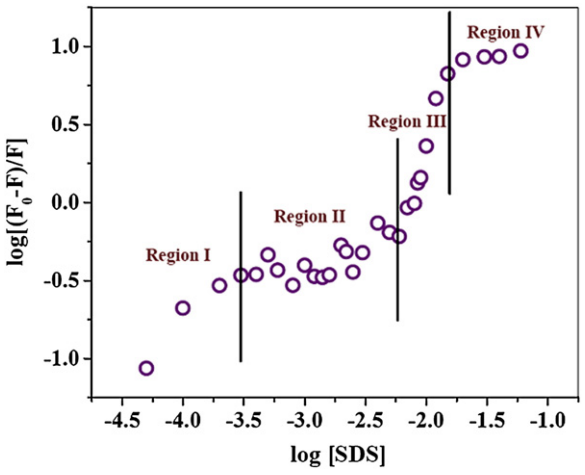

Fig. 1. A plot of log [(F_0 − F) / F] against log[SDS] as per the modified Stern–Volmer equation. F_0 and F are the peak fluorescence intensities of HSA in the absence and presence of quencher, SDS. The regions (I–IV) represent the various binding stages of SDS to HSA. Figure reproduced from Ref. [24].