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Antagonist effect between violaxanthin and de-epoxidated pigments in nonphotochemical quenching induction in the qE deficient brown alga *Macrocystis pyrifera*

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ABSTRACT

Nonphotochemical quenching (NPQ) of Photosystem II fluorescence is one of the most important photoprotection responses of phototropic organisms. NPQ in *Macrocystis pyrifera* is unique since the fast induction of this response, the energy dependent quenching (qE), is not present in this alga. In contrast to higher plants, NPQ in this organism is much more strongly related to xanthophyll cycle (XC) pigment interconversion. Characterization of how NPQ is controlled when qE is not present is important as this might represent an ancient response to light stress. Here, we describe the influence of the XC pigment pool (ΣXC) size on NPQ induction in *M. pyrifera*. The sum of violaxanthin (Vx) plus antheraxanthin and zeaxanthin (Zx) represents the ΣXC. This pool was three-fold larger in blades collected at the surface of the water column (19 mol mol^{-1} Chl $a \times 100$) than in blades collected at 6 m depth. Maximum NPQ was not different in samples with a ΣXC higher than 12 mol mol^{-1} Chl $a \times 100$; however, NPQ induction was faster in blades with a large ΣXC. The increase in the NPQ induction rate was associated with a faster Vx to Zx conversion. Further, we found that NPQ depends on the de-epoxidation state of the ΣXC, not on the absolute concentration of Zx and antheraxanthin. Thus, there was an antagonist effect between Vx and de-epoxidated xanthophylls for NPQ. These results indicate that in the absence of qE, a large ΣXC is needed in *M. pyrifera* to respond faster to light stress conditions.

1. Introduction

The nonphotochemical quenching (NPQ) of Photosystem II (PSII) chlorophyll (Chl) *a* emission is a proxy to measure the thermal dissipation in the photosynthetic apparatus of plants and algae [1]. Dissipation of excess energy as heat is one of the most important photoprotection mechanisms of phototropic organisms. This process confers strong fitness to plants grown under field conditions [2] and provides resistance to environmental stress [3]. NPQ is a complex and finely regulated process that in higher plants consists of four different components: (1) qE, energy or ΔpH-dependent quenching; (2) qT, state transition quenching; (3) qI, photoinhibitory quenching and, (4) qZ, a zeaxanthin (Zx) dependent quenching [4]. The carotenoid Zx plays an important role in the qE, qZ and qI components of NPQ, either as a direct quencher or as a modulator of these processes in the photosynthetic apparatus of higher plants [5,6]. Zx is formed under saturating light conditions. A pH lower than 6 in the thylakoid lumen activates the violaxanthin de-epoxidase enzyme (VDE) that converts violaxanthin (Vx) into antheraxanthin (Ax) and then into Zx. The back conversion reaction takes place in darkness and is mediated by the Zx epoxidase enzyme [7]. The Vx to Zx conversion in high light and the back reaction in darkness are known as the xanthophyll cycle (XC) [7]. The XC is present in higher plants, mosses, lichens, green algae and brown algae [6,8,9], while in diatoms, xanthophytes, haptophytes and dinoflagellates a homologous cycle exists in which diadinoxanthin is interconverted into diatoxanthin, known as diadinoxanthin cycle [6,10].

Of the different components involved in photoprotection, qE is the fastest component of NPQ since it is induced in high light and

Abbreviations: Ax, antheraxanthin; Chl, chlorophyll; Dd, diadinoxanthin; DPS, de-epoxidation state of the xanthophyll cycle pigment pool; Dt, diatoxanthin; ΔpH, proton gradient across the thylakoid membrane; FCP, fucoxanthin-chlorophyll protein; HL, high light; HPLC, high performance liquid chromatography; LHC, light harvesting complex; LL, low light; MGDG, monogalactosyldiacylglycerol; NPQ, nonphotochemical quenching of PSII florescence; PSII, photosystem II; qE, energy-dependent quenching; qI, photoinhibitory quenching; qZ, zeaxanthin-dependent quenching; qT, state transition quenching; Vx, violaxanthin; VDE, violaxanthin de-epoxidase; XC, xanthophyll cycle; ΣXC, xanthophyll cycle pigment pool; Zx, zeaxanthin; ZE, zeaxanthin epoxidase

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disappears in darkness in seconds to minutes [1]. This component is activated by a drop in the pH of the thylakoid lumen (formation of a transthylakoid proton gradient, ΔpH) that is sensed by the PsbS protein in higher plants [11] or LHCSR proteins in green algae [12]. Protonation of PsbS induces conformational changes of PSII antenna complexes that promote thermal dissipation in this system [11]. Zx modulates the PsbS-dependent quenching since the presence of this pigment enhances the formation of qE and retards its relaxation [13]. Also, lutein accelerates qE formation in a similar way as Zx [14] in some tropical plants that have a lutein cycle (formation of lutein from lutein epoxide) involved in photoprotection [15]. In contrast to qE, qZ is a slowly developing (10 to 30 min after light exposure) and slowly relaxing (10 to 60 min in darkness) component of NPQ that depends on the formation of Zx and is indirectly dependent on ΔpH since a low pH is necessary to activate the VDE [4,5]. qI is the longest lasting component of all the NPQ components [16]; it has been associated with PSII damage and its relaxation requires the repair of this system [17]. Zx also participates in qI since any sustained down regulation or inactivation of PSII is accompanied by the retention of this pigment [3]. The relative contribution of each component to NPQ development depends on the intensity and the duration of the exposure of the organism to light [5]. In higher plants, qE is the most important response to short term (min) exposure to saturating light since it allows a flexible and rapid switching between a light harvesting and energy dissipation function of the light harvesting antenna system [2].

The brown alga *Macrocystis pyrifera* has a slow induction of NPQ upon exposure to high light, and the fast NPQ relaxation phase that takes place in the first few minutes after light to dark transition is absent in this alga [18]. In contrast to higher plants, preillumination of this alga does not accelerate NPQ induction [18]. NPQ follows the accumulation of Zx upon illumination in any pre-acclimation condition, and the disruption of the ΔpH in light does not collapse NPQ in *M. pyrifera* [19]. Thus, qE is not present here and NPQ is strongly associated with XC activity [18,19]. In other Chl *c* containing organisms such as diatoms, NPQ is also closely related to XC pigment interconversion [6,20]. However, diatoms show a ΔpH related NPQ formed immediately upon illumination [21]. The lack of qE has been reported only for *M. pyrifera* [18] but several other brown algae show similar characteristics of NPQ control [22,23].

There are important evolutionary implications of the differential control of NPQ between higher plants and brown algae [18]. Probably the xanthophyll cycle appeared before the separation of the green and red algae (brown algae) lineages and light harvesting evolutionary events must have influenced the mechanisms of photoprotection related to this cycle [18]. It is not known how NPQ is controlled when it is independent of the ΔpH, but strongly related to the XC. *M. pyrifera* is a good model to investigate the response to light stress of an organism in which NPQ is independent of the ΔpH (absence of qE that has a fast response). In this work, we characterize the effect of the xanthophyll cycle pigment pool (ΣXC) on the rate and the extent of NPQ induction and its relaxation in *M. pyrifera*. The size of the ΣXC in this alga varies significantly in blades exposed to different light conditions [24]. An increase in the concentration of XC pigments is characteristic of sun-acclimated organisms [25–27]. In higher plants a high concentration of XC pigments slows the formation and relaxation of NPQ [28]. A 40% decrease in the rate of NPQ induction was detected in *Arabidopsis* mutants that overexpress XC pigments [28]. Similar reduction in NPQ induction rate has been reported for organisms grown in intermittent light that possess a large ΣXC [29]. In an organism that lacks the fast ΔpH-dependent NPQ component, a large ΣXC might represent a physiological constraint if this pool negatively affects the rate of NPQ induction. Here we have investigated how the size of the ΣXC affects the development of NPQ in *M. pyrifera*. We show that a large ΣXC is associated with an increase in the rate but not in the amplitude of NPQ induction. We also found that this process is related to the depoxidation state of the xanthophyll cycle and not to the absolute concentration of the photoprotective pigments zeaxanthin and antheraxanthin.

2. Materials and methods

2.1. Plant material

Macrocystis pyrifera (L) C. Agardh samples were collected by scuba diving from Campo Kennedy kelp forest (31° 41.96 N; 116° 40.90 W), near Ensenada Baja California, México in November of 2009. Three blades were collected from the surface, and then at depths of 1, 2, 3 and 6 m in the water column. Blades were collected from a single organism early in the morning, tagged, kept in darkness and transported in coolers with seawater to the laboratory. Tissue discs (1.2 cm diameter) from each blade were cut with a cork borer 10 cm above the pneumatocyst, along the central axis of each blade. The discs of the three blades from the same depth were pooled and maintained in 250 mL Erlenmeyer flasks with 200 mL of filtered seawater at 17 ± 0.5 °C. The discs were maintained under illumination (75 μmol photons m^{-2} s^{-1}; cool-white fluorescent bulbs) for 4 h, followed by at least 8 h of dark incubation. This light to dark treatment favored epoxidation of xanthophyll cycle pigments. After this treatment, the discs showed a maximum Photosystem II (PSII) quantum efficiency higher than 0.7, which is characteristic of non-stressed photosynthetic tissue of *M. pyrifera* [24]; this efficiency was estimated from the F_v/F_m ratio, where F_v is the variable chlorophyll (Chl) fluorescence and F_m is the maximal Chl fluorescence, see e.g. [1].

At the sampling site, irradiance in the 400 to 700 nm range was measured in the water column with a quantum scalar PAR (Photosynthetic Active Radiation) irradiance sensor QSPL-2101 (Biospherical Instruments, San Diego CA, USA). Three profiles of the irradiance changes with the depth were measured during the sampling period. Irradiance data were fitted using the Marquardt–Levenberg algorithm of Sigma Plot software (Jandel SSP Scientific SPSS, Chicago, IL, USA) to the Lambert–Beer model for the reduction of irradiance in the water column: $E_z = E_0\ e^{-k_d\ z}$, where E_z is the irradiance at depth z, E_0 is the irradiance at surface and the light attenuation coefficient is represented by k_d [30].

2.2. Experimental setup

To investigate the effect of the xanthophyll cycle pool size (ΣXC) and light intensity on the de-epoxidation and NPQ formation in *M. pyrifera*, the algal discs, collected from different depths, were exposed to five different light intensities. PSII florescence and pigment concentration were measured in samples exposed for 10 min to 45, 90, 550, 950, and 1550 μmol photons m^{-2} s^{-1}. Light was provided by 50-Watt halogen dichroic lamps (Techno Lite) and the intensity was adjusted by using homemade neutral density filters (plastic sieves) placed between the lamp and the sample. During the light period, algal discs were placed in homemade acrylic chambers with continuous flow of seawater maintained at 17 ± 0.5 °C and supplemented with 2 mM $NaHCO_3$ to avoid any possible CO_2 limitation. The light intensity was measured in situ with a cosine-type quantum sensor of a pulse amplitude modulated fluorometer (Diving-PAM; Heinz Walz, Effeltrich, Germany).

To evaluate NPQ relaxation and XC epoxidation, an independent experiment was performed. Surface samples were collected in June of 2010 from a different organism than the one in which induction experiments were performed. The samples were acclimated as mentioned above (maintained at 75 μmol photons m^{-2} s^{-1} during 4 h, followed by 8 h of dark incubation) and then exposed to 1000 μmol photons m^{-2} s^{-1} for 60 min. Further samples were taken at 4, 6, 10, 20, 50 min of light exposure and maintained in darkness for up to 60 min. NPQ and the concentrations of the XC pigments concentration were monitored during light and dark treatments in each subsample. According to