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Review

Thiol-based redox switches ☆

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ABSTRACT

Regulation of protein function through thiol-based redox switches plays an important role in the response and adaptation to local and global changes in the cellular levels of reactive oxygen species (ROS). Redox regulation is used by first responder proteins, such as ROS-specific transcriptional regulators, chaperones or metabolic enzymes to protect cells against mounting levels of oxidants, repair the damage and restore redox homeostasis. Redox regulation of phosphatases and kinases is used to control the activity of select eukaryotic signaling pathways, making reactive oxygen species important second messengers that regulate growth, development and differentiation. In this review we will compare different types of reversible protein thiol modifications, elaborate on their structural and functional consequences and discuss their role in oxidative stress response and ROS adaptation. This article is part of a Special Issue entitled: Thiol-Based Redox Processes.

1. Reactive oxygen species and oxidative stress response

Life in an aerobic environment inevitably leads to the formation of reactive oxygen species (ROS) [1], including hydrogen peroxide (H_2O_2) and superoxide (O_2^-). These oxidants are endogenously generated as metabolic by-products from processes such as oxidative phosphorylation, or are directly produced by enzymes like NADPH oxidases (i.e., Nox) [2]. Whereas low concentrations of ROS play important roles in cell signaling processes, large amounts of ROS cause irreversible modification and damage to virtually every cellular macromolecule, including lipids, DNA and proteins [3–5]. A number of conserved enzymatic and non-enzymatic systems are present in pro- and eukaryotic organisms that detoxify excess ROS, prevent and repair oxidative damage and maintain redox homeostasis. However, when biological systems are no longer able to deal with ROS, they experience a stress situation commonly known as oxidative stress, which becomes quickly lethal if it is let to run its course.

To protect themselves against oxidative damage, organisms have developed a number of different response systems, whose main function is to sense and rapidly respond to changing levels of specific oxidants. Most of the responses involve transcriptional changes, mediated by oxidative modification of specific transcription factors [6]. The transcriptional activator OxyR, for instance, directly senses increased peroxide levels in *Escherichia coli* [7]. Upon its activation, OxyR induces the expression of catalase and peroxiredoxin to detoxify hydrogen peroxide and organic peroxides, and also induces the expression of thioredoxin and glutaredoxin in order to reduce oxidative thiol modifications and restore redox homeostasis [8]. In yeast, the transcriptional regulator Yap1p takes over this function, sensing the presence of reactive oxygen species and responding to them with the upregulation of antioxidant genes [9]. In addition to these transcriptional changes, organisms also respond to increased oxidant levels with the instantaneous activation of stress-specific chaperones, which protect proteins against oxidative protein aggregation [10,11]. ROS-mediated change in the function of select metabolic enzymes further serves to redirect metabolic pathways from energy production towards NADPH generation [12]. In concert, these responses provide considerable protection against oxidative damage.

2. Cysteine oxidation — a sensitive and (mostly) reversible way to respond to oxidants

One of the most commonly employed ways to sense alterations in cellular oxidant levels or the redox environment is through the reversible modification of thiol-containing cysteine side chains in redox-sensitive proteins [6,13–16]. While most cysteines have pK_a values between 8 and 9 and are therefore fully protonated and largely non-reactive under physiological pH conditions, oxidation-sensitive thiols are often (but not always) characterized by much lower pK_a values [17]. These low pK_a-values result in the deprotonation of these cysteines under physiological pH conditions. The resulting thiolate anions exhibit much higher reactivity than their protonated thiol counterparts [18]. Reactivity among different thiolate anions also depends on the local protein environment, and currently there is no accurate way to predict thiolate anion reactivity [19]. Upon exposure to oxidants, thiolate

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anions rapidly form sulfenic acids, which are important intermediates in the thiol oxidation process (Fig. 1) [20,21]. Due to their high reactivity towards nearby thiol groups, sulfenic acids are generally very short-lived [22]. So far, only few redox-regulated proteins, such as the FAD-containing NADH peroxidase from *Streptococcus faecalis* and matrix metalloproteinase MMP-7 [23,24] have been shown to use this fully reversible oxidative modification to control their functional activity [20,25]. Most sulfenic acids, however, rapidly react with other protein thiols to form intra- or intermolecular disulfide bonds, interact with non-protein thiols such as the small redox compound glutathione (GSH) or cysteine to form mixed disulfide bonds (i.e., S-glutathionylation, S-cysteinylation), or react with nearby amino groups to form cyclic sulfenamides (Fig. 1). Further oxidation of sulfenic acid or sulfenamides leads to sulfinic acid and sulfonic formation, which are typically irreversible oxidation processes. One exception is the formation of sulfinic acid at the active site cysteine of select eukaryotic peroxiredoxins [26]. This overoxidation product, which forms part of a functional redox switch, is reduced by sulfiredoxin, a specialized ATP-dependent sulfinic acid reductase [27].

Most oxidative thiol modifications are reduced by the thioredoxin or glutaredoxin systems [28,29]. At the core of these systems are small, highly conserved oxidoreductases, thioredoxin (e.g., TrxA) and glutaredoxin (e.g., Grx1, Grx3), which use direct thiol–disulfide exchange reactions to reduce oxidized protein thiols. Both oxidoreductases have the characteristic thioredoxin fold, harboring a conserved Cys-X-Y-Cys motif that facilitates the reduction of client proteins [30]. In the initial step, the first Cys of the thioredoxin twin cysteine motif attacks the oxidized thiol of the client protein. In the case of TrxA, this nucleophilic attack leads to the formation of a mixed disulfide bond between TrxA and the client protein. To resolve this intermolecular disulfide bond and fully reduce the client protein, the second cysteine in TrxA launches another nucleophilic attack at the mixed disulfide bond, forming an intramolecular disulfide bond in TrxA thereby fully reducing the client protein [31]. Reversibility of the thioredoxin system is achieved by thioredoxin reductase (TrxB), which uses the reductive power of NADPH to reduce the disulfide bond in TrxA [28,32]. Glutaredoxin (Grx) is different from Trx in that it preferentially attacks S-glutathionylated proteins, forming a mixed disulfide with GSH in this process [29,33]. A second molecule of GSH attacks this intermolecular disulfide bond, reducing Grx and releasing oxidized GSSG, which in turn is reduced by the NADPH-dependent glutathione reductase (GR) [28,34]. Despite Grx's preference for S-glutathionylated proteins and Trx's preference for sulfenic acids, in vivo studies revealed that the two systems can replace each other in the cell [35–37]. This redundancy emphasizes their important roles in maintaining cellular redox homeostasis and preventing higher and typically irreversible thiol modifications.

3. Redox-regulation — a powerful posttranslational mechanism to regulate protein activity

Select proteins involved in photosynthetic processes have long been known to be inactivated by disulfide bond formation under dark conditions, and activated upon disulfide bond reduction once the organism is photosynthetically active and starts producing NADPH [16,38,39]. Moreover, numerous prior studies have reported thiol-containing proteins, which loose their activity during the purification in the absence of reducing agents. Many of these results were historically dismissed as in vitro artifacts, explained by the random and non-specific disulfide bond formation, which alters the structure and hence the function of the proteins. What was more difficult to dismiss as an artifact was the discovery of cytosolic proteins that gain activity when oxidized. One of the first examples of this was OxyR, the peroxide sensitive transcription

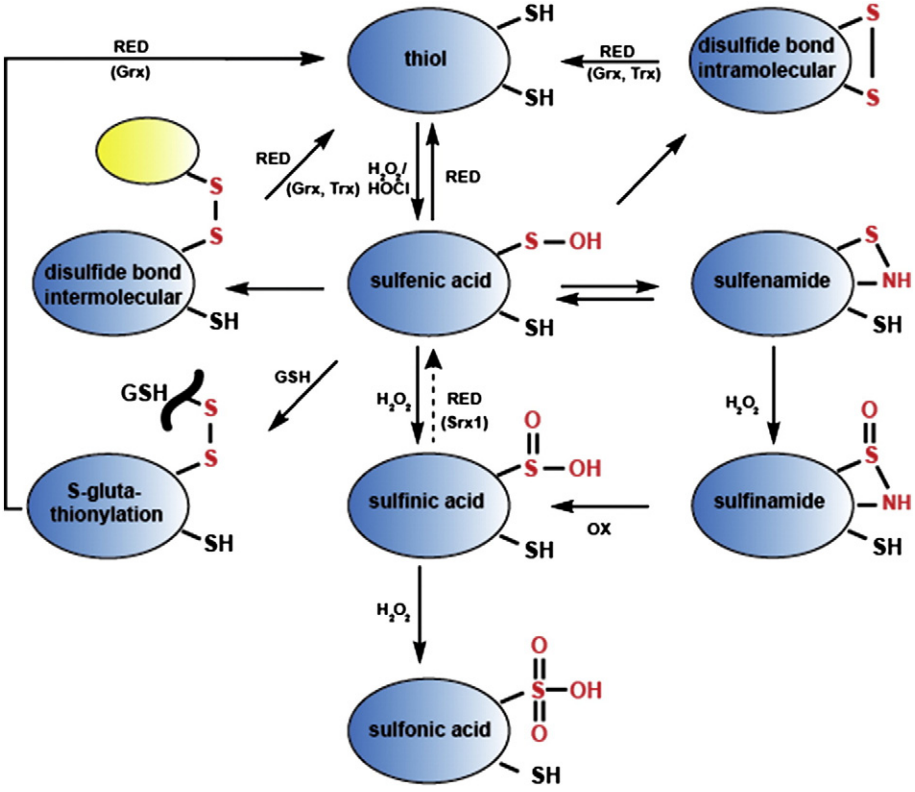

Fig. 1. Oxidative thiol modifications commonly found in redox-regulated proteins. Upon reaction with peroxide (H_2O_2) or hypochlorous acid (HOCl), redox-sensitive thiol groups (RSH) rapidly form sulfenic acids (RSOH). These sulfenates are highly reactive and tend to quickly react with nearby cysteine thiols to form inter- or intramolecular disulfide bonds (RSSR). Alternatively, they form mixed disulfides with the small tripeptide glutathione (GSH) (RSSG), or undergo cyclic sulfenamide formation (RSNHR). These oxidative thiol modifications are fully reversible, and reduction (RED) is catalyzed by members of the glutaredoxin (Grx) or thioredoxin (Trx) system. Further oxidation of sulfenic acid to sulfinic acid (RSO_2H), sulfinamide (RSONHR) or sulfonic acid (RSO_3H) is irreversible. One exception is the active site sulfinic acid in peroxiredoxin, whose reduction is mediated by the highly specialized ATP-dependent sulfiredoxin Srx1.