thalassemia reports

Perspective

What Is the Relevance of Murburn Concept in Thalassemia and Respiratory Diseases?

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Abstract: Murburn concept is a novel perspective for understanding cellular function, deeming cells as simple chemical engines (SCE) that are powered by redox reactions initiated by effective charge separation (ECS). The 1-electron active diffusible reactive (oxygen) species, or DR(O)S, equilibriums involved in these processes are also crucial for homeostasis, coherently networking cells, and rendering electromechanical functions of sensing and responding to stimuli. This perspective presents the true physiological function of oxygen, which is to enable ECS and the generation of DR(O)S. Therefore, DR(O)S must now to be seen as the quintessential elixir of life, although they might have undesired effects (i.e., the traditionally perceived oxidative stress) when present in the wrong amounts, places and times. We also elaborated that tetrameric hemoglobin (Hb) is actually an ATP-synthesizing murzyme (an enzyme working via murburn concept) and postulated that several post-translational modifications (such as glycation) on Hb could result from murburn activity. Murburn perspective has also enabled the establishment of a facile rationale explaining the sustenance of erythrocytes for 3–4 months, despite their lacking nucleus or mitochondria (to coordinate their various functions and mass-produce ATP, respectively). Although thalassemia has its roots in genetic causation, the new awareness of the mechanistic roles of oxygen-hemoglobin-erythrocyte trio significantly impacts our approaches to interpreting research data and devising therapies for this malady. These insights are also relevant in other clinical manifestations that involve respiratory distress (such as asthma, lung cancer, COVID-19 and pneumonia) and mitochondrial diseases. Herein, these contexts and developments are briefly discussed.

Keywords: murburn concept; murzyme; hemoglobin; erythrocyte; thalassemia; superoxide; respiratory/mitochondrial diseases

Citation: Manoj, K.M. What Is the Relevance of Murburn Concept in Thalassemia and Respiratory Diseases? *Thalass. Rep.* **2023**, *13*, 144–151. https://doi.org/10.3390/thalassrep13020013

Academic Editor: Aurelio Maggio

Received: 28 February 2023
Revised: 3 May 2023
Accepted: 10 May 2023
Published: 12 May 2023

1. Introduction

Erythrocyte defects/disorders of genetic origin such as thalassemia lead to anemia, respiratory and several other diseases, which pose a significant burden in human society [1]. Murburn concept is a term I originally introduced into the scientific jargon in 2015, while presenting the interpretation of experimental works on heme-enzymes cytochrome P450 (CYP) at the 35th Midwest Enzyme Chemistry Conference (Chicago) and 20th North American ISSX Meeting (Orlando) [2]. In toto, about four dozen articles in mainstream research journals, books and popular web (Internet) portals [2–49] have featured murburn concept. They also include invited reviews [10,14,16,39,45] and cover-page credited pieces or special editorial mentions [7,11,12,17,19,27,45]. The first workshop on murburn concept was conducted in March 2023 at IIT Bombay [50].

2. What Is Murburn Concept?

The coining of the term murburn stems from the fusion of "mured" (closed) and "burning" (a rather chaotic redox process that usually involves oxygen) [10]. This terminology is an effort to capture the essentially stochastic scheme of reactions/processes that could involve a DRS such as superoxide (an oxygen-centered ionic radical) or singlet oxygen

and derivatives thereof (such as hydrogen peroxide, hydroxyl radical, hydroxide ion, etc.). Murburn concept is as an evidence-based rationale that vouches for the intermediacy of diffusible reactive species (DRS) in routine cellular metabolism and physiology (Figure 1).

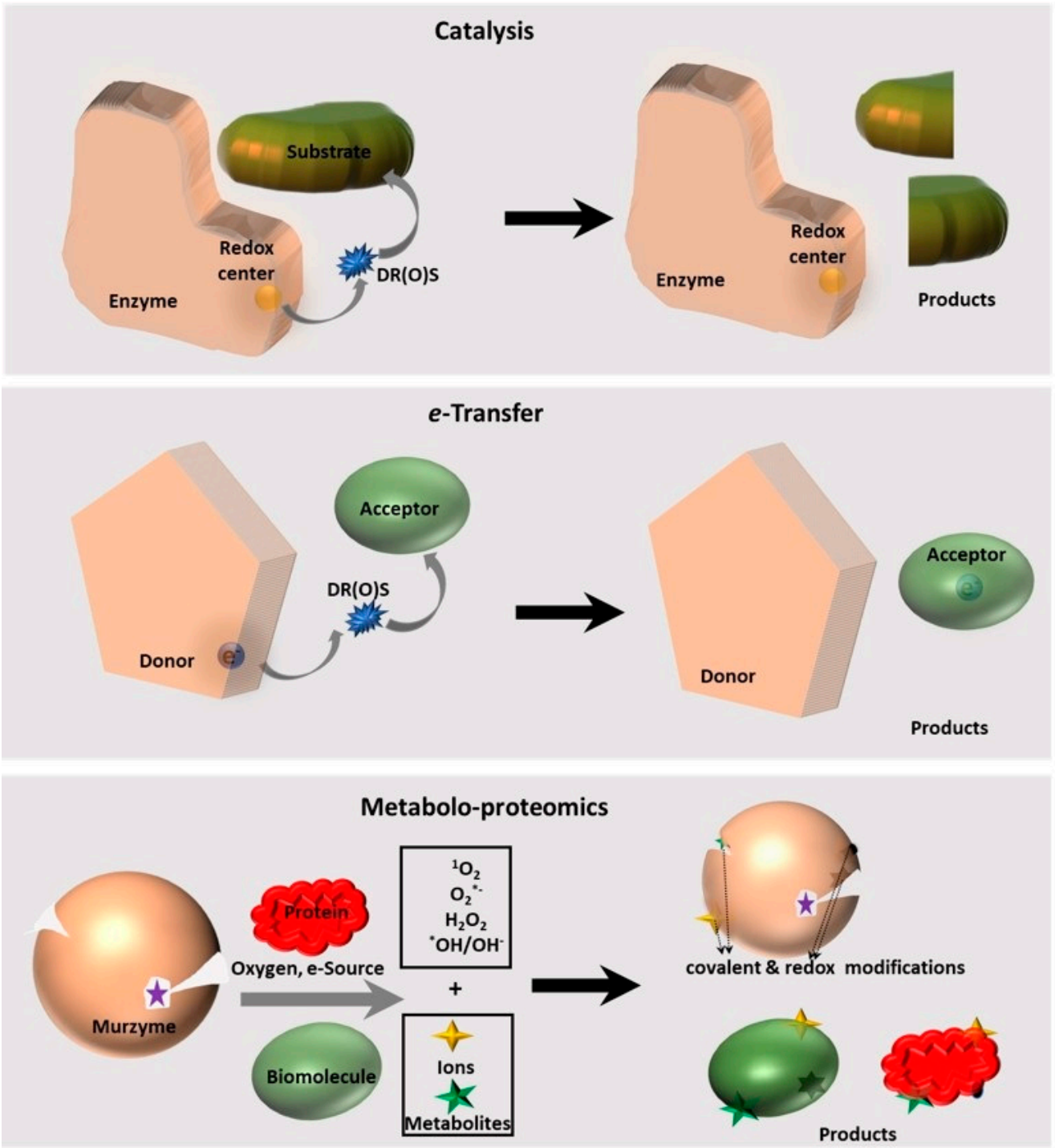

Figure 1. The essential principles of murburn concept are depicted graphically. Topological complementation of the enzyme–substrate as a lock–key or induced fit complex is not necessary in murburn catalytic (**top panel**) or e-transfer (**mid-panel**) schemes between the donor and acceptor, as it involves the intermediacy of enzyme-produced or enzyme-stabilized DR(O)S. The DR(O)S generated in milieu can also move moieties onto proteins/biomolecules, thereby enlarging the metabolo-proteomic landscape of the cell (**bottom panel**).

This new perspective explains the anomalous kinetics (generic activations, inhibitions, multi-phasic substrate dependence, etc.) and unusual mechanistic signatures (diversity of substrates, kinetic isotope effects, conversion of active-site excluded molecules, etc.) seen in diverse forms of hemo/flavo-protein-mediated catalysis leading to oxygen insertions, bond breakages, and other inter- and intra-molecular electron or moiety/group transfer reactions [51–61]. Questioning the acclaimed and long-standing explanations, this new insight was applied to elaborate upon a bevy of fundamental metabolic and physiological contexts. DRS-mediated oxygen–water-equilibrium-centric murburn models were provided for the roles of biomolecules and processes involved in: powering (respiration, photosynthesis, thermogenesis), homeostasis (xenobiotic clearance, ion differentials, volume constancy, etc.), electro-mechanical activities (water mobilization), sensing and response to stimuli (vision), metabolo-proteomics, physiological dose responses, trans-membrane potential fluctuations, inflammatory immune responses, etc. [2–61]. In murburn perspective, cells are seen as simple chemical engines (SCE) that are initiated by effective charge separation (ECS) [40]. That is, proteins with cofactors that contain a *d*-electron or extensively conjugated π-electron system (e.g., heme and flavo proteins, respectively) may enable oxygen activation, particularly in the presence of reduced nicotinamides (which contain two electrons but only one hydrogen atom equivalent). This simple system enables flavin- and oxygen-based ECS and heme-based spin conversions and high potential radical generation. Several redox proteins can stabilize the DRS [23,26] and even proteins that lack redox active centers can utilize DRS [37,40], thereby qualifying upon several poorly understood aspects of bioenergetics and electrophysiology. As a consequence, the stochastic principle of murburn concept serves as a supplementary/complementary principle to the deterministic central dogma for affording a satisfactory explanatory paradigm for the origin, sustenance and termination of cellular activities [44]. Under the new perspective, murzymes are seen as those proteins that work via murburn concept, generating, modulating, stabilizing or utilizing DRS.

3. How Is Murburn Concept Relevant in Thalassemia and Respiratory/Mitochondrial Diseases?

The pathophysiology of thalassemia stems from mutation(s) in hemoglobin (alpha-beta gene(s), leading to poor assembly/function of functional hemoglobin (Hb) and underproduction or poor maturation of erythrocytes [62,63]. The currently adopted clinical approach centers around basic strategies involving: (a) the administration of small/large molecules such as hydroxyurea to enhance Hb production and erythrocyte osmolarity/turgor [64], folate to aid erythropoiesis [65], chelation agents to counter Fe-overload [66], growth hormones to alleviate the limited development of body [67], and recombinant proteins (e.g., luspatercept or Reblozyl) aiding better erythrocyte population [68]; (b) blood transfusion [69]; (c) bone marrow/stem cell transplantation [70,71]; (d) gene therapy (e.g., CRISPR-methodology and Zynteglo) [72,73], etc. Murburn concept is relevant in all of these contexts and also in a bevy of other redox/respiratory and mitochondrial diseases (which are also gene-based ailments) that are supposed to involve "oxidative stress". Elucidation of the routes and details of the physiological function of DR(O)S shall provide a strong etiology to differentiate the pathological symptoms and enable us to fine-tune the care measures provided in clinical settings. This is because all cellular activities depend also on murzyme/murburn-based activities, and an understanding of pivotal aspects such as redox homeostasis, oxygen utilization by cells/mitochondria, ATP-synthesis and energy metabolism, the functioning of heme proteins such as hemoglobin, etc., is absolutely essential for understanding, detecting and treating such diseases.

3.1. The Modality of Oxygen Utilization by Proteins/Cells

In the classical purview of respiratory physiology, molecular oxygen is primarily needed to serve as the terminal electron acceptor, staying wedded to Complex IV (also called cytochrome oxidase complex, found in the inner membrane of mitochondria), ultimately

making two molecules of water. In other metabolic schemes (such as that of CYPs in endoplasmic reticulum mediated xenobiotic clearance), once again, oxygen was supposed to stay bound at the heme center of proteins, hydroxylating or oxidizing molecules tightly bound to the heme protein. In such classical schemes, DRS such as superoxide and hydroxyl radicals (or even molecules such as hydrogen peroxide and singlet oxygen) (Figure 2) were deemed as unavoidable toxic waste products. Although the binding of oxygen at heme centers and O-atom insertion thereafter cannot be denied or refuted, my group's pursuits have conclusively demonstrated [2–61] that without the ECS and form of catalysis afforded by DRS, several of the routine metabolic/physiological functions would not transpire. This is a profound paradigm-shifting perception in biological science, which explains why we need oxygen so critically and how/why aerobic life forms thrive on Planet Earth now [44]. So, when DRS are experimentally observed in erythrocytes or other cells, it should no longer be deemed as purely a manifestation of pathophysiology! The contextual (spatial, temporal and quantitative) aspects are more important, and the purely aesthetic disposition of deeming DRS as unwanted is unwarranted.

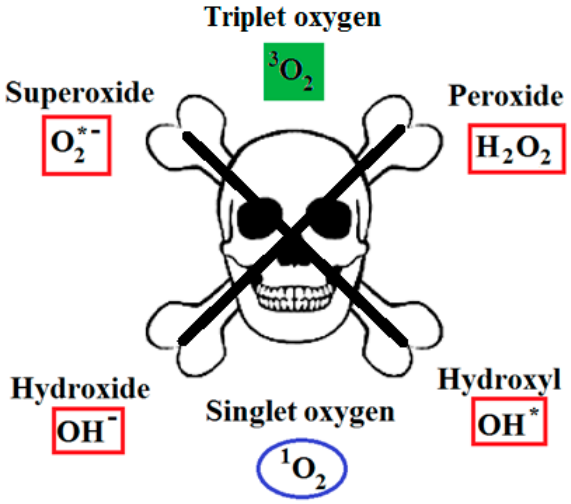

Figure 2. Murburn concept postulates that DRS produced in oxygen–water equilibriums are not to be seen purely as "oxidative stress" agents (dangerous and toxic waste products), but they serve as obligatorily required intermediates essential for cellular powering, coherence, homeostasis, electromechanics, etc.

3.2. The Novel Function of Hemoglobin as a Murzyme ATP-Synthase

Tetrameric hemoglobin is perhaps one of the most studied proteins and has been recognized to have multiple functions in addition to transporting oxygen [74]. However, it is unclear why the main oxygen binding protein is hetero-tetrameric in blood, whereas in muscle tissues, the oxygen binding protein of myoglobin is monomeric. We found that the highly packed hetero-tetrameric Hb serves as an ATP-synthase in erythrocytes, by virtue of Fe(II)-O_2 and Fe(III)-O_2^{*-} binding and dissociation equilibriums, and their stochastic nature and statistical outcomes. This role of Hb makes up for the inadequate output of glycolytic ATP-synthesis in RBC and explains the hetero-tetrameric structure of Hb, with the pore on the beta globin monomer [25]. In this connection, it must also be noted that the DR(O)S production ability of Hb enables it to catalyze several auto- and hetero- post translational modifications such as glycations, phosphorylations, etc., which are also important markers in clinical research [42].

3.3. Erythrocyte Sustenance without Mitochondria and Nucleus

The classical perspectives require the nucleus to maintain protein levels and these proteins regulate cellular concentrations of metabolites and ions via purely affinity-driven measures. Furthermore, given the high amounts of energy expense for the ion-pumping

perception to maintain Na-K differentials, mitochondria are also expected for energy supplementation in RBCs. Murburn concept obviates these predicaments to explain coherent and homeostatic functions [24,27,30,32,44], and this aspect is also relevant (and explored further) for cellular morpho-mechanics.

4. Future Research Agenda and Therapeutic Regimen

Thalassemia and mitochondrial diseases owe their etiology to genetic causations. Yet, in light of the impacting realities (Sections 3.1–3.3) unveiled recently, the future research agenda and clinical care in respiratory diseases area should be reoriented to enhance the efficacy of oxygen-aided functionalism, as it is evident that murburn perspective governs the physiological interaction scheme of redox proteins, biomolecules and oxygen. The long-standing aesthetic stigma suggesting that DR(O)S are merely disruptive and unavoidable agents should give way to a more realistic outlook on the viability and obligatory requirement for their necessary roles in the sustenance of life.

To reiterate: given the fact that: (a) NO (nitric oxide, a DROS!) is already recognized as a molecular messenger; (b) the classical bioenergetics paradigm of electron transport chains, proton-pumps and rotary ATP synthesis, etc., are untenable [9]; (c) the DROS-based murburn concept provides a thermodynamically/kinetically and evolutionarily viable explanation for cellular powering [27,44]; and (d) the global and acute toxicity of small doses of cyanide cannot be explained without invoking murburn concept [14], it is highly opportune to reorient redox biomedical research and clinical therapy efforts for respiratory diseases. It is now imperative to understand the contexts of DR(O)S playing Dr. Jekyll and Mr. Hyde, and to accommodate the murburn perspective.

Funding: This research received no external funding.

Data Availability Statement: All data required to peruse this manuscript are provided herein.

Acknowledgments: The pursuit was powered by Satyamjayatu: The Science & Ethics Foundation.

Conflicts of Interest: The author declares no conflict of interest.

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