



Homeostasis of Oxidative-Related Metabolism in Human Erythrocyte: An Overview of the Hemoglobin S Presence Implications



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Abstract

Sickle erythrocytes are characterized by the presence of sickle hemoglobin (HbS), instead of normal one (Hb). HbS suffers recurrent polymerization/de polymerization, it has tendency to autoxidise faster than Hb, leading to formation of hemi-chromes, and increased and unremitting pro oxidant generation. As consequence, sickle erythrocytes show a hyper-oxidative state that appears to be involved in the rupture of the metabolic homeostasis in this cell. This rupture causes a series of clinical complications in a vicious cycle in which many different oxidative processes play a central role in the patho physiology of sickle cell diseases. Thus, in this review, we briefly overview the implications of the HbS presence in the disruption of erythrocyte metabolic homeostasis, addressing the integration of some erythrocyte metabolic pathways, such as redox, glycolytic, pentose phosphate, methemoglobin reductive and Rapoport-Luebering ones.

Key words: Hemoglobin S; Oxidative processes; Antioxidants; Metabolic integration

Introduction

Sickle Cell Disease (SCD) is one of the most important hemoglobinopathies and is the most common severe monogenic disorders worldwide [1]. The term SCD embraces a group of genetic conditions in which pathology results by substitution of the 20th nucleotide, adenine by thymine, resulting in the exchange of glutamic acid (hydrophilic characteristics) by valine (hydrophobic characteristics), in the sixth position of the chain (HBB_{glu6val}) [1-3]. Thus, results in different structural and biochemical characteristics of normal hemoglobin (Hb) and formation of hemoglobin S (HbS) [4,5]. This amino acid exchange favours interactions between hydrophobic residues of the protein under conditions of low oxygen tension, dehydration or acidosis, forming polymers [5]. These grow, break the cytoskeleton and fill the erythrocyte, causing modifications in its architecture and flexibility, culminating in the formation of sickle erythrocytes, which are less flexible, more adherent to the endothelium and prone to hemolysis [6,7].

The clinical complications in SCD include hemolytic anemia, endothelial dysfunction, inflammation, and hypercoagulability, effects of ischemia-reperfusion injury, hypoxemia and damage to multiple organs [7-10]. These complications have a cyclic nature and the oxidative stress process acts as cause and consequence [11]. In addition to the relationship with patho-physiological

events that may imply in more severe clinical outcomes, the hyper-oxidative state of the sickle erythrocyte appears to be involved in the rupture of the metabolic homeostasis in this cell [7]. Once it has been demonstrated alterations in the erythrocyte redox metabolism [11], as well as in the activities of the enzymes of the glycolytic and the phosphate pentose pathways, resulting in function impairments of these pathways [12,13], and altered levels of pyridine nucleotides and purine metabolites [13].

Many studies have elucidated oxidative stress in SCD or in red blood cell (RBCs). On the other hand, to the authors' knowledge, there are no works addressing the implications of the HbS presence in the disruption of erythrocyte metabolic homeostasis, regarding oxidative-related processes. Thus, in this review, we briefly overview the integration of some erythrocyte metabolic pathways, such as redox, glycolytic, pentose phosphate, methemoglobin reductive and Rapoport-Luebering ones.

Redox-Related metabolism of Human HbA-Containing Erythrocyte

RBCs are highly specialized and the most abundant cells in the human organism [14,15]. Their primary function is oxygen (O₂) transportation from the lungs to the tissues [14,15]. Thus, these cells are highly susceptible to oxidative damage, due to the high concentration of O₂ and Hb, which undergoes auto-oxidation,

producing methemoglobin (MetHb) and superoxide radical ($O_2^{\bullet-}$) that is an important trigger of the oxidative processes [16-18]. Although it is a normal physiological process, even a small rate of Hb autoxidation can produce substantial oxidant specie levels, since more than 95% of erythrocyte cytoplasm protein content is composed of Hb, whose concentration bound to O_2 is approximately 5mM [19].

In addition to dealing with an internal environment in constant oxidant specie production, during human RBC lifespan of about 120 days, erythrocytes are exposed to a large number of stressful oxidative situations [17,20]. For instance, RBCs pass through the lungs at least one time per minute, where they face a highly pro-oxidative environment [21]. Furthermore, human erythrocytes are exposed to oxidants produced in the circulation [22], as well as to a wide range of oxidative xenobiotics. Thereby, the integrity of erythrocyte redox metabolism is constantly challenged, as well as the related metabolic pathways.

In this scenario, in order to prevent or attenuate the oxidative stress generated in the cell, RBCs are equipped with an effective and self-sustaining antioxidant system that makes them mobile free radical scavengers, providing antioxidant protection, both enzymatic and non-enzymatic [11]. Activity of antioxidant defence enzymes, including superoxide dismutase (SOD), which enables the radical $O_2^{\bullet-}$ from the auto-oxidation of Hb to be disputed into O_2 and hydrogen peroxide (H_2O_2), posterior, catalase (CAT) neutralizes the H_2O_2 , transforming it in H_2O and O_2 [23]. Other enzymes that contribute to the reduction of lipid/alkyl peroxides as well as other organic hydroperoxides are glutathione per-oxidase (GPx) and per-oxiredoxins (Prx), using reduced glutathione (GSH) as cofactor [23,24]. GSH also acts as cofactor for glutathione-S-transferase (GST) in the detoxification of xenobiotics and of glutaredoxin (Grx), responsible for the reduction of oxidized proteins and as corbate [23]. In addition, GSH protects important membrane proteins against oxidation, such as spectrin, favouring the maintenance of the integrity and flexibility of the erythrocyte membrane [25], and it is an integral part of the sulfhydryl group pool that keeps Hb in its reduced state [26].

Under conditions associated with excessive oxidant generation, high levels of oxidized GSH obtained, e.g. glutathione disulfide (GSSG) can be externalized in order to prevent cytotoxicity. This mechanism may be responsible for the decrease of GSH levels or decreased GSH/GSSG ratio in erythrocytes [27,28]. However, GSSG can be reverted to its reduced form by the action of glutathione reductase (GR), which uses NADPH, as a reducing agent [27,28]. Likewise, thioredoxin reductase (TR) uses NADPH from the pentose phosphate pathway (PPP) to reduce oxidized thioredoxin (TrxS2) to its dithiol form (Trx (SH)₂), the latter being responsible for reducing PrxS2 [29,30]. Undoubtedly the per-oxidase activity of Prx enzymes, a very large and highly conserved family of peroxidases, is critical to protect RBCs from oxidative damage [31], since Prxs are the third most abundant protein in the erythrocyte [29,31]. In addition to their peroxidase function,

Prx2 (one of the six isoforms found in humans) appears to act as Hb molecular chaperone under conditions of oxidative stress [32]. For instance, during erythropoiesis by maintaining adequate Hb folding and after erythrocyte maturation, by preventing Hb denaturation [29,33,34]. Furthermore, Prx2 binds to free heme with high affinity, probably in order to avoid oxidative reactions triggered by it [35].

Another constituent of the intra-erythrocyte sulfhydryl group pool is ergothioneine (Ergo), which, curiously, is the second most abundant thiol in RBCs [26]. Thus, it seems logical to assume that Ergo is an antioxidant specialized in the protection of this cell type, but the reasons for such abundance have not yet been sufficiently investigated. Furthermore, RBCs have a plasma membrane redox system (PMRS) that transfers electrons from intracellular substrates to extracellular electron acceptors which may be NAD^+ or/and dehydroascorbic acid [36]. Therefore, RBCs uniquely function to protect Hb via a selective barrier, allowing gaseous and other lig and transport as well as providing enzymatic mechanisms to maintain Hb in a functional nontoxic state [37], and antioxidant protection not only to themselves but also to other tissues and organs in the body [38].

In addition to the antioxidant system, erythrocytes have an efficient energy production, which derives exclusively from glucose degradation through the glycolytic pathway and subsequent lactate production, due to the absence of mitochondria, producing ATP and NADH [39,40], while ATP is involved in the maintenance of erythrocyte integrity, NADH is the main reducing agent of met Hb, keeping Hb in the ferrous state. This last reaction is catalyzed by NADH-cytochrome b5 reductase, also known as the meta Hb reductase [41]. Human erythrocytes express very high levels of insulin-independent glucose transporter (GLUT1) that ensures, through passive transport, high glucose concentration in the erythrocyte cytosol, normally close to that in the plasma [42,43]. Approximately 90% of that glucose is metabolized through the glycolytic pathway to produce ATP [23]. Moreover, RBCs have a nucleotide metabolism that assists in the maintenance of the energy balance in erythrocytes, through the purine metabolic cycle [44]. The remaining glucose is directed to the PPP, whose main function is the production of redox potential in the form of NADPH [40], by successive oxidation reactions catalyzed by glucose-6-phosphate dehydrogenase (G6PDH) and phosphor gluconate dehydrogenase [23,40].

The close relationship between the glycolytic and PPP is evident, once the glycolytic flux is modulated by a competition between glycolytic enzyme, phosphofructokinase-1 (PFK-1) and deoxygenated Hb (deoxy-Hb) by the cytoplasm domain of the protein band 3 (CDB3) [45]. Briefly, conditions of low concentration of O_2 promote the release of PFK-1 from CDB3, induced by deoxy-Hb binding, increasing the flow of glucose to glycolytic, consequently the production of 2, 3-BPG, through Luebering - Rapoport pathway, which allosterically regulates O_2 release from Hb [46]. On the other hand, since glucose consumption is constant, in erythrocytes exposed to high O_2 concentrations,

PFK-1 is inhibited by its binding to CDB3, favouring glucose directing to PPP, in order to ensure adequate levels of NADPH needed to protect the erythrocytes of lesions triggered by oxidant [45]. Furthermore, Zhang et al. [43] demonstrated in vitro that under induced oxidative stress situations, erythrocytes shifted glucose metabolism towards the oxidative PPP, seeking NADPH production for oxidant mitigation. However, perturbations in these pathways involved in RBC cellular function and survival can lead to an enhanced flow of pro-oxidant generation, culminating in oxidative stress, consequently in loss of metabolic homeostasis and premature senescence.

Disruption of Redox-Related Metabolism by Hbs

Sickle erythrocytes are characterized by the presence of HbS, instead of normal HbA. As mentioned previously, HbA undergoes autoxidation at normal physiological rate, while HbS is highly unstable thus altering this rate. According to Hebbel et al. [47], HbS has tendency to autoxidise 1.7 times faster than HbA. This accelerated autoxidation causes premature HbS denaturation, leading to formation of hemi chromes, which have high affinity for CDB3 [48,49], mediating the oxidative cross-linking of CDB3 by disulfide bonds [50]. The result is the band-3 clustering and its dissociation of cytoskeleton proteins by ankyrin binding rupture [50,51]. As mentioned before, one of the band-3 functions is to bind to glycolytic enzymes and organize them into the membrane, thereby regulating the glucose flux between glycolysis and PPP [52]. Therefore, the release of glycolytic enzymes from the oxidized band-3 might be responsible for the alterations in the activities of glycolytic and PPP enzymes [12], as well as in the levels of NADH and NADPH [53], previously reported in the literature, in sickle erythrocytes.

Moreover, upon de-oxygenation, HbS molecules expose hydrophobic contacts formed between valine of one HbS molecule and alanine, phenylalanine and leucine from an adjacent HbS [54,55]. This crystallization produces a polymer nucleus, which grows and fills the erythrocyte, disrupting its architecture and flexibility and promoting cellular dehydration, with physical and oxidative cellular stress [56]. Nevertheless, HbS polymerization is reversible; fibers “melt” as oxygen is taken up by the HbS and the normal discoid shape returns [10]. This re-oxygenation phase might be considered the major source of pro-oxidant production in SCD [18,57]. Hence, sickle erythrocytes have been reported to generate two fold greater extent of O₂•⁻, H₂O₂, hydroxyl radical (HO•) and lipid oxidation products compared with HbA-containing erythrocytes [18,47,58]. The increased and unremitting pro-oxidant generation in sickle erythrocytes results in excessive antioxidant consumption and thus antioxidants deficiency [11,59,60] that may also trigger glucose metabolism shifting.

The repeated polymerization/de-polymerization process can also lead to blood cell adhesion, vaso occlusion, ischemia-reperfusion injury and hemolysis [5,10]. During periods of cellular hypoxia or stress, adenosine is released from cells along with the

adenine nucleotides, ATP, ADP, and AMP, which are converted to adenosine by ectonucleotidases [61,62]. In addition, Zhang et al. [63] demonstrated that adenosine can enhance 2,3-BPG production via A2B receptor activation, suggesting that elevated adenosine had an unrecognized role in normal RBCs to promote O₂ release and prevent acute ischemic tissue injury. However, this response is even more pronounced in SCD patients due to increased amounts of ATP in the circulation derived from chronic sickle red cell hemolysis and tissue damage from vaso occlusion [64]. Thus, in sickle erythrocytes, the beneficial role of excessive adenosine-mediated 2,3-BPG induction becomes detrimental by promoting deoxygenating, HbS polymerization and subsequent sickling [63].

Hemolysis increases the concentration of free plasma Hb, which in the ferrous (Fe²⁺) valence state, is readily available to participate in Fenton-based redox reactions [65]. Under normal conditions, this potential source of oxidative stress is minimized by haptoglobin (Hp) [66]. However, in SCD people, plasma Hp levels are low due to chronic hemolysis [67] that overwhelms endogenous plasma Hp levels and other scavenging mechanisms. Moreover, with extracellular Hb the body not only need to deal with the autoxidation reaction but also with the heme that dissociates from met Hb [68].

Heme is a low molecular weight hydrophobic molecule that can be taken up by cell membranes, plasma proteins, and lipids. In the same manner as Hp-Hb scavenging system, plasma hemopexin (Hpx) sequesters heme in an inert, non-toxic form and transports it to the liver for catabolism and excretion, preventing heme's pro-oxidant and pro-inflammatory effects [66,69]. However, once more the characteristic hemolytic condition of SCD persons overwhelms endogenous scavenging capacity of plasma Hpx [67], enabling further deleterious effects of heme and by-products. Taken the above observations into consideration, the presence of HbS generates a series of Consequences for individuals in a vicious cycle in which many different oxidative processes play a central role in the path physiology, thus in the further clinical consequences.

Conclusion

Human erythrocytes are normally exposed to continuous pro-oxidant sources, both intra and extra cellular, as a consequence of their physiological role. Although reductive capacity of an erythrocyte is 250 times higher than its potential oxidizing agents are, HbS is capable of several alterations that overwhelm the antioxidant defence system, leading to the loss of metabolic homeostasis due to the integration of biochemical pathways briefly depicted in this review. Therefore, more studies regarding the main enzymes and by products involved in erythrocyte metabolic homeostasis remains a worthy and promising goal, which might lead to more specific and effective prognostic biomarkers, as well as to new therapeutic targets or agents capable of improve the sickle erythrocyte metabolism.

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Conflict of Interest

The authors declare no competing financial or relationship with other people or organizational interests. Furthermore, the authors have materially participated in the article preparation and approved the final article version.

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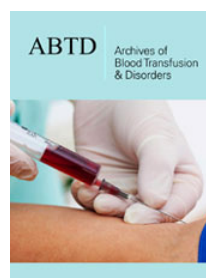
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