Overoxidation of chloroplast 2-Cys peroxiredoxins: balancing toxic and signaling activities of hydrogen peroxide

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INTRODUCTION

Oxygenic photosynthesis is an essential process for life on Earth because it allows the use of light and water to produce biomass and oxygen. However, it is also a process potentially harmful due to the transport of electrons in the presence of oxygen, which inevitably produces reactive oxygen species (ROS). Several environmental challenges such as drought, low or high temperature, high light intensity, or salinity, alter chloroplast ROS homeostasis producing oxidative stress (Miller et al., 2010). To adequately respond to these stressful conditions chloroplasts are equipped with different antioxidant systems both enzymatic and non-enzymatic. It should be taken into account that besides their harmful effect, ROS play an important second messenger. In order to balance the toxic and signaling activities of hydrogen peroxide its level has to be tightly controlled. To this end, chloroplasts are equipped with different antioxidant systems such as 2-Cys peroxiredoxins (2-Cys Prxs), thiol-based peroxidases able to reduce hydrogen and organic peroxides. At high peroxide concentrations the peroxidase function of 2-Cys Prxs may become inactivated through a process of overoxidation. This inactivation has been proposed to explain the signaling function of hydrogen peroxide in eukaryotes, whereas in prokaryotes, the 2-Cys Prxs of which were considered to be insensitive to overoxidation, the signaling activity of hydrogen peroxide is less relevant. Here we discuss the current knowledge about the mechanisms controlling 2-Cys Prx overoxidation in chloroplasts, organelles with an important signaling function in plants. Given the prokaryotic origin of chloroplasts, we discuss the occurrence of 2-Cys Prx overoxidation in cyanobacteria with the aim of identifying similarities between chloroplasts and their ancestors regarding their response to hydrogen peroxide.

Keywords: chloroplast, hydrogen peroxide, peroxiredoxin, redox regulation, thioredoxin, oxidative stress

Photosynthesis, the primary source of biomass and oxygen into the biosphere, involves the transport of electrons in the presence of oxygen and, therefore, chloroplasts constitute an important source of reactive oxygen species, including hydrogen peroxide. If accumulated at high level, hydrogen peroxide may exert a toxic effect; however, it is as well an important second messenger. In order to balance the toxic and signaling activities of hydrogen peroxide its level has to be tightly controlled. To this end, chloroplasts are equipped with different antioxidant systems such as 2-Cys peroxiredoxins (2-Cys Prxs), thiol-based peroxidases able to reduce hydrogen and organic peroxides. At high peroxide concentrations the peroxidase function of 2-Cys Prxs may become inactivated through a process of overoxidation. This inactivation has been proposed to explain the signaling function of hydrogen peroxide in eukaryotes, whereas in prokaryotes, the 2-Cys Prxs of which were considered to be insensitive to overoxidation, the signaling activity of hydrogen peroxide is less relevant. Here we discuss the current knowledge about the mechanisms controlling 2-Cys Prx overoxidation in chloroplasts, organelles with an important signaling function in plants. Given the prokaryotic origin of chloroplasts, we discuss the occurrence of 2-Cys Prx overoxidation in cyanobacteria with the aim of identifying similarities between chloroplasts and their ancestors regarding their response to hydrogen peroxide.
Arabidopsis is composed of ten members (Dietz, 2003). The first Anabaena organisms (Karplus and Poole, 2012; Rhee et al., 2012). A notion confirm the relevant role of the hydrogen peroxide-dependent having a less important function in signaling. Different reports gen peroxide is efficiently reduced and does not accumulate, thus 2-Cys Prxs of which were considered to be insensitive, hydro-...
enough to initiate the redox homeostasis exclusively in chloroplasts, by expressing NTRC in the background mutant under the RbcS promoter. Therefore, whether or not chloroplast 2-Cys Prxs undergo overoxidation and the mechanisms controlling the redox status of the enzyme are relevant questions to determine their antioxidant and/or signaling function.

Two-dimensional gel electrophoresis analysis of 2-Cys Prxs from wild type and mutants deficient in either 2-Cys Prx A or 2-Cys Prx B from Arabidopsis revealed the overoxidation of both enzymes (Kirchsteiger et al., 2009). Surprisingly, the NTRC knock out mutant showed lower level of 2-Cys Prx overoxidation than wild type plants, despite the fact that the deficiency of NTRC may cause oxidative stress. This was a first indication suggesting that the reduction of the enzyme, as a pre-requisite for the formation of the sulfenic acid intermediate, is required for the subsequent overoxidation to sulfonic acid, as outlined in Figure 2. The other component affecting the level of 2-Cys Prx peroxidation in chloroplasts is Srx, which is encoded in plants by a single gene, the protein showing dual targeting to chloroplast and mitochondria (Liu et al., 2006; Iglésias-Barna et al., 2011). Chloroplast Srx was shown to effectively reverse 2-Cys Prx overoxidation (Rey et al., 2003b). Though initially it was thought that overoxidation was an irreversible process, it was then found that sulfiredoxin (Srx) is able to reverse the overoxidized form to the reduced form of the enzyme in a reaction that required ATP and Mg²⁺ (Biteau et al., 2003; Woss et al., 2003). Overoxidation favors the formation of the HMW form of 2-Cys Prxs, which promotes the chaperone activity of these enzymes (Figure 2). All these data, obtained from analyses with yeast and human enzymes, indicated that the redox status of 2-Cys Prx is essential to determine their peroxidase or chaperone activity, making them efficient sensors and key components of the response to oxidant conditions (Karplus and Poole, 2012).

In plants, the chloroplast is an essential organelle not only because of photosynthesis, but also because it is the site of synthesis of a variety of compounds, such as hormones, which play a role in signaling. The role of the chloroplast as an important source of hydrogen peroxide is well known (Mubarakshina et al., 2010). Indeed, we have recently shown that restitution of the redox homeostasis exclusively in chloroplasts, by expressing NTRC in the ntrc background mutant under the RbcS promoter, was necessary and sufficient to recover wild type growth and development of lateral roots regardless of the impaired redox homeostasis in root amyloplasts (Ferrández et al., 2012; Kirchsteiger et al., 2012). Therefore, whether or not chloroplast 2-Cys Prxs undergo overoxidation and the mechanisms controlling the redox status of the enzyme are relevant questions to determine their antioxidant and/or signaling function.

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According to the floodgate hypothesis, the signaling activity of hydrogen peroxide in eukaryotic organisms is based on the inactivation of 2-Cys Pxs by overoxidation, which allows the transient increase in the peroxide necessary to act as second messenger to be efficiently catalyzed by Srx in a reaction that requires ATP and Mg$^{2+}$. According to this scheme, two enzymes, NTRC and Srx, seem to play a central role in controlling the redox status of 2-Cys Pxs in chloroplasts. It has been proposed that 2-Cys Pxs may exert a critical function by balancing antioxidant and signaling activities of chloroplast produced hydrogen peroxide (Dietz et al., 2006). A recent study has further demonstrated that the double knock out mutant lacking both 2-Cys Pxs seems not viable, providing higher resistance though, as it is rapidly reduced, the peroxide can be used for signaling. In contrast, the Arabidopsis Srx knock out mutant revealed a function of the enzyme in the response to photooxidative stress (Rey et al., 2003). Chloroplast 2-Cys Pxs are sensitive to overoxidation (Briand and Rey, 2005; Kirchsteiger et al., 2009; Iglesias-Buena et al., 2010), thus behaving as expected for enzymes of a eukaryotic organelle. Because it is well established that chloroplasts evolved from a prokaryotic endosymbiont (Gould et al., 2008), it arises the question whether 2-Cys Pxs sensitivity was already present in the prokaryotic endosymbiont or was a gain-of-function of these enzymes that occurred during chloroplast evolution. To address this question, Pascual et al. (2010) analyzed the presence of the GG(L/V/I)G and YF motifs in the genes encoding 2-Cys Pxs from different sources. This search confirmed the presence of sensitive 2-Cys Pxs, characterized by the presence of both motifs, in eukaryotes. However, it revealed an unexpectedly large number of cyanobacterial strains similar to present day cyanobacterial strains to respond to oxidative stress. While Arabidopsis showed high sensitivity, Synechocystis survived higher concentrations of hydrogen peroxide. The strategy based on high efficiency of hydrogen peroxide detoxification provides a trade-off, as it is rapidly reduced, the peroxide cannot be used for signaling. In contrast, the Arabidopsis strategy, based on low capacity of detoxification, causes the increase of hydrogen peroxide required to act as second messenger, though it may have as well a harmful effect. Interestingly, the strategy of chloroplasts, which are equipped with sensitive 2-Cys Pxs and lack catalase, is very similar to the Arabidopsis strategy. This is in agreement with the proposal that chloroplasts originated from cyanobacterial strains similar to present day Anabaena species (Deusch et al., 2008).
CONCLUDING REMARKS AND FUTURE PROSPECTS

The inactivation of the peroxidase activity of 2-Cys Prxs, caused by the overoxidation of their cysteine residues, has been proposed to be essential for the signaling function of hydrogen peroxide in eukaryotic organisms. In chloroplasts, which constitute an important source of hydrogen peroxide and have a prominent signaling function [8], 2-Cys Prxs are among the most abundant proteins. Despite the prokaryotic origin of the plant chloroplast, the 2-Cys Prxs of this organelle undergo peroxide-mediated overoxidation, thus behaving as eukaryotic-type enzymes. The redox status of chloroplast 2-Cys Prxs, mostly controlled by NTRC and Srx, may balance the antioxidant and signaling functions of chloroplast-produced hydrogen peroxide and, thus, its activity as second messenger. Although much progress has been made on the biochemical properties of 2-Cys Prxs, little is yet known about the mechanisms explaining their function in signaling. The identification of the targets of these enzymes may be of aid to establish these functions.

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