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Non-photochemical quenching and xanthophyll cycle activities in six green algal species suggest mechanistic differences in the process of excess energy dissipation

Theresa Quaas[a,1], Silvia Berteotti[b,1], Matteo Ballottari[b], Kerstin Flieger[a], Roberto Bassi[b], Christian Wilhelm[a,c], Reimund Goss[a,*]

[a] Department of Plant Physiology, University of Leipzig, Johannisallee 23, D-04103 Leipzig, Germany
[b] Department of Plant Physiology, University of Verona, Strada le Grazie 15, 37134 Verona, Italy
[c] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

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ABSTRACT

In the present study the non-photochemical quenching (NPQ) of four biofilm-forming and two planktonic green algae was investigated by fluorescence measurements, determinations of the light-driven proton gradient and determination of the violaxanthin cycle activity by pigment analysis. It was observed that, despite the common need for efficient photoprotection, the structural basis of NPQ was heterogeneous in the different species. Three species, namely *Chlorella saccharophila*, *Chlorella vulgaris* and *Bracteacoccus minor*, exhibited a zeaxanthin-dependent NPQ, while in the three other species, *Tetracystis aeria*, *Pedinomonas minor* and *Chlamydomonas reinhardtii* violaxanthin de-epoxidation was absent or unrelated to the establishment of NPQ. Acclimation of the algae to high light conditions induced an increase of the NPQ activity, suggesting that a significant part of the overall NPQ was rather inducible than constitutively present in the green algae. Comparing the differences in the NPQ mechanisms with the phylogenetic position of the six algal species led to the conclusion that the NPQ heterogeneity observed in the present study was not related to the phylogeny of the algae but to the environmental selection pressure. Finally, the difference in the NPQ mechanisms in the different species is discussed within the frame of the current NPQ models.

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Introduction

Due to daily changes in the light intensity, all oxygenic photosynthetic organisms need strategies for maintaining the balance between efficient light harvesting, photochemistry and photoprotection from excess light, which may cause damage to the photosynthetic apparatus due to the formation of reactive oxygen species. This is especially true for algae which are found in biofilms where they are exposed to extreme environmental conditions, i.e. high light intensities in combination with drought and/or cold stress. High light intensities do not cause light stress per se, but the cell can be severely damaged when the absorbed excitation energy exceeds the energy that can be utilized by photosynthesis (Ruban et al., 2012). The fastest response to high light stress is provided by the so-called non-photochemical quenching (NPQ) of chlorophyll a (Chl a) fluorescence, which prevents the over-excitation of the photochemical reactions via the dissipation of the excessive excitation energy as heat (Baker, 2008). The overall NPQ consists of at least three components that can be distinguished by their different relaxation times after a period of high light illumination, i.e. high-energy-state-quenching (qE), quenching due to state transitions (qT) and photoinhibitory quenching (qI) (Müller et al., 2001). Photoinhibitory quenching is either caused by the inactivation or damage of PSII reaction centres or by a stable quenching in the PSII antenna, which is related to the slow conversion of the xanthophyll cycle pigment zeaxanthin (Zx) to violaxanthin (Vx) as shown by Adams et al. (2002) for overwintering plants and by Nilkens et al. (2010) for *Arabidopsis thaliana*. In contrast to Zx-dependent qE (see below) this stable antenna quenching is independent from the light-driven ΔpH once Zx is accumulated. Both processes contributing to qI result in an enhanced heat dissipation, which is characterized by very slow

* Corresponding author at: Universität Leipzig, Institut für Biologie I, Johannisallee 21–23, 04103 Leipzig, Germany. Tel.: +49 341 9736873; fax: +49 341 9736899.
E-mail address: rgoss@rz.uni-leipzig.de (R. Goss).
[1] These authors contributed equally to the paper.

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relaxation kinetics in the range of hours after the exposition to excess light. In the process of State 1–State 2 transitions a fraction of the peripheral antenna proteins dissociates from PSII and interacts with PSI. In vascular plants and green algae state transitions form and relax on a timescale of tens of minutes and are responsible for the balancing of excitation energy between PSII and PSI and the regulation of linear and cyclic electron flow in the chloroplasts (Allen, 1995). High-energy-state-quenching (qE) is usually the main component of NPQ. According to the current knowledge qE is triggered by the formation of a ΔpH across the thylakoid membrane (Horton et al., 1996). Furthermore, qE was found to depend on the existence of special xanthophyll molecules bound to the PSII antenna proteins, i.e. lutein and zeaxanthin (Demmig-Adams and Adams, 1996; Horton et al., 2005). The work of Demmig-Adams and Adams (1996) showed that the conversion of Vx to Zx, together with the formation of a ΔpH, leads to qE during illumination with high light intensities. Based on the observation that qE selectively quenches the emission of fluorescence bands originating in the LHCII (Ruban et al., 1991) and the very low NPQ in ch1 mutants of *A. thaliana* lacking antenna proteins (Havaux et al., 2007), the process of qE was assigned to components of the PSII antenna system. However, the exact localization and nature of the quenching site within the PSII antenna is still a matter of debate and different structural models for qE have been proposed. According to studies by Horton and co-workers it is possible that high qE values can be induced in the absence of Zx if the lumenal pH is lower than 4.5–5.0 (Noctor et al., 1991). Zx is thought to modulate the relationship between qE and the ΔpH and quenching in the presence of Zx can be activated at a lower, light-driven ΔpH. This led to the proposal of the "LHCII aggregation model" by Horton et al. (2005). Zx is suggested to facilitate the aggregation of LHCIIb trimers which, however, mainly depends on the protonation of specific amino acid residues exposed to the lumenal surface of the Lhcb proteins. In the model of Horton et al. (2005) the actual quenching site within the LHCIIb is represented by a Chl a-Chl a or a Chl a-lutein dimer. Studies by Holt and coworkers (2005) showed the selective formation of a carotenoid radical cation upon excitation of chlorophyll molecules under conditions of a maximum steady-state feedback de-excitation. The radical cation $Zx^{\bullet+}$ was found in the monomeric Lhcb complexes Lhcb4, Lhcb5 and Lhcb6 (Avenson et al., 2008) and was correlated with qE. A special Chl-Zx heterodimer was shown to be responsible for the quenching of the excited bulk chlorophyll molecules (Ahn et al., 2008).

After the discovery of a novel PSII-related protein in vascular plants, PsbS (Funk et al., 1995), it was shown that qE is almost completely absent in *A. thaliana* mutants that lack this protein (Li et al., 2000). PsbS is essential for qE both in the absence and presence of Zx, but, due to the absence of associated pigments, does not represent the actual quenching site (Bonente et al., 2008). Thus, PsbS was proposed to act as pH sensitive switch inducing a conformational change of the Lhcb proteins associated with qE (Bonente et al., 2008; Ruban et al., 2012). The protonation of PsbS was shown to induce a reorganization of PSII subunits in grana partitions with a segregation of the outer antenna from the inner PSII section (Betterle et al., 2009; Johnson et al., 2011). The new NPQ model by Holzwarth and co-workers (2009) suggests the presence of two different quenching sites termed Q1 and Q2. These quenching sites are supposed to be associated with detached LHCIIb aggregates and minor LHCII antenna proteins, which are tightly attached to the PSII core complex, respectively (Dong et al., 2015). According to the model, only quenching at the Q2 site requires the presence of Zx while NPQ at site Q1 is independent of Vx de-epoxidation (Jahns and Holzwarth, 2012). This is consistent with the formation of two quenching sites as detected by time-resolved spectroscopy (Miloslavina et al., 2011).

A protein related to PsbS, the LhcSR protein, was shown to play a comparable role in qE formation in the green alga *Chlamydomonas reinhardtii* (Koziol et al., 2007; Peers et al., 2009; Bonente et al., 2011). In contrast to PsbS, LhcSR is a chlorophyll-xanthophyll binding protein and might thus act as a direct quencher of excitation energy. LhcSR is an ancient member of the light-harvesting complex superfamily and orthologues are found throughout the photosynthetic eukaryotic taxa, with the exception of the red algae and vascular plants (Peers et al., 2009). Interestingly, NPQ induction in *C. reinhardtii* depends on the presence of LhcSR3 proteins, but not on Vx de-epoxidation, since the *npq1* mutant, which is unable to accumulate Zx, exhibits a similar NPQ as the wild type (Bonente et al., 2011). Evidence has been accumulated that both PsbS- and LhcSR-dependent NPQ depends on the interaction with other Lhcb subunits. In *C. reinhardtti* the subunit Lhcbm1 was reported to be necessary for NPQ induction, likely directly interacting with LhcSR3 (Elrad et al., 2002; Ferrante et al., 2012). Further studies on *A. thaliana* showed that the absence of Lhcb4 reduced both the kinetics and the amplitude of NPQ, while the absence of the major antenna proteins Lhcb1 and Lhcb2 led to a reduction of qE (Andersson et al., 2003). However, *in vitro* investigations with recombinant Lhcb proteins suggested that any Lhcb subunit could represent the potential site of qE quenching (Ballottari et al., 2010; Ruban et al., 2012).

The aim of the present study was to analyze NPQ in different green algae, which are either planktonic or can form biofilms and thus have a different need for photoprotection in their natural environment. In nature, biofilms are frequently exposed to strong spatial and temporal variations of light, heat/cold and CO_2 availability and therefore the presence of an efficient photoprotection mechanism, i.e. NPQ, seems crucial for the survival of biofilm forming algae. To determine whether these algae show a uniform NPQ or if differences occur in the mechanism of enhanced heat dissipation, the individual NPQ components qI and qE, and state transitions were studied in detail. To further characterize the main component of NPQ, qE, the essential factors for qE formation, i.e. the light-driven ΔpH and the concentration of the Vx cycle pigments, were determined. It was a further intention of the present study to understand, if the different NPQ components are under control of the environmental selection pressure or if they are related to the phylogenetic position of the different algal species. Finally, the relevance of the present measurements for the current NPQ models should be discussed on the background of the biodiversity of photoprotective mechanisms (Goss and Lepetit, 2015).

Materials and methods

Plant material

All cultures (Fig. 1) were cultivated as sterile air-lift or batch cultures at a temperature of 21 °C. BBM (Bold's Basal Medium; Bischoff and Bold, 1963), ESFL (Basal Medium; according to the instructions provided by the Sammlung von Algenkulturen Göttingen, SAG, 2007) and HSM medium (Ferrante et al., 2012) were used as cultivation media. Cultures were either grown at a control light intensity of 70 μmol photons m^{-2} s^{-1} (PAR) or at a high light intensity of 400 μmol photons m^{-2} s^{-1} (PAR) with a light dark cycle of 14:10 h.

Measurement and determination of NPQ via pulse-amplitude-modulation (PAM) fluorometry

NPQ was calculated according to van Kooten and Snel (1990): NPQ = (Fm − Fm′)/Fm′. The chlorophyll fluorescence was measured with a chlorophyll fluorometer PAM-101 (Walz, Effeltrich, Germany). Saturating light pulses with a duration of 0.8 s were

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