Journal of Plant Physiology 167 (2010) 761–770

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Iron stabilizes thylakoid protein–pigment complexes in Indian mustard during Cd-phytoremediation as revealed by BN-SDS-PAGE and ESI-MS/MS

M. Irfan Qureshi [a,1], Gian Maria D'Amici [b,1], Marco Fagioni [b], Sara Rinalducci [b], Lello Zolla [b,*]

[a] Department of Biotechnology, Jamia Millia Islamia, New Delhi 110025, India
[b] Department of Environmental Sciences, University of Tuscia, Largo dell'Università snc, 01100 Viterbo, Italy

ARTICLE INFO

Article history:
Received 12 November 2009
Received in revised form
30 December 2009
Accepted 5 January 2010

Keywords:
Blue-native PAGE
Brassica juncea
Cadmium stress
Iron deficiency
Thylakoidal proteomics

ABSTRACT

Two-dimensional BN-SDS-PAGE, ESI-MS/MS and electron microscopy (EM) were used to study the role of iron (Fe) under cadmium (Cd) stress in retention of thylakoidal multiprotein complexes (MPCs) and chloroplast ultrastructure of Indian mustard, a moderate hyperaccumulator plant. Mustard was grown hydroponically with or without iron for 17 days and then exposed to $CdCl_2$ for 3 days. Fe deficiency led to an increase in oxidative stress and damage to chloroplast/thylakoids accompanied by a decrease in chlorophyll content; exposure of plants to Cd further enhanced the oxidative stress and Cd accumulation (more in −Fe plants). However, the presence of iron aided plants in the suppression of oxidative stress and retention of chloroplasts and chlorophylls under Cd stress. Proteomic analyses by 2D BN-SDS-PAGE and mass spectrometry showed that Fe deficiency considerably decreased the amount of LHCII trimer, ATPase-F1 portion, cyt b6/f and RuBisCO. No or less reduction, was observed for PSI(RCI+LHCI), the PSII-core monomer, and the PSII subcomplex, while an increase in the LHCII monomer was noted. Under iron deficiency, Cd proved to be very deleterious to MPCs, except for the PSII subcomplex, the LHCII monomer and free proteins which were increased. Iron proved to be very protective in retaining almost all the complexes. MPCs showed greater susceptibility to Cd than Fe deficiency, mainly at the level of RuBisCO and cyt b6/f; an increase in the amount of the PSII subcomplex, LHCII monomer and free proteins indicates differences in the mechanisms affected by Fe deficiency and Cd stress when compared to Fe-fed plants. This study furthers our understanding of the sites actually damaged in MPCs under Fe deficiency and Cd stress. A role emerges for iron in the protection of MPCs and, hence, of the chloroplast. The present study also indicates the importance of iron for efficient phytoextraction/phytoremediation.

Introduction

Metal ions such as iron, copper, manganese and zinc are essential micronutrients for all forms of life and play important roles in numerous biochemical processes, including electron transfer reactions. Iron is used as a cofactor and is present in heme- and iron–sulfur proteins (Van Hoewyk et al., 2007), which play important roles in photosynthesis and N and S assimilation (Abdel-Ghany et al., 2005) in chloroplasts. Despite the vast abundance of iron on earth, iron deficiency is the most common nutritional deficiency in the world. Iron deficiency is thus a serious problem in numerous crops and a major concern for plants growing on calcareous or alkaline soils (Marschner, 1995). Plants grown under Fe-deficient conditions develop a characteristic 'chlorosis' due to a decrease in the amounts of light-harvesting pigments, chlorophylls and carotenoids. Fe deficiency is accompanied by a marked decrease in the rates of photosynthesis and electron transport (Spiller and Terry, 1980), loss in number of the granal and stromal-lamellae per chloroplast and loss of many thylakoid membrane components (Andaluz et al., 2006).

Cadmium is well known for its phytotoxicity, which is associated with a number of morphological, physiological, and biochemical events. Important sources of Cd contamination are atmospheric decomposition derived from mining, smelting, and fuel combustion, as well as the use of phosphate fertilizers and sewage sludge (Jensen and Bro-Rasmussen, 1992; Lugon-Moulin et al., 2004). In addition to the consequences of competition with essential metals, Cd can exert toxic effects through its high affinity for sulfhydryl groups in proteins and other biological molecules (Sanità di Toppi and Gabbrielli, 1999; Fagioni et al., 2009), and may also induce oxidative stress (Qadir et al., 2004) by

Abbreviations: ACN, acetonitrile; ATPase, ATP synthetase; BN, blue native; cyt b6/f, cytochrome b6/f; EM, electron microscopy; ESI-MS/MS, electrospray ionization-tandem mass spectrometry; FA, formic acid; LHC, light-harvesting complex; MPCs, multiprotein complexes; PAGE, polyacrylamide gel electrophoresis; PS, photosystem; ROS, reactive oxygen species; RP-HPLC, reversed-phase-high performance liquid chromatography; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; SDS, sodium dodecyl sulfate; SOD, superoxide dismutase

* Corresponding author. Tel.: +39 0761 357100; fax: 39 0761 357179.
E-mail address: zolla@unitus.it (L. Zolla).

[1] These authors contributed equally to the work.

0176-1617/$ - see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2010.01.017

inhibiting photosynthetic light reactions (electron leaching) and/or by inhibiting reactive oxygen species (ROS)-detoxifying enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), etc. Cd also induces Fe-deficiency-like chlorosis in plants and is capable of ionic imbalance (Sanità di Toppi and Gabbrielli, 1999) and of forming a Cd–Chl complex. In the shoots, Cd may induce a series of alterations that can influence chlorophyll content, photochemical quantum yield of photosynthesis (Baryla et al., 2003), light-harvesting complex (LHC) I and II (Sanità di Toppi and Gabbrielli, 1999), etc. Cd, in fact, strongly inhibits the synthesis of chlorophylls (Sanità di Toppi and Gabbrielli, 1999) and their stable binding to proteins, thereby decreasing the accumulation of pigment–lipoprotein complexes, including photosystem I (PSI) (Sárvári et al., 1999) and PSII (Küpper et al., 2007). A severe decrease was noted with 150 μM of Cd in the content of large and small subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), as well as other enzymes of photosynthesis and chlorophyll biosynthesis (Gillet et al., 2006). Cd can bind competitively to the essential Ca-binding sites in PSII during photoactivation of the water-splitting system (Faller et al., 2005), and direct inhibition of the oxygen evolution is also possible (Pagliano et al., 2006).

The thylakoid membranes are the subcompartments in which the primary reactions of photosynthesis occur. In these reactions, about 100 proteins are involved and are organized in four major multisubunit protein complexes: the PSI, PSII, the ATP synthase complex and cytochrome b6/f (cyt b6/f) complex (Hippler et al., 2001). Proteomics of the thylakoid membrane is an excellent approach to establish the number and identity of the proteins localized to this subcompartment in pigment–multiprotein complexes (MPCs), and to study the impact of nutrients and toxic metals on them. Numerous effects of Cd on photosynthesis strongly resemble those of Fe deficiency, and are characterized by inhibition and disorganization of Chl–protein complexes (Küpper et al., 2007; Timperio et al., 2007). Cd-induced chlorosis and loss of photosynthetic components, as well as their recovery, have been shown to be linked to the Fe status of nutrient media (Solti et al., 2008), as high Cd content in the growth medium suppresses Fe uptake by the plants. The use of metal-accumulating plants to remove toxic metals, including Cd, from soil and aqueous streams has been proposed as a possible solution to soil metal contamination (Chaney et al., 2005). The process of using plants for environmental restoration is termed phytoremediation. Cd is a particularly favorable target metal for this new technology because it is readily transported and accumulated in the shoots of several plant species (Wagner, 1993). Because Indian mustard is known for accumulation and high tolerance of elevated Cd concentrations (Qadir et al., 2004; Alvarez et al., 2009), we aimed to investigate the impact of Cd on thylakoidal MPCs and the role of iron under Cd stress. Pusa Jai Kisan was selected among *Brassica juncea* cultivars for its high Cd accumulation potential (Qadir et al., 2004). This model could help to understand the changes in thylakoidal protein complexes. This study helps us to understand the role of iron in stabilizing or maintenance of thylakoidal complexes under Cd stress and in a Cd-hyperaccumulator plant. The obtained data will hopefully help to design a strategy for efficient phytoremediation. The majority of the work to date tends to correlate the beneficial effects of Fe to a balancing process which, on the one hand, counteracts Cd-induced Fe deficiency, and on the other, relieves the negative effects induced by Cd by lowering its uptake, as a result of competition between the metals. A gap still persists in literature about the actual role of Fe itself, either in the presence or absence of Cd on components of thylakoids. This information, when provided, could finally shed light on the differential mechanism of damage/impairment by Cd and Fe deficiency. This study was designed to bridge this gap. With respect to large-scale thylakoid proteomics, direct effects of iron deficiency and cadmium on thylakoidal Chl–protein complexes and a protective role of iron have never been reported. However, several techniques, including LC/MS-based proteomics as well as 1D and 2D gel separation methods, have been used to resolve different plant proteomes, including those of plastids (Peltier et al., 2004), though with different approaches. The 2D gel proteomics and LC-MS have proven useful to resolve the thylakoid proteome in different plant species, as well as to study post-translational modifications of specific thylakoid proteins (Zolla et al., 2003). Membrane proteins, however, are poorly solubilized with the detergents commonly used for 2D proteomics due to their high hydrophobicity (Santoni et al., 2000), and this technique may result in significant exclusion of integral membrane proteins. To tackle this limitation, another 2D technique (blue-native-polyacrylamide gel electrophoresis (BN-PAGE)) can be adopted, which is based on the use of the anionic dye Coomassie Brilliant Blue G-250 to transfer negative charges to membrane–protein complexes, while keeping them in a structurally intact form, thus making them more soluble for 2D analysis (Schägger and von Jagow, 1991). The BN-PAGE technique has the advantage over common 2D techniques of permitting a direct quantitative assessment of differential changes in a given proteome. Complexes are maintained in a native form and any rearrangement or damage can be visualized.

The aim of this study was to examine the role of iron under cadmium stress on the quantity and quality of thylakoidal complexes and their subunits. The concentration of Cd used in our experiment (125 μM) is higher than naturally occurring Cd, but at the same time it is not lethal for the plant used in this study, *B. juncea* cv. Pusa Jai Kisan, and for plants adapted to retain turgor. To follow our objectives, two-dimensional electrophoresis (2DE), i.e. BN-PAGE in the first dimension, and SDS-PAGE (SDS – sodium dodecyl sulfate) in the second dimension were used. The map of the thylakoidal proteome was scanned and images analyzed after the 1st and 2nd dimensions, to record qualitative changes. The bands from BN-PAGE were identified using an ESI-Ion Trap coupled with MASCOT software to match the obtained peptide profile with the protein database. Changes in the ultrastructure of chloroplasts examined with an electron microscope (EM), content of chlorophylls (a and b) and thiobarbituric acid reactive substances (TBARS, indicator of oxidative stress magnitude) were also investigated.

Materials and methods

Plant material and growth conditions

Healthy, authentic and physically uniform seeds of *Brassica juncea* L. cv. Pusa Jai Kisan, generously provided by Dr. R.K. Katiyar (National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute (IARI), New Delhi, India) were used in this study. Experiments were conducted using 250 mL beakers in a controlled environment chamber with a 16 h photoperiod (under 100 μmol m^2 s^{-1} light). A day/night temperature and relative humidity regimes of 25 ± 2 °C and 70% were adopted, respectively. Seeds were washed with detergent for 15 min and surface sterilized with 1% sodium hypochlorite in double distilled water (DDW) for 10 min followed by 10 washings with DDW. Sterilized seeds were sown in the dark on wet paper towels in covered Petri dishes, where seedlings were grown for 3 days on quarter-strength Hoagland nutrient media containing iron. Seedlings were then transferred to quarter-strength Hoagland nutrient solution (Timperio et al., 2007) with iron (+Fe, 40 μM as iron sulphate) or without iron (−Fe −Cd). The seedlings on both