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Review Article

Thiol chemistry and specificity in redox signaling

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ABSTRACT

Exposure of cells to sublethal oxidative stress results in the modulation of various signaling pathways. Oxidants can activate and inactivate transcription factors, membrane channels, and metabolic enzymes, and regulate calcium-dependent and phosphorylation signaling pathways. Oxidation and reduction of thiol proteins are thought to be the major mechanisms by which reactive oxidants integrate into cellular signal transduction pathways. This review focuses on mechanisms for sensing and transmitting redox signals, from the perspective of their chemical reactivity with specific oxidants. We discuss substrate preferences for different oxidants and how the kinetics of these reactions determines how each oxidant will react in a cell. This kinetic approach helps to identify initial oxidant-sensitive targets and elucidate mechanisms involved in transmission of redox signals. It indicates that only those proteins with very high reactivity, such as peroxiredoxins, are likely to be direct targets for hydrogen peroxide. Other more modestly reactive thiol proteins such as protein tyrosine phosphatases are more likely to become oxidized by an indirect mechanism. The review also examines oxidative changes observed during receptor-mediated signaling, the strengths and limitations of detection methods for reactive oxidant production, and the evidence for hydrogen peroxide acting as the second messenger. We discuss areas where observations in cell systems can be rationalized with the reactivity of specific oxidants and where further work is needed to understand the mechanisms involved.

Contents

Abbreviations: DCF, dichlorodihydrofluorescein; DPI, diphenylene iodonium; DuOx, dual oxidase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NAC, *N*-acetylcysteine; NOX, NADPH oxidase; PTP, protein tyrosine phosphatase; ROS, reactive oxygen species.

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Introduction

Aerobic cells continually encounter reactive oxidants from a variety of sources and neutralize their effects with an intricate array of antioxidants. Whereas high oxidant exposure or low antioxidant defence can result in damage to critical cellular constituents and ultimately be cytotoxic, moderate alterations to redox homeostasis commonly initiate a signaling response. Redox signaling is a well-recognized stress response that leads to a variety of downstream effects including increased expression of protective and repair enzymes. There is also mounting evidence that redox signaling is a part of normal metabolism in nonstressed cells. In this situation, endogenously generated oxidants act as second messengers for receptor agonists such as growth factors and hormones, signaling the proliferative or metabolic changes associated with these ligands. Oxidants can activate and inactivate transcription factors, membrane channels, and metabolic enzymes, and modulate calcium-dependent and phosphorylation signaling pathways. These processes incorporate the major regulatory networks of cells, giving redox signals the capacity to stimulate and tune most aspects of cell physiology.

Oxidation and reduction of thiol proteins is thought to be the major mechanism by which reactive oxidants integrate into cellular signal transduction pathways. Thiol proteins are well suited as targets because cysteine residues are sensitive to oxidation, and changes in enzymatic activity or binding characteristics due to oxidation provide a mechanism for transmission of the signal. However, to meet signaling criteria there needs to be preferential oxidation of specific proteins, and the process should be fast and reversible. The focus of this review is on how a redox signal is sensed and transmitted. We consider the kinetic properties that help identify likely targets for specific oxidants, and highlight areas where this knowledge is yet to rationalize experimental findings. These same concepts are explored in receptor-mediated models of redox signaling. Our emphasis is mainly on mammalian cells; bacterial systems are considered in more detail elsewhere [1], and other reviews give broader coverage of cellular responses to oxidants and the downstream effects of redox signaling [2,3]. The recent review by Janssen-Heininger and associates [4], which focuses on the impact of protein thiol oxidation and nitrosylation in cell signaling, complements this paper which emphasizes mechanisms of thiol modification.

Principles of redox signaling

There are two general mechanisms proposed for redox regulation (Fig. 1). One is based on the thermodynamic principle that all thiol/disulfide couples are in equilibrium, with the ratio of oxidized to reduced forms determined by the redox potential of the cell [5,6]. The tripeptide glutathione acts as the main redox buffer [7]. When the cell becomes more oxidizing the ratio of GSH to GSSG decreases and exchange reactions enable equilibration with thiol proteins to increase their disulfide content (Fig. 1, Pathway 1). Protein thiols vary in their redox potential. This means that some are more reduced than others at a given GSH:GSSG ratio and they show different sensitivities to changes. However, direct thiol disulfide exchange reactions are slow and there is increasing evidence that cells do not maintain thermodynamic equilibrium, especially during a dynamic signaling process [6]. Exchange reactions catalyzed by glutaredoxin or thioredoxin [8,9] are more likely to be involved in returning the cell toward equilibrium following a signaling event.

The alternative mechanism is a more specific cellular response to an oxidant, based on the kinetic properties of a relatively few sensitive targets (Fig. 1, Pathway 2). These target proteins would be transiently oxidized to enable transmission of the signal and then enzymatically reduced to their basal oxidation state. The existence of proteins that vary in their oxidant sensitivity would enable activation of different pathways depending on the level of oxidant exposure. For signaling via this mechanism, only a small number of highly sensitive proteins would be modified in oxidant stressed cells. These thiols must be sufficiently reactive to undergo oxidation in the presence of cellular antioxidants, or competing antioxidants must be depleted before oxidation of the target occurs. A variation on this mechanism, which is discussed in more detail below, is that there may be a small subset of very reactive thiol proteins, termed sensor proteins. Once oxidized, they facilitate the oxidation of other target proteins through selective protein–protein interactions and thiol exchange (Fig. 1, Pathway 3). We will examine the evidence for these mechanisms in redox signaling by first describing what has been observed in oxidant-treated cells and then considering how this can be rationalized with the chemical reactivity of thiols with different oxidants.

Detection of oxidant-sensitive cellular thiol proteins

With the rapid advancement of proteomic technologies there has been an increasing number of reports assessing global changes in redox state of thiol proteins in oxidatively stressed cells [10–18]. Various methodologies are used, but they are generally based on quantifying the incorporation of labeled iodoacetamide or maleimide derivatives that alkylate cysteine residues, or monitoring structural modifications of oxidized proteins (reviewed in [19]). Oxidation can be detected as a decrease in probe incorporation, but the sensitivity of detection in complex mixtures where many thiol proteins remain unmodified can be improved by first blocking all reduced cysteines

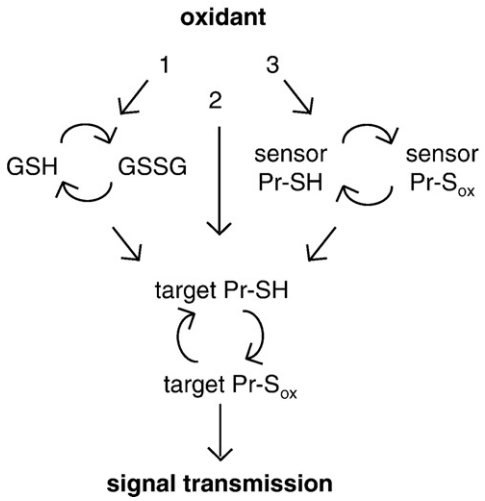

Fig. 1. General mechanisms of redox signaling. Increased exposure of cells to an oxidizing species can cause selective thiol oxidation and signal transmission. We illustrate three pathways that could be responsible for the oxidation of a select subset of thiol proteins. (1) Thermodynamic model. Alterations in cellular redox buffers; e.g., GSH results in oxidation of thiol proteins with a hierarchy dependent on the redox potential of target cysteines. (2) Direct targeting. The local environment of specific target cysteines considerably enhances their reactivity to the oxidant. (3) Facilitated targeting. A variation of direct targeting, where extremely reactive sensor proteins scavenge the signaling oxidant and then facilitate the oxidation of target proteins through specific protein interactions and thiol transfer reactions.