BBA - Bioenergetics 1860 (2019) 102–110

Contents lists available at ScienceDirect

BBA - Bioenergetics

journal homepage: www.elsevier.com/locate/bbabio

Light-adaptive state transitions in the Ross Sea haptophyte *Phaeocystis antarctica* and in dinoflagellate cells hosting kleptoplasts derived from it

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ARTICLE INFO

Keywords:
Kleptoplasts
Phaeocystis Antarctica
Ross Sea dinoflagellates
Secondary plastids
Light state transitions

ABSTRACT

Light state transitions (STs) is a reversible physiological process that oxygenic photosynthetic organisms use in order to minimize imbalances in the electronic excitation delivery to the reaction centers of Photosystems I and II, and thus to optimize photosynthesis. STs have been studied extensively in plants, green algae, red algae and cyanobacteria, but sparsely in algae with secondary red algal plastids, such as diatoms and haptophytes, despite their immense ecological significance. In the present work, we examine whether the haptophyte alga *Phaeocystis antarctica*, and dinoflagellate cells that host kleptoplasts derived from *P. antarctica*, both endemic in the Ross Sea, Antarctica, are capable of light adaptive STs. In these organisms, Chl *a* fluorescence can be excited either by direct light absorption, or indirectly by electronic excitation (EE) transfer from ultraviolet light absorbing mycosporine-like amino acids (MAAs) to Chl *a* (Stamatakis et al., Biochim. Biophys. Acta 1858 [2017] 189–195). Here we show that, on adaptation to PS II-selective light, dark-adapted *P. antarctica* cells shift from light state 1 (ST1; more EE ending up in PS II) to light state 2 (ST2; more EE ending up in PS I), as revealed by the spectral distribution of directly-excited Chl *a* fluorescence and by changes in the macro-organization of pigment-protein complexes evidenced by circular dichroism (CD) spectroscopy. In contrast, no STs are clearly detected in the case of the kleptoplast-hosting dinoflagellate cells, and in the case of indirectly excited Chls *a*, via MAAs, in *P. antarctica* cells.

1. Introduction

Photosynthetic organisms are continuously exposed to random changes in the quantity and the quality of ambient light. To protect themselves from possible photodamage and to optimize photosynthesis they employ various processes that are generally distinguished as short-term light adaptations, if no de novo syntheses are involved, and as long-term light acclimations, if such syntheses are involved. The principle light adaptive processes of photosynthetic organisms are high energy quenching (qE) and state transitions (STs). Both are linked to and regulated by the rate of oxygenic photosynthetic electron transport from Photosystems II (PS II) to Photosystem I (PS I; see e.g., chapters in [1,2]).

High energy quenching (qE) is triggered when the thylakoid lumen becomes acidic by protons released in the photo-oxidation of H_2O by the O_2-evolving complex of PS II and by protons translocated across the thylakoid membrane from the stroma by the coupled intersystem electron transport. It occurs at high light intensities when the light-induced proton influx from the stroma space into the lumen spaces exceeds the dark proton efflux via the ATP synthase complex of the thylakoid membrane. Cyanobacteria dissipate the excess electronic excitation (EE) in Chl *a* at the level of phycobilisomes by direct quenching by means of the orange carotenoid protein process (reviewed by Kirilovsky and Kerfeld, ref. [3]) and by the ST1-to-ST2 transition that diverts excess EE away from PS II [4]. On the other hand, plants and algae dissipate the excess EE in Chl *a* using both reversibly de-epoxidized xanthophylls in the thylakoid membrane (xanthophyll cycle, XC; see e.g. chapters in [1,2]), and by the ST1-to-ST2 transition.

STs, is the reversible physiological process that ensures a balanced supply of EE to the reaction centers of PS I and PS II. It was discovered

Abbreviations: Chl, chlorophyll; Dd, diadinoxanthin; Dt, diatoxanthin; EE, electronic excitation; EEE, excess electronic excitation; MAAs, mycosporine-like amino-acids; PS I, Photosystem I; PS II, Photosystem II; qE, high energy non-photochemical quenching; STs, state transitions; ST1, state 1; ST2, state 2; VAZ, violaxanthin - antheraxanthin - zeaxanthin; XC, xanthophyll cycle; Δλ, bandwidth at half-maximum

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https://doi.org/10.1016/j.bbabio.2018.11.016
Received 15 May 2018; Received in revised form 11 September 2018; Accepted 7 November 2018
Available online 08 November 2018
0005-2728/

by N. Murata [5] in the red alga *Porphyridium cruentum* and by C. Bonaventura and J. Myers [6] in the green alga *Chlorella pyrenoidosa* and has been since studied quite extensively, mostly in cyanobacteria (e.g., [7–9]) and plants and algae with primary plastids (e.g., [10–14]) and less so in algae with secondary plastids [1]. The transition from state 1 to state 2 (ST1-to-ST2) occurs when the intersystem electron transporting intermediates are reduced, as a result of an over-driven PS II, while the state 2 to state 1 transition (ST2-to-ST1) occurs when they are oxidized by an over-driven PS I. It is established that, PS II emits stronger Chl *a* fluorescence in ST1 and weaker in ST2, while PS I emits stronger Chl *a* fluorescence in ST2 and weaker fluorescence in ST1.

STs involve considerable rearrangements of the light-harvesting proteins that are embedded in thylakoid membranes, so their mechanism relates to the particular structural features of these membranes. Thus, thylakoids can be unstacked (as in cyanobacteria), partly stacked and unstacked (as in plants and algae with primary plastids) and unstacked but banded (as in algae with secondary plastids). In the prokaryotic cyanobacteria, the delivery of EE is regulated at the level of the megacomplex formed by the attachment of the phycobilisome (PBS), the phycobiliprotein-containing light harvesting organelle, to stroma-exposed sites of the PS II and PS I core complexes [15]. In the primary plastids of plants and green algae are characterized by the presence of unstacked thylakoids (stroma lamellae) and stacked thylakoids (grana). Unstacked thylakoids, in particular, house the PS I and the ATP synthase protein complexes, while the stacked thylakoids in the grana house preferentially the PS II complexes [16–19]. In these organisms, STs involve palindromic movements of the Chl a/b containing light-harvesting proteins between stacked and unstacked thylakoids [20].

Whether the regulatory process of light-adaptive STs is restricted to cells with primary plastids (E1), which have both grana and stroma lamellae, or it occurs also in cells with secondary plastids (E2) is a question under debate (see e.g., C. Büchel [21]. On the basis of F_{Chla} and O_2 evolution kinetics, measured at room temperature (RT), TG Owens [22] concluded that STs do not occur in the diatom *Phaeodactylum tricornutum*. In contrast, PB Gibbs & J Biggins [23], using F_{Chla} fluorometry (at RT and 77 K) proved that STs do occur in the chrysophyte *Ochromonas danica* (E2, red line). The assertion of TG Owens has been questioned by R. Goss & B. Lepetit [24] on the basis of results by Y. Fujita & K. Okhi [25]) who also suggested a re-evaluation by means of different experimental approaches. In fact, the light state of photosynthetic cells can be recognized, at room temperature by kinetic fluorometry and circular dichroism and at low temperature by spectrofluorometry.

In this research we examined whether the haptophyte *Phaeocystis antarctica* and a Ross Sea dinoflagellate (RSD; ref. [26]) that hosts kleptoplasts derived from *P. antarctica*, both endemic and ecologically dominant in Ross Sea, Antarctica, are capable of light-adaptive STs. Recently, we succeeded in resolving the Chl *a* fluorescence spectrum of *P. antarctica* at 77 K into a PS II contribution (peaking at 683 nm) and a PS I contribution (peaking at 689 nm [27]). This possibility enabled us to adapt these algae to PS II-selective light, or to PS I-selective light, and to examine whether these adaptations do lead to STs.

2. Materials and methods

2.1. Cell cultures and preparations

A unialgal culture of *P. antarctica* was created by picking algal colonies and transferring them, after two washes with filter sterilized culture medium (f/2 + silica, ref. [28]) to fresh sterile medium. A culture enriched in RSD cells was created by collecting cells from near the surface of a mixed culture of RSD and *P. antarctica*. The resulting culture was > 95% enriched in RSD dinoflagellate cells. *P. antarctica* and RSD cells were re-cultivated from the enriched cultures by inoculating cells into culture medium at 0–4 °C in an illuminated incubator with a photoperiod of 12 h light, 12 h dark. Total Chl (Chl a, Chl c_2, Chl c_3) concentration was determined according to Ritchie [29].

2.2. Absorption and fluorescence measurements

Absorption spectra of cell suspensions were measured with a Hitachi U-3010 UV–visible scanning spectrophotometer (Hitachi High Technologies Corporation, Japan) that was equipped with a 60 mm integrating sphere, layered on the inside with $BaSO_4$. The spectra were scanned from 300 nm to 700 nm, at a speed of 200 nm/ min. Displayed spectra are normalized to equal peak heights (= 1) at 675 nm.

Assay samples for fluorometry were prepared by injecting 200 µl cell suspension into quartz capillary tubes (2.5 mm internal diameter). Chl *a* fluorescence emission spectra were measured with a Hitachi F-2500 spectrofluorometer (Hitachi High Technologies Corporation, Japan), which was equipped with liquid-nitrogen sample housing and a red-sensitive photomultiplier. The spectra were scanned using a detection bandwidth of $\Delta\lambda = 2.5$ nm, and a Corning CS 2–60 cut-off filter to prevent stray exciting light below 670 nm from entering the measuring monochromator. Presented fluorescence spectra are normalized at 690 nm.

Cells that had been adapted to darkness for 30 min at 2–4 °C were subsequently illuminated for 4 min either at 670 nm ($\Delta\lambda = 5$ nm, 63 µmol photons·m^{-2}·s^{-1}; PS II-selective light), or at 683 nm light ($\Delta\lambda = 5$ nm; 65 µmol photons·m^{-2}·s^{-1}; PS I-selective light) obtained from the exciting monochromator of the Hitachi F-2500 fluorometer. Additional light adaptations were performed by illuminating dark adapted cells for 4 min either with ultraviolet light A (UVA; ~335–415 nm, max. 375 nm, $\Delta\lambda$ ~45 nm, 1 µmol photons·m^{-2}·s^{-1}; transmitted by combined Corning glass filters CS 4-70 and CS-7-51), as well as with broad blue-green band light (~350–650 nm, max 630 nm, $\Delta\lambda$ ~210 nm, 150 µmol photons·m^{-2}·s^{-1}; transmitted by Corning glass filter CS 4-70). Immediately after the light adaptations, cell samples were frozen to 77 K with liquid nitrogen and their fluorescence spectra were recorded with excitation either at 436 nm ($\Delta\lambda = 10$ nm; Chl *a*-selective), or at 345 nm ($\Delta\lambda = 10$ nm; MAAs-selective). Three independent measurements were performed for each experiment.

2.3. Treatment with DBMIB and DCMU

Dark-adapted (276 K, 30 min) *P. antarctica* cells were treated either with 1 µM of 2, 5-dibromo-3-methyl-6-isopropylbenzoquinone (DBMIB) [30,31] or with 20 µM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). The cells were subsequently light adapted for 4 min at 670 nm or at 683 nm, then either fluorescence spectra were recorded at 77 K, or CD spectra recorded at 276 K.

2.4. Circular dichroism spectra

CD spectra of cell suspensions (prepared as described in 2.1, 10 µg/ml Chl) were recorded in the range 400–800 nm at 3 °C (276 K) on a JASCO J-715 spectropolarimeter equipped with Peltier temperature control system. Each displayed spectrum is the average of three scans recorded with quartz cuvettes of 1 mm path length, 50 nm/min scan speed, 8 s response time, and resolution of 0.5 nm. The CD spectra are presented in mdeg and are baseline corrected.

To observe CD spectral changes caused by light treatment, samples were illuminated for 3 min at 683 nm and at 670 nm in the sample holder of the spectropolarimeter using the CD lamp (OSRAM Xenon Short Arc XBO) with a slit width of 3000 µm (with 3 µmol photons·m^{-2}·s^{-1}, in either case), followed by the recording of their CD spectra under the usual settings. Three independent measurements were performed for each experiment.

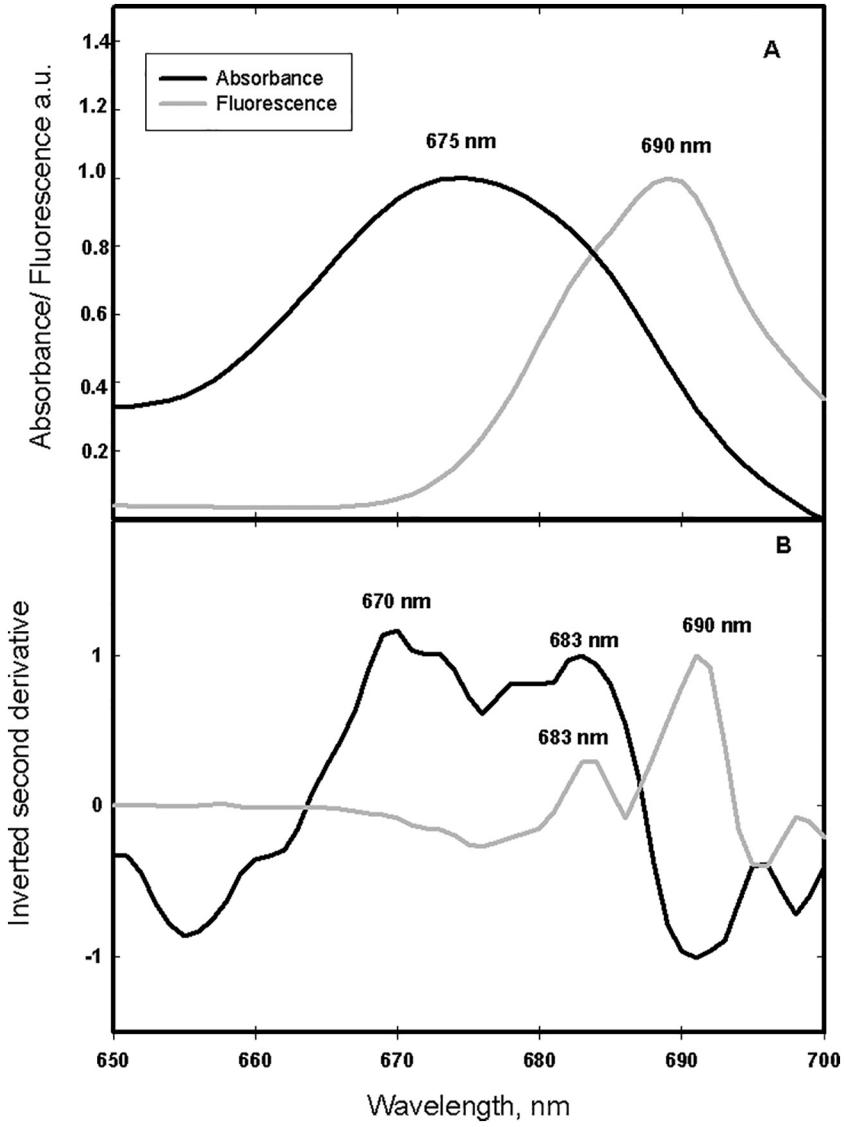

Fig. 1. (A) Absorption (black lines) spectra and fluorescence spectra (grey lines; 650 nm to 750 nm) of *P. antarctica*. (B) Inverted 2nd derivative absorption and fluorescence spectra of *P. antarctica* (650 nm to 700 nm). Absorption spectra were recorded at 277 K and fluorescence spectra were recorded at 77 K.

3. Results

Fig. 1A displays the red absorption band (black line) and the fluorescence emission band (grey line) of *P. antarctica* at 277 K and 77 K, respectively, and Fig. 1(B) displays the inverted 2nd derivatives of these two bands. In the latter spectra, each primary band splits into two main sub-bands peaking at 670 nm (A670) and at 683 nm (A683) in the case of absorption, and at 683 (F683) and 690 (F690) in the case of fluorescence. As shown in ref. [27], F683 originates from Chls *a* in PS II and F690 from Chls *a* in PS I, indicating thus that A670 originates from Chls *a* in PS II and A683 originates from Chls *a* in PS I.

After dark adaptation, eukaryotic photosynthetic cells (plants and algae) are known to shift to state 1 (ST1) due to the auto-oxidation of the PQ-pool by free O_2 [32]. Illumination of such ST1 cells with PS II-selective light reduces their PQ-pools and shifts them to ST2. In that state the light harvesting antenna of PS I becomes enlarged in comparison to that in ST1. Since the 670 nm light is preferentially absorbed by the PS II Chls *a*, we expect that illumination of dark-adapted *P. antarctica* cells with this light will cause the reduction of their PQ pools and will shift them to ST2. This is expected to be reported by an enhancement of PS I fluorescence (F690) and a depression of PS II fluorescence (F683). In contrast, illumination with PS I-selective light (683 nm) is expected to exert a small effect, at the most, because the PQ pools of dark-adapted *P. antarctica* cells are already oxidized. In this case, only marginal changes in the F683 and F690 Chl *a* fluorescence bands at 77 K are expected.

In the experiment depicted in Fig. 2, *P. antarctica* cells were first dark-adapted for 30 min at 277 K and then light-adapted for 4 min either to PS II-selective light (670 nm) or to PS I-selective light (683 nm). Immediately after, they were frozen to 77 K and Chl *a* fluorescence spectra were recorded. It is clearly illustrated in Fig. 2A, that the emission spectra of dark-adapted cells (black line) and of cells adapted to 683 nm light (grey line) are nearly the same. In contrast, adaptation of *P. antarctica* cells to PS II-selective light (670 nm), which drives the reduction of the PQ pool, results in a conspicuous depression of fluorescence emission band (black line). More detailed information on the role of selective light adaptation on the oxidation-reduction state of the PQ pool, and on the spectral distribution of Chl *a* fluorescence is provided by the inverted 2nd derivative spectra in Fig. 2B. As shown in Stamatakis et al. [27], directly recorded fluorescence emission bands (as those in Fig. 2A) split into individual PS II (F683) and PS I (F690) emissions in the inverted 2nd derivative fluorescence spectra. Fig. 2B shows that on illuminating dark-adapted *P. antarctica* cells (ST1, PQ pool oxidized) with PS I-absorbed (683 nm) which oxidizes the PQ pool,

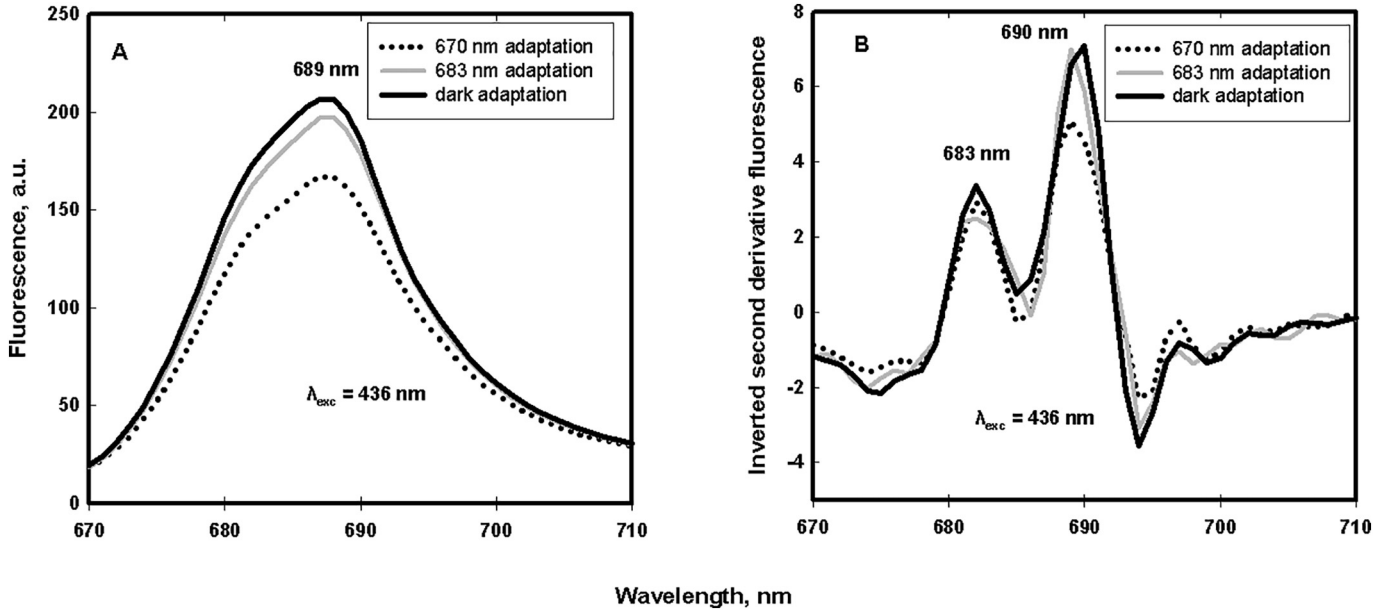

Fig. 2. Fluorescence spectra (A) and inverted 2nd derivative fluorescence spectra (B) of *P. antarctica* cells after 30 min dark adaptation (black lines) and followed by 4 min adaptations to PS II-selective light (670 nm; dotted lines) or to PS I-selective light (683 nm; grey lines). All dark and light adaptations were carried out at 277 K. Immediately after, the cell suspensions were frozen to 77 K and fluorescence was recorded by exciting Chl *a* at 436 nm.

the Chl *a* fluorescence emission of PS I (F690) is almost unchanged while that of PS II is slightly depressed. In contrast, when the dark-adapted cells are illuminated PS II-selective light (670 nm) which reduces the PQ-pool, F690 is strongly depressed. These changes indicate clearly a ST1-to-ST2 transition of the *P. antarctica* cells upon the reduction of the PQ-pool.

Mycosporine-like aminoacids (MAAs) are water-soluble, low molecular weight (< 400 kDa) compounds that are found in various cyanobacteria, algae and animals. They absorb maximally in the UVA range (310–362 nm) and serve primarily as protective UV screens and as antioxidants (Rosic NN et al. [33] and citations). As shown in ref. [27], UV light absorbed by MAAs does also sensitize Chl *a* fluorescence both in *P. antarctica* and in RSD cells. This indicates that a fraction of the MAAs exists within the chloroplast and in close proximity to Chls *a* to which it transfers EE. In the Fig. 3 experiment we asked whether STs induced by adaptations to PS II-selective (670 nm) or to PS I-selective (683 nm) light which are detectable by directly excited Chl a fluorescence (see Fig. 2) can be also detected by means of MAAs-sensitized Chl *a* fluorescence. Dark and light adaptations of *P. antarctica* cells were performed as in the Fig. 2 experiment but their effects on the sizes of the PS II and PS I light-harvesting antennae were tested by exciting Chl *a* fluorescence at 345 nm. This excitation band is strongly absorbed by the MAAs of *P. antarctica* (shinorine and mycosporine glycine-valine [33]), and weakly by the short-wave tails of Chls *a*, c_2, and c_3 that are present in *P. antarctica* ([33] and citations). According to Fig. 3A, the MAAs-sensitized Chl *a* fluorescence of cells adapted to 670 nm (reduced PQ pools) is slightly lower than that of cells adapted to 683 nm (oxidized PQ pools). The inverted second derivatives in Fig. 3B show the same to be true both for the PS II emission (F683) and the PS I emission (F690). These results show that the light-adaptive redox shifts of the PQ-pool and the attendant state transition shifts are not detectable by indirectly excited (via MAAs) Chl *a* fluorescence.

DBMIB (2,5-dibromo-3-methyl-6-isopropylbenzoquinone) is a selective electron transport inhibitor that prevents the oxidation of the PQ-pool by the cytochrome b_6f complex (see e.g., [30,31,34]). In its presence, the reduction of the PQ pool with PS II-selective light and the ST1-to-ST2 transition are allowed, while the oxidation of the PQ pool, and the attendant ST2-to-ST1 transition by illumination with PS I-selective light are inhibited. The phenomenology displayed in Fig. 4 confirms the involvement of the PQ pool in the selective light adaptive phenomena observed in the Fig. 2 experiment. Specifically, panel B which presents the inverted 2nd derivatives of the fluorescence spectra, shows that, in the absence of DBMIB, Chl *a* F690 (i.e., Chl *a* fluorescence emitted from PS I) is high after light adaptation to 670 nm (that causes the reduction of the PQ pool) and low after adaptation to 683 nm (that causes the oxidation of the PQ pool). In contrast, in panel D, F690 remains high at both light adaptations, indicating the failure of the PS I-selective light (683 nm) to drive the oxidation of the PQ pool and cause the ST2 to ST1 shift. In the presence of DCMU (Supplementary Material Fig. 1A) the 77 K fluorescence spectra of dark-adapted cells (black line) and of DCMU-treated dark adapted cells (dashed black line) are nearly the same, indicating thus that the oxidation of the PQ pool is prevented in the dark, irrespectively of the presence or the absence of DCMU. Moreover, in the presence of DCMU, and after illumination either with PS II-selective light or with PSI- selective light, the inverted 2nd derivatives of the fluorescence spectra (Supplementary Material Fig. 1B), show that F690 remains high at both light adaptations, as in the dark. These data indicate that ST1-to-ST2 transitions are not allowed, while the reduction of the PQ pool and the attendant ST1-to-ST2 transition by illumination with PS II-selective light are inhibited.

In the Fig. 5 experiment, we asked whether *P. antarctica* can undergo light-induced STs at the light conditions that prevail in its natural sea habitats, namely in the deeper mixed layers of the Ross Sea [35]. Blue and green wavelengths are known to penetrate the sea down to about 200 m while yellow and red wavelengths do not go below 50 m [36]. In this experiment, dark-adapted *P. antarctica* cells were subsequently light adapted either to UVA light (332 nm–418 nm, max at 375 nm) that approximates the photic environment in the shallower range of the sea, or to broad band blue-green light (330 nm to 655 nm, max. at 457 nm) that approximates the photic environment in the deeper range. UVA light is absorbed mainly by the MAAs and less by the absorption tails of Chls (*a*, c_2, and c_3) while the broad band blue-green light is absorbed strongly both by the Chls and the MAAs. Chl *a* fluorescence of cell suspensions was excited at 436 nm. As shown in Fig. 5A, the adaptation to blue-green light caused a small depression of Chl *a* fluorescence while the adaptation to UVA light caused a much larger depression. The inverted 2nd derivative spectra in Fig. 5B show that UVA light affected mostly the Chl a fluorescence emitted by PS I (i.e.,

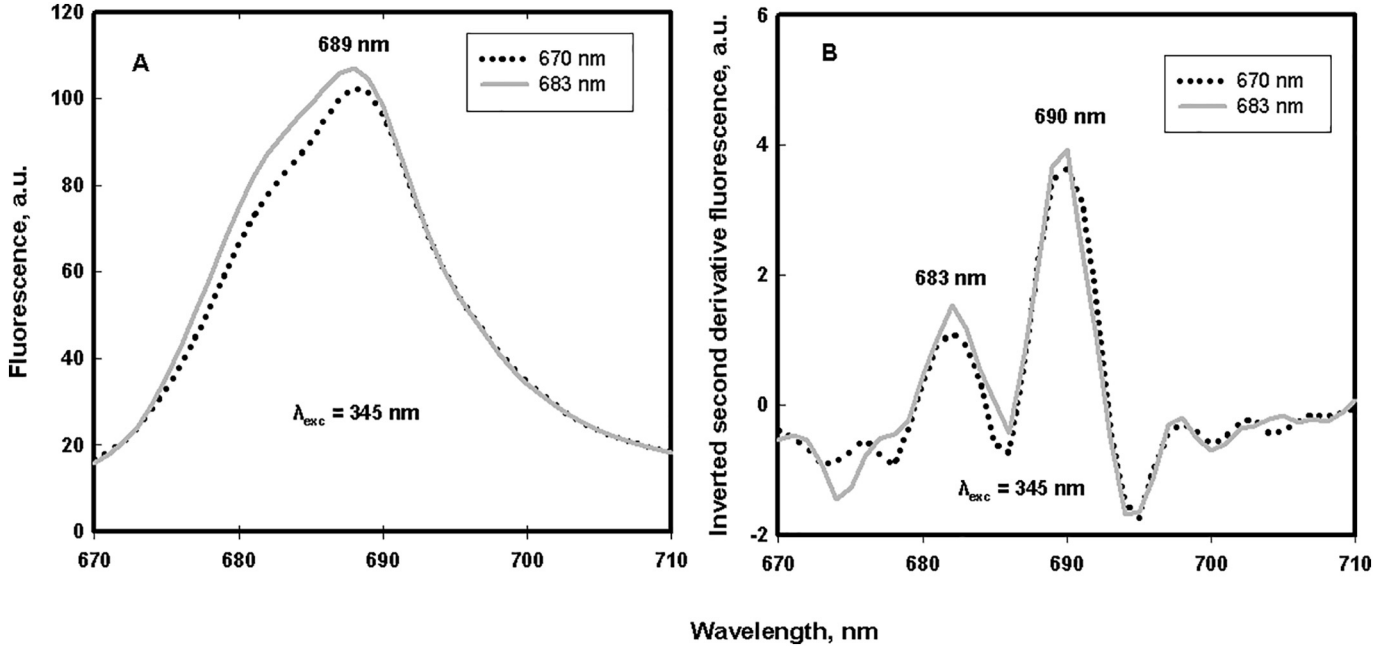

Fig. 3. 77 K fluorescence spectra (A) and inverted 2nd derivative fluorescence spectra (B) of *P. antarctica* after adaptation to PS II-selective light (670 nm; dotted line) and to PS I-selective light (683 nm; grey line). Fluorescence was excited at 345 nm (MAAs-selective light).

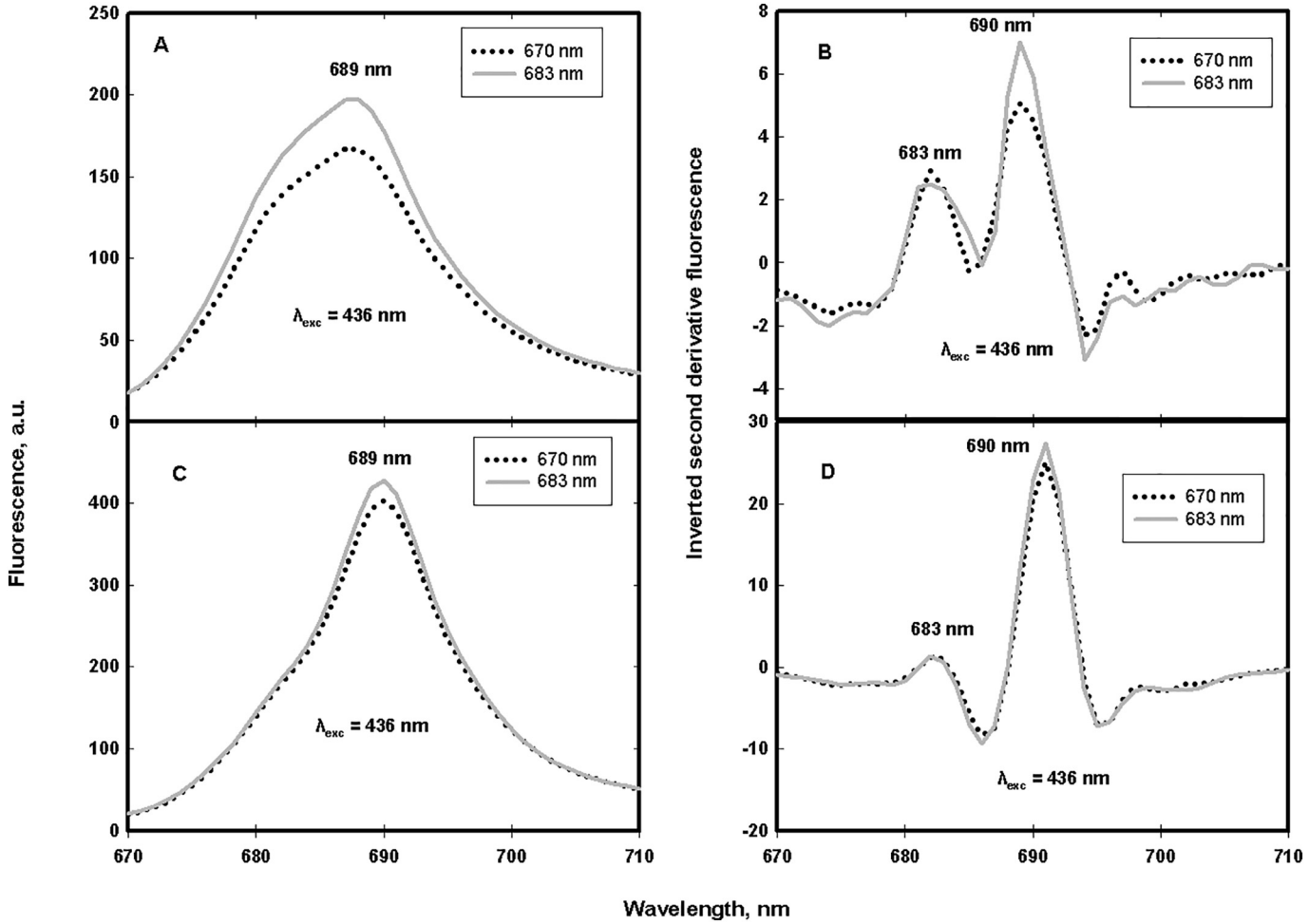

Fig. 4. Effect of DBMIB on the ST2-to-ST1 transition of *P. antarctica* cells. A and B, 77 K fluorescence spectra and inverted 2nd derivative spectra of control cells. C and D, 77 K fluorescence spectra and inverted 2nd derivative spectra of DBMIB-treated cells. Chl *a* fluorescence was excited at 436 nm. All other details as in the Fig. 2 legend.

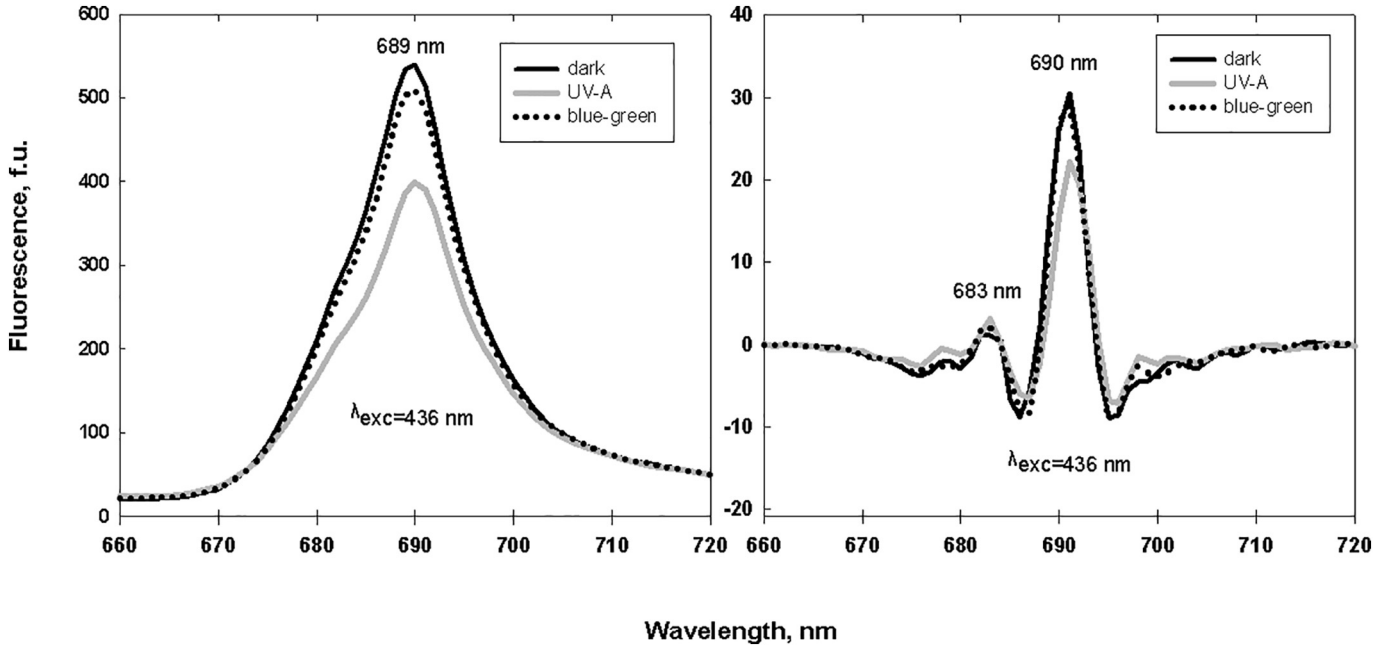

Fig. 5. Fluorescence spectra (A) and inverted 2nd derivative fluorescence spectra (B) of *P. antarctica* cells after dark adaptation (black lines) followed by adaptations to broad band UVA light (332 nm–418 nm, max at 375 nm; grey lines) and to broad band blue-green light (330 nm–655 nm, max. at 457 nm; dotted lines). Fluorescence spectra were recorded at 77 K. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

F690), while blue-green light has nearly no effect. These results indicate that *P. antarctica*, which inhabits all depth ranges of the ocean photic zone is capable of light adaptive STs.

A characteristic feature of STs is the substantial reorganization of the arrays of photosystem complexes that are embedded in the thylakoid membranes of cyanobacteria and algae. In general, these arrangements are more orderly in ST1 and less orderly in ST2, a situation which has been visualized by various methodologies including freeze fracture electron microscopy [37], small angle neutron scattering and circular dichroism (CD) spectroscopy [38,39]. In this research, we used CD spectroscopy as an independent approach to explore the question whether *P. antarctica* cells are capable of light-adaptive state transitions. After adaptation to ST1 in the dark the CD spectrum in Fig. 6A contains excitonic bands at (−)474 nm and (+)499 nm that may be attributed to interactions involving Chl *α* and Chl *c* or carotenoids, and at (−)666 and (+)692 nm evidently originating only from Chl *α* molecules [39,40]. The intensity and the shape of the bands are consistent [41] with the organization of thylakoid membranes of *P. antarctica* in banded layers without grana stacks, as revealed in the electron microscopy study of the morphology of this species [42]. Cells were subsequently illuminated for 3 min with the CD spectropolarimeter light, either at 670 nm (PS II selective) or at 683 nm (PS I selective), within the sample holder. Immediately after illumination, the CD spectra were recorded and are also presented in Fig. 6A. It is

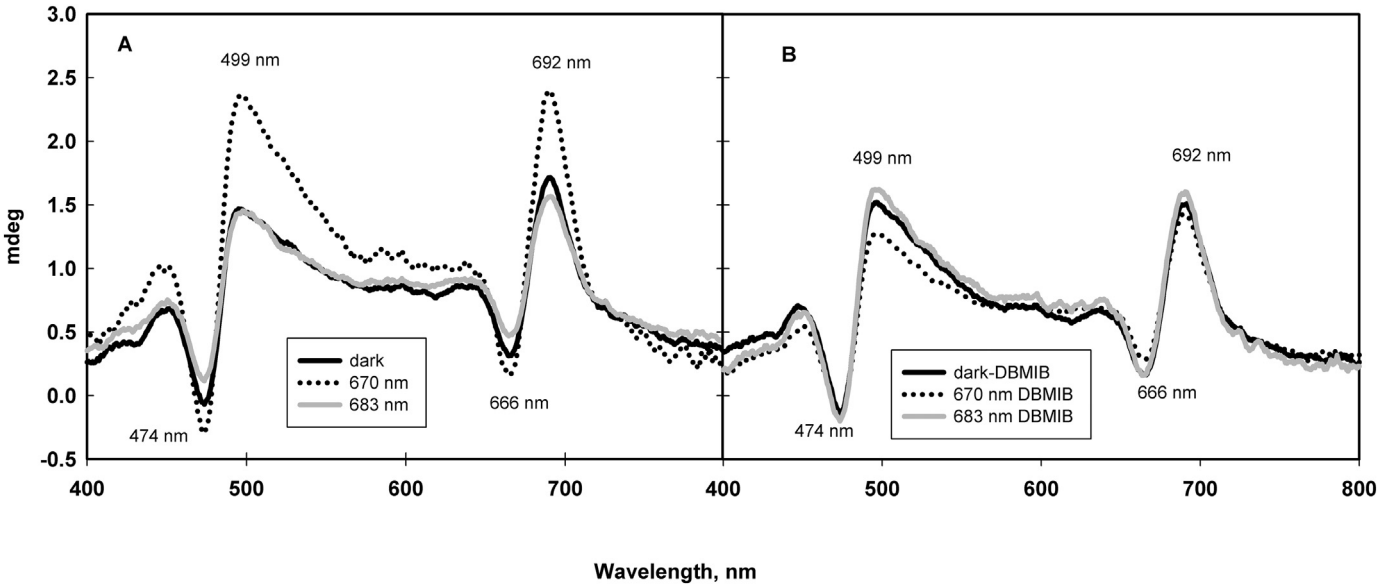

Fig. 6. Circular dichroism spectra of control *P. antarctica* cells (A) and of DBMIB-treated (+DBMIB) cells (B) after adaptation to dark (black lines), PS II-selective light (670 nm, dotted lines) or to PS I-selective light (683 nm, grey lines).

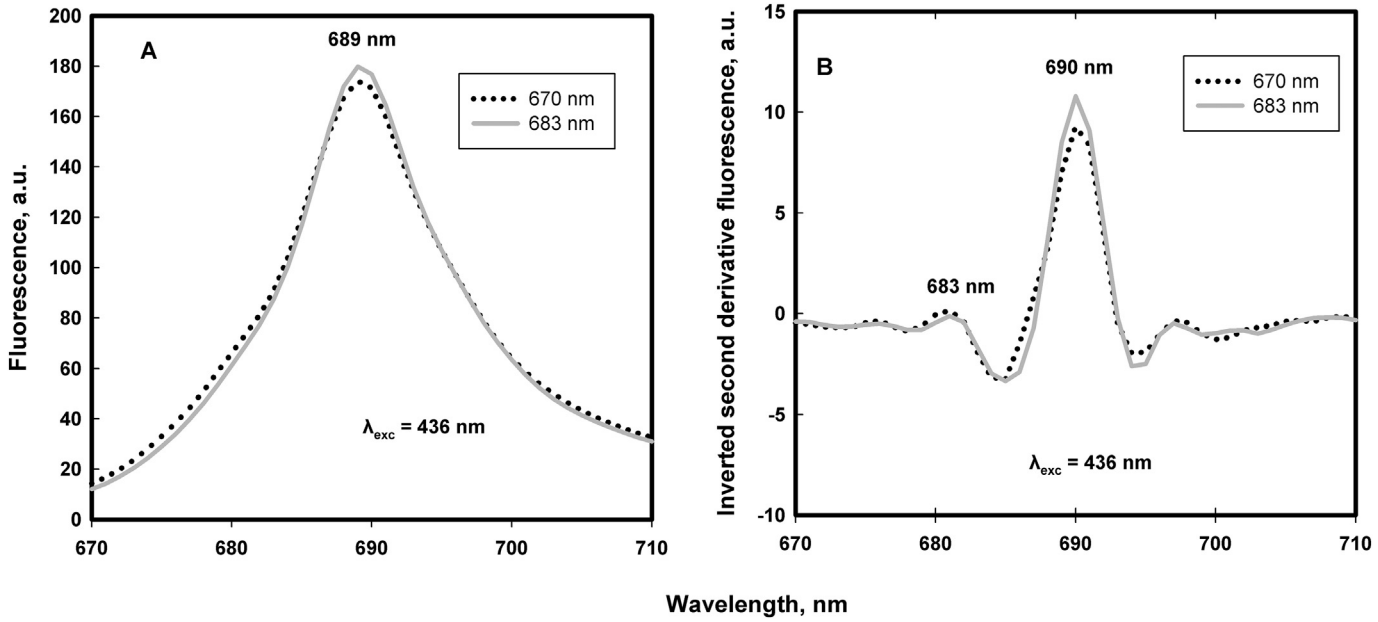

Fig. 7. 77 K fluorescence spectra (A) and inverted 2nd derivative fluorescence spectra (B) of RSD cells after adaptations to PS II-selective light (670 nm, dotted lines) and to PS I-selective light (683 nm; grey lines). Fluorescence was excited at 436 nm (Chl *a* -selective light).

obvious that selective illumination generates spectra with different amplitudes of the characteristic peaks at 474/499 nm and 666/692 nm, suggesting that modifications are taking place in the arrangement of the photosynthetic complexes in thylakoid membranes [38]. In the presence of DBMIB, however, which inhibits the PS II to PS I electron transport by blocking electron flow after the PQ pool [43], no difference is noted in the CD spectra after selective illumination with PS II or PS I light (Fig. 6B). The fact that no changes associated with the photosynthetic complexes are noted in the CD spectra in the presence of DBMIB, suggests that a dynamic communication of the photosystems through the PQ pool exists in *P. antarctica*, in agreement with the fluorescence findings.

Kleptoplasts hosted within RSD cells are characterized by a diminished presence of PS II holochromes and by a weaker photosynthetic O_2 evolution activity compared to the parent chloroplasts of the algal cells [27]. Essentially, the RSD-kleptoplast system is an efficient photoheterotroph that operates primarily its PS I. In the Fig. 7 experiment we asked whether these dinoflagellate-hosted kleptoplasts are capable of state transitions upon selective light adaptation like the chloroplasts within the *P. antarctica* cells. Light adaptations, in this experiment, were carried out as described in Materials and Methods and in the legend of Fig. 2. Chl *a* fluorescence was excited at 436 nm. Overall, as Fig. 7A shows, the Chl *a* fluorescence of ST2 kleptoplasts (adapted to 670 nm light) is slightly higher than of the ST1 kleptoplasts (adapted to 683 nm light). An inverted 2nd derivative analysis of the latter spectra (Fig. 7B) shows that the light adaptations affected nearly exclusively the Chl *a* fluorescence of PS I, as it could have been anticipated by the diminished presence of PS II in kleptoplasts.

The Fig. 8 experiment performed with RSD cells is similar to the Fig. 7 experiment with respect to the light adaptation treatments. Here, however, we asked whether the ST1-to-ST2 transition, can be detected by exciting Chl *a* fluorescence indirectly, via MAAs-selective excitation at 345 nm, and not directly at 436 nm, the Chl *a*-selective excitation, as in the Fig. 7 experiments. As in the analogous experiment with *P. antarctica* cells (see Fig. 3), no STs were evident with this excitation.

4. Discussion

Acclimative responses of *P. antarctica* to prolonged exposures to super-optimal light (*high light*), or to sub-optimal light (*low light*) have been investigated extensively. To high light, *P. antarctica* cells respond by reducing their content of light harvesting pigment-proteins and by increasing the presence of the photo-protective xanthophylls diadinoxanthin (Dd) and diatoxanthin (Dt; [44,45]). This proves, indirectly, the ability of *P. antarctica* to operate the light-adaptive process of high energy quenching (qE) via the diadinoxanthin-diatoxanthin (DD-DT) cycle which is typical for haptophytes [46]. To low light, *P. antarctica* cells respond by increasing their content of light harvesting pigment-proteins [47]. For optimal results, however, this low light acclimative process will be effective only if accompanied by a light-adaptive process that ensures the balanced delivery of electronic excitation to the reaction centers of PS II and PS I (PS II-RC, PS I-RC), namely by the STs process.

STs allow balancing of the absorption capacity of the two photosystems by reversible relocation of the light-harvesting complexes between PSII and PSI [11]. STs are well documented in cyanobacteria [7] as well as in plants and green algae [12]. In these organisms, the non-random distribution of PS II and PS I (lateral heterogeneity, LH) is important because it minimizes excitation spillover losses from PS II to PS I and to heat. LH was first discovered and extensively documented in primary plastids that contain both unstacked (lamellae) and stacked thylakoids (grana; see [48], and citations) and only quite recently shown to be present in secondary plastids of diatoms whose thylakoids are banded but not stacked grana [49,50]. There is no experimental evidence of this phenomenon in the photosynthetic membranes of other oxygen evolvers: red algae, heterokonts, haptophytes or dinoflagellates [48]. Specifically, the chromophytes and haptophytes (comprising the two classes *Coccolithophyceae* and *Pavlovophyceae*), are considered not to have LH of PSII and PSI [51]. Moreover, the thylakoids of all haptophytes are unstacked and arranged in groups of parallel bands along most of their length [51]. Given that both the presence of LHCII and the ability to effect a high degree of LH of LHCII and PSII complexes from PSI complexes appear to be prerequisites for the occurrence of a state transition [52], it was suggested that STs could not be included in the photosynthetic machinery of secondary endosymbiosis taxa belonging to the red lineage.

The occurrence of STs has never been demonstrated in *P. antarctica*, and in fact, doubts have been expressed whether it may be possible in algae with secondary plastids in which thylakoids exist in parallel bands, rather than in stacked and unstacked regions [21]. Our present

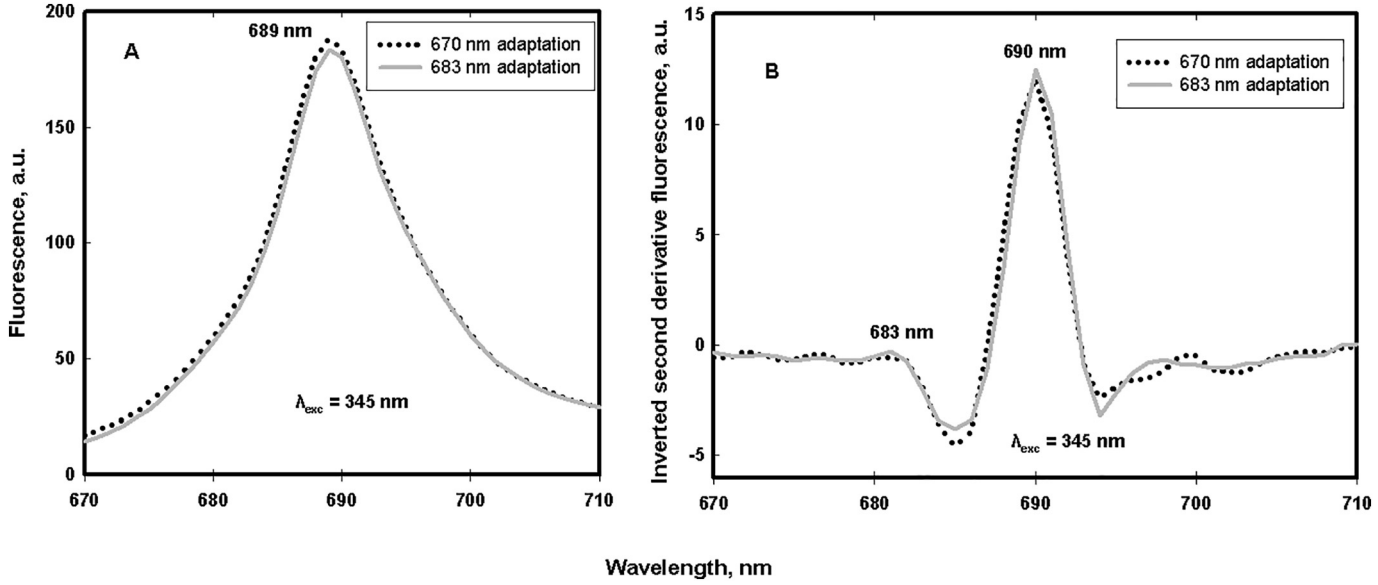

Fig. 8. 77 K fluorescence spectra (A) and inverted 2nd derivative fluorescence spectra (B) of dark-adapted RSD cells followed by light adaptations to PS II-selective light (670 nm; dotted line) and to PS I-selective light (683 nm; grey line). Fluorescence was excited at 345 nm (MAAs-selective light).

research provides three lines of evidence that *P. antarctica* cells are indeed capable of light-adaptive STs. First, the reduction of the PQ-pool by illuminating cells with PS II-selective light depresses the F_{Chla} emitted by PS I, as it would be expected for a ST1-to-ST2 transition (cf. Fig. 2). Second, when, the oxidation of the reduced PQ-pool of ST2 cells, upon light activation of by PS I, is prevented in the presence of the electron transport inhibitor DBMIB, no ST2-to-ST1 transition takes place transition (cf. Fig. 4). And third, by the observation of changes in the protein-pigment CD signal upon the transition ST1-to-ST2, which become minimal in the presence of DBMIB.

The PS II activity of the kleptoplasts that are housed within dinoflagellate cells (RSD cells) is substantially lower (~1/5) compared to the PS II activity of parent chloroplasts within *P. antarctica* cells [25]. Since these RSD cells are essentially PS I-operating photo-heterotrophs, it is expected that their PQ-pool is maintained in the oxidized state and the RSD cells are stably in ST1. This explains the very weak difference in F690 between RSD cells adapted to PS I-selective light (683 nm) and PS II-selective light (670 nm) displayed in Fig. 7.

P. antarctica is known to synthesize MAAs which are distributed throughout its cytoplasm and which screen off UV light [52]. The fraction of the MAAs that exists within its chloroplasts and in proximity to Chl *a* has been shown to be capable sensitizing Chl *a* fluorescence, therefore of harvesting light for photosynthesis [27]. The Fig. 8 experiment shows that light state shifts to ST1 or to ST2, following light-adaptive treatments with PS I-selective, or PS II-selective light, respectively, are not visualized via MAAs-excited Chl *a* fluorescence. This result is consistent with the expectation that the distribution of the MAAs in the various chloroplast compartments must be determined chiefly by their solubility and not by light-induced shifts of antenna proteins.

In conclusion, our results show that *P. antarctica* is capable of optimizing the use of light in its dimly illuminated underwater habitat, while kleptoplast-housing dinoflagellates are not. It would be interesting to see if this difference is somehow reflected in the distribution of *P. antarctica* and RSD cells in the vertical sea column.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbabio.2018.11.016.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgements

This work was supported in part by a National Science Foundation grant (PLR-1341362) to RJG.

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