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Expression of genes involved in redox homeostasis and antioxidant defense in a marine macroalga *Ulva fasciata* by excess copper

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ABSTRACT

The expression of genes involved in the control of redox homeostasis and antioxidant defense was studied in macroalga *Ulva fasciata* Delile in response to 5 and 50 μM $CuSO_4$. Redox-related genes, methionine sulfoxide reductase A (*UfMsrA*), thioredoxin (*UfTrx*), cyclophilin (*UfCyp*), and ferritin (*UfFer*) that were up-regulated by excess Cu [Wu, T.M., Lee, T.M., 2008. Regulation of activity and gene expression of antioxidant enzymes in *Ulva fasciata* Delile (Ulvales, Chlorophyta) in response to excess copper. Phycologia 47, 346–360] were cloned and their expression was compared to superoxide dismutase (*UfMnsod* and *UfFesod*), ascorbate peroxidase (*UfApx*), glutathione reductase (*UfGr*), and catalase (*UfCat*). Transcripts of *UfMsrA*, *UfCyp*, and *UfFer* were increased by excess Cu with a peak at 3 h and that of *UfTrx* increased after 6–9 h, but not affected by 4-day exposure to excess Cu, except an increase in *UfMsrA* transcript. Transcripts of *UfMnsod*, *UfFesod*, *UfApx*, *UfGr* and *UfCat* can be increased by 4-day exposure to Cu excess [Wu, T.M., Lee, T.M., 2008. Regulation of activity and gene expression of antioxidant enzymes in *Ulva fasciata* Delile (Ulvales, Chlorophyta) in response to excess copper. Phycologia 47, 346–360] but not by short-term excess Cu treatment, except *UfGr* whose transcript increased after 3 h. Reactive oxygen species involved in up-regulation of antioxidant defense enzymes genes. These results suggest that the expression of genes of antioxidant defense enzymes and *UfMsrA* are associated with long-term adaptation of *U. fasciata* to Cu excess and transcription of redox-related genes and *UfGr* is up-regulated for short-term acclimation.

1. Introduction

Heavy metals, depending on their oxidation states, could be highly reactive and, as a result, toxic to most organisms. The toxic effect of heavy metals appears to be related to the production of reactive oxygen species (ROS) (Winterbourn, 1982), reduction of the cellular antioxidant capacity (Sies, 1999), and the resulting unbalanced cellular redox status (Pinto et al., 2003). Antioxidants and redox buffers are present in order to minimize the risk of detrimental effects (Scheibe et al., 2005). Tight control of redox homeostasis is fundamental in cell metabolism because ROS act at subtle levels as signalling molecules in development and stress perception. Plants express a battery of enzymes that contribute to the control of cellular ROS levels, redox status, and cellular repair. Ferritin (Fer) plays a role in maintaining cellular redox status by controlling the levels of free iron species Fe^{2+} and Fe^{3+}. The role of Fer in the regulation of redox reactions involving iron may turn out to be especially important in plants subjected to stress influences (Paramonova et al., 2007) (Table 1).

Elevated ROS lead to oxidative damage of macromolecules (Levine et al., 1996; Mittler et al., 2004). For example, proteins are prone to ROS-induced modification processes, particularly at thiol groups, which are oxidized to sulfenic or sulfinic forms or undergo disulfide formation (Davies, 2005). Oxidative modification decreases the integrity of amino acids, in turn, changes in structural formation of their individual side chains. Fortunately, some types of protein damage are reversible through the action of cellular repair mechanisms. In the case of methionine (Met), it would be easily oxidized to form methionine sulfoxide (MetSO), which results in the modification of activity and conformation for proteins (Davies, 2005). To rescue the protein function, MetSO is readily reversed by enzymes present in most organisms, methionine sulfoxide reductase (Msr) that utilizes Trxs to catalyze the reduction of free and peptide-bound MetSO back to the correct Met residue (Brot et al., 1981; Romero et al., 2006).

Our previous study has obtained fragments of several redox-related genes from suppression subtractive hybridization (SSH) library of a marine macroalga (*Ulva fasciata* Delile) by short-term exposure to 50 μM $CuSO_4$, in which they are homologous to *Fer* (SSH-11, EY255109), *Trx* (SSH-12, EY255110), and *Cyp* (SSH-10, EY255108) (Wu and Lee, 2008). In this SSH library, we have found a clone that is homologous to *Arabidopsis thaliana* MsrA (GenBank accessory number BAB09008), but its *E* value is only

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Table 1
Real-time quantitative PCR primers.

Gene	Nucleotide sequence (5′ → 3′)		Melting temperature of product	Size of product
UfCyp	Forward Reverse	CATCATCATTCATGGACGTTGT GCAGCACCACTGCGATAA	76.8	81
UfFer	Forward Reverse	GTCAGAGACATAGATGCCATATACT ATGCTCCACATCCAACCCTTA	76.0	117
UfTrx	Forward Reverse	TTCGCACTGAGGGCTTG GTGAACATCTTACGTTACGCT	81.1	109
UfMsrA	Forward Reverse	GCACGACCCAGCATACTGAGGC GTGCTGCAACGAGTTGTCGC	82.4	82
UfFeSOD1	Forward Reverse	TGCACGCCGAAGGACATA CCAAAGCCATTGTGAATCGAG	75.5	81
UfFeSOD2	Forward Reverse	ATTGAGTCGGTGAGCCT TGCACACAAGCGTTGTTAC	81.4	110
UfMnSOD	Forward Reverse	TGCACGCCGAAGGACATA CCAAAGCCATTGTGAATCGAG	79.8	83
UfApx	Forward Reverse	GTTTCAGGCAGGCA GCA ATTCGCATTGTTCTGGGAATC	79.0	83
UfGr	Forward Reverse	GATTTAGGCCAGGCGGA TCATTTCATCTGATCATATAACAGAACCC	80.5	90
UfCat	Forward Reverse	GAA TACCTTGACCAAAGTGGTT GTAAGTGCAGTCTACGTCG	76.7	95
UfTub	Forward Reverse	GTGGGCTATTAAATGGAGTATTGTT ACAGATAGGGTATCAAAGCGAA	76.2	94

$3e^{-4}$. Because MsrA is responsible for Trx-mediated repairing of oxidized methionine residue of protein, it was also examined in this study. The full-length cDNAs of redox-related genes (*UfMsrA*, *UfFer*, *UfTrx*, and *UfCyp*) were cloned using rapid amplification of cDNA ends (RACE). The characteristics of these cDNAs and deduced amino acid sequences were identified by a series of bioinformatic softwares. Then, the dynamics in transcript abundances of these genes in responses to short-term exposure (12 h) to 50 μM $CuSO_4$ were examined by real-time polymerase chain reaction. Because the transcripts of antioxidant defense enzymes (Mn superoxide dismutase (SOD) (*UfMnsod*), FeSOD (*UfFesod*), ascorbate peroxidase (*UfApx*), glutathione reductase (*UfGr*), catalase (*UfCat*)) can be increased by long-term exposure to 50 μM $CuSO_4$ (4 days) (Wu and Lee, 2008), and because ROS generation and scavenging are associated with cellular redox balances, changes in the transcripts of *UfMnsod*, *UfFesod1*, *UfFesod2*, *UfApx*, *UfGr*, and *UfCat* were determined within 12 h after exposure to 5 or 50 μM $CuSO_4$ and compared to those of redox-related genes. Additionally, because we have previously shown that exposure to Cu excess can induce H_2O_2 accumulation (Wu and Lee, 2008), the role of ROS on the regulation of redox-related genes and genes for antioxidant defense enzymes, the short-term changes (0.5 h treatment) in the transcripts of redox-related genes and genes for antioxidant defense enzymes to different H_2O_2 concentrations (0.01, 0.2 and 2.5 mM H_2O_2) under light and dark condition were compared. The responses of redox-related genes and genes for antioxidant defense to $O_2^{\bullet-}$ (menadione treatment) were also determined 0.5 h after exposure to 100 μM menadione under light and dark condition. Differences in the expression profile of genes involved in redox homeostasis and antioxidant defense between short-term acclimation and long-term adaptation will be discussed.

2. Materials and methods

2.1. Algal culture and treatments

U. fasciata was maintained and treated as described by Wu and Lee (2008). For all treatments, thalli of 1 g wet weight (w. wt.) were cultured in a beaker containing 300 mL of 35‰ ASW. Carbon and N and P nutrients were provided by adding $NaHCO_3$, $NaNO_3$ and Na_2HPO_4 in the final concentrations of 3 mM, 400 μM and 20 μM, respectively, and other nutrient elements were provided by adding N-free (NH_4^+ and NO_3^-) and P-free (PO_4^{3-}) Provasoli nutrient solution (Provasoli, 1968) in ASW. For the short-term treatment, thalli treated with and without extra 5 or 50 μM $CuSO_4$ were sampled at 0, 3, 6, 9, and 12 h (temperature was 25 °C and the photosynthetically active radiation (400–700 nm) was set at 150 μmol photon m^{-2} s^{-1} in the absence of algae). For the long-term treatment, $CuSO_4$ of 0, 5, 10, 20, and 50 μM was prepared by adding extra Cu in the ASW enriched with N-free (NH_4^+ and NO_3^-) and P-free (PO_4^{3-}) Provasoli nutrient solution. The temperature was 25 °C and the photoperiod was 12 h with photosynthetically active radiation (400–700 nm) of 150 μmol photon m^{-2} s^{-1} in the absence of algae. Seawater was changed everyday. After 4 days, thalli were fixed in liquid nitrogen and stored in −70 °C for further analyses.

The short-term responses in the transcripts of redox-related genes and genes for antioxidant defense enzymes to different H_2O_2 concentrations were treated with thalli with 0.01, 0.2 and 2.5 mM H_2O_2 under light (150 μmol photon m^{-2} s^{-1}) and dark condition for 0.5 h treatment, and then thalli were sampled. In an attempt to know the responses of redox-related genes and genes for antioxidant defense to $O_2^{\bullet-}$, thalli were treated with 100 μM menadione (initially dissolved in 1 mL 95% ethanol) for 0.5 h under light and dark condition.

After treatments, thalli were immediately frozen in liquid nitrogen and stored at −70 °C freezer prior to extraction of total RNA.

2.2. Total RNA isolation, SSH library construction, and rapid amplification of cDNA ends

Total RNA was extracted using the TRIZOL reagent (Invitrogen Life Technologies, CA, USA) according to the manufacturer's instructions. Total RNA for SSH and RACE libraries was extracted from thalli at 6, 9, and 12 h post Cu treatment. The subtracted library was constructed using the PCR-Select™ cDNA Subtraction Kit (Clontech, CA, USA). RNA from thalli treated with extra Cu (50 μM $CuSO_4$) was