Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 188 (2018) 675–683

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Alteration of human serum albumin binding properties induced by modifications: A review

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ARTICLE INFO

Article history:
Received 30 March 2017
Received in revised form 23 April 2017
Accepted 9 May 2017
Available online 10 May 2017

Keywords:
Albumin
Modification
Oxidation
Glycation
Ageing

ABSTRACT

Albumin, a major transporting protein in the blood, is the main target of modification that affects the binding of drugs to Sudlow's site I and II. These modification of serum protein moderates its physiological function, and works as a biomarker of some diseases. The main goal of the paper was to explain the possible alteration of human serum albumin binding properties induced by modifications such as glycation, oxidation and ageing, their origin, methods of evaluation and positive and negative meaning described by significant researchers.

1. Introduction

Human serum albumin (HSA) is well known multifunctional protein in the intravascular compartment. Its main function is a transport of many endo- and exogenous compounds like drugs, steroids, fatty acids and thyroid hormones [1]. HSA, is organized in I, II and III domains and A and B subdomains. It contains single polypeptide chain of 585 amino acids containing *i.e.* 17 tyrosil residues and one tryptophan – Trp-214. HSA owns 35 cysteine residues, where 34 form intramolecular disulfide bonds. A single thiol, free Cys-34, constitutes almost 80% of the all thiol groups in plasma. Because Cys-34 is located in subdomain IA, it is probable that plays an important role in drugs binding. For HSA two major drug specific binding sites have been identified – Sudlow's site I and site II [2]. Site I is found in subdomain IIA while site II in IIIA [3]. It is noteworthy that drugs binding with serum proteins (*i.e.* albumin) is involved not only in the pharmacological effects of a drug but also in its pharmacokinetics.

A main protein in the blood, albumin, is the main goal of chemical stresses during physiological states like oxidative stress occurring in the ageing degenerative diseases (Alzheimer's and Parkinson's disease), and glucose stress in diabetes mellitus [4,5]. Because glycation and oxidation co-occur with each other, reinforcing negative effects, therefore they are referred to common name – glycoxidative processes [6]. *In vivo* and *in vitro* studies of albumin and other proteins oxidation and glycation allowed to identify markers used in the diagnosis and monitoring of diseases. From the recent studies it has become obvious that these modifications have an influence on albumin actions and impact on functioning of cell. In addition long lasting glycoxidative processes lead to the isomerization to aged form of serum albumin and formation of aggregates. Both, the ageing process and interactions of ligands with proteins induce variations in the properties of the macromolecules. This phenomenon modifies the biological activities and conformations of albumin and contributes to the appearance of unexpected abilities [7]. It is noteworthy that the reduction in serum albumin biological activity has a medical significance, because it affects drug metabolism and particularly drug tolerance in the elderly [8].

Large amounts of spectral data that contain useful information concerning albumin modification are usually obtained from different analytical techniques including chromatography, spectrometry, spectrophotometry [9], spectroscopy [10], *etc.* The present paper describes based on the literature the albumin modification, its influence on albumin physiological functions and practical use of modified proteins.

2. Modification of Human Serum Albumin by Oxidation

Oxidation, glycation and *S*-nitrosylation are the major posttranslational modifications of albumin [11]. These modifications are distributed to the body through the circulatory system and albumin oxidized forms increases with the increase of pathophysiological states [12]. According to Scopus database, oxidative modification since more than 30 years are the subject of over than hundred researches. Works that

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http://dx.doi.org/10.1016/j.saa.2017.05.023
1386-1425/

described chemical modifications of various amino acid residues in BSA appeared in the late 70s of the previous century. Yoshikawa et al. showed the photoreaction to prove that the produced oxidation product reacts with protein and one of the reaction sites is tyrosine-OH [9]. Meucci et al. said that no gross alteration can be observed in the electrophoretic and chromatographic patterns of albumin, whereas localized modifications are indicated by the changes in absorption and fluorescence spectra and in polarization degree [8]. Oxidative stress contributes to the pathophysiology of a variety of diseases, and circulating biomarkers of its severity remains a topic of great interest for researchers [13]. With the modification due to the oxidation of plasma proteins, especially human serum albumin (HSA), an oxidative stress increases in hepatocellular carcinoma (HCC) [14] and in patients with type 1 diabetes mellitus [15]. Endogenously generated reactive oxygen species (ROS) causes an oxidative destruction of macromolecules like DNA, lipids and proteins [16]. As it was written by Yan and Sohal, proteins oxidative modification increases their carbonyl content that can be widely employed as markers of protein oxidative damage. Sancataldo et al. wrote that changes in protein structure may be induced due to the protein and reactive oxygen species interaction. This phenomenon leads to the protein aggregation process inhibition or/and modification that determine the formation of types with different inherent toxicity [17]. Using high-performance liquid chromatography (HPLC) and spectroscopic methods Takahashi et al. examined the oxidation and carbonylation of thiol that are oxidative chemical modifications [18]. By the use of differential scanning calorimetry (DSC) and circular dichroism (CD) they characterized the protein structure and observed the increase in denaturation curve midpoint temperature caused by the addition of albumin-specific stabilizers.

Many studies have discovered numerous types of proteins modifying agents *i.e.* Khan et al. using peroxynitrite or other nitrating agents studied tyrosine residues modification [19]. The next potent oxidant, that was the subject of Pattison et al. studies, is a hypochlorous acid (HOCl), produced *in vivo* by activated phagocytes [20]. Pattison et al. have proved that proteins are the major goals for this modification agent and the oxidation reaction results in side-chain alteration, fragmentation of backbone and cross-linking. It is worthy to note that not only non-enzymatic glycation but also oxidation (oxidative stress) is responsible for the formation of Advanced Glycation End-products (AGEs). For research purposes Rodriguez-Ayala et al. studied advanced oxidation products (AOPP), myeloperoxidase (MPO)-activity, pentosidine and carboxymethyl lysine (CML) as markers for oxidative stress. AOPP are closely related to Advanced Glycation End-products (AGEs)-pentosidine. They are treated as a marker of AGEs and increase with the progress of chronic renal failure and [21]. With the oxidative modifications, the formation of amino acids tyrosine and tryptophan derivatives occurs [22]. Besides of popular oxidant agents, Salavej et al. studied the myeloperoxidase changes amino acid residues in human serum albumin [23]. They investigated the changes of eight albumin amino acid residues using separation techniques. Tempe et al. suggested that the nature of the oxidant influence on site of modification and the process is based on protein specific structural subjects to direct the oxidation [24].

As measure of oxidation the most popular is a detection of the free thiol group at Cys-34 in albumin, because decreased levels of albumin-Cys-34 are correlated with increased oxidation [25]. The main popular oxidant agent is chloramine-T (CT). Both chloramine-T and *N*-chlorosuccinimide, mild oxidizing reagents, oxidize methionine residues in proteins and peptides to that methionine sulfoxides at neutral and slightly alkaline pH [26]. Shechter et al. [26], Maciążek-Jurczyk et al. [27], Anraku et al. [28] and other scientists presented a method for cysteine sulfhydryl groups selective oxidation under neutral conditions using chloramine-T. Human serum albumin was also mildly oxidized using metal-catalyzed oxidation system or H_2O_2 (mildly oxidation). Using calorimetric, chromatographic, electrophoretic and spectroscopic techniques they analyzed the treatments influence on the structural, drug-binding and esterase-like properties. In order to quantitatively determine the modification of free thiol group in HSA Cys residue, the Ellman's reagent was used [29]. Based on the spectrophotometric measurement Shechter et al. wrote that using CT only cysteine was oxidized to cystine [26]. Kouno et al. in order to mimic oxidative stress oxidized HSA with CT in the absence and in the presence of *N*-acetyl-L-methionine (*N*-AcMet) or *N*-acetyl-L-tryptophan (*N*-AcTrp) [30]. They examined whether *N*-AcMet is better that *N*-AcTrp to protect albumin against oxidation and found promising results with the use of *N*-AcMet that can be treated as an effective albumin stabilizer and antioxidant.

Cysteine represents the SH group that is essential to the redox regulation of protein structure and function. Serum protein redox modification alters its physiological properties and can be used as a biomarker of oxidative stress [31].The goal of protein oxidation may be also its binding sites. Dubinina et al. have analyzed oxidation of tryptophan and tyrosine by the analysis of the level of tryptophan and tyrosine [32]. The production of dityrosine crosslinks and the oxidation of tryptophan residues were recorded by fluorescence. They discovered that the degree of oxidative alteration of the amino acid residues of the protein depended on the incubation time and the kind of proteins. As Annibal et al. discovered, cysteine, methionine, tryptophan and tyrosine are the most protein residues that are sensitive to oxidation [33]. It is noteworthy that their oxidized form are treated as biomarkers of oxidative protein damages [33]. Goto et al. also studied chemical modifications of proteins [34]. They presented a comprehensive LC/MS/MS-based strategy in order to screen HSA several chemical modifications. Conducted by them database, 7 types of 36 modification sites, containing oxidation of Met-87, Trp-214 and Cys-34 and glycation of Lys-525, were established. Eckardstein et al. found also oxidized Met-112 and Met-148 [35]. Furthermore Met-111 and Met-147, two methionine residues in serum albumin, are highly oxidized to methionine sulfoxide, especially in patients with diabetes and renal failure and in healthy smokers. This data have been demonstrated by Suzuki et al. using label-free mass spectrometry approach to quantify redox changes in methionine residues that should facilitate the identification of additional circulating biomarkers suitable for predicting the development or progression of human disease [13]. Anraku et al. also have imitated oxidative damage of HSA using CT and their findings using HPLC combined with fluorescence detection, capillary electrophoresis and spectroscopic techniques showed that site II is more susceptible than site I and Cys-34, during the HSA exposition to oxidative stress [36].

Most of studies have been dedicated to the analysis of oxidation influence on ligands binding to albumin. Reactive oxygen species (ROS) resulted in oxidative modification of serum albumin. This phenomenon makes a number of fundamental changes in the three-dimensional structure that influences the binding and cause major drug interactions, especially in polytherapy [27]. Since the 1980s, researchers studied binding mechanisms of medical substances and other ligands to modified protein in ligand-albumin complex and also in the presence of both endo- and exogenous substances. Silva et al. presented the data based on the different analytical techniques that oxidation increases the albumin iron binding capacity [37]. Sancataldo et al. wrote that oxidation of single residues affects whole HSA tertiary structure making the protein compaction, increasing thermal stability, and reducing association tendency [17]. Barreca et al. emphasized that HSA structure that is responsible for ligands binding can be modified due to the complexation, causing the protein structure much more resistant to modification like oxidation and fibrillation [7]. By the analysis of α-helix structure, hydrophobic cavity of subdomain IIA and hydrogen bindings studies, using fluorescence, UV–visible, FTIR spectroscopy, native electrophoresis, protein ligand docking studies, scanning electron microscopy, they investigated the interactions of phloretin with HSA. Maciążek-Jurczyk et al. have analyzed with spectroscopy the impact of oxidative stress on HSA structure using CT as a oxidation modifier [27]. They investigated that two drugs used together in multidrug therapy influence on HSA