H$_2$O$_2$-induced acclimation of photosystem II to excess light is mediated by alternative respiratory pathway and salicylic acid


College of Life Science, Northwest Normal University, Lanzhou, 730070, Gansu Province, China*
Institute of Modern Physics, Chinese Academy of Sciences, 730000 Lanzhou, China**

Abstract

Acclimation to excess light is required for optimizing plant performance under natural environment. The present work showed that the treatment of Arabidopsis leaves with exogenous H$_2$O$_2$ can increase the acclimation of PSII to excess light. Treatments with H$_2$O$_2$ also enhanced the capacity of the mitochondrial alternative respiratory pathway and salicylic acid (SA) content. Our work also showed that the lack in alternative oxidase (AOX1a) in AtAOX1a antisense line and the SA deficiency in NahG (salicylate hydroxylase gene) transgenic mutant attenuated the H$_2$O$_2$-induced acclimation of PSII to excess light. It indicates that the H$_2$O$_2$-induced acclimation of PSII to excess light could be mediated by the alternative respiratory pathway and SA.

Additional key words: excess light; hydrogen peroxide; photosystem II; salicylic acid.

Introduction

Light energy is absorbed by photosynthetic organs of plants and is used for photosynthetic CO$_2$ assimilation. In natural environments, however, plants often experience high irradiance stress generated by excess light (EL), which causes the amount of absorbed light energy to exceed that needed for photosynthesis. Excess light energy can lead to the overproduction of electrons, which may damage the reaction center of PSII and cause the perturbation and inhibition of photosynthetic electron transport, consequently, damaging plants and limiting crop production (Karpinski et al. 1999, 2000; Li et al. 2009).

Karpinski et al. (1999), Karpinska et al. (2000) found that treatment of leaves with H$_2$O$_2$ before EL can efficiently provoke plant acclimatory responses to subsequent EL. And, it is revealed that plant acclimatory responses to EL are controlled (at least in part) by the redox status of the QA (quinone A)–QB (quinone B)–PQ (plastoquinone) pool, and the H$_2$O$_2$-induced the acclimatory responses to EL could be related to the ability of H$_2$O$_2$ to regulate the redox status of QA–QB–PQ pool by increasing the oxidation of QA (Karpinski et al. 1997, 1999, Karpinska et al. 2000, Pfannschmidt et al. 1999).

Since EL exerts oxidative stress in both chloroplasts and cytosol, the chloroplastic and cytosolic antioxidant defenses play important roles in the plant acclimatory responses to EL (Karpinska et al. 2000; Mullineaux et al. 2000; Hernández et al. 2004). The data obtained from the current studies also suggest that the H$_2$O$_2$-induced EL acclimation could be attributed to the activation of cytosolic and chloroplastic antioxidant enzymes, such as cytosolic ascorbate peroxidase (Karpinski et al. 1997, 1999, Karpinska et al. 2000). However, the physiological processes or components that are involved in the plant EL acclimation are multiple. In the last decades, there has been increasing number of reports about the function of mitochondria in plant light acclimation, and these reports found that the mitochondrial alternative oxidase (AOX) is a main contributor to such a function of mitochondria (Yoshida et al. 2006, 2007; Zhang et al. 2010; Florez-Sarasa et al. 2011). Mitochondrial AOX is located in the mitochondrial inner membrane and catalyses the alternative respiratory pathway (or cyanide-resistant respiration). In higher plants, AOX branches from the main respiratory chain and makes the electrons flow from...
ubiquinone directly to AOX conservation (complexes III and IV). Thus, the presence of AOX makes plants to dissipate the redox energy into heat instead of ATP production (Millenaar and Lambers 2003). Although AOX is not an antioxidant and thus bypasses two of the three sites of energy enzyme, it has been demonstrated that AOX has important benefits for dissipation of excess reduced equivalents in chloroplasts and thus functions in plant acclimatory responses to EL (Yoshida et al. 2006, 2007, Zhang et al. 2010, Florez-Sarasa et al. 2011). More importantly, it has been found that a treatment with exogenous H$_2$O$_2$ can enhance the capacity of the alternative respiratory pathway or induce the expression of AOX (Wagner 1995, Feng et al. 2008). These characteristics of AOX invoke a hypothesis that the alternative respiratory pathway, acting as a non-antioxidant component in mitochondria, might play a role in the H$_2$O$_2$-induced EL acclimation. However, whether such a role of the alternative respiratory pathway actually exists it has not been studied.

Another question worth further studying is the cellular signaling cascade in the H$_2$O$_2$-induced EL acclimation. It is known that H$_2$O$_2$ can induce the accumulation of many important intracellular signaling molecules, including calcium, salicylic acid (SA), nitric oxide, and ethylene (Quan et al. 2008). However, the lack of knowledge about whether these downstream signals of H$_2$O$_2$ could be involved in the H$_2$O$_2$-induced EL acclimation is remarkable. Mateo et al. (2006) reported that SA is required for optimal photosynthesis and regulating redox homeostasis of plant cells. Thus, it is speculated that SA could act as a downstream signal of H$_2$O$_2$ and be involved in the H$_2$O$_2$-induced acclimation to EL. However, no data about the role of SA in H$_2$O$_2$-induced EL acclimation is given so far.

In the present work, by using AtAOX1a antisense line and NahG transgenic mutant, we investigated the effects of the alternative respiratory pathway and SA on the H$_2$O$_2$-induced acclimation of PSII to EL. This work may contribute to the understanding of the mitochondria-dependent mechanism and the cellular signaling cascade in H$_2$O$_2$-induced EL acclimation.

### Material and methods

**Plant materials and growth conditions:** Seeds of *Arabidopsis* ecotype Columbia (Col-0) wild type, AtAOX1a antisense lines (AS-12), and NahG transgenic mutant, which encodes the enzyme salicylate hydroxylase that inactivates salicylic acid, were sown on a mixture of loam soil, vermiculite, and perlite (2:1:1, v/v) and were maintained at 4°C for 2 d. Then, the plants were grown in a growth chamber with 10-h day [100 ± 10 μmol(mol photon)$^{-1}$ m$^{-2}$ s$^{-1}$ at 22°C] and 14-h night (18°C) cycle and 70% relative air humidity. Eight-week-old plants were used for experimental treatments.

**Treatment:** Leaves of seedlings were detached and were vacuum infiltrated for 4 min with 0, 0.1, 1, or 10 mM H$_2$O$_2$ solutions prepared by dissolving in H$_2$O. Subsequently these leaves were floated on H$_2$O$_2$ solutions or H$_2$O for additional 2 h as described. All of these treatments were executed under low light [100 ± 10 μmol(photon)$^{-1}$ m$^{-2}$ s$^{-1}$ at 22°C]. Thereafter leaves were removed from the solutions, wiped, and left in the air. The leaves with the petiole were still placed in a small tube with some water to prevent desiccation and were exposed to excess light [1,500 ± 200 μmol(photon)$^{-1}$ m$^{-2}$ s$^{-1}$ PAR] for 1 h.

**Capacity of mitochondrial alternative respiratory pathway:** Mitochondria were isolated and purified as described by Keech et al. (2005). The isolated mitochondria were maintained in the dark for 1 h and the oxygen uptake by the isolated mitochondria was measured with a Clark-type oxygen electrode at 22°C in the dark. The reaction medium contained 10 mM TES (pH 7.5), 300 mM sucrose, 5 mM KH$_2$PO$_4$, 10 mM KCl, 2 mM MgSO$_4$, and 0.1% (w/v) bovine serum albumin. Malate (10 mM) and 1 mM glutamate were added into the reaction medium as substrates for mitochondrial respiration. To ensure complete activation of AOX, 10 mM dithioretil and 1 mM pyruvate were supplemented. To measure the capacity of the mitochondrial alternative respiratory pathway ($V_{alt}$), 1 mM KCN was added and $V_{alt}$ was defined as the stable O$_2$ uptake sensitive to salicylhydroxamic acid (SHAM) in the presence of 1 mM KCN; it was obtained when the rate of O$_2$ uptake was linear for at least 10 min after addition of KCN (Fig. 1S; supplement available online). $V_{alt}$ were expressed as mmol(O$_2$) mg$^{-1}$ (protein) min$^{-1}$. The residual respiration (O$_2$ uptake in the presence of both KCN and 5 mM SHAM) were undetectable. The concentrations of KCN and SHAM were used according to previous reports (Niewiadomska et al. 2004).

**Chlorophyll (Chl) fluorescence parameters** of the detached leaves treated with H$_2$O$_2$ or H$_2$O were measured by using a portable Chl fluorometer (*Walz, Effeltrich, Germany*), as described previously by Demmig-Adams et al. (1996). The Chl fluorescence parameters were calculated by the formulas according to Genty et al. (1989). In brief, the $F_F$/$F_m$, the maximal efficiency of PSII, was defined as $(F_m - F_0)/F_m$, where $F_0$ is the maximum fluorescence emission from the dark-adapted state (30 min of dark adjustment by covering the leaf with a black cloth) measured with a pulse of saturating light [1-s saturating flash of 8,000 μmol(photon)$^{-1}$ m$^{-2}$ s$^{-1}$ ] and $F_m$ is the minimal fluorescence emission from the dark-adapted state. $F_{PSII}$, the PSII operating efficiency, was defined as $(F_m - F_{0}')/F_m'$, where $F_{0}'$ is the steady-state level of fluorescence emission at the given irradiance, and $F_m'$ is the maximum fluorescence emission from the light-adapted state measured with