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Modulation of Various Redox State of Human Serum Albumin, Content of Carbonyl and Thiol Group, and Pseudo-Esterase Activity

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Abstract

Understanding the based-on drug or drug conjugates to reach the beneficial optimally recognized by the immune system requires multidisciplinary approaches and detailed of albumin as the key circulating a transporting/transmission and antioxidant protein of blood drug interaction. Albumin, in reduced form (mercaptoalbumin, HMA), with antioxidant ability and alterations/deteriorations in the redox status of human serum albumin (HSA) under oxidative stress formation in infection diseases and its complications strongly modifies albumin antioxidant capacity. The aim of this study was the investigation of carbonyl/oxidative stress and pseudo-esterase activity of mercaptolbumin and oxidized HSA models. HSA (P. pastoris) purchased from MedChemExpress (USA) was used for study to model oxidative stress, HSA in reduced (intact) form was treated with H_2O_2, tert-butylhydroperoxide (t-BHP) and chloramine T (CT). The content of HSA-bound carbonyl groups decreased in under treatment with t-BPH- and CT-reduced HSA and more less extent in case of H_2O_2-treated. Fatty acid-free HSA and mercaptoalbumin (HMA) advanced oxidation protein products (AOPP) concentrations were significantly lower than in H_2O_2 loading reduced HSA by 123% and 235%, respectively. The total thiols level was lower in HMA + CT compared to reduced HMA by 51% and even increased after treatment of HMA with H_2O_2. Pseudo-esterase activity of HMA maintains >65% in the presence of hydroperoxide and occurs pronounced loss in the presence of chloramine T. Hydrogen peroxide at physiological concentration about 10 μM occurs less damage of reduced from of HSA then t-BPH and CT, and unlike t-BPH and CT, without significantly changes in pseudo-esterase activities.

Keywords

Human Serum Albumin, Mercaptoalbumin, Thiols, Carbonyl Group, Advanced Oxidative Protein Products, Pseudo-Esterase Activity

1. Introduction

Malaria is a life-threatening disease induced by parasites of the *Plasmodium falciparum* species, exacerbated by resistant strains of Plasmodium and lacks effective vaccines and drugs. Survival, growth and multiplication of malaria parasites are strongly dependent on redox balance and oxidative stress, hydrogen peroxide (H_2O_2) redox signaling in pathogen-host cell interactions and involved in the target action mechanism of various antimalarial drugs [1] [2]. Moreover, degradation of hemoglobin as a result of P. falciparum induced oxidative stress during its intraerythrocytic life cycle, leads to the generation, significantly higher amount of H_2O_2, with the ability to selectively oxidize reactive cysteine residues in protein, and albumin as particularly. Thus, H_2O_2-dependent redox-signaling mechanism and molecular targets of changes H_2O_2-mediated signaling in P. falciparum cellular damage and tissues injury can contribute to deciphering parasite-host cell interactions and mechanisms of drug action and resistance. Moreover, it was suggested that oxidation modification of plasma proteins, predominantly albumin and fibrinogen, and as a result hyperproduction of its advanced oxidation protein products (AOPP), as one of the inflammatory markers, could be a candidate for biomarkers of malaria progression, anemia development [3]. One of the pathways of development of new drug for rational therapy and to overcome of the spread of parasites resistance is elaborated the strategy management based on susceptibility of the Plasmodium and others parasities, viruses and infections insights into disturbances, the redox balance and oxidative insults induction. Continuous strengthening of oxidative stressors substances during its intraerythrocytic cycle internal prooxidants derived in cytoplasm, degraded the hemoglobin to peptides and amino acids, and releases heme (Fe^{2+}) which converts into hemin (Fe^{3+}), acted as a long-range and fast acting signaling molecule for generation of superoxide anion that dismutase's to H_2O_2 [4] [5]. Peroxidative actions depended on soluble free hemin which then accumulated of chloroquine into the digestive vacuole of *Plasmodium* and resulting in oxidative damage to membranes, proteins, and DNA, etc. [6] [7]. Furthermore, generation of H_2O_2, the most important reactive oxygen species (ROS) in cells controls protein functions of redox-sensitive proteins and enzymes, usually by selectively oxidizing of cysteine (Cys) reactive residues. Antioxidant properties of HSA with normal serum concentration about 35 - 50 g/L and 70% - 80% of one redox active Cys 35 (from 34 Cys residues in BSA) in reduced/sulfhydryl form [8] [9] assumed that H_2O_2 diffused from the source to the target protein, where it collides with the target thiol and directly introducing a mildly pro-oxidative state (hydrogen peroxide at a low physiological concentration

of 10 nM on the susceptibility of protein (albumin) to glycation, particularly at physiological) [10]. This oxidation-driven H_2O_2-induced oxidation hypothesis was also tested on reduced albumin (mercaptalbumin) on the assumption that commercially source native albumin would be already partly oxidized and whether *in vitro* modification of albumin by low level of radicals lead to loss thiol group content. Moreover, the well-known antimalarial drug, chloroquine increases oxidative effects in the parasite through increased soluble free hemin and the most active prooxidant as 8-hydroxyquinoline (a phenolic compound), thus did not promote oxidation. Increased peroxidation produced by chloroquine depended on hemin and it was evidenced in presence of H_2O_2 [5]. However, neither molecular targets nor regulatory mechanisms and dynamics changes of H_2O_2-mediated signaling in P. falciparum have been clearly understanding, including the differences in the mechanisms of H_2O_2 or other agents oxidative and/or carbonyl stress modified in key transporting and more abundant protein of plasma, human serum albumin, and its relation to functioning of HSA efficacy. Overall, present work defined the different posttranslational modifications of main antioxidant molecule in human blood albumin, generated by thiol oxidation and revise its properties and possible biological function, pseudo-esterase activity, and advanced oxidation protein products (AOPP) formation, as oxidative stress biomarker.

2. Materials and Methods

All materials (reagent) were purchased in form of analytical grade, and all solutions were prepared in deionized and/or bidistilllated water. Serum Albumin/ALB Protein, Human (P. pactoris) (HSA) was purchased from Med Chem Express MCE) USA. HSA dissolved in sodium phosphate buffer (1 M, pH 7.4) and 0.02% NaN_3 as a preservative. 1,4-Dithiothreitol (DTT), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, 99%), Sigma, 2,4-Dinitrophenylhydrazine (DNPP), hydrogen peroxide 30% (Meck Mellipore), Ascorbic acid and tert-butylhydroperoxide (t-BHP) were purchased from Merck, Germany. The salts of Phosphate buffered saline (PBS) were of analytical grade (8.77 g/L NaCl, 1.28 g/L Na_2HPO_4, 1.36 g/L KH_2PO_4, adjusted with 50% NaOH to pH 7.2 - 7.4). Highly purified water was obtained by deionization and filtration with a Millipore purification apparatus (18.2 MΩ·cm at 25˚C).

2.1. Preparation of Reduced Form of HSA (Mercaptoalbumin)

In nondenaturating conditions in which about one third of cysteine-34 of HSA is disulfide-bonded to glutathione or cysteine and this can be reversed by mild reduction with DTT [11] [12]. To HSA solution added of one equivalent of dithiothreitol (10 μmol). The reaction was carried out for 1 h at 37˚C and DTT washed/dialyzed away from HSA with 0.1 M potassium phosphate buffer, pH 7.4, using an Ultracel 30 K device (Millipore, USA). Mercapto-HSA prepared by this procedure contains 0.84 ± 0.05 mol-SH/mol HSA, as determined with 4.4 dithiodipyridine (DTDP) using ε_{324} = 19,800 [13].

2.2. Preparation of oxidized HSA

2.2.1. Induction of Oxidative Stress by Strongly Pro-Oxidant Molecule, Chloramine T

300 μM of HSA was reacted with 10 mM CT in phosphate buffer, pH 7.4 for 2 h at 37˚C. All samples were incubated darkly in the closed vials at a temperature of 37˚C with continuous shaking (50 rpm). The incubation mixtures contained HSA at a final concentration of 0.08 mM. For measurements of the effects of additive on protein oxidation, HSA and investigated drug were incubated with 20 mM chloramine T for 60 min [14].

2.2.2. Induction of Oxidative Stress by Pro-Oxidant Molecule, Hydrogen Peroxide

The concentration of H_2O_2 stock solution (0.4 M), typically 30% (v/v) H_2O_2 (Sigma Aldrich, Sweden) was determined spectrophometrically accurately using a molar extinction coefficient ε = 39.4 M^{-1} cm^{-1} at 240 nm. 0.4 M of H_2O_2 was diluted with PBS to the concentration 5 mM. Then 2 μl of H_2O_2 was added to 1 ml of albumin (100 μM) (final concentration of hydrogen peroxide in albumin solution was 10 μM, as a physiologically relevant concentration at inflammatory conditions [15] and 300 μ of H_2O_2 oxidized all thiols in HSA [16]). After 1 h incubation HSA was dialyzed once (cellulose dialysis tubes membrane, MWCO 14,000) against PBS (5 mM, pH 7.4) (4 h at 4˚C on automatic stirrer) to eliminate H_2O_2. At the end of dialysis, the concentration of proteins was measured again (3 times in each sample) according to Thermo Scientific™ Pierce™ BCA Protein Assay Kits instruction manual and expressing data as HSA equivalents (HSA; mg mL^{-1}) or determined spectrophotometrically on the assumption that the $E^{1\%}_{cm}$ was 5.30 at 278 nm and the molecular weight of HSA was 66,000 [17].

2.2.3. Induction of Oxidative Stress by Pro-Oxidant Molecule, Tert-Butyl Hydroxyperoxide (t-BHP)

Oxidative stress was induced by the addition of 2 μl *t*-BPH (7.778 M was diluted with PBS to the concentration 50 mM) to 1 ml HSA (100 μM) solution at 37˚C (final concentration of t-BHP in albumin solution was 100 μM). After 1 h incubation HSA was dialyzed at the same manner as in case of hydroperoxide studying.

2.3. Analysis of Thiol Group Content

The sulfhydryl contents in 1 μM HSA determined at pH 7.4 in 0.1M potassium phosphate buffer with equimolar levels of Ellman's (DTNB-5,5'-dithio-bis-(2-nitrobenzoic acid) reagent). HSA with or after chemical modification was mixed with phosphate buffer (10 mM, pH 8.0) and the absorbance at 412 nm was measured (A_0). Then the DTNB 1 M in phosphate buffer (10 mM, pH 8.0) was added to the mixture and incubated for 1 h at 37˚C in the dark. At the end of incubation, the absorbance of samples measured again at 412 nm (A_1). The absorbance differences were calculated as follows $A = A_1 - A_0$. The sulfhydryl concentrations measured spectrophotometrically at 412 nm using molar absorption coefficient ε =

$1.415 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ against blank after 30 min at t = 25˚C temperature [18] and expressed as nmol/mg protein or mol thiols/mol protein.

2.4. Analysis of Carbonyl Content

The antioxidant capacity of albumin was measured in accordance with the method of [19] by measurement of carbonyl content, as an oxidative stress marker. The HSA carbonyl residues performed by 2,4-dinitrophenylhydrazine (2,4-DNPH) assay [Protein Carbonyl Assay Kit according to manufactures instruction (Cayman Chemical Co., SA)]. Samples were submitted to 10 mM 2,4-DPNH in 2.5 M HCl for 1 h, followed by deproteinization with 20% TCA. The pellet washed three times in ethanol/ethyl acetate and solubilized in guanidine 6 M. The amount of protein hydrazine produced is quantified as the absorption at 370 nm, and the molar extinction coefficient of 22,000 $M^{-1} \cdot cm^{-1}$ was used to interpret the data as mol DNPH reacted/mg protein [20] [21]. The concentration of C = O is expressed per total protein content (nmol/mol protein) and normalized absorbance of protein and calculated carbonyl concentration by Beer-Lambert Law.

2.5. Advanced Oxidation Protein Products Assay (AOPPs) as Oxidative Stress Markers

The level of AOPPs formation determinates via a spectrophotometric detection performed by Witkko-Sarsat *et al.* [22] 100 µL of protein were acidified with 20 µL of acetic acid and then added 1 mM CT (n-chloro-p-toluene sulfonimide sodium salt) in volume of 1 ml, time of incubation 60 min. The 4.0 fold excess of sodium thiosulfate (20 µL) was used to eliminate the residue of unreacted CT hydrate. AOPP-HAS was also prepared by exposing red HSA (100 mg/ml) to hydrogen peroxide (100 mM) and dialyzed overnight against PBS. The assay was calibrated using chloramine-T and absorbance read at 340 nm. AOPPs' concentrations were expressed as µMol of chloramine-T equivalents.

2.6. Pseudo-Esterase Activity Measurement

HSA at final concentrations 15 µM in 67 mM potassium phosphate buffer, pH 7.4 preincubated at 25˚C for 5 min, and then added 500 µM of p-nitrophenyl acetate for 5 min in this condition acetylation of only Tyr 411 sites occurs [23]. Reaction terminated using chilled ethanol (0.5 mL) [24] and monitoring the absorbance of the released product, p-nitrophenol, spectrophotometrically at λ_{abs} = 400 nm, molar absorption coefficient ε = 17,700 $M^{-1} \cdot cm^{-1}$.

2.7. Statistical Analysis

The experiment was performed in three series, each time in duplicate. SPSS v.22.0 was used for statistical analysis of the data. Data are shown as average values from at least three experiments ± standard deviation (SD). Experiments containing two groups of data were analyzed using Student's t-test for independent samples;

differences were considered significant when $p < 0.05$.

3. Results

Albumin, human or bovine, is protein responsible for maintaining the redox state of the plasma, regulating osmotic pressure and distributing fluids and transporting function of various ligands. Albumin in its redox form, mercaptoalbumin (HMA), exerts antioxidant effect by binding of many types of biological active molecules and ions to prevent ROS (such as H_2O_2, O_2^-, HOCl, $ONOO^-$ and etc.) and which capable of trapping several reactive species by one active free sulfhydryl groups of Cys 34, for example. Markers of HMA oxidative damage, significantly decreased antioxidant activity greater in the presence of chloramine T > tBHP > H_2O_2. Early it was shown H_2O_2, one of the major oxidants generated in neutrophils, and a substrate of myeloperoxidase, did not, unlike HOCl, convert HSA into chaperone like holdases, and thus occurs potentially less detrimental positive feedback loop for neutrophil protection and highly immunogenic foreign antigens formation [25].

3.1. Relationship between Specify of Oxidation and Thiols and Carbonyl Content in Reduced HSA

Induction of oxidative stress in HMA *in vitro* by incubated with H_2O_2, t-BHP (10^5 mol·L^{-1}) and Chloramine T caused a significant increase in carbonyl content after 10 h of incubation, reached a plateau after 72 h 72 h treatment (Table) and longer times of incubation resulted in the development of turbidity, indicating the denaturation of the protein that's failed to increase the amount of these groups. The clearly detectable increase in the amount of albumin-bound carbonyl groups was found for t-BPH-HMA and CT-HMA and to a lesser extent, in case of H_2O_2-HMA. In contrast, the total thiols level was lower in HSA_{red} + chloramine T compared to reduced HSA (mercaptoalbumin) by 51%.

The effects hydrogen peroxide, chloramine T and t-BHP on albumin oxidation and esterase activities.

Modifications state of HSA	Mol Carbonyl groups/mol HSA	Total thiols, mol SH/mol protein	AOPP, μmol/mg protein	Esterase-like activities μmol NP/min/μmol HSA
HSA, free of fatty acids	0.06 ± 0.01	0.25 ± 0.05	30 ± 5	1.54 ± 0.14
HSA_{red} (+DTT 1 mM)-mercapto-HSA	0.04 ± 0.04*	0.88 ± 0.08***	20 ± 4*	1.28 ± 0.08*
HSA_{red} + H_2O_2 (10 μmol)	0.08 ± 0.01$^{\#\#}$	1.9 ± 0.1$^{***\#\#\#\Delta\Delta}$	67 ± 7$^{***\#\#\#}$	1.42 ± 0.08
HSA_{red} + tBHP	0.12 ± 0.02$^{***\#\#\#\Delta}$	0.65 ± 0.08$^{***\#\#\Delta\Delta\Delta}$	98 ± 10$^{***\#\#\#\Delta\Delta}$	0.78 ± 0.04$^{***\#\#\#\Delta\Delta\Delta}$
HSA_{red} + Chloramine T	0.26 ± 0.06$^{***\#\#\#\Delta\Delta\Delta\square\square\square}$	0.43 ± 0.06$^{***\#\#\#\Delta\Delta\Delta\square\square\square}$	131 ± 11$^{***\#\#\#\Delta\Delta\Delta\square\square\square}$	0.28 ± 0.03$^{***\#\#\#\Delta\Delta\Delta\square\square\square}$

Note: significance of difference in comparison: *with HSA free fatty acid, $^{\#}$with HAS red, $^{\Delta}$with HSA_{red} + H_2O_2, $^{\square}HSA_{red}$+ tBHP; one symbol-$p < 0.05$, two-$p < 0.01$, three-$p < 0.001$.

3.2. Relationship between Specify of Oxidation and Advanced Oxidation Protein Products AOPP of Reduced HSA

Treatment of HSA_{red} (HMA) with CT induced pronounced increased in AOPP and protein carbonyl concentrations (rise in 6.5 fold and 6.55 fold respectively), while under treatment with tBPH only in 3.0 and 4.9 fold respectively.

At baseline, FA-free HSA and mercaptoalbumin AOPP concentrations were significantly lower than in H_2O_2 loading reduced HSA by 123% and 235% respectively and strongly correlated with the carbonyl content ($r = 0.81$, $p < 0.001$) (Table).

3.3. Relationship between Specify of Oxidation and Pseudo-Esterase Activities of HSA

The serum albumin exhibits an esterase activity which can hydrolyze p-nitrophenyl acetate and convert it to p-nitrophenol [23] due to acetylating of highly reactive negatively charged tyrosine group at position 411 in HSA (410 in BSA). The pseudo-esterase activity practically not affected in the case of treatment HSA with H_2O_2, whereas significantly depressed in HSA + tBHP and more pronounced in HSA + Chloramine T (Table).

4. Discussion

Prolonged and persistent *Plasmodium falciparum* infection with or without symptoms involves a strong interrelationship between oxidative stress and pathogenesis, and oxidative stress strengthening could result in adverse malaria outcomes and may lead to complications. The malarial parasite augments oxidative stress to weaken the host and exerts antioxidant effects against host defense mechanisms. However, the anti-malarial drugs induce oxidative insult to reduce parasitic load and exert antioxidant activity in hosts and/or pro-oxidant activity in parasites. Using cell imaging with the cytosolically and mitochondrially expressed H_2O_2 biosensors biotechnology, in asexual blood stages of stably transfected P. falciparum NF54-attB parasites [26], were provided results, that the mode of action of some antimalarial drugs and some of the novel antimalarial compounds includes disturbance of H_2O_2 homeostasis and in addition, degradation of haemoglobin leads to the generation of H_2O_2 within the malaria parasite and in blood. Carbonyl (CO) groups (aldehydes and ketones) are produced on protein side chains (especially of Pro, Arg, Lys, and Thr) when they are oxidized. These moieties are chemically stable, which is useful for both their detection and storage. The key pathophysiological relevance of the non-oncotic properties of blood albumin as a major target-protein for oxidative modifications [27]-[32] and stronger than ascorbic acid free radical's scavenger properties. Free radical's induces post-translation alterations that in sum expressed as AOPP, can further induced ROS production in cells and tissues, which coupled to, and promotes, inflammation and immune cell activation, thus sustaining the existence of a feedback loop and circulated in the body oxidized albumin acts as a damage-associated molecular

pattern, with immunogenic activity [33]. From the table, it can be seen that the activity of H_2O_2-HSA is the same as that of native HSA ($p > 0.1$). In contrast, the activities of CT-HSA were much reduced and to the same level ($p > 0.1$). HSA was mildly oxidized by treatment with a H_2O_2 (H_2O_2-HSA), t-BPH (t-BPH-HAS) or chloramine-T (CT-HSA) in *in vitro* oxidation model. According to the detailed study of Finch *et al.* [34], the SH-group of ^{34}Cys and Met residues have been oxidized in the case of H_2O_2-HSA. According to these authors, chloramine-T also oxidizes Cys and Met residues. Tested the effect of chloramine-T on the Met residues of albumin preparations by CNBr fragmentation and SDS-PAGE showed that Met residues were oxidized to methionine sulfoxide in CT-HSA and to a lesser extent in H_2O_2-HSA. It was reported that the catalytic activity of HSA is exhibited by the reactive residue Tyr 411 present in subdomain IIIA with different catalytic cleavage mechanisms. When ligand molecules bind to the subdomain region, they may interfere with the catalytic activity of HSA. The t-BPH, as greater hydrophobicity agent than hydroperoxide, penetrates/conjugates more easily with Cys 34 located in 10 angstrom wide hydrophobic pocket in HSA, and thus occurs more damaging of this region of albumin. Early was shown that, t-BPH in contrast to H_2O_2, completely inhibited peroxiredoxin-4 activity of HSA, however, both ligands/oxidants resulted in the same oxidative damage leading to sulfinic acid formation in Cys residues [35]. Moreover, was demonstrated that AOPP-HSA possesses the ability to activate isolated monocytes *in vitro* as predictor of respiratory burst, is dependent of the chlorinated nature of the antioxidants and initiated by CT and hypochlorite, but not H_2O_2 in range 10 – 100 μ [22] [25]. Plasma AOPP levels were predictive for anaemia and oxidative stress markers for clinical malaria infection in children [3]. Hydrogen peroxide at sublethal concentrations affects S.aureus general fitness and leads to a downregulated genes, predominantly included in pyrimidine [36]. Moreover, obtained data that treated of mercato-HSA with H_2O_2 practically did not change the catalytic activity towards p-nitrophenyl acetate, which is predominantly associated with the conformation state of subdomain IIIA of HSA close proximity to ^{410}Arg and ^{411}Tyr [37], unlike induced by CT pronounced decrease in pseudo-esterase activity could indicate that hydroperoxide-inducible alterations in mercapto-albumin caused conformational changes which increase the distance between the ^{410}Arg and ^{411}Tyr active groups [38] in less extent than chlorination modification. From the other side, on the model of oxidative modified HSA induced by the changes in microenviroutment could lead to disturbances in port-translational modification of HSA, which act as the primary carrier for various exogenous and endogenous compounds and free radicals, and alter the pharmacokinetic properties of several drugs. Beside the already known and pharmacologically used antimalarial drugs such as quinolines and artemisinins, the development of new medicines with dual-action increases oxidative stress in malaria parasites by elevating intracellular H_2O_2 levels and decreases the damage oxidative associated complications for blood proteins whchi remain the actual problem and medical parasitology and pharmacology [27], the most

important reactive oxygen species (ROS) in cells.

5. Conclusion

The implications of hyperproduced free radicals in physiopathogenesis of various infectious diseases and its complications, and malaria as particularly, in which erythrocytes infected with *P. falciparum* produced $OH^{\bullet}$ radicals and H_2O_2 about twice as much compared to normal erythrocytes [27], gave ground to suggest a crucial role of oxidative stress in clinical manifestation and systemic complications of parasite-induced diseases. Long regarded as a toxic byproduct that can damage macromolecules including DNA, proteins and lipids [28], H_2O_2 is increasingly recognized as an important cellular signaling molecule with regulatory functions [29] [30]. H_2O_2 selectively oxidizes reactive cysteine residues and thereby controls functions of redox-sensitive protein—HSA. The esterase-like property seems especially useful in converting prodrugs to active drugs in plasma and its activities significantly decrease under treatment of HSA with t-BPH and CT, but not hydrogen peroxide. Thus, research efforts aim to expand reduced HAS's ability to interact with more different drugs in order to improve the delivery of various pharmacological drugs remained as important problem in rational medicine and diagnostics.

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Ethics Approval

The authors date that the experimental protocol of the study was revised and approved by the Interinstitutional Animal Care and Use Committee of the Tbilisi State Medical University and International Centre of Introduction of New Biomedical Technology, Tbilisi, Georgia (No. 11-819012; date: March 11, 2021).

Conflicts of Interest

The authors have no competing interests to declare that are relevant to the content of this article.

References

[1] Egwu, C.O., Pério, P., Augereau, J., Tsamesidis, I., Benoit-Vical, F. and Reybier, K. (2022) Resistance to Artemisinin in Falciparum Malaria Parasites: A Redox-Mediated Phenomenon. *Free Radical Biology and Medicine*, **179**, 317-327. https://doi.org/10.1016/j.freeradbiomed.2021.08.016

[2] Rahbari, M., Rahlfs, S., Przyborski, J.M., Schuh, A.K., Hunt, N.H., Fidock, D.A., *et al.* (2017) Hydrogen Peroxide Dynamics in Subcellular Compartments of Malaria Parasites Using Genetically Encoded Redox Probes. *Scientific Reports*, **7**, Article No. 10449. https://doi.org/10.1038/s41598-017-10093-8

[3] Zhang, G., Skorokhod, O.A., Khoo, S., Aguilar, R., Wiertsema, S., Nhabomba, A.J., *et al.* (2014) Plasma Advanced Oxidative Protein Products Are Associated with Anti-Oxidative Stress Pathway Genes and Malaria in a Longitudinal Cohort. *Malaria Journal*, **13**, Article No. 134. https://doi.org/10.1186/1475-2875-13-134

[4] Klonis, N., Crespo-Ortiz, M.P., Bottova, I., Abu-Bakar, N., Kenny, S., Rosenthal, P.J., *et al.* (2011) Artemisinin Activity Againstplasmodium Falciparumrequires Hemoglobin Uptake and Digestion. *Proceedings of the National Academy of Sciences*, **108**, 11405-11410. https://doi.org/10.1073/pnas.1104063108

[5] Herraiz, T., Guillén, H., González-Peña, D. and Arán, V.J. (2019) Antimalarial Quinoline Drugs Inhibit B-Hematin and Increase Free Hemin Catalyzing Peroxidative Reactions and Inhibition of Cysteine Proteases. *Scientific Reports*, **9**, Article No. 15398. https://doi.org/10.1038/s41598-019-51604-z

[6] Acharya, P., Garg, M., Kumar, P., Munjal, A. and Raja, K.D. (2017) Host-Parasite Interactions in Human Malaria: Clinical Implications of Basic Research. *Frontiers in Microbiology*, **8**, Article 889. https://doi.org/10.3389/fmicb.2017.00889

[7] Kumar, R., Adams, B., Musiyenko, A., Shulyayeva, O. and Barik, S. (2005) The Fk506-Binding Protein of the Malaria Parasite, Plasmodium Falciparum, Is a Fk506-Sensitive Chaperone with Fk506-Independent Calcineurin-Inhibitory Activity. *Molecular and Biochemical Parasitology*, **141**, 163-173. https://doi.org/10.1016/j.molbiopara.2005.02.007

[8] Peters, T. (1996) All about Albumin: Biochemistry, Genetics and Medical Applications. Academic Press. Inc.

[9] Roche, M., Rondeau, P., Singh, N.R., Tarnus, E. and Bourdon, E. (2008) The Antioxidant Properties of Serum Albumin. *FEBS Letters*, **582**, 1783-1787. https://doi.org/10.1016/j.febslet.2008.04.057

[10] Mueller, S., Riedel, H. and Stremmel, W. (1997) Direct Evidence for Catalase as the Predominant H_2O_2 Removing Enzyme in Human Erythrocytes. *Blood*, **90**, 4973-4978. https://doi.org/10.1182/blood.v90.12.4973

[11] Pan, J. and Carroll, K.S. (2013) Persulfide Reactivity in the Detection of Protein S-Sulfhydration. *ACS Chemical Biology*, **8**, 1110-1116. https://doi.org/10.1021/cb4001052

[12] Sogami, M., Nagoka, S., Era, S., Honda, M. and Noguchi, K. (1984) Resolution of Human Mercapt and Nonmercaptalbumin by High-Performance Liquid Chromatography. *International Journal of Peptide and Protein Research*, **24**, 96-103. https://doi.org/10.1111/j.1399-3011.1984.tb00933.x

[13] Grassetti, D.R. and Murray, J.F. (1967) Determination of Sulfhydryl Groups with 2, 2'- or 4, 4'-dithiodipyridine. *Archives of Biochemistry and Biophysics*, **119**, 41-49. https://doi.org/10.1016/0003-9861(67)90426-2

[14] Grzebyk, E. and Piwowar, A. (2014) The Tibetan Herbal Medicines Padma 28 and Padma Circosan Inhibit the Formation of Advanced Glycation Endproducts (AGE) and Advanced Oxidation Protein Products (AOPP) *in Vitro*. *BMC Complementary and Alternative Medicine*, **14**, Article No. 287. https://doi.org/10.1186/1472-6882-14-287

[15] Mueller, S., Riedel, H. and Stremmel, W. (1997) Determination of Catalase Activity at Physiological Hydrogen Peroxide Concentrations. *Analytical Biochemistry*, **245**,

55-60. https://doi.org/10.1006/abio.1996.9939

[16] Carballal, S., Radi, R., Kirk, M.C., Barnes, S., Freeman, B.A. and Alvarez, B. (2003) Sulfenic Acid Formation in Human Serum Albumin by Hydrogen Peroxide and Peroxynitrite. *Biochemistry*, **42**, 9906-9914. https://doi.org/10.1021/bi027434m

[17] Ohkubo, A. (1969) On the Conformation around the Sulfhydryl Group in Human Serum Albumin. *The Journal of Biochemistry*, **65**, 879-888. https://doi.org/10.1093/oxfordjournals.jbchem.a129092

[18] Riener, C.K., Kada, G. and Gruber, H.J. (2002) Quick Measurement of Protein Sulfhydryls with Ellman's Reagent and with 4,4'-Dithiodipyridine. *Analytical and Bioanalytical Chemistry*, **373**, 266-276. https://doi.org/10.1007/s00216-002-1347-2

[19] Levine, R.L., Williams, J.A., Stadtman, E.P. and Shacter, E. (1994) Carbonyl Assays for Determination of Oxidatively Modified Proteins. In: *Methods in Enzymology*, Elsevier, 346-357. https://doi.org/10.1016/s0076-6879(94)33040-9

[20] Reznick, A.Z. and Packer, L. (1994) Oxidative Damage to Proteins: Spectrophotometric Method for Carbonyl Assay. In: *Methods in Enzymology*, Elsevier, 357-363. https://doi.org/10.1016/s0076-6879(94)33041-7

[21] Sadowska-Bartosz, I., Galiniak, S., Skolimowski, J., Stefaniuk, I. and Bartosz, G. (2014) Nitroxides Prevent Protein Glycoxidation *in Vitro*. *Free Radical Research*, **49**, 113-121. https://doi.org/10.3109/10715762.2014.982113

[22] Witko-Sarsat, V. (1999) Advanced Oxidation Protein Products as a Novel Molecular Basis of Oxidative Stress in Uraemia. *Nephrology Dialysis Transplantation*, **14**, 76-78. https://doi.org/10.1093/ndt/14.suppl_1.76

[23] Lockridge, O., Xue, W., Gaydess, A., Grigoryan, H., Ding, S., Schopfer, L.M., *et al.* (2008) Pseudo-Esterase Activity of Human Albumin. *Journal of Biological Chemistry*, **283**, 22582-22590. https://doi.org/10.1074/jbc.m802555200

[24] Ikeda, K., Kurono, Y., Ozeki, Y. and Yotsuyanagi, T. (1979) Effects of Drug Bindings on Esterase Activity of Human Serum Albumin. Dissociation Constants of the Complexes between the Protein and Drugs Such as N-Arylanthranilic Acids, Coumarin Derivatives and Prostaglandins. *Chemical and Pharmaceutical Bulletin*, **27**, 80-87. https://doi.org/10.1248/cpb.27.80

[25] Ulfig, A., Schulz, A.V., Müller, A., Lupilov, N. and Leichert, L.I. (2019) N-Chlorination Mediates Protective and Immunomodulatory Effects of Oxidized Human Plasma Proteins. *E Life*, **8**, e47395. https://doi.org/10.7554/elife.47395

[26] Rahbari, M., Rahlfs, S., Przyborski, J.M., Schuh, A.K., Hunt, N.H., Fidock, D.A., *et al.* (2017) Hydrogen Peroxide Dynamics in Subcellular Compartments of Malaria Parasites Using Genetically Encoded Redox Probes. *Scientific Reports*, **7**, Article No. 10449. https://doi.org/10.1038/s41598-017-10093-8

[27] Atamna, H. and Ginsburg, H. (1993) Origin of Reactive Oxygen Species in Erythrocytes Infected with Plasmodium Falciparum. *Molecular and Biochemical Parasitology*, **61**, 231-241. https://doi.org/10.1016/0166-6851(93)90069-a

[28] Storz, G. and Imlayt, J.A. (1999) Oxidative Stress. *Current Opinion in Microbiology*, **2**, 188-194. https://doi.org/10.1016/s1369-5274(99)80033-2

[29] Veal, E.A., Day, A.M. and Morgan, B.A. (2007) Hydrogen Peroxide Sensing and Signaling. *Molecular Cell*, **26**, 1-14. https://doi.org/10.1016/j.molcel.2007.03.016

[30] Neill, S.J. (2002) Hydrogen Peroxide and Nitric Oxide as Signalling Molecules in Plants. *Journal of Experimental Botany*, **53**, 1237-1247. https://doi.org/10.1093/jexbot/53.372.1237

[31] Meyer, A.J. and Dick, T.P. (2010) Fluorescent Protein-Based Redox Probes.

Antioxidants & Redox Signaling, **13**, 621-650. https://doi.org/10.1089/ars.2009.2948

[32] Winterbourn, C.C. (2008) Reconciling the Chemistry and Biology of Reactive Oxygen Species. *Nature Chemical Biology*, **4**, 278-286. https://doi.org/10.1038/nchembio.85

[33] Wezena, C.A., Krafczyk, J., Staudacher, V. and Deponte, M. (2017) Growth Inhibitory Effects of Standard Pro and Antioxidants on the Human Malaria Parasite Plasmodium Falciparum. *Experimental Parasitology*, **180**, 64-70. https://doi.org/10.1016/j.exppara.2017.02.017

[34] Finch, J.W., Crouch, R.K., Knapp, D.R. and Schey, K.L. (1993) Mass Spectrometric Identification of Modifications to Human Serum Albumin Treated with Hydrogen Peroxide. *Archives of Biochemistry and Biophysics*, **305**, 595-599. https://doi.org/10.1006/abbi.1993.1466

[35] Ikeda, Y., Nakano, M., Ihara, H., Ito, R., Taniguchi, N. and Fujii, J. (2011) Different Consequences of Reactions with Hydrogen Peroxide and T-Butyl Hydroperoxide in the Hyperoxidative Inactivation of Rat Peroxiredoxin-4. *Journal of Biochemistry*, **149**, 443-453. https://doi.org/10.1093/jb/mvq156

[36] Buvelot, H., Roth, M., Jaquet, V., Lozkhin, A., Renzoni, A., Bonetti, E., *et al.* (2021) Hydrogen Peroxide Affects Growth of S. Aureus through Downregulation of Genes Involved in Pyrimidine Biosynthesis. *Frontiers in Immunology*, **12**, Article 673985. https://doi.org/10.3389/fimmu.2021.673985

[37] Ferrer, M.L., Duchowicz, R., Carrasco, B., de la Torre, J.G. and Acuña, A.U. (2001) The Conformation of Serum Albumin in Solution: A Combined Phosphorescence Depolarization-Hydrodynamic Modeling Study. *Biophysical Journal*, **80**, 2422-2430. https://doi.org/10.1016/s0006-3495(01)76211-x

[38] Watanabe, H., Tanase, S., Nakajou, K., Maruyama, T., Kragh-Hansen, U. and Otagiri, M. (2000) Role of Arg-410 and Tyr-411 in Human Serum Albumin for Ligand Binding and Esterase-Like Activity. *Biochemical Journal*, **349**, 813-819. https://doi.org/10.1042/bj3490813