Monodehydroascorbate reductase (MDAR) accounts for the tolerance of a green alga *Chlamydomonas reinhardtii* P. A. Dang to high light stress

Abstract

Monodehydroascorbate reductase (MDAR) is an antioxidant enzyme that converts oxidized ascorbate (MDA) to ascorbate to maintain high ascorbate concentration for algae against oxidative stress. This study examined the role of MDAR in *Chlamydomonas reinhardtii* P. A. Dang in the defense of oxidative stress by over-expression of *MDAR* gene. The *MDAR* gene was cloned and constructed in pGEMT vector (KpnI/NdeI), and then ligated to the expression vector, pChlamy3 (HSP70A/RBCS2 fusion promoter) with hygromycin resistance (hygromycin phosphotransferase (*aphIV*)). The resulting plasmid, pChlamy3-*CrMDAR*, was transformed in *Chlamydomonas reinhardtii* by electroporation, then screened for hygromycin resistant colonies. After verification with the insertion of *MDAR* gene fragments in the genome using genomic DNA PCR (Kpn-*MDAR*-NdeI fragment), three transformants (MDAR overexpressing transformant) were obtained. To test their resistance to high light induced oxidative stress, the MDAR-overexpressing transformants were illuminated under 1,600 mmol $\cdot m^{-2} \cdot s^{-1}$ condition. These transformants exhibited high tolerance to high light stress. The present findings reveal a role of MDAR in *Chlamydomonas reinhardtii* against high light induced oxidative stress. The expression of *MDAR* gene expression (mRNA abundance), protein amount (westdern blot), and enzyme activity in these transformants as well as their response to H_2O_2 challenge will be studied. The downregulatoon of MDAR expression using amiRNA is now undertaken.