Chapter 1
The Concept of Oxidative Stress After 30 Years

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Abstract *Oxidative stress is an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage* (Sies H, Jones DP, Oxidative stress. In: Fink G (eds) Encyclopedia of stress. 2nd edn, vol 3, Elsevier, Amsterdam, pp 45–48, 2007). The concept of oxidative stress, first formulated in 1985, is presented and discussed in the context of current developments. The role of hydrogen peroxide in oxidative stress and redox signaling has come into focus, with attempts to explore spatio-temporal control. Special aquaporins serve as peroxiporins. Research on molecular redox switches governing oxidative stress responses is in full bloom. On the more practical side, cautious use of terminology and methods regarding the so called ROS, reactive oxygen species, is recommended. The major role in antioxidant defense is fulfilled by antioxidant enzymes, not by small-molecule antioxidant compounds. The field of oxidative stress research embraces chemistry, biochemistry, cell biology, physiology and pathophysiology, all the way to health and disease research, ultimately providing a scientific basis for a modern redox medicine.

Keywords Oxidative stress • Redox homeostasis • Stress responses • Redox code • Hydrogen peroxide • Redox signalling • Molecular redox switches • Redox medicine

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1 Introduction

The concept of oxidative stress has been introduced for research in redox biology and medicine in 1985, now over 30 years ago, in an introductory chapter [1] in the book entitled *Oxidative Stress*. A concurrent comprehensive review entitled "Biochemistry of Oxidative Stress" presented the knowledge on prooxidants and antioxidants and their endogenous and exogenous sources and metabolic sinks [2]. Since then, redox biology as a research area has found fulminant development in a wide range of disciplines, starting from chemistry and radiation biology through biochemistry and cell physiology all the way into general biology and medicine. A recent commentary dealt with the merits and pitfalls of this concept: "Among the merits is the notion which is elicited by the two terms, namely (i) that aerobic metabolism is a steady-state characterized by a redox balance, as denoted by the term oxidative (and implicitly, reductive), and (ii) that strains in the balance can occur, as denoted by the term "stress". This latter concept evokes the occurrence of biological stress responses" [3]. Research on oxidative stress responses is in full bloom, concerning the functioning of central master switches, for example NF-kappaB or Nrf2/Keap1 in eukaryotes, or OxyR in prokaryotes. Much of the redox signaling occurs through molecular thiol redox switches, with significance of particularly reactive cysteines in specialized proteins [4, 5]. Regarding pitfalls of the concept, the term oxidative stress has been over-stressed, both in the public perception and in research circles. Indeed, the very first sentence in Ref. [1] is: "As a biochemist, one may wonder whether Selye's term should be stressed as it is in the present context". At the beginning of 2016, the number of hits in PubMed for "oxidative stress" is more than 1,000 entries per month, currently totalling over 153,000.

2 Concept of "Oxidative Stress"

The definition of oxidative stress as a global concept, in 1985, was [1]: *A disturbance in the prooxidant-antioxidant balance in favor of the former*. It was an important point, from the beginning, that there is a diversity of prooxidants and a diversity of antioxidants, operating with vastly different chemical and biological reactivities. Likewise, it may be mentioned that there is also a huge diversity of molecular targets: DNA, RNA, proteins, lipids, carbohydrates and other biomolecules. Prooxidants include free-radical species and non-radical species generated by enzymes or non-enzymatically, and antioxidants include powerful enzymes and also low-molecular mass compounds. A noteworthy insight was the perception that oxidation-reduction (redox) reactions in living cells are utilized in fundamental processes of redox regulation, collectively termed "redox signaling" and "redox control". The concept of oxidative stress was updated in 2007 to include the role of redox signaling [6]: *Oxidative stressis an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage*. The main idea underlying this global concept is that in

living biological systems there is a maintenance of a redox balance, that is also called redox homeostasis. This is not an equilibrium as defined in thermodynamics, but it is a steady-state, away from thermodynamic equilibrium, *i.e*. a non-equilibrium [7]. As for a global definition, specific redox systems are not directly and specifically addressed, but they need to in given cases and conditions. Numerous redox systems of different nature coexist in cells and tissues, and not all of them are directly related or connected, contingent on the presence of redox catalysts, i.e. redox-active enzymes or redox-active compounds.

3 The Redox Code

These relationships have recently been conceptualized under four principles which, together, make up the *"Redox Code"* [8]: The *first* principle is the use of the reversible electron accepting and donating properties of nicotinamide in NAD and NADP to provide organization of metabolism, operating at near-equilibrium. Substrate oxidations are linked to reduction of NAD^+ and $NADP^+$, which in turn are linked to ATP production, catabolism and anabolism, respectively. The *second* principle is that metabolism is linked to structure through kinetically controlled redox switches in the proteome, which determine tertiary structure, macromolecular interactions and trafficking, activity and function. The abundance of proteins and reactivity of sulfur switches with oxidants vary over several orders of magnitude to support specificity in biological processes. The *third* principle is that of redox sensing, in that activation/deactivation cycles of redox metabolism, especially involving H_2O_2, support spatio-temporal sequencing in the differentiation and life cycles of cells and organisms.

The *fourth* principle is that redox networks form an adaptive system to respond to environment from microcompartments through subcellular systems to the levels of cell and tissue organization. This adaptive redox network structure is required to maintain health in a changing environment and, if functionally impaired, contributes to disease and organism failure. The term "Redox Code" applies to the redox organisation of cells, tissues and organisms. It is not confined to mammalian cells, and it extends ultimately to all living matter.

4 Specific Forms of Oxidative Stress

Given the enormous variety and range of prooxidant and antioxidant enzymes and compounds and of targets, as mentioned above, subforms of oxidative stress were identified [6], as shown in Table 1.1. Attempts were made to introduce intensity scales ranging from physiological oxidative stress to excessive and toxic oxidative burden [9]. A useful introduction to oxidative stress in biomedical and biological research is available, collecting basic concepts, definitions and currently employed methods used in this field [10].

Table 1.1 Oxidative Stress: definition, specific forms, and classification according to intensity

Category	Term	References
Definition	Oxidative stress	[1, 3, 6]
Specific form	Nutritional oxidative stress	
	Dietary oxidative stress	
	Postprandial oxidative stress	
	Physiological oxidative stress	
	Photooxidative stress	
	Ultraviolet (UV-A, UV-B)	
	Infrared-A	
	Radiation-induced oxidative stress	
	Nitrosative stress	
	Reductive stress	
Related terms	Oxidant stress, Pro-oxidant stress	
	Oxidative stress status (OSS)	
Classification	Basal oxidative stress	[9]
	Low intensity oxidative stress	
	Intermediate intensity oxidative stress	
	High intensity oxidative stress	

Modified from Refs. [3] and [6]

5 Role of Hydrogen Peroxide in Oxidative Stress and Redox Signaling

Hydrogen peroxide, the two-electron reduction product of oxygen, was identified in 1970 as a normal metabolite under aerobic conditions in living cells [11], occurring at about 10 nM intracellular concentration [12]. A major contributor is the mitochondrial respiratory chain [13, 14], notably Complexes I and III, but also Complex II [15]. Important further mitochondrial sources of hydrogen peroxide are given by several dehydrogenases, notably 2-oxoacid dehydrogenases [16, 17], as reviewed in [18]. In liver, H_2O_2 is produced at a rate of 50 nmol/min × g of tissue, which is about 2 % of total oxygen uptake in the steady state of physiological conditions [19]. Metabolically generated H_2O_2 emerged from recent research as a central hub in redox signaling and oxidative stress (see [20]). Hydrogen peroxide is well suited for redox signaling [21], and the role of peroxiredoxins is of paramount importance [22]. A novel aspect of hydrogen peroxide signaling relates to its diffusion properties. While it has long been assumed that hydrogen peroxide diffusion through lipid-containing membranes occurs at a sufficient rate, the discovery of facilitated diffusion through specific aquaporins provides for spatio-temporal control [23]. This discovery opened an exciting field on membrane transport of hydrogen peroxide by peroxiporins [24–26] and its significance for redox signaling [27–29]. See also Ref. [20] for discussion.

6 Molecular Redox Switches

The term "oxidative stress" encompasses the implicit notion of *adaptation*, coming from the general association of stress with stress response. This goes back to Selye's concept of stress as the "general adaptation syndrome" [30, 31]. The role of molecular *redox switches* came into focus in recent years, foremost the dynamic role of cysteines in proteins, opening the field of the redox proteome, currently flourishing because of advances in mass spectrometry and imaging methodology [32]. A bridge between phosphorylation/dephosphorylation and protein cysteine reduction/oxidation is given by the redox sensitivity of critical cysteinyl residues in protein phosphatases, opening the molecular pathway for signaling cascades as fundamental processes throughout biology. Much is yet to be learned in this area of research, as pointed out in a critical review on the impact of thiol peroxidases in redox regulation [33].

What was particularly exciting to many researchers was the discovery of master switch systems [34], prominent examples being OxyR in bacteria [35] and NFkappaB [36] and Nrf2/Keap1 [37] in higher organisms. That batteries of enzyme activities are mustered by activation of gene transcription through a "simple" redox signal is still an exciting strategy [38]. Much of current effort in redox biology is addressed towards these response systems. Obviously, medical and pharmacological intervention attempts are a consequence.

7 Redox Medicine

There are implications of the disruption of redox homeostasis for health and disease processes [39]. As outlined recently [8], the very functioning of cell metabolism is governed by redox processes. Fine-tuning of reaction cascades involves redox-active metabolites such as hydrogen peroxide (see above), nitric oxide, hydrogen sulfide and carbon monoxide. Biomedical research has addressed these aspects in overwhelming detail, beyond the scope of the present article. Just to name a few major topics: aging [40, 41], neurodegenerative diseases [42, 43], cardiovascular diseases/atherosclerosis [44], wound healing [45], cancer [46], immunology [47], and metabolic diseases such as diabetes [48].

There has been considerable effort in identifying potential therapeutic targets, biomarkers and pharmacological and clinical drugs applicable in redox medicine, as recently compiled [49–51].

Nutrition is another huge area impinging on health and disease, with important redox implication. Carbohydrate metabolism, in particular insulin signaling and postprandial oxidative stress, have a major impact [52–54]. Likewise, micronutrients and bioactives play a role. One example is the micronutrient element, selenium, which is present in selenoproteins in the form of selenocysteine. The selenoproteome in humans comprises 25 members with various functions [55, 56]. Viral and

bacterial infections are often associated with deficiencies in micronutrients, including selenium, and the selenium status may affect the function of cells in both innate and adaptive immunity [57]. Our group has addressed other aspects of selenium homeostasis and health [58–60], a large field of current interest.

Physical exercise is another lifestyle factor with implications to redox processes. Principles for integrating reactive species into exercise physiology have been examined [61].

8 Cautionary Words

In contemporary research, to talk simply of "exposing cells or organs to oxidative stress" should be discouraged. Instead, the exact molecular conditions and specific compounds need to be identified. Even more important, in transposing redox considerations into medicine, concrete molecular oxidation-reduction descriptions are to be preferred.

A related pitfall is the use of ROS, which stands for reactive oxygen species, or RNS, which stands for reactive nitrogen species, where again the chemical species involved should be focused and defined whenever it is possible. This issue has been dealt with in detail recently [62]; this guide to free radical research terminology and methodology is highly recommended for perusal by researchers.

This "one-size-fits-all" mentality pervades also into the analytic methodology: measuring the so called "total antioxidant capacity (TAC)" in a blood plasma sample will not give useful information on the state of the organism, and should be discouraged [63]. Rather, individual relevant enzyme activities and patterns of antioxidant molecules need to be assessed.

The use of genetically encoded fluorescent protein indicators has permitted noninvasive studies in cells and organs, an early landmark being the hydrogen peroxide probe HyPer [64]. This field has developed impressively [65, 66], and specificity and sensitivity are being improved [67]. However, these probes need to be carefully applied with appropriate controls and calibration.

9 Conclusions

Useful as the term "oxidative stress" may be in research, there has been an inflationary development in research circles and more so in the medical field and, even more than that, in the public usage outside scientific endeavors. Obviously, a general term describing a global condition cannot be meant to depict specific spatiotemporal chemical relationships in detail and in specific cells or organ conditions. Recent progress in methodology has permitted insight in these topics.

Thus, the subcellular compartmentation of redox processes and redox components and their regulation is being studied at a new level in mammalian cells [68].

Oxidative stress and stress responses can now be studied in a more refined fashion at molecular detail. This gives us hope for exciting new insight and discoveries for the next 30 years of oxidative stress research.

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