Adaptations to Hypoxia and Redox Stress
Essential Concepts Confounded by Misleading Terminology

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Cells and organisms have developed a remarkable array of responses to adapt to hypoxic environments. In this Viewpoint, the author discusses the definitions and determinants of redox stress in hypoxia, the relationships among the changes in glucose metabolism, and the alterations in cell redox state.

The ability to adapt to hypoxia is a fundamental survival strategy for all living organisms. In part, these adaptive mechanisms likely evolved from primordial metabolic programs in the hypoxic environment of the Proterozoic Eon† within which ATP is generated from atypical energy sources, such as hydrogen sulfide. The best-studied adaptive mechanism is, of course, stabilization of the transcription factor, hypoxia-inducible factor-1, which leads to changes in gene expression that enhance glycolysis and attenuate oxidative phosphorylation. In recent years, the transition from oxidative phosphorylation to glycolysis has also been demonstrated in normoxic environments and shown to be induced by mediators such as D(R)-2-hydroxyglutarate, an oncometabolite product of the neomorphic activity of mutant isocitrate dehydrogenases (IDH1 and IDH2), fumarate, and hydrogen sulfide. These and other related mechanisms decrease the cell’s metabolic dependence on molecular oxygen for ATP synthesis.

In addition to its effect on cellular energetics, the shift from oxidative phosphorylation to glycolysis in hypoxic environments serves another key purpose: it decreases the generation of potentially injurious reactive oxygen species (ROS). With hypoxia (but not anoxia), the mitochondrial electron transport chain becomes inefficient and leaks electrons to molecular oxygen, generating superoxide anion and hydrogen peroxide (by spontaneous or enzyme-catalyzed dismutation). The determinants of superoxide generation in these conditions include high proton motive force, increased ubiquinol/ubiquinone, and increased NADH/NAD⁺ in the setting of decreased ATP synthesis. The shift from oxidative phosphorylation to glycolysis offsets these adverse effects, in part, by decreasing carbon flux through the tricarboxylic acid cycle to limit further NADH generation, the essential source of reducing equivalents for the electron transport chain. In addition, as we have shown, the action of the hypoxamir 3 miR-210 impairs the assembly of nonheme, iron-sulfur cluster-containing proteins by suppressing expression of the scaffold proteins ISCU 1/2.† These proteins facilitate incorporation of nonheme iron–sulfur clusters into apoproteins, such as aconitase and the cytochromes, thereby decreasing redox-active iron sources of reducing equivalents for superoxide generation. A central consequence of these adaptive responses that limit electron leak is preservation of the optimal NADH/NAD⁺, especially in mitochondria. Our work on highly specific fluorescent sensors that can be used in live cells to record NADH and NADH/NAD⁺ demonstrates that the cell defends mitochondrial NADH/NAD⁺ at the expense of its cytosolic counterpart. Yet, even with this wide range of metabolic modifications in hypoxia, NADH continues to be generated through glycolysis, albeit at a reduced rate.

How, then, does the cell deal with the mounting pool of reducing equivalents to minimize ROS generation and optimize NADH/NAD⁺? To begin to address this question, I must digress a bit to discuss the distinctions among oxidative (oxidant) stress, reductive stress, and redox stress, and how the use of these terms differs between biologists and chemists. The term “oxidative stress” was first used by Paniker et al 7 in 1970 in studies of the response of the erythrocytic glutathione–glutathione disulfide system to hydrogen peroxide. Because of its effect on the oxidation of erythrocytic glutathione to glutathione disulfide, exogenous hydrogen peroxide was viewed as imposing an oxidative (ie, oxidizing) stress on the cell. Since that time, the notion of oxidative stress as a mechanism for inducing a unique set of adaptive homeostatic responses in cells and organisms has evolved, both as an experimental method for exploring adaptive redox responses and as a putative determinant of aging and of many pathobiological processes. To biologists, oxidative stress implies that a cell or organism produces or is exposed to an excess of (highly) reactive molecules, usually oxygen-centered, nitrogen-centered, or both, that exceeds endogenous antioxidant capacity (oxidoreductase enzyme activities or small-molecule antioxidants). From the chemical perspective, however, oxidative stress as defined by biologists is a vague, imprecise concept that fails to recognize explicitly what the oxidants are in a given set of circumstances, how their reactivity is countered or controlled, and, most important, what the reductants are to which the oxidants must be coupled for the redox reactions to proceed. Achieving chemical precision, however, is not without its challenges in complex biological redox systems for the following reasons: (1) The specific ROS are difficult to identify because of incomplete knowledge of their sources, sinks, and fluxes. (2) Their chemical reactions are often rapid compared with the time scale of typical measurement techniques. (3) Free radical...
ROS participate in a wide range of potential propagating and terminating reactions. (4) There is compartmental heterogeneity in the distribution of ROS within the cell. (5) They are difficult to track in real time because of the lack of ideal reporter molecules for each ROS. (An ideal reporter is one that reacts with the ROS of interest with exquisite specificity, with sufficient rapidity, and at low enough concentrations so as not to affect the steady-state or transient concentration of the specific ROS it is designed to track.)

Regardless of the definition of oxidative stress and its quantitative determination, its biological consequences can only be recognized in light of the reductants that have been oxidized and the effects of those coupled redox reactions on the functional pathways in which the redox-active molecules are involved. Clearly, these are complicated biochemical systems processes that defy explication and are not served well either by overly simplistic biological generalizations or by unrealistic expectations of chemical precision in a statistically complex ensemble of reactions.

Most biologically important oxidants are derivatives of molecular oxygen or molecular oxygen itself. Partially reduced forms of molecular oxygen, including superoxide anion and hydrogen peroxide, are viewed as ROS that contribute oxidant stress. However, from a simple chemical perspective, these molecules can both oxidize molecules of the appropriately matched (ie, more negative) redox potential and reduce other molecules of the appropriately matched (ie, more positive) redox potential. For example, superoxide anion can reduce disulfides and oxidize α-tocopherol, leading to its oxidation to molecular oxygen and its reduction to hydrogen peroxide, respectively, whereas hydrogen peroxide can reduce ferrylhemoglobin and oxidize methionine, leading to its oxidation to superoxide anion and its reduction to water, respectively. Why, then, do we typically view an excess of superoxide or hydrogen peroxide as providing simply an oxidative stress? As partially reduced forms of molecular oxygen able to participate in redox reactions in which they reduce molecules with more positive redox potential, they could just as well be viewed as imposing a reductive stress on the system from a chemical perspective. The answer to this question can be found in terminology. By convention, biologists focus on the oxygen-centered nature of ROS when they define oxidative stress without regard for the precise chemistry of the redox reactions in which these species can engage or for the precise change in the local redox microenvironment in which they are generated. By contrast, chemists tend to focus on the flow of electrons (or hydride anion) in the redox-coupled reactions in which ROS participate.

How does reductive stress fit into this discussion, and what adaptive changes have evolved to accommodate it? The term reductive stress was first used by Gores et al31 in 1989 in studies of hypoxia in hepatocytes. From a biological perspective, reductive stress recognized in this setting is the complement of oxidative stress by virtue of its providing an excess of reducing equivalents that cannot be adequately accommodated by endogenous oxidoreductases or existing oxygen tensions in the local environment (oxygen being a terminal electron acceptor in electron transport and redox reactions in living systems). From a chemical perspective, reductive stress suffers from the same misleading implications as oxidative stress, often confusing readers and reviewers in the process. For example, even in the absence of reoxygenation or reperfusion, sustained hypoxia leads to an increase in ROS, which suggests an oxidative stress from the biological perspective; however, as the above discussion indicates, these ROS are partially reduced forms of molecular oxygen that clearly can evolve in the setting of increased reducing equivalents, their consequences again depending on the redox-coupled reactions in which they are engaged. Given these complexities, erroneous oversimplifications, and chemical imprecision, if biologists or chemists need to use a generic term to discuss disturbances in oxidation–reduction reactions that arise from an excess of oxidants or reductants and their functional consequences in a biological system, the term “redox stress” should be used. Equally important, however, is the need to be as explicit as possible about the particular molecular species involved in redox reactions that modify biological phenotype.

With these concepts in mind, let us return to the redox adaptations that accompany hypoxic metabolism and consider how the cell accommodates the increasing production of reducing equivalents. Remember that while redirecting glucose metabolism to glycolysis decreases NADP production by the tricarboxylic acid cycle and decreases leaky electron transport chain flux, glycolysis continues to produce NADH (2 moles per mole of glucose). How the cell handles this mounting pool of reducing equivalents remained enigmatic until recently. Two groups, ours9 and Intlekofer et al,10 showed that in the setting of hypoxia, all cells reduce 2-oxoglutarate to the L(S)-enantionimer of 2-hydroxyglutarate. Furthermore, we showed that L(S)-enantionimer of 2-hydroxyglutarate has 3 important functions: (1) it serves as a reservoir for reducing equivalents and buffers NADH/NAD+ in that NADH provides the reducing potential required to synthesize it from 2-oxoglutarate; (2) it decreases glycolysis (by a mechanism yet to be determined), distinguishing it from its oncometabolite enantiomeric counterpart, D2HG, which increases glycolysis; and (3) it increases pentose phosphate pathway activity. Redirecting carbon flux from glycolysis to the pentose phosphate pathway further decreases NADH generation shifting reducing equivalents into the NADPH pool. The increase in NADPH, in turn, provides the reducing equivalents needed to maintain cellular erythrocytic glutathione for ROS elimination, especially peroxides via the action of the glutathione peroxidases, with glutathione peroxidase-1 being the greatest consumer of cellular glutathione under redox stress conditions. The increase in NADPH also fuels NADPH oxidase(s) activity, which is important in attenuating the consequences of the redox stress of hypoxia, perhaps by the extracellular disposition of superoxide and hydrogen peroxide.11

Other cellular adaptations to redox stress exploit the pleiotropic chemistry of the sulfhydryl group (especially protein
cysteinyl residues), its variable redox potential as governed by the protein microenvironment within which it resides, and the modification of that redox potential by local conformational or redox changes. These dynamic changes in the redox state of protein cysteinyl residues have led us to propose the concept of functional, as opposed to structural, disulfides, the reduction of which modifies protein function rather than alters the structural integrity of protein conformation.12,13

Given the ubiquity of the stress of hypoxia throughout evolution, there are, no doubt, other adaptive mechanisms yet to be discovered. For example, recent work suggests that fermentative molecular hydrogen production is used by some facultative aerobes to survive reductive stress during hypoxia14 and that hydrogen sulfide has evolved to serve as an oxygen sensor in certain organisms. As these mechanisms are explored, it will be important to consider their consequences both for cellular energetics and for cellular redox state. Although these two metabolic processes are inextricably linked at several levels, adaptive homeostatic responses that address them are often distinct. Understanding this complexity in all of its dimensions from both chemical and biological perspectives offers unique therapeutic strategies by which cellular function and phenotype can be preserved and hypoxic injury mitigated.

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References
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