Plant Science xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Review

Mini-review: Glutathione peroxidases as redox sensor proteins in plant cells

Q1 Gisele Passaia, Márcia Margis-Pinheiro*

Department of Genetics, Federal University of Rio Grande do Sul, RS, Brazil

ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form 27 January 2015
Accepted 29 January 2015
Available online xxx

Keywords:
Glutathione peroxidases
Oxidative stress
Redox signaling
Redox regulation
Reactive oxygen species

ABSTRACT

Glutathione peroxidases are thiol-based enzymes that catalyze the reduction of H_2O_2 and hydroperoxides to H_2O or alcohols, they mitigate the toxicity of these compounds to the cell mainly using thioredoxin as an electron donor. Additionally, certain redox sensor and signaling functions are being ascribed to these enzymes in prokaryotes, fungi, and plants. We review the evolutionary history, enzymatic and biochemical evidence that make GPX proteins, in addition to being peroxiredoxins, important candidates for acting as redox sensor proteins in plants: (i) the lower peroxidase activity of Cys-GPX; (ii) the thiol catalytic center; (iii) the capacity to interact with regulatory proteins. All these characteristics suggest that at the basal level, plant GPXs have an important role in redox signal transduction in addition to their peroxidase activity.

Contents

1. Introduction

Q2 The generation of reactive oxygen species (ROS) is particularly enhanced when plants are subjected to abiotic and/or biotic stresses. The produced ROS participate in a range of important signaling processes during growth, development, acclimation to stress, cell cycle and programmed cell death. These molecules have to be sensed to control the concentration of ROS. It has been established that hydrogen peroxide (H_2O_2) in particular acts as a signaling molecule that diffuses across membranes and triggers specific signal transduction pathways (for a review see [1,2]). The dual role of H_2O_2 as a damaging compound and as a messenger requires a tightly balanced defense system. Accordingly, this molecule should be maintained at a very low micromolar level: if the organism is subjected to increased levels, the signal must be propagated to the regulatory targets, resulting in the appropriate activation of responses (reviewed by [3]). Reactive oxygen species are produced during reactions such as photosynthesis and respiration (reviewed by [4]). Furthermore, ROS levels are elevated after biotic or abiotic stresses such as drought, salinity, chilling, high light, mechanical stress and pathogen attack (for a review, see [5,6]). To manage an uncontrolled oxidation status cells possess a set of hydrogen peroxide-decomposing enzyme: catalases, ascorbate peroxidases (APX), glutathione peroxidases (GPX), peroxiredoxins, and type-III peroxidases (for a review, see [7,8]). Moreover, GPX, GSTs (glutathione S-transferases) with GPX activity and peroxiredoxins also decompose alkyl hydroperoxides in addition to H_2O_2 [9–11]. The two chloroplastic Poplar GPXs exclusively use thioredoxin instead of glutathione as electron-donor substrate. On the other hand,

* Corresponding author at: Universidade Federal do Rio Grande do Sul, Avenida Bento Gonçalves, 9500, CEP: 91510-970, Porto Alegre, RS, Brazil.
Tel.: +55 51 3308 9814.
E-mail addresses: marcia.margis@ufrgs.br, marcia.p.margis@gmail.com (M. Margis-Pinheiro).

http://dx.doi.org/10.1016/j.plantsci.2015.01.017
0168-9452/

Poplar GPXs are not specific for one electron-accepting substrate, they can reduce H_2O_2 and lipid hydroperoxides [8]. The GPX family of proteins is found in virtually all kingdoms of life [12] and is being increasingly studied in plants [13–18]. This enzyme family is included in the heme-free thiol peroxidase class and can use other reducing substrates in addition to glutathione and thioredoxin [8,15,19]. The majority of vertebrate GPX sequences harbor selenocysteine in their active site, while plant GPX possesses cysteine [10,12]. In phylogenetic studies, plant GPXs clustered together with animal class 4, also named phospholipid GPX (PHGPX4 or GPX4) [12]. Mammalian GPX7 is also cysteine based and has a low glutathione peroxidases activity [20]. Moreover, rice PHGPX showed affinity for GSH and thioredoxin as electron donors [21]. PHGPXs have all the aminoacid residues required for the binding with glutathione mutated or deleted, reducing the affinity for this peptide for the catalysis (for a review see [22]). The cysteine that forms the catalytic site of non-selenium GPXs (NSGPX) is oxidized by H_2O_2 or by an organic hydroperoxide and then reacts with a second cysteine in the same protein. The resulting intramolecular disulfide bridge is then reduced by thioredoxin. This and the lack or substitutions in the aminoacid residues responsible for glutathione affinity may explain plant GPX higher affinity for thioredoxin than glutathione.

These enzymes have additional properties that make them strong candidates to assume the function of sensors during oxidative stress [23]. In this review, we highlight that plant GPXs, in addition to their role as ROS scavengers, are also redox sensor proteins and propose that this ability should be further investigated.

2. Protein sensor and redox regulation

The post-translation redox process is defined as being "a reversible post-translational alteration in the properties of a protein, typically the activity of an enzyme, as a result of change in its oxidation state" [24]. The mechanistic principles in redox regulation are well reviewed [25]. Proteins containing highly reactive thiol groups, such as GPX, are strong candidates for the sensing mechanism in redox regulation [25]. Most CysGPXs but not all of them are considered relatively weak peroxidase scavengers compared with catalase and seleno-based GPXs (Hoffman 2002). The high cysteine reactivity for H_2O_2 makes CysGPX and peroxirredoxins ideal enzymes for H_2O_2 sensing and signaling providing the specificity required for this processes [23].

Such a signaling function has been ascribed to GPX from yeast and Arabidopsis [15,26,27]. Yeast GPX3, for example, activates yeast activation protein 1 (Yap-1), a bZIP transcription factor, via an oxidation-induced event that promotes the activation of genes encoding thiol-reducing and antioxidant proteins [26]. Yeast GAPDH2 also interacts with GPX3 through the carboxy-terminal domain reducing S-nitrosylation of GAPDH under nitric oxide stress conditions consequently increasing cellular viability [27]. In *Arabidopsis thaliana*, GPX3 protein physically interacts with phosphatase type 2C (PP2C) proteins such as abscisic acid insensitive (ABI) 1 and ABI2, leading to stomatal closure via the activation of plasma membrane Ca^{2+} and K^+ channels [15]. Indeed, some peroxiredoxins are also considered to act as redox sensor enzymes in a similar way as that of GPX. The peroxiredoxin 2-Cys Tsa1 activates Yap1 in an analogous way as Orp1 (yeast GPX3) in another yeast strain (Y700, having a nonsense mutation), but the specificity of GPX3 is restored when wild-type Ybp1 is introduced [28,29]. Peroxiredoxins and cysteine glutathione peroxidases share some characteristics: both classes of enzymes have cysteine in their catalytic site. In addition, the use of thioredoxin as a reducing substrate and the biochemical feature classification of GPX indicate that these enzymes constitute a fifth group of peroxiredoxins and are misnamed [8,30]. In the flagellate protozoa kinetoplastid group

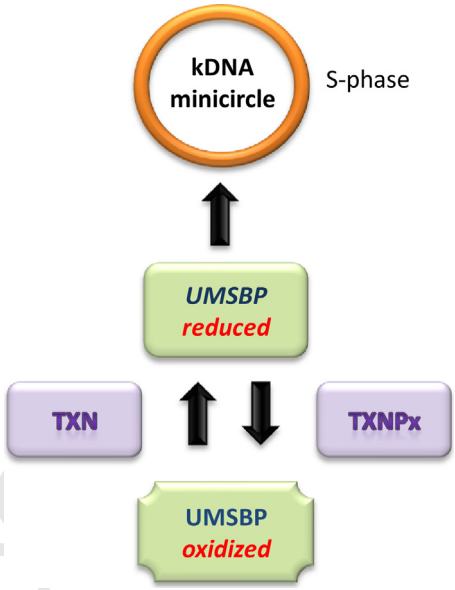

Fig. 1. Redox regulation of UMSBP protein. Reduced UMSBP is the only form that binds kinetoplastid DNA minicircle throught the zinc finger thiol group. UMSBP is reduced by tryparedoxin (TRX). Oxidized UMSBP do not bind the KDNA minicircle and its oxidative state is promoted by tryparedoxin peroxidase (TXNPx).

(the group includes *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania* species), a peroxiredoxin perceives H_2O_2 and directly transduces the oxidant equivalents to CxxC motifs of a Zn-finger domain of the UMCSBP protein (universal minicircle sequence binding domain), which binds to kinetoplastid DNA minicircles and regulate its replication [31]. The trypanosoma kinetoplastid developed distinct redox molecular mechanism using trypanothione ($T[SH]_2$)-based tryparedoxin (TXN) and tryparedoxin peroxidases (TXNPx). TXN reduces UMCSB protein through the zinc finger domain allowing UMCSB to bind kDNA minicircle during the S-phage and initiate replication [31] (Fig. 1).

Schizosaccharomyces pombe Pap1 is the homolog of Yap1 and responds to H_2O_2 at low concentrations accumulating in the nucleus, but its activation is delayed at higher concentrations of the compound. The peroxiredoxin Tpx1 is the upstream activator of Pap1; at low H_2O_2 concentrations, the antioxidant scavenger transfers a redox signal to the transcription factor. Interestingly, at higher concentrations, this protein–protein interaction delays the temporal activation of Pap1 [32]. The *S. pombe* Pap1 transcription factor activates antioxidant and drug resistance genes. It is interesting to note that oxidized Pap1 associates with Prr1 (constitutive nuclear transcription factor) and activates catalase, sulfiredoxin and thioredoxin reductase [33].

The biochemical and enzymatic characteristics of GPX and peroxiredoxins include high reactivity toward H_2O_2, overall low catalytic efficiency (discussed in more detail in the next section) and the ability to reversibly inactivate substrates. These enzymes can scavenge low levels of endogenous H_2O_2 as well as restrict scavenging to these low concentrations. Peroxiredoxins and GPXs can oxidize protein-thiols in addition to their physiological reductant (thioredoxin). This specific characteristic is very important because it allows the oxidation of regulatory proteins that are otherwise not directly reactive with peroxide [23]. Instead, GPXs and peroxiredoxins act as intermediates that transfer the redox signal.

GPX sensors are targeted by the redox state of the cell (H_2O_2) in yeast, which leads to their oxidation. Oxidized GPXs can then interact with the target protein (the YAP1 transcription factor) and oxidize two cysteines, forming an intramolecular disulfide bridge. This causes the target protein (YAP1) to accumulate in the nucleus

Please cite this article in press as: G. Passaia, M. Margis-Pinheiro, Mini-review: Glutathione peroxidases as redox sensor proteins in plant cells, Plant Sci. (2015), http://dx.doi.org/10.1016/j.plantsci.2015.01.017