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Review Article

Hydrogen peroxide sensing, signaling and regulation of transcription factors

H. Susana Marinho[a], Carla Real[a], Luísa Cyrne[a], Helena Soares[a,b,c], Fernando Antunes[a,*]

[a] Departamento de Química e Bioquímica, Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal
[b] Instituto Gulbenkian de Ciência, Oeiras, Portugal
[c] Escola Superior de Tecnologia da Saúde de Lisboa, IPL, Lisboa, Portugal

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ABSTRACT

The regulatory mechanisms by which hydrogen peroxide (H_2O_2) modulates the activity of transcription factors in bacteria (OxyR and PerR), lower eukaryotes (Yap1, Maf1, Hsf1 and Msn2/4) and mammalian cells (AP-1, NRF2, CREB, HSF1, HIF-1, TP53, NF-κB, NOTCH, SP1 and SCREB-1) are reviewed. The complexity of regulatory networks increases throughout the phylogenetic tree, reaching a high level of complexity in mammalians. Multiple H_2O_2 sensors and pathways are triggered converging in the regulation of transcription factors at several levels: (1) synthesis of the transcription factor by upregulating transcription or increasing both mRNA stability and translation; (ii) stability of the transcription factor by decreasing its association with the ubiquitin E3 ligase complex or by inhibiting this complex; (iii) cytoplasm–nuclear traffic by exposing/masking nuclear localization signals, or by releasing the transcription factor from partners or from membrane anchors; and (iv) DNA binding and nuclear transactivation by modulating transcription factor affinity towards DNA, co-activators or repressors, and by targeting specific regions of chromatin to activate individual genes. We also discuss how H_2O_2 biological specificity results from diverse thiol protein sensors, with different reactivity of their sulfhydryl groups towards H_2O_2, being activated by different concentrations and times of exposure to H_2O_2. The specific regulation of local H_2O_2 concentrations is also crucial and results from H_2O_2 localized production and removal controlled by signals. Finally, we formulate equations to extract from typical experiments quantitative data concerning H_2O_2 reactivity with sensor molecules. Rate constants of 140 M^{-1} s^{-1} and $\geq 1.3 \times 10^3$ M^{-1} s^{-1} were estimated, respectively, for the reaction of H_2O_2 with KEAP1 and with an unknown target that mediates NRF2 protein synthesis. In conclusion, the multitude of H_2O_2 targets and mechanisms provides an opportunity for highly specific effects on gene regulation that depend on the cell type and on signals received from the cellular microenvironment.

Introduction

Hydrogen peroxide (H_2O_2) is a ubiquitous oxidant present in all aerobic organisms. Since its first identification in a living cell, H_2O_2 was considered a toxic byproduct of aerobic metabolism, something that cells had to remove [1]. If H_2O_2 detoxification catalyzed by catalases and peroxidases was not adequate, H_2O_2 would diffuse and oxidize biological targets causing cellular malfunctions responsible for several pathologies and aging. Favoring this paradigm was the discovery that neutrophils use H_2O_2 toxicity and produce massive amounts of H_2O_2 during the oxidative burst to kill invading pathogens. In the 70s some isolated observations already supported a role for H_2O_2 as a signaling molecule, *e.g.* H_2O_2 was found to mimic insulin action [2] or to activate guanylate cyclase [3]. Apparently, these observations remained mostly unnoticed in the field of oxidative stress, but at the end of the 80s some key discoveries built up on them. In 1987, it was found that H_2O_2 at micromolar levels elicits arterial pulmonary relaxation mediated by the activation of guanylate cyclase [4] and in 1989, H_2O_2 was found to potentiate tyrosine phosphorylation during insulin signaling [5] and to stimulate cell proliferation at low concentrations [6]. Also in 1989, OxyR was identified as the transcription factor (TF) targeted by H_2O_2 in the adaptive response of *Escherichia Coli* (*E. coli*) and *Salmonella typhymurium* (*S. typhymurium*) [7], and in 1990 NF-κB was identified as a redox regulated TF [8]. In the following year, the activation of NF-κB by H_2O_2 was discovered in a publication [9] that had a profound impact in the field, with near 3500 citations so far. Also in 1991, NADPH oxidases were identified in non-phagocytic cells as H_2O_2 producing systems [10,11]. If H_2O_2 was

Abbreviations: AD, activation domain; ER, endoplasmic reticulum; GPx, glutathione peroxidases; NES, nuclear exporting signal; NLS, nuclear localization signal; PHD, prolyl hydroxylase; Prxs, peroxiredoxins; TF, transcription factor; Ub, Ubiquitin.

* Corresponding author.
E-mail address: fantunes@fc.ul.pt (F. Antunes).

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a toxic species, why were cells intentionally producing this species by a complex regulated mechanism? Concomitantly, several clinical trials based on the notion that oxidants were toxic and antioxidants were beneficial for cancer prevention were largely unsuccessful as reviewed in [12]. Nowadays, redox biology is an established field and the essential regulating role played by H_2O_2 *in vivo* with important implications in health and disease is unquestionable. However, there are still a lot of unanswered questions regarding our understanding of redox-dependent regulation of gene expression. What makes a good H_2O_2 sensor? What are the common chemical and kinetic principles that govern H_2O_2 signaling? Is it possible to obtain an integrative view of H_2O_2 regulation of TFs?

In this review, we will start by discussing what characteristics an H_2O_2 sensor should have; we review the chemistry of H_2O_2, mainly its reaction with thiols. The aim is to give a brief overview of basic chemical and kinetic principles that govern H_2O_2 signaling. Next, we describe briefly the TFs reviewed here, which include bacterial (OxyR and PerR), yeast (Yap1, Msn2/4, Maf1, and Hsf1), and mammalian (AP-1, NRF2, CREB, TP53, NOTCH, NF-kB, SP1, HIF-1, SREBP-1 and HSF1) TFs. The main body of this article describes the redox regulation of these TFs by H_2O_2. A detailed review on each of the TFs listed is not intended, as there are many excellent reviews that do so. We aim to give an integrative review of their regulation by H_2O_2 at several steps: synthesis and stability of the TF, cytoplasm-nuclear trafficking and DNA binding and transactivation, so that the reader is made aware of the diversity of mechanisms by which H_2O_2 regulates TFs and also what the common themes in H_2O_2-regulated signaling pathways are.

What makes a good sensor for H_2O_2?

The characteristics of a good sensing molecule for H_2O_2 can be derived from basic concepts taken from information theory and chemistry. Low-molecular weight thiols react slowly with H_2O_2, as exemplified by the rate constants for H_2O_2 reaction with cysteine and reduced glutathione (GSH), which are respectively 2.9 $M^{-1}\,s^{-1}$ and 0.87 $M^{-1}\,s^{-1}$ (pH 7.4, see Table 1). The reaction of thiols with H_2O_2 involves a nucleophilic attack of the thiolate on H_2O_2 and, as such, thiol reactivity is driven by the pKa of the sulfhydryl (−SH) group. Since the pKa of the SH group in cysteine is 8.3 only about 10% of free cysteine is ionized at the physiological pH. In proteins, the electrostatic environment around the SH group of cysteine residues may render these groups more acidic and, therefore, they may have an increased reactivity towards H_2O_2, since a higher fraction will be in the thiolate form. Nucleophilicity is also an important factor and, in several proteins, a lower stabilization of the thiolate in cysteine residues increases nucleophilicity of the thiolate [13] and increases, by several orders of magnitude, the rate constants with H_2O_2 (see Table 1). The concept of redox signaling by H_2O_2 was proposed following the discovery of proteins involved in signaling, such as phosphatases, kinases and transcription factors, that contain cysteine residues whose SH groups are oxidized (Fig. 1) causing a change of their biological activity. According to this paradigm upon an increase in the concentration of H_2O_2, these proteins are specifically oxidized, and a cascade of molecular events ensues. Unfortunately, the wealth of data identifying reactive SH groups, *i.e.* groups that are oxidized upon exposure to an oxidant, contrasts with the near absence of quantitative kinetic data characterizing this reactivity. The few rate constants listed in Table 1 show that the reactivity with H_2O_2 of signaling proteins like the phosphatases Cdc25B and PTB1B is much lower than the reactivity of peroxiredoxins (Prxs), of the selenocysteine residues present in glutathione peroxidases (GPx), or of the heme center present in catalase. In addition, the cellular abundance of antioxidant enzymes like GPx, Prxs and catalase is much larger than that of signaling proteins like phosphatases or TFs. This is important since in the reaction of H_2O_2 with thiols we are dealing with second order rate constants, *i.e.* the rate of reaction is proportional to the concentrations of H_2O_2 and the thiol. The consequence is that signaling molecules cannot compete with known protein antioxidant systems that remove H_2O_2. In addition, existing data show that several types of GPx (at least eight isoenzymes) and Prxs (six isoenzymes) coexist [14,15]. If these enzymes had only an antioxidant function, why is there such a variety? For all these reasons, it was concluded that a signaling protein like PTP1B that is redox regulated by H_2O_2 [16–18] but has a low reactivity towards H_2O_2 [19], could not be a direct sensor of H_2O_2 [13,20–22]. Also, antioxidant systems like Prxs and GPx would constitute a kinetic bottleneck that avoids any significant reaction of H_2O_2 with signaling low-reactive proteins [13]. Instead, a high reactive protein, like a peroxiredoxin or a glutathione peroxidase, would be the initial H_2O_2 sensor, which through a thiol-disulfide reshuffling transfer reaction would then oxidize the target protein. This paradigm was inspired in the activation mechanism of the OxyR TF in bacteria [23]. However, these kinetic considerations do not tell the whole story.

(1) Different H_2O_2 signaling pathways are triggered by different H_2O_2 concentrations and occur with different kinetics. For example exposure of H4IIEC hepatocytes to extracellular H_2O_2 (25–50 μM) for 3 h decreased insulin-stimulated AKT phosphorylation, and increased the phosphorylation of both JNK and its substrate c-JUN, while lower concentrations of H_2O_2 (5–10 μM) enhanced insulin-stimulated phosphorylation of AKT [24]. In addition, H_2O_2 exerts often biphasic responses in which one effect is reversed in a narrow range of concentration such as in H_2O_2 regulation of fatty acid synthase [25–27]. If the initial target is a high-reactive molecule, it is hard to imagine such quantitative diversity in H_2O_2 response.

(2) More importantly, information is not mass. That antioxidant systems impose a kinetic bottle-neck for the flux of H_2O_2, and that a rate of oxidation of a sensor is vastly outcompeted by the rate of oxidation of antioxidant systems is irrelevant for a sensing mechanism. In Fig. 2, we simulate a situation where an antioxidant system outcompetes the reaction of H_2O_2 with PTP1B by nine orders of magnitude and, in spite of that, PTP1B is oxidized with a half-life of 5.7 min, a time scale typical of a signaling response. The role of a sensor is to interact selectively with the signaling molecule and to produce an effect that can be measured by a transducer. So, its main role is to transmit information and not, *e.g.* to be a bulk catalyst in a biochemical pathway. What is important is that a variation of H_2O_2 concentration is sensed and this information is transmitted downstream the signaling cascade. By sensing we mean the rate of oxidation of the sensor increases/decreases upon an increase/decrease in the H_2O_2 concentration (the signal). If the rate of oxidation of the sensor is many orders of magnitude lower than the rate of production of H_2O_2 or the rate of H_2O_2 consumption by antioxidant systems, this is actually a good characteristic for a sensor. An ideal sensor does not change the intensity of the signal, it just responds to a change in the signal. For example, a thermometer in a water bath senses changes in the temperature, and does not decrease or increase the temperature of the water. One biochemical illustration of this is the HIF system sensing O_2. In this system, a prolyl hydroxylase (PHD) catalyzes the hydroxylation of the subunit HIF-1α by O_2, which is then subsequently marked for degradation [28]. The fraction of O_2 consumed by PHD compared with the overall cellular O_2 consumption is small, but this does not prevent it from being an O_2 sensor.

Thus, a putative target with reactivity towards H_2O_2 much lower than other molecules also present in the system does not, *per se*, exclude it from being a sensor. Next, we evaluate whether the known characteristics of low-reactivity thiol proteins are compatible with a