OA1.3 - Molecular mechanism for NPQ induction in Chlamydomonas reinhardtii

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Plants and algae in their natural environment are constantly exposed to rapid change in quality and ntensity of light. Overexcitation of photosynthetic apparatus as consequence of electron transport chain saturation after high light stress has the potential to induce the production of toxic reactive oxygen species (ROS). To prevent damage by action of ROS a wide range of photoprotective mechanisms has been developed by plants and algae. Non Photochemical Quenching (NPQ) is the most rapid one and leads to the dissipation of excitation energy into heat. In the green alga *C. reinhardtii* NPQ is triggered by LHCSR proteins, two transmembrane pigment binding proteins able to sense lumenal pH whose accumulation is increased by high light exposure. Modulating LHCSR level and, as consequence, NPQ, has been proposed as a strategy to enhance biomass productivity of algal strains and thus the knowledge of its mechanism is fundamental. The identification and the characterization of binding chromophores is a keystone for the comprehension of quenching mechanism. Different version of LHCSR3 mutated in chlorophyll binding sites was produced and investigated using both an *in-vivo* and *in-vitro* approach. Models proposed for excitation energy quenching involve the formation of carotenoid radical cation (Ahn et al 2008), or the population of S1 state of carotenoids (Ruban et al 2007), or the formation of Chl-Chl strongly coupled dimers (Muller et al 2010). According to these models, specific chlorophyll binding residues were mutated *in vivo* and *in vitro* considering the proximity to carotenoids and the formation of excitonic coupling with other chlorophylls. In particular Chl a 612 and Chl a 613 were chosen due to their interaction with Car L1 site while chlorophyll Chl a 603 (A5) due to the proximity to Car L2. In addition, Chl 612, Chl 613 and Chl a 603 are strongly coupled with Chl 610, Chl 614 and Chl 609 respectively. *In-vitro* approach involves the expression of protein in *E. Coli* followed by refolding with pigments and allows to establish the presence of pigments bind to these conserved sites. Interestingly, Chl a 612 as reported for other LHC proteins (i.e. Lhcb1, Lhcb4,Lhcsb5 and Lhcb6) is a Chl a and shows the lowest energy of Chl herein investigated. Involvement of Chl 612, Chl 613 and Chl 603 in quenching mechanism was evaluated *in vitro* measuring fluorescence lifetime in aggregation state at low pH to miming quenching conditions: the results obtained demonstrate that only in absence of Chl 613 and Chl 603 LHCSR3 mutated holoproteins were characterized by a reduced quenching efficiency. The role of the different Chl binding residues herein investigated was then analysed *in vivo* by complementation of *npq4 lhcsr1* mutant, lacking all LHCSR proteins, with WT and mutated LHCSR3 gene on Chl 612, Chl 613 and Chl 603 binding residues. The results obtained demonstrate that, correlating NPQ and LHCSR3 protein level, in absence of Chl 612 the induction of NPQ was similar to the WT case, while in the case of Chl 613 only a slight reduction of specific LHCSR quenching activity was evinced. For Chl a 603, considering also *in-vitro* study, a possible structural role was proposed