Phytochemistry 72 (2011) 1081–1091

Contents lists available at ScienceDirect

Phytochemistry

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

Alteration of mitochondrial protein complexes in relation to metabolic regulation under short-term oxidative stress in Arabidopsis seedlings

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ARTICLE INFO

Article history:
Available online 10 December 2010

Keywords:
Arabidopsis thaliana
Mitochondria
Oxidative stress
Blue native/SDS–PAGE
Protein complex
Metabolite profiling
Tricarboxylic acid cycle
Glycolysis
Ascorbate
Amino acid metabolism

ABSTRACT

Plants reconfigure their metabolic network under stress conditions. Changes of mitochondrial metabolism such as tricarboxylic acid (TCA) cycle and amino acid metabolism are reported in Arabidopsis roots but the exact molecular basis underlying this remains unknown. We here hypothesise the reassembly of enzyme protein complexes to be a molecular mechanism for metabolic regulation and tried in the present study to find out mitochondrial protein complexes which change their composition under oxidative stress by the combinatorial approach of proteomics and metabolomics. Arabidopsis seedlings were treated with menadione to induce oxidative stress. The inhibition of several TCA cycle enzymes and the oxidised NADPH pool indicated the onset of oxidative stress. In blue native/SDS–PAGE analysis of mitochondrial protein complexes the intensities of 18 spots increased and those of 13 spots decreased in menadione treated samples suggesting these proteins associate with, or dissociate from, protein complexes. Some spots were identified as metabolic enzymes related to central carbon metabolism such as malic enzyme, glyceraldehyde-3-phosphate dehydrogenase, monodehydroascorbate reductase and alanine aminotransferase. The change in spot intensity was not directly correlated to the total enzyme activity and mRNA level of the corresponding enzyme but closely related to the metabolite profile, suggesting the metabolism is regulated under oxidative stress at a higher level than translation. These results are somewhat preliminary but suggest the regulation of the TCA cycle, glycolysis, ascorbate and amino acid metabolism by reassembly of plant enzyme complexes.

1. Introduction

Abiotic and biotic stresses adversely affect growth and productivity of plants and are serious threats to the sustainability of crop yields. Reactive oxygen species (ROS) are accumulated in plant cells when plant metabolism is perturbed by various stresses. It leads to oxidative damages of cellular components such as DNA, proteins and lipids (Møller et al., 2007). The metabolic network of plant cells must, therefore, be reconfigured both to allow the maintenance of metabolism and to produce reducing agents for the scavenging of ROS and thus ameliorate the damage they can do. In previous studies, we have conducted comprehensive analysis of metabolic regulation under oxidative stress in Arabidopsis roots and cultured cells and documented changes of mitochondrial metabolism including those in tricarboxylic acid (TCA) cycle and amino acid metabolism (Baxter et al., 2007; Lehmann et al., 2009). Our previous studies thus confirmed the importance of mitochondrial metabolism in stress response as is also indicated by many studies of other researchers (Dutilleul et al., 2003; Pastore et al., 2007). The results also highlighted a complex relationship between the levels of transcripts, metabolites, and metabolic flux suggesting the participation of post-transcriptional especially post-translational regulation of enzyme activity in the regulation of primary metabolism (Lehmann et al., 2009). An important role of post-transcriptional regulation in stress–response is also suggested from poor statistical correlation of protein expression data with microarray results especially in the short term response (Jiang et al., 2007; Koussevitzky et al., 2008). There are, furthermore, explicit indications that considerable metabolic control is executed at the metabolite and on the protein levels including protein modifications (Morgenthal et al., 2007). It is therefore critical, for a full

Abbreviations: 2D, two dimensional; AlaAT, alanine aminotransferase; BN, blue-native; CSY, citrate synthase; FUM, fumarase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLDH, L-galactono-1,4-lactone dehydrogenase; IDH, NAD^+ dependent isocitrate dehydrogenase; IVD, isovaleryl-CoA dehydrogenase; MDAR, monodehydroascorbate reductase; MDH, malate dehydrogenase; NAD-ME, NAD^+ dependent malic enzyme; PDH, pyruvate dehydrogenase; ROS, reactive oxygen species; TCA, tricarboxylic acid; TPI, triosephosphate isomerase.

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doi:10.1016/j.phytochem.2010.11.003

understanding of metabolic regulation under oxidative stress, to elucidate the post-translational regulation of enzymes involved in mitochondrial metabolism.

Metabolic enzymes are well known to be regulated allosterically by the substrates and/or the products of the pathway (Lal et al., 2005; Slocum, 2005). Many other post-translational modification of the enzyme proteins such as phosphorylation, glutathionylation and nitrosylation could be involved in metabolic regulation (Dalle-Donne et al., 2007; Lindermayr and Durner, 2009; Schulze, 2010). Among them we hypothesised in this study the reconfiguration of enzyme protein complexes as a molecular mechanism for mitochondrial metabolic regulation under oxidative stress. Proteins do not diffuse freely but behave in a highly organised manner in living cells. Enzyme proteins can form multienzyme protein complexes that serve to microcompartment metabolic pathways and thus allow operation of more efficient metabolic transitions (Saks et al., 2008). The organisation of metabolic pathways by enzyme protein complexes has been discussed as the main molecular-scale organisation units to orchestrate the multiple metabolic processes (Degenring et al., 2004; Durek and Walther, 2008; Graham et al., 2007; Ro and Douglas, 2004; Srere, 2000). Additionally transient complexes offer the possibility of fast exchange of some of the polypeptide components upon reassembly and thus can be a molecular basis for rapid and fine tuning or redirection of metabolism. Following this reasoning, a protein complex which changed its composition under oxidative stress would likely be an important component of metabolic regulation under these conditions.

Two dimensional (2D) blue-native (BN)/SDS–PAGE was used in the present study in an attempt to identify candidate protein complexes in mitochondria. By employing BN–PAGE for the first dimension protein complexes can be separated largely on the basis of their molecular weight without their dissociation into the constituent polypeptides. Subsequently, each component of the complex is separated in the second dimension with SDS–PAGE. BN–PAGE has been extensively used to investigate the structure of mitochondrial and chloroplast electron transport chains in plants. There are now a growing number of studies employing this method for the investigation of other hydrophobic and hydrophilic high molecular weight protein complexes (Eubel et al., 2005). We used this technique here to survey proteins associated to or dissociated from protein complexes under menadione-induced oxidative stress in Arabidopsis seedlings. Using this approach we identified candidate proteins involved in rearrangement of protein/enzyme complexes. Taken together with the results from metabolite profiling, BN/SDS–PAGE analysis suggested a novel regulatory mechanism of several metabolic pathways which was mediated by rearrangement of protein/enzyme complexes of plant mitochondria.

2. Results

2.1. Metabolic response against oxidative stress in liquid culture seedlings

We chose seedlings in liquid culture as a material because of the convenience for mitochondrial isolation from this system (Sweetlove et al., 2007). We analysed the metabolic response of seedlings under menadione-induced oxidative stress to compare it with that of the root system reported previously (Lehmann et al., 2009). Menadione is a redox active quinone known to induce production of superoxide radicals at the mitochondrial electron transport chain by competing with the ubiquinone pool for electrons (Thor et al., 1982). It is documented to affect chloroplast as well and to induce rapid oxidation of mitochondria, chloroplasts and the cytosol (Lehmann et al., 2009). By using menadione, oxidative stress under stress condition in which the ROS generation from cellular electron transport chains are enhanced could be mimicked. Due to the short term nature of the experiment no visible phenotype was observed. The total activities of TCA cycle enzymes known as markers for the onset of oxidative stress (Verniquet et al., 1991) were measured (Fig. 1). Aconitase was strongly inhibited by the treatment with 120 μM of menadione for 2 h as were pyruvate dehydrogenase (PDH) and fumarase (FUM), indicating the onset of oxidative stress. Citrate synthase (CSY), NAD^+ dependent isocitrate dehydrogenase (IDH) and NAD^+ dependent malate dehydrogenase (MDH) were not affected significantly in this condition. We also analysed some parameters related to ROS accumulation and redox state in plants namely the contents of pyridine nucleotides, protein and chlorophyll (Fig. 2). The content of NADPH was dramatically decreased in the menadione treated sample down to 11% of that in control sample. $NADP^+$ also decreased in menadione treated sample. By contrast, NAD^+ increased following menadione treatment. As a result of the decrease in NADPH, the redox state of the pyridine nucleotide pool shifted considerably toward the oxidative state (Fig. 2A). Moreover, the total pool size decreased to 77% of that observed for the control. The contents of protein and both chlorophyll *a* and *b* were invariant following the treatment (Fig. 2B and C). GC–MS based metabolite profiling was next conducted to gain a more comprehensive view of metabolic changes (Table 1). Many changes in marker metabolites were observed; a dramatic decrease in sugar phosphates of the glycolytic pathway such as glucose-6-phosphate and fructose-6-phosphate, an increase of pyruvate and citrate in the first half of TCA cycle and a decrease of fumarate and malate contents in the latter half, accumulation of γ-aminobutyric acid, a striking increase of uracil, decrease of aspartate and glutamate and an accumulation of *O*-acetyl serine in combination with a decrease of methionine. However, in contrast to our previous study, a general increase of amino acids was not observed. We did additionally observe a decrease of the dehydroascorbate level.

2.2. BN/SDS–PAGE analysis of mitochondrial protein under menadione-induced oxidative stress

Mitochondrial protein complexes were analysed by 2D-BN/SDS–PAGE in order to determine proteins which preferentially attached to, or detached from, protein complexes under oxidative

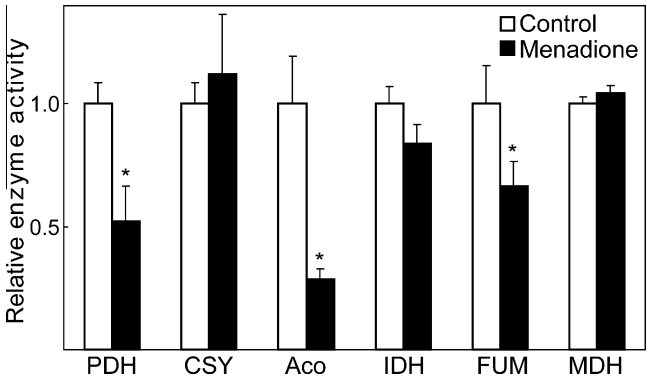

Fig. 1. Effect of menadione on the activities of TCA cycle enzymes. Seedlings were treated with menadione for 2 h and enzyme activities of pyruvate dehydrogenase (PDH), citrate synthase (CSY), aconitase (Aco), NAD^+ dependent isocitrate dehydrogenase (IDH), fumarase (FUM) and malate dehydrogenase (MDH) in whole cell extract were measured. Relative enzyme activity was determined by dividing enzyme activity by that of control sample. Each values represent means ± standard deviation of three biological replicates. Asterisks showed significant differences between control and menadione treated samples in *t*-test ($p < 0.05$). Open bars, control; Closed bars, menadione treated seedlings.