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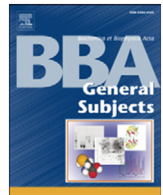

Oxidized albumin. The long way of a protein of uncertain function ☆

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ABSTRACT

Background: Proteins are extremely reactive to oxidants and should represent a potential target of instable reactive oxygen. This may represent a problem for plasma proteins since they may be directly modified *in vivo* in a compartment where antioxidant enzymatic systems are scarcely represented. On the other hand, it is possible that some plasma components have evolved over time to guarantee protection, in which case they can be considered as anti-oxidants.
Scope of review: To present and discuss main studies which addressed the role of albumin in plasma antioxidant activity mainly utilizing *in vitro* models of oxidation. To present some advances on structural features of oxidized albumin deriving from studies carried out on *in vitro* models as well as albumin purified *in vivo* from patients affected by clinical conditions characterized by oxidative stress.
Major conclusions: There are different interaction with HOCl and chloramines. In the former case, HOCl produces an extensive alteration of ^{238}Trp and ^{162}Tyr, ^{425}Tyr, ^{47}Tyr, while thiol groups are only partially involved. Chloramines are extremely reactive with the unique free SH group of albumin (^{34}Cys) with the formation of sulfenic and sulfinic acid as intermediates and sulfonic acid as end-product. Oxidized albumin has a modified electrical charge for the addition of an acidic residue and presents α-helix and random coil reorganization with subtle changes in domain orientation.
General significance: Albumin, is the major antioxidants in plasma with a concentration (0.8 mM) higher than other antioxidants by an exponential factor. Functional and protective roles in the presence of oxidative stress must be defined. This article is part of a Special Issue entitled Serum Albumin.
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1. Introduction

Albumin is the most abundant serum protein in mammalians and represents the oldest marker of phylogeny from primates to humans. While evolutionary stability suggests a critical role of the protein, its functions are generally confined to the transport of ions and drugs. The role of albumin as ion transporter is allowed by its plasticity that is the conformational change this protein may achieve in relation to pH. The regulation of plasma levels of Ca^{++} is the most remarkable example, in which case albumin-Ca^{++} binding varies from 10% in the case of acidosis to 80% for alkalosis and represents a reservoir of disposable Ca^{++} that can satisfy rapid requests [1].

Several *in vitro* models of oxidation indicate that albumin also plays key anti-oxidant functions [2–6] that strengthens its biological role and makes it necessary to review anti-oxidant functions in human livings. In the following sections we present different aspects of albumin as an anti-oxidant protein including the definition of major anti-oxidant groups inside the protein, biochemistry and structural features of the oxidized derivate. The possibility that oxidized metabolites may serve as surrogate biomarker of oxidative damage will be finally discuss. This review would represent a starting point on the way to deepen the knowledge of the role of oxidants as mediators of organ damage in selected diseases.

2. Considerations on extracellular anti-oxidant systems

Reactive oxygen species may play tremendous impact in human livings [7,8]. They are physiologically generated at low levels inside cells during oxidative phosphorylation where are easily integrated by specialized anti-oxidant defenses. The physiological response to the oxidative burst is less definite outside cells where the levels of both glutathione and antioxidant enzymes are low and probably inadequate to blunt a severe stress [9]. Generally speaking, all kinds of oxygen radicals may induce translational modifications on most proteins [10]. The list includes several oxygen (O^-, OH^-, RO^-, $RO2^-$, H2O2, HOCL, HOBr) and nitrogen (NO −, HNO2, ONOO −, *etc.*) species [11] that modify almost all amino acid residues [10,12]. In blood, the generation of free radicals, mainly hypochlorous acid

Abbreviations: HOCl, hypochlorous acid; AOPPs, advanced oxidation products; ESI-MS, electrospray ionization mass spectrometry; LC–MS–MS, liquid chromatography with tandem mass spectrometry; DSC, differential scanning calorimetry

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(HOCl) and chloramines, by polymorphonuclear leukocytes (neutrophils and monocytes) is crucial for defense against exogenous factors (infectious agents, *etc.*)[9,13]. HOCl derives from chloride and hydrogen peroxide in a reaction that is catalyzed by myeloperoxidase, an enzyme generated by leukocytes during the oxidative burst. Chloramines are produced by the oxidation of circulating aminoacids by HOCl and are to be considered a second order oxidation product that retains an oxidative activity for biological molecules. Actually, it seems paradoxical that levels of anti-oxidant enzymes are much lower in blood than in the intracellular compartment since blood is comparably, if not more, exposed to the oxidative stress by leukocytes. Proteins are target of oxygen radicals in this setting and may function as scavengers of the majority of reactive compounds [6,14]. Owing to their stability, oxidation products of plasma proteins can retain the fingerprint of the initial modification and be utilized as markers of oxidative stress [12,15]. In spite that several biomarkers of oxidative stress are available for analytical strategies only few data are available on protein oxidation *in vivo* that report structural analysis and most of our knowledge are derived from the characterization of proteins in artificial oxidation models [16,17]. It is likely that only the definition of chemical structure of oxidized proteins as they occur in humans may serve as a trace for any evolution in pathology or be utilized as surrogates for clinical outcome. The starting point is to consider which protein and which reactive oxygen species interact.

The unique descriptions of oxidized proteins *in vivo* are related to the so called AOPPs (advanced oxidation protein products) that have been detected in several clinical conditions such as diabetes mellitus, IgA glomerulonephritis and more in general in diseases affecting the kidney and leading to uremia [18–21]. In these cases AOPP levels seem to predict the progression of atherosclerotic cardiovascular events, renal lesions and death [18,22,23]. More in general, AOPPs play critical roles in basic physiologic functions as potent inducers of oxidative burst *in vitro* and *in vivo* suggesting that oxidized proteins may contribute by themselves to the inflammatory process [24]. In spite these seemingly important pathologic implications, we still lack a clear structural characterization of AOPPs that is mandatory for any evolution. Based on indirect techniques, Capeillere-Blandin et al. [25] have identified albumin as the main AOPP product in plasma, a finding that confirmed the previous data indicating albumin as the major target of oxidant stress in serum [20,21,26].

3. Albumin as an antioxidant protein

In early 2000, preliminary studies based on the separation of oxidized albumin by HPLC had shown its presence in the serum of patients with renal dysfunction mainly affected by uremia [20,21,26]. The indication of albumin as the most important plasma/serum component undergoing oxidation seemed to be the logical consequence of the fact that it retains a myeloperoxidase activity and is, for this reason, vulnerable to oxidation by HOCl. Data deriving from studies on albumin oxidized '*in vitro*' [6] showed different mechanisms of reaction with HOCl and chloramine [16,17]. In fact, HOCl reactivity with albumin produces an extensive alteration of ^{238}Trp and ^{162}Tyr, ^{425}Tyr, ^{47}Tyr, while thiol groups are only partially involved [14,16]. On the contrary, the unique free SH group of albumin (^{34}Cys) is extremely reactive with chloramines which induce the formation of two instable intermediates (*i.e.* sulfenic and sulfinic acid) and terminate with the end-product sulfonic acid [27] (see scheme in Fig. 1). It seems of note that the same ^{34}Cys scavenges also other electrophilic compounds and/or substances such as heterocyclic aromatic amines [28] that may generate confusion in the characterization of oxidized albumin. Therefore, studies on albumin oxidized *in vivo* that define its precise structure were crucial to address the role of this molecule as an anti-oxidant. Due to the very low-level of oxidized albumin in normal plasma structural studies addressing these aspects could not be done considering normal clinical conditions. This possibility turned up when Musante et al. [29–31] showed an extensive sulfonation of albumin ^{34}Cys SH in patients affected by primary nephrotic syndrome, a clinical condition characterized by renal lesions leading to urinary waste of proteins. In the following sections we give an outline of the main features of oxidized albumin including the description of main biochemistry features and laboratory techniques for its determination.

4. Biochemistry of oxidized albumin

4.1. Modification of Cys residues

The chemistry of oxidized residues of serum albumin has been evaluated *in vivo* in clinical conditions that are now known to be associated with an oxidative stress (see the above description) and were confronted with normal serum albumin. To our knowledge, studies performed by Musante et al.. [29,30] were the first and still unique to address this aspect from a structural point of view. The analysis of the exact mass of albumin was carried out by ESI-MS showing a modest (+48 Da) albeit constant increment from 66.555 to 66.507 kDa that corresponds to the addition of 3 oxygen radicals (Fig. 2a). LC–MS–MS of tryptic digest of albumin was utilized to identify potential sites of oxidation. The major finding was that the oxidation of the unique free SH of albumin sequence at ^{34}Cys (peptide LVLIAFAQQCPFEDHV) that is transformed into a sulfonic group (m/z 511.71 in triply charge and 1610.5 in double charge); two intermediate products of oxidation (*i.e.* sulfenic -SO^- and/or sulfinic derivates SO_2^-) at ^{169}Cys and ^{265}Cys were, in parallel, observed. The same peptide above in normal albumin showed instead two precursor ions (*i.e.* m/z 831.21 and 1245.67) at ^{34}Cys that are compatible with alkylation by iodoacetamide utilized for LC analysis. Normal albumin was presented in two $C_5H_9NO_2S$ groups involving ^{329}Met and ^{446}Met that probably represent a signal of physiologic aging of the protein. Comparison of results deriving from the analysis of *in vivo* and *in vitro* oxidized albumin [16,17,27] demonstrated that modifications in the later situation are far more extensive than what we can observe *in vivo*. In fact, in the later condition (*i.e.* albumin oxidized *in vitro*) an extensive sulfonic transformation (SO_3^-) of several free SH residues was observed together with several methionine sulfoxide ($C_5H_9NO_2S$) derivatives. The finding of sulfonation 13 Cys residues suggests conformational derangement with the rupture of disulfide bonds that in a normal conformation are liked in disulfide bonds. This aspect was confirmed by differential calorimetry (see below).

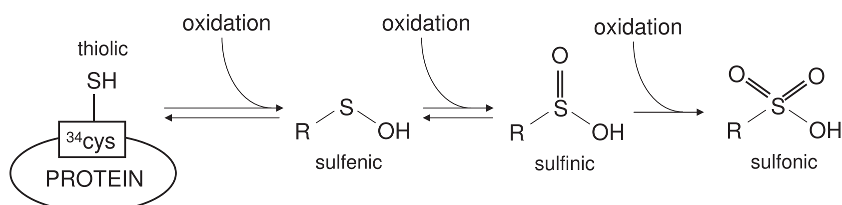

Fig. 1. Proposed scheme for albumin oxidation. Oxidation of a free SH leads to the formation of a stable sulfonic acid as end product; two intermediates of the reaction are sulfenic and sulfinic acids.

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