RESEARCH PAPER

Presence of state transitions in the cryptophyte alga Guillardia theta

Otilia Cheregi1, Eva Kotabová2, Ondřej Prášil2, Wolfgang P. Schröder1, Radek Kaňa2,* and Christiane Funk1,*

1 Department of Chemistry, Umeå University, SE-90187 Umeå, Sweden
2 Institute of Microbiology, Centre Algatech, Laboratory of Photosynthesis, Opatovický Mlýn, Třeboň 379 81, Czech Republic

* To whom correspondence should be addressed. E-mail: christiane.funk@chem.umu.se; kana@alga.cz

Received 2 April 2015; Revised 10 June 2015; Accepted 29 June 2015

Editor: Tracy Lawson

Abstract

Plants and algae have developed various regulatory mechanisms for optimal delivery of excitation energy to the photosystems even during fluctuating light conditions; these include state transitions as well as non-photochemical quenching. The former process maintains the balance by redistributing antennae excitation between the photosystems, meanwhile the latter by dissipating excessive excitation inside the antennae. In the present study, these mechanisms have been analysed in the cryptophyte alga Guillardia theta. Photoprotective non-photochemical quenching was observed in cultures only after they had entered the stationary growth phase. These cells displayed a diminished overall photosynthetic efficiency, measured as CO₂ assimilation rate and electron transport rate. However, in the logarithmic growth phase G. theta cells redistributed excitation energy via a mechanism similar to state transitions. These state transitions were triggered by blue light absorbed by the membrane integrated chlorophyll a/c antennae, and green light absorbed by the lumenal biliproteins was ineffective. It is proposed that state transitions in G. theta are induced by small re-arrangements of the intrinsic antennae proteins, resulting in their coupling/uncoupling to the photosystems in state 1 or state 2, respectively. G. theta therefore represents a chromalveolate algae able to perform state transitions.

Key words: Blue/low light adaptation, chlorophyll a/c antenna, cryptophytes, growth stage, non-photochemical quenching, state transitions.

Introduction

Harvesting of sun light is the first step in the photosynthetic process. In the photosynthetic light reaction, antenna complexes (reviewed by Jansson, 1999; Dall’Osto et al. 2015) rapidly transfer absorbed light energy to a reaction center, which is located in a special membrane system called thylakoid membrane. While the reaction centers of Photosystem II (PSII) and Photosystem I (PSI) remained highly conserved during evolution, various antenna systems have evolved in photosynthetic organisms. Prokaryotic cyanobacteria and eukaryotic red algae contain phycobilisomes with covalently bound phycobilipigments as their major antennae (Bailey et al., 2008). These structures are extrinsically associated to the outside of the thylakoid membrane. In higher plants and green algae, the most abundant antenna is the chlorophyll alh-binding light harvesting complex (referred to as LHC), which is inserted into the thylakoid membrane (Neillson and Durnford, 2009). Cryptophytes acquired their chloroplasts by secondary endosymbiogenesis (Gibbs, 1981) and, similarly to red algae, the
progenitor of their chloroplasts, use two different light-harvesting systems: phycobiliproteins and chlorophyll a/c-binding proteins. The Chl a/c-binding proteins are homologous to the Chl a/b of plants (Green and Durnford, 1996; Durnford et al., 1999). Even though the phycobiliproteins originated from the red algal ancestor, in cryptophytes they are not organized into phycobilisomes bound to the stromal face of the thylakoids, but are located in the thylakoid lumen (Spear-Bernstein and Miller, 1989). Variations in the lumen-exposed domains of the Chl a/c proteins may have implications for interaction between the intrinsic light-harvesting antennas and the lumen-located, soluble phycobiliprotein (Broughton et al., 2006).

Light harvesting provides a major challenge in optimizing photosynthesis; while light provides the energy necessary for carbon fixation, at the same time it damages the cells. When more light is absorbed than can be used for photochemistry, the excess energy can potentially lead to the production of highly reactive oxygen species that cause photo-oxidative damage and inhibit photosynthesis (Li et al., 2009). Photosynthetic organisms have adapted to evolve a variety of direct and indirect protection mechanisms. Long-term acclimations to the prevalent light intensity involve regulation of the antenna size (Kopečná et al., 2012; Kouřil et al., 2013). However, besides adjusting light absorption, photosynthetic organisms can dissipate excess light energy in a process called non-photochemical quenching (NPQ) (de Bianchi et al., 2010). The molecular mechanisms of NPQ vary among cyanobacteria, algae, and higher plants depending on their different light-harvesting antennae (reviewed in Niyogi and Truong, 2014). Another rapid physiological mechanism for adaptation to light quality and intensity are state transitions. They allow balancing of the absorption capacity of the two photosystems by reversible relocation of the light-harvesting complexes between PSII to PSI (Dietzel and Pflanzschmidt, 2008). Generally, light leading to excess excitation of PSII induces a transition to state 2, in which excitations are diverted towards PSI. On the contrary, when PSII is over-excited the state 1 is induced, transferring light energy to PSII. State transitions are well documented in plants and green algae (reviewed by Minagawa, 2011) as well as in cyanobacteria (Campbell et al., 1998; Kirilovsky et al., 2014), while state transitions in algae from the red clade have been analysed less extensively (Owens, 1986; Snyder and Biggins, 1987; Gibbs and Biggins, 1989; Delphin et al., 1996). The first proposal of state transition in red algae was made by the Norio Murata (Murata, 1969), however its mechanism is still not clear (Delphin et al., 1995). A state-transition-‘like’ behavior in cryptophytes has been observed in Cryptomonas ovata; energy delivering to the photosystems was controlled by small conformational changes/uncoupling of the pigment-proteins and considered to be the major photoprotective mechanism in cryptophytes (Snyder and Biggins, 1987). Recently, other photoprotective mechanisms have been studied in various cryptophytes (Funk et al., 2011; Kaňa et al., 2012b). Cryptophytes are adapted to very low light and are able to take advantage of quantum coherence to improve the efficiency of energy capturing (Collini et al., 2010; Harrop et al., 2014). In Rhodomonas salina a new type of NPQ has been described (Kaňa et al., 2012b) and recently it was shown that Guillardia theta is able to perform non-photochemical quenching (Funk et al., 2011). Here we performed in-depth analysis of the photoprotection mechanisms and photochemistry of G. theta. It is shown that the mechanisms of photoprotection and photosynthetic efficiency change significantly with culture age. In the logarithmic growth phase, light harvesting is accompanied by a state transitions-like mechanism and the absorbed light is efficiently used for carbon fixation. Blue light absorbed by the chlorophyll a/c antennae induces these state transitions, while green light, exciting the lumen-localized phycoerythrin, has no stimulating effect. In the stationary growth phase no state transitions take place, instead non-photochemical quenching is induced, leading to a drastic decrease of photosynthetic activity. The results show that the cryptophyte algae G. theta displays a growth phase-dependent photoprotection mechanism and highlight the presence of state transitions in cryptophytes.

Materials and methods

Culturing and cell counting

G. theta cells (CCMP2712) were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton. Cultures were grown in Fernbach culture flasks in h/2 media (Guillard, 1975) shaken (120rpm) at 18 °C under white light (30 μmol photons m⁻² s⁻¹) with light-dark cycle (12 h:12 h). Rhodomonas salina (strain CCAP 978/27) was grown in the same media and at the same conditions as G. theta.

Cultures of one litre were started with the same number of cells (~10⁵). Every second day cell number and size were determined using a calibrated Coulter Counter (Beckman Multisizer III) equipped with a 70 μm aperture. Samples were measured in triplicate.

Pigment determination

Chlorophyll a and c concentrations in the cells were determined by absorption using an UV/VIS spectrophotometer (Unicam UV 550, Thermo Spectronic, UK) and calculated according to the equations of Jeffrey and Humphrey (1975). Triplicates of 10–15 ml of the algal suspension were filtered onto Whatman GF/F filters; the pigments were extracted by 90% acetone for 24 h at 4 °C in darkness.

Absorption and fluorescence spectra

Absorption spectra were recorded by a spectrophotometer (Unicam UV 550, Thermo Spectronic, UK) equipped with an integrating sphere. Cells were collected on nitrocellulose membrane filters (Pragochema, Czech Republic) and the filters were then positioned in the integrating sphere. Absorbance was measured between 400 and 800 nm, with a bandwidth of 4 nm.

Fluorescence emission spectra (77 K) were measured using an Aminco-Bowman Series 2 spectro-fluorometer (Thermo Fisher Scientific, USA). Cells (3 ml) adapted at room temperature either to white low light (2.5 μmol photons m⁻² s⁻¹) for
intensity and $n_{PSII}$ gives the ratio of functional PSII to total chlorophyll a. The value $1/n_{PSII} = 500$ [mol chla/mol RCII] was used, which had been previously estimated for cryptophytes [see Suggett et al., (2010) for details].

State transitions were studied using a Double-modulation FL100 fluorimeter (Photon System Instruments, Brno, Czech Republic). Before the measurement, cell suspensions (2ml) were placed in a stirred cuvette and dark adapted for 20 min to ensure transition to low fluorescence state 2. The minimal value of fluorescence ($F_0$) was then measured immediately after dark adaptation in low-intensity blue measuring light. Dark adapted cells were illuminated with low-intensity blue light (7 $\mu$mol photons $m^{-2}$ s$^{-1}$) to induce the transition from state 2 to state 1. Afterwards, the reverse process (transition from state 1 to state 2) was induced by keeping cells in dark, the maximal fluorescence $F_m$ was measured by the same multiple turnover flashes (464 nm, duration 350 ms, intensity 4200 $\mu$mol photons $m^{-2}$ s$^{-1}$) applied every 20 s or on the end of the dark period. The ability to perform state transition during growth was measured every 2 d from the ratio $F_v(\text{LL})/F_v(\text{D})$ or $F_v(\text{D})/F_v(\text{D})$ . Parameters $F_v(\text{LL})$ and $F_v(\text{D})$ represent variable fluorescence values measured with cells adapted to 2 $\mu$mol photons $m^{-2}$ s$^{-1}$ of white low-light for 10 min (LL) and cells taken from dark (D), respectively. To remove the effect of increasing culture density during the growth, the $F_v$ values were normalized by dividing by respective minimal fluorescence levels, $F_0(\text{LL})$ or $F_0(\text{D})$.

**Kinetic fluorescence spectroscopy**

Changes in fluorescence emissions during state transitions were measured using the spectrally resolved fluorescence induction (SRFI) method (see e.g. Kaňa et al., 2012a; Kotabová et al., 2014). In this method the diode-array spectrophotometer SM-9000 (Photon System Instrument, Brno, Czech Republic) was combined with a double-modulation FL100 fluorimeter (Photon System Instruments, Brno, Czech Republic) that was used for actinic light excitation and spectra synchronization. Before the measurement, cell suspensions were dark-adapted for 20 min to ensure transition to the low fluorescence state 2. The spectral changes in maximal fluorescence were then detected before and after application of low-intensity blue light (464 nm, 7 $\mu$mol photons $m^{-2}$ s$^{-1}$) for 80 s causing the transition from state 2 to state 1. Maximal fluorescence was induced by multiple-turnover flashes (464 nm, duration 350 ms, intensity 4200 $\mu$mol photons $m^{-2}$ s$^{-1}$).

**Photosynthesis**

The photosynthetic carbon fixation was determined using the small volume $^{14}$C incubation method of (Lewis and Smith, 1983). Sample aliquots of 1 ml were incubated for 40 min at 18 °C with $^{14}$C-sodium bicarbonate (MP Biochemicals, USA) at a final concentration of 1 µCi m$^{-2}$ in the laboratory-built photosynthesotron (Bruyant et al., 2005). Samples for triplicate background counts (with 50 µl of formaldehyde) and total counts (with 50 µl of ethanolamine) were prepared. Samples with 50% HCl (1 ml) were left overnight to purge off unincorporated label before 10 ml of Ecolume scintillation cocktail was added and disintegrations per minute were counted on
a calibrated Tri-Carb 2810 TR Liquid Scintillation Analyser (Perkin Elmer, USA). Dissolved inorganic carbon concentrations were determined in a cell-free medium by the Gran titration technique described by (Butler, 1982). The carbon fixation rate was normalized to the Chl content.

**Results**

**Characterization of growth phases in G. theta cultures**

Growth of *G. theta* cells in shaking batch culture (12h/12h day/night irradiance rhythm) was monitored over a period of 13 d; *G. theta* had a doubling time of 34 h in its logarithmic phase (Days 2–6). However, already from Day 6 the growth rate slowed down and the culture entered the stationary phase (Fig. 1); characteristic time points are Day 2 (early logarithmic phase), Day 6 (turning point to stationary phase), Day 9 (early stationary phase), and Day 13 (late stationary phase). Cultures that were not shaken, but instead bubbled with air, had reduced growth rate and entered the stationary phase 2–3 d later, however, at roughly the same cell density compared with the shaken culture (Fig. 1).

The growth curve of *G. theta* was compared with *Rhodomonas salina*, a related cryptophyte, often referred to as the model cryptomonad. Under the same growth conditions like *G. theta*, the growth of *R. salina* remained in logarithmic phase throughout the whole experimental period of 14 d (data not shown).

The diameter of individual *G. theta* cells increased gradually with culture age (Fig. 2A); when the cells entered the stationary phase at Day 6, with decreased doubling time the *G. theta* cells continued to grow in size. The total amount of chlorophyll per cell decreased during the logarithmic phase, but remained constant in the stationary phase (Fig. 2A). The amount of Chl *a* decreased by 43% from 1.34 pg/cell (Day 2 after culture started) to 0.750 pg/cell (in 13-d-old cultures), meanwhile Chl *c* only decreased from 0.234 pg/cell (Day 2) to 0.156 pg/cell (Day 13), resulting in a decreased Chl *a*/Chl *c* ratio by 13% during the experiment.

Changes in pigment composition and antennae organization during the growth phases were monitored by whole cell absorption spectra. Figure 2B shows whole cell absorption spectra of the culture in dependence of the growth phases: early logarithmic phase (Day 2), turning point to stationary phase (Day 6), and stationary phase (Day 13). The spectra were characterized by absorption maxima at 436 nm (mainly Chl *a*), 465 nm (Chl *c*), 495 nm (alloxanthin), 545 nm (phycoerythrin), and 680 nm (Chl *a*). The carotenoid:Chl *a* ratio increased from 0.9 (logarithmic phase) to 1.2 (stationary phase), while the amount of phycoerythrin decreased in stationary phase.

Changes in the functional organization of phycoerythrin (PE) in *G. theta* cells were analysed by low temperature (77 K) fluorescence emission spectra (Fig. 3). De-convoluted spectra of dark adapted cells in the early logarithmic growth phase revealed two main PE emission bands, F576 and F589, and two additional minor bands, F607 and F644 (Fig. 3C). F576 almost disappeared in the stationary phase (Fig. 3A), while the fluorescence bands at 694 nm (PSII) and 705 nm (PSI) increased, indicating that F576 PE is energetically stronger coupled to PSII and PSI during the stationary growth phase.
Light harvesting and photoprotective mechanisms of G. theta change age-dependently

The effective PSII antenna size (oPSII) and connectivity of PSII reaction centers (p) was measured by fast repetition rate fluorometry (FRRF). In the logarithmic growth phase, the p-value was high (around 0.3, see Fig. 4), indicating that PSII centres share a common antenna; this interconnection was lost in the stationary phase (p<0.1 in Fig. 4). At the same time, the effective antenna size of PSII significantly increased by 25% from G. theta cultures growing in logarithmic phase (oPSII of ~330 Å²) to stationary phase (oPSII of ~430 Å²) (Fig. 4); more chlorophyll molecules were effectively connected with PSII in the stationary phase.

Detailed analysis of NPQ in G. theta cultures was performed with respect to the culture age (Fig. 5B). Surprisingly, in the logarithmic phase, G. theta cells showed rather low NPQ (below 0.5, see Fig. 5A, Day 2), while in the stationary phase NPQ steadily increased (Fig. 5A, Day 13). These results are in contrast to NPQ measurements performed in R. salina, which displayed quite high NPQ values (~1.4 at Day 2, see Supplementary Fig. S1, available at JXB online) already in very young cultures. Further, the presence of state transition was quantified in G. theta at different culture ages. During logarithmic growth phase, characterized by low NPQ, a phenomenon similar to state-transitions was detected in G. theta cells (Fig. 6A); pretreatment with low-light (2 µmol photons m⁻² s⁻¹) was required to reach maximal fluorescence. When dark-adapted G. theta cells were exposed to low-intensity blue light (7 µmol photons m⁻² s⁻¹) for 300 s the fluorescence increased, this process was reversed in darkness (Fig. 6A). This dark recovery was also observed during treatment with saturating blue flashes (see red curve in Fig. 6A), therefore it was independent of fluorescence quenching. Instead, the initial fluorescence increase, Fm, in low blue light, was attributed to state 2 to state 1 transition; the following decrease in darkness then corresponded to state 1 to state 2 transition. State 2 to state 1 transitions were observed in G. theta only after exposure to low-intensity blue light, green light had no effect, neither on state 2 to state 1 transition nor on its recovery (see Supplementary Fig. 2A, 2B). Thus, state transitions in G. theta are exclusively controlled by chlorophyll-binding proteins and not by the lumen-located phycoerythrin antenna.

Room temperature fluorescence emission spectra were recorded on dark-adapted cells either exposed to blue light for 5 min or kept in darkness (Fig. 7). Dark-adapted cells had a substantially lower maximal variable fluorescence (Fv/Fm) (see Fig. 7A). The difference spectra between Fm in State 2 and Fm in State 1 (Fig. 7B) displays a pronounced increase of chlorophyll α emission in Fm originating from PSII. Interestingly to note, state transitions were only observed in G. theta cultures in the logarithmic growth phase (Figs 6A, B and 7 and Supplementary Fig. S2); while these low light-induced changes in variable fluorescence disappeared in the stationary phase (after Day 6, see ΔFv/Fo in Fig. 6B), NPQ increased (Fig. 5B).

Presence of state transitions correlates with maximal photosynthetic performance in G. theta

Growth phase-dependent photosynthetic activity of primary (PSII efficiency, electron transport rate) and secondary (CO₂ fixation) photosynthetic reactions was analysed in G. theta.

Interestingly, the largest F576 emission was observed in young cells that were dark adapted for 20 min, fluorescence emission decreased in cells adapted to low light (Fig. 3B).
During lag and logarithmic growth phases, maximal efficiency of PSII photochemistry ($F_{V}/F_{M}$) was detected (Fig. 8). It then gradually declined as the culture progressed to the stationary phase (Day 6–9, Fig. 1). $F_{V}/F_{M}$ decreased by 30% from its value of 0.7 during logarithmic phase (up to Day 6) to 0.5 in stationary phase (day 13) (Fig. 8A). The absolute electron transport rate (ETR), quantifying the maximal rate of electron generation in PSII and thus the maximal capacity of primary photosynthetic reactions rapidly declined between Days 6 and 9, when the cultures were entering the stationary phase (Fig. 8B). Within the stationary phase (Days 9–13) an additional small gradual decline of ETR was observed. Changes in ETR correlated with the overall capacity of photosynthetic carbon fixation measured from $^{14}$C incorporation rate (Fig. 8C). As expected, carbon fixation rapidly declined between Days 6 and 9, consistent with the lowered PSII maximal efficiency (Fig. 8A) and electron transport rate (Fig. 8B). The transition from logarithmic to stationary phase was therefore accompanied by pronounced changes in the overall photosynthetic capacity.

**Discussion**

In the present study, it was shown that the cryptophyte alga *G. theta* is able to perform state transitions and non-photochemical quenching (NPQ) to efficiently modulate...
photosynthesis. Interestingly, *G. theta* uses these two mechanisms in different growth stages. In the logarithmic growth phase, state transitions are performed, photosynthetic activity as well as CO$_2$ fixation are high, cells contain a high amount of chlorophyll, meanwhile NPQ is very low. However, when the cells enter the stationary phase, instead of state transition they use NPQ; the cells grow in size, meanwhile their chlorophyll content per cell drops. The reason for this separation between the two types of photo-protection, which has not been reported in higher plants, is not known at present time. This work demonstrates that *G. theta* represents a chromalveolate algae that is able to perform state transitions.

**State transitions**

Previously it has been shown that the cryptophyte alga *R. salina* can perform fast and flexible NPQ (qE type) in the logarithmic growth phase (Kaňa et al., 2012b). Also *G. theta* is capable of performing NPQ (Funk et al., 2011), however, this mechanism is restricted to the stationary growth phase. In the logarithmic growth phase, under conditions when *R. salina* uses NPQ, *G. theta* performs instead state transitions to regulate the amount of energy arriving at the reaction centers. State transitions dependent on the culture age have been reported before for the chromophyte alga *Ochromonas danica* (Gibbs and Biggins, 1989). Dark adapted *G. theta* cells were in the low-fluorescence state 2, transition to the high-fluorescence state 1 was induced by low intensity blue (7 µmol m$^{-2}$ s$^{-1}$) or very low intensity white light (2 µmol m$^{-2}$ s$^{-1}$; not shown). At first view, the mechanism of state transitions performed by *G. theta* resembles the process observed in cyanobacteria (Campbell et al., 1998); when dark-adapted cyanobacterial cells are irradiated with low blue light (the light preferentially absorbed by PSI) PQ oxidation increases due to their high PSI:PSII ratio. Antenna redistribution towards PSII results then in the high-fluorescent state 1 (for review see e.g. Kirilovsky et al., 2014). This process is observed in dark-adapted cyanobacteria cells, when respiration leads to a reduced PQ pool (Mullineaux and Allen, 1990), causing efficient energetic coupling of the phycobilisomes to PSI (see e.g. Papageorgiou et al., 2007; Kaňa et al., 2014). Organization of the light-harvesting antenna in cryptophytes, however, is very different from the one in cyanobacteria. The large cyanobacterial phycobilisomes are stromal protein complexes and transfer light energy directly to the reaction centres; state transition seem to be connected with phycobilisome mobility (Joshua and Mullineaux, 2004; Kaňa et al., 2014) or with their slight re-arrangement state 2 is stimulated by phycobilisome-absorbed orange light (see...
Kiriłovsky et al. (2014)) for recent review). Cryptophytes, on the other hand, contain lumen-located phycoerythrin (PE) biliproteins as well as intrinsic chlorophyll *a/c* antenna complexes. The PE antenna seems to be rigid and immobile (Kaća et al., 2009). In *G. theta* green light, absorbed by phycoerythrin, did not induce state transition like changes (see Supplementary Fig. S2); state transitions were solely induced by light absorbed by chlorophylls (blue light, Fig. 6A) of the membrane integrated Chl *a/c* antenna. Since the low-temperature (77K) emission peaks of PSI (F705) and PSII (F694) in cryptophytes are very close to each other (Supplementary Fig. S3), no antenna redistribution was revealed between PSI and PSII during state transitions. Still, the room temperature measurements of maximal fluorescence (Fig. 7) clearly demonstrate that thylakoid membranes reorganize during state transitions and involve the intrinsic Chl *a/c* binding antennae, although the extent to which the Chl *a/c* antennae of *G. theta* are preferentially coupled to PSII (state 1, exposure to blue light) or to PSI (state 2, darkness) cannot be estimated. In higher plants and green algae, state transitions involve phosphorylation of the light harvesting antennae (Minagawa, 2011). This is in contrast to red algae, where the light induced protein phosphorylation patterns were the same in state I and state II (Biggins and Bruce, 1989) and inhibitors of phosphorylation had no effect on state transitions (Delphin et al., 1995). In cryptophytes phosphorylation of the Chl *a/c* binding intrinsic antenna has been demonstrated in *Chroomonas sp.* (Janssen and Rhiel, 2008). The role of phosphorylation for state transitions of cryptophytes will have to be demonstrated in future experiments.

The PE antenna of cryptophytes was recently shown to be extremely efficient in light harvesting due to their quantum coherence (Collini et al., 2010). Even though state transitions in *G. theta* cultures growing in the logarithmic phase were light absorbed by the intrinsic chlorophyll *a/c* antenna, they induced re-arrangements of the PE antennae within the thylakoid lumen (Fig. 3): in dark-adapted logarithmically growing cells bilins with a ‘typical’ emission maximum at 589 nm (F589) were observed as well as an additional pool displaying the unusual maximum at 576 nm (F576). Upon low-light treatment (Fig. 3B), however, F576 decreased. In the stationary culture phase, when state transitions were absent, F576 was negligible and only F589 was observed, independent of the pretreatment (see Day 9 in Fig. 3A). Using *in vitro* measurements on isolated PE, a blue shifted emission maxima (like F576) was attributed to a loss of exciton splitting in exciton-coupled pairs of bilins (MacColl et al., 1999b). These coupled bilin-pairs were described to be the lowest-energy chromophores receiving energy from other bilins and more importantly, delivering the energy to the next chromo-protein (for more details see MacColl et al., 1999a). The exciton-coupling/splitting of the bilin pairs therefore might control the energy transfer from the PE antenna to the chlorophyll *a/c* antenna and explain the observed changes in the F589/F576 ratio. Blue light seems to induce excitonic coupling of PE to the intrinsic chlorophyll *a/c* antennae, which in turn is coupled to PSII as evident from the increased maximal fluorescence in state I (Figs 6 and 7). Such a mechanism would not require any physical movements of the PE biliproteins, very small re-arrangements of the antenna proteins would be sufficient. In the cryptophytes *R. salina* (Kača et al., 2009) and *Cryptomonas ovata* (Snyder and Biggins, 1987) the luminal-located PE antennae were observed to be immobile.

Non-photochemical quenching replaces state transitions in stationary growth phase

While state transitions disappeared in the stationary growth phase of a *G. theta* culture (Fig. 6B), photoprotective NPQ increased (Fig. 5B). At the same time the PE content per cell diminished in the stationary phase (Fig. 2B), most likely the degraded phycobiliproteins were used as an additional N source (Lewitus et al., 1990). Owing to the decreased amount of PE in the stationary phase, NPQ in *G. theta* can be assigned to the Chl *a/c* antenna, similar to *R. salina* (Kača et al., 2012b). However, in *R. salina* NPQ was found to be high already in very young cultures (Supplementary Fig. S1), while NPQ in *G. theta* was restricted to the stationary growth phase (Fig. 5B). 14C incorporation experiments measured at increasing light intensities have shown that NPQ in *R. salina* occurs when the Calvin-Benson cycle is saturated (Kača et al., 2012b). In *G. theta* the CO2 fixation rate was drastically decreased in the stationary phase (Fig. 8C). Under these conditions, ATP regeneration is expected to slow down and as a consequence the lumen should become acidified. In *R. salina* it was shown that lumen acidification triggers NPQ, low pH induced the quenching state in the Chl *a/c* antenna via their reversible protonation (Kača et al., 2012b). Here, it is proposed that also in *G. theta* NPQ is a pH-dependent process resembling the described mechanism in *R. salina*.

The photo-protective capacity seems to be higher in *R. salina* (Supplementary Fig. S1) compared with *G. theta* (Fig. 5). Differences in the photo-protective capacity in diatoms have been explained by their adaption to different ecological niches (Lavaud et al., 2007; Lavaud and Lepetit, 2013). Analysing the phylogenetic relation of the two cryptophyte species based on SSU rDNA sequences (Hoef-Emden, 2008), indeed they were grouped into two distant evolutionary clades. Moreover they occupy different ecological niches with *R. salina* being estuarine (Hammer et al., 2002), while *G. theta* was isolated from coastal regions (Rappé et al., 1998). Estuarine species are known to possess a higher and more flexible capacity for photoprotection than oceanic and coastal ones (Strzepek and Harrison, 2004; Lavaud et al., 2007). The two algae therefore perform different strategies: to survive in fluctuating and dynamic light conditions *R. salina* requires efficient NPQ, while *G. theta* rather optimizes its light-harvesting capacity for efficient photosynthesis using state transitions; NPQ is only induced as a feedback reaction, when photosynthesis is down-regulated in the nutrient-limited stationary phase.

Physiological role of state transitions in *G. theta*

Equilibrating the distribution of excitation energy between the photosystems, state transitions are known to be important not only under limiting light conditions, but also to protect
against photoinhibition by minimizing PSII antenna size at high light intensities (Minagawa, 2011). While they are less significant in higher plants (Lunde et al., 2003), as much as 80% of the LHCII was found to be mobile during state transitions of green algae (Finazzi et al., 2001; Drop et al., 2014). In cyanobacteria, their importance in photoprotection still is a matter of debate (for recent review see e.g. Kirilovsky et al., 2014), however, they maximize the light-harvesting efficiency during low light exposure (Mullineaux and Emlyn-Jones, 2009). In green algae it was observed that part of the mobile PSI antenna is detached from PSII in state 2 and without docking to PSI is switched into a quenched state (Ünlü et al., 2014). Such coupling/decoupling of the (phycobilins) antenna has also been proposed for cyanobacteria (Kirilovsky et al., 2014). Here it is proposed that even in G. theta the antennae seem to attach to the photosystems under low light conditions and detach under excess light. While these state transitions are preferentially triggered in the Chl ale antenna, they involve both, the membrane integrated Chl ale antennae as well as the PE antennae in the thylakoid lumen. The process optimizes photosynthesis in the logarithmic growth phase and thus maximizes photosynthetic capacity (see PSII, ETR and Pmax in Fig. 8) under light-limiting conditions. It therefore represents a physiological advantage of G. theta in situ.

Supplementary data

Supplementary data are available at JXB online.

Fig. S1. Non-photochemical quenching capacities of R. salina cells during logarithmic phase of growth.

Fig. S2. Effect of green light on state-transitions of G. theta cells.

Fig. S3. Low temperature (77 K) fluorescence emission of G. theta cells after excitation of chlorophylls at 436 nm.

Funding

This work was supported by the Swedish Energy Agency (2012–005889) (to C.F.), Umeå University and the Artificial Leaf and Solar Fuel Project (KAW 2011-0055) (to O.C., WPS, and C.F.). E.K. O.P. and R.K. were supported by projects GAČR P501/12/G055 and GAČR P501/12/0304 financed by the Czech Science Foundation and by the project Algatree (CZ.1.05/2.1.00/03.0110) and Algatree Plus (MSMT LO1416) provided by Czech Ministry of Education, Youth and Sport.

Acknowledgements

The authors would like to thank Prof. Beverley R. Green for excellent scientific discussions and suggestions on this manuscript and Eva Žišková for her technical assistance.

References

Bailey S, Melis A, Mackey KRM, Cardol P, Finazzi G, van Dijken G, Berg GM, Arrigo K, Shrager J, Grossman A. 2008. Alternative photosynthetic electron flow to oxygen in marine Synechococcus. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1777, 269–276.

Biggins J, Bruce D. 1989. Regulation of excitation energy transfer in organisms containing phycobilins. Photosynthesis Research 20, 1–34.

Broughton MJ, Howe CJ, Hiller RG. 2006. Distinctive organization of genes for light-harvesting proteins in the cryptophyte alga Rhodomonas. Gene 369, 72–79.

Bruyant F, Babin M, Genty B, et al. 2005. Diel variations in the photosynthetic parameters of Prochlorococcus strain PCC 9511: Combined effects of light and cell cycle. Limnology and Oceanography 50, 850–863.

Butler NB. 1982. Carbon dioxide equilibria and their applications . New York: Addison-Wesley Publishing Company Inc.

Campbell D, Hurry V, Clarke AK, Gustafsson P, Öquist G. 1998. Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. Microbiology and Molecular Biology Reviews 62, 667–683.

Collini E, Wong CY, Wilk KE, Curmi PMG, Brumer P, Scholes GD. 2010. Coherently wired light-harvesting in photosynthetic marine algae at ambient temperature. Nature 463, 644–647.

Dall’Osto L, Bressan M, Bassi R. 2015. Biogenesis of light harvesting proteins. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1847, 861–871.

de di Bianchi S, Ballottari M, Dall’Osto L, Bassi R. 2010. Regulation of plant light harvesting by thermal dissipation of excess energy. Biochemical Society Transactions 38, 651–660.

Delphin E, Duval J-C, Kirilovsky D. 1995. Comparison of state 1-state 2 transitions in the green alga Chlamydomonas reinhardtii and in the red alga Rhodella violacea: effect of kinase and phosphatase inhibitors. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1232, 91–95.

Delphin E, Duval J-C, Etienne A-L, Kirilovsky D. 1996. State transitions or Aph-dependent quenching of photosystem II fluorescence in red algae. Biochemistry 35, 9435–9445.

Dietzel L, Pfannschmidt T. 2008. Photosynthetic acclimation to light gradients in plant stands comes out of shade. Plant Signaling & Behavior 3, 1116–1118.

Drop B, Yadav K.N.S, Boekema EJ, Croce R. 2014. Consequences of state transitions on the structural and functional organization of Photosystem I in the green alga Chlamydomonas reinhardtii. The Plant Journal 78, 151–191.

Durnford DG, Deane JA, Tan S, McDadden Gl, Gantt E, Green BR. 1999. A phylogenetic assessment of the eukaryotic light-harvesting antenna proteins, with implications for plastid evolution. Journal of Molecular Evolution 48, 59–68.

Finazzi G, Barbagallo RP, Bergo E, Barbato R, Forti G. 2001. Photoinhibition of Chlamydomonas reinhardtii in State 1 and State 2: damages to the photosynthetic apparatus under linear and cyclic electron flow. Journal of Biological Chemistry 276, 22251–22257.

Funk C, Alami M, Tibiletti T, Green BR. 2011. High light stress and the one-helix LHC-like proteins of the cryptophyte Guillardia theta. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1807, 841–846.

Gibbs P, Biggins J. 1989. Regulation of the distribution of excitation energy in Ochromonas danica, an organism containing a chlorophyll-A/C carotenoid light harvesting antenna. Photosynthesis Research 21, 81–91.

Gibbs SP. 1981. The chloroplast of some algal groups may have evolved from endosymbiotic eukaryotic algae. Annals of the New York Academy of Sciences 361, 193–208.

Goss R, Lepeit B. 2015. Biodiversity of NPD. Journal of Plant Physiology 172, 13–32.

Green BR, Durnford DG. 1996. The chlorophyll-carotenoid proteins of oxyrogenic photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 47, 685–714.

Guillard RL. 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith W, Charley M, eds. Culture of Marine Invertebrate Animals . New York: Springer US, 29–60.

Hammer A, Schumann R, Schubert H. 2002. Light and temperature acclimation of Rhodomonas salina (Cryptophyceae). photosynthetic performance. Aquatic Microbial Ecology 29, 237–256.

Harrop SJ, Wilk KE, Dinshaw R, et al. 2014. Single-residue insertion switches the quaternary structure and exciton states of cryptophyte
