The extraordinary longevity of kleptoplasts derived from the Ross Sea haptophyte *Phaeocystis antarctica* within dinoflagellate host cells relates to the diminished role of the oxygen-evolving Photosystem II and to supplementary light harvesting by mycosporine-like aminoacids

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*Abbreviations: DMF, N,N'-dimethylformamide; DCMU, 3-(3,4-dichloro-phenyl)-1,1-dimethylurea; MAA, mycosporine-like aminoacid; p-NTP, p-nitrothiophenol; PBQ, phenyl-p-benzoquinone; ROS, reactive oxygen species; RSD, kleptoplasts-hosting Ross Sea dinoflagellate; Δλ, full bandwidth at half-maximum;
ABSTRACT

The haptophyte *Phaeocystis antarctica* and the novel Ross Sea dinoflagellate that hosts kleptoplasts derived from *P. antarctica* (RSD; R.J. Gast et al., 2006, J. Phycol. 42 233–242) were compared for photosynthetic light harvesting and for oxygen evolution activity. Both chloroplasts and kleptoplasts emit chlorophyll *a* (Chl *a*) fluorescence peaking at 683 nm (F683) at 277 K and at 689 (F689) at 77 K. Second derivative analysis of the F689 band at 77 K revealed two individual contributions centered at 683 nm (F*\_i*683) and at 689 (F*\_i*689). Using the *p*-nitrothiophenol (*p*-NTP) treatment of Kobayashi *et al.* (*Biochim. Biophys. Acta* 423 (1976) 80-90) to differentiate between Photosystem (PS) II and I fluorescence emissions, we could identify PS II as the origin of F*\_i*683 and PS I as the origin of F*\_i*689. Both emissions could be excited not only by Chl *a*-selective light (436 nm) but also by mycosporine-like aminoacids (MAAs)-selective light (345 nm). This suggests that a fraction of MAAs must be proximal to Chls *a* and, therefore, located within the plastids. On the basis of second derivative fluorescence spectra at 77K, of *p*-NTP resolved fluorescence spectra, as well as of PSII-driven oxygen evolution activities, PS II appears substantially less active (~ 1/5) in dinoflagellate kleptoplasts than in *P. antarctica* chloroplasts. We suggest that a diminished role of PS II, a known source of reactive oxygen species, and a diminished dependence on nucleus-encoded light-harvesting proteins, due to supplementary light-harvesting by MAAs, may account for the extraordinary longevity of RSD kleptoplasts.
1. Introduction

Chloroplasts are commonly known as the permanent, semi-autonomous, photosynthetic organelles of plants and algae. In addition, functional algal chloroplasts exist symbiotically in the cytoplasm of some protozoans (ciliates, foraminifera, dinoflagellates) and of a single taxon of metazoans (sacoglossan sea slugs) that feed on unicellular algae [1]. The process of engulfing algae and using their chloroplasts for photosynthesis by non-photosynthetic host cells has been termed kleptoplasty [2]. It is a remarkable type of symbiotic association, involving the maintenance of the chloroplasts—the ‘kleptoplasts’—in a functional state within a non-photosynthetic host cell. This is noteworthy because in plant or algal cells the majority of genes involved in regulation of the chloroplast have been transferred to the nucleus over the long-term evolution of endosymbiosis, which is not likely to occur in short term kleptoplast associations.

Dinoflagellates are a group of unicellular eukaryotes (protists) that includes autotrophic, heterotrophic, and mixotrophic species. Kleptoplasty, the temporary retention of functional chloroplasts derived from algal prey, has been described for several heterotrophic dinoflagellates [3-7]. A novel and abundant dinoflagellate group, related to the ichyotoxic genera *Karenia* and *Karlodinium*, but without sharing evolutionary history with its plastids, was discovered by Gast et al. [8] in the Ross Sea, Antarctica. The kleptoplasts of the novel Ross Sea dinoflagellates (RSD) are closely related to the free-living unicellular photosynthetic haptophyte *Phaeocystis antarctica*, a species that often dominates phytoplankton blooms in the Ross Sea. The RSD do not grow indefinitely in clonal (uniprotistan) culture, and can be maintained for a long-term only in mixed cultures with *P. antarctica*. When deprived of *P. antarctica* cells, RSD gradually lose their kleptoplasts over a period of 5–8 months, or longer [9, 10]. Other kleptoplastidic dinoflagellates generally need to acquire new plastids within 1 month, presumably because the acquired kleptoplasts do not maintain photosynthetic functionality over longer time periods [3, 6, 11]. On the other hand, chloroplasts sequestered within the sacoglossan sea slugs, are capable of photosynthesis for approximately 10-months in the absence of algal nuclei [12, 13].

Chloroplast functionality depends on a continual signal crosstalk between the chloroplast and the cell nucleus and on the importation by the chloroplast of nucleus-encoded and cytosol-synthesized proteins [14]. Particularly important among the latter are the Chl-carotenoid-binding proteins of the light harvesting complexes (LHC) of photosystem II (PS II) and photosystem I (PS I). The finding that the RSD-hosted kleptoplasts are active for ex-
traordinarily long times stimulated our interest in exploring the causes behind it. Toward that
end, we used fluorometry to compare light harvesting characteristics and PS II-driven O2
evolution to compare photosynthetic activities of RSD and \textit{P. antarctica} cells.

Chl \textit{a} fluorescence \textit{in vivo} has been designated as “the signature of photosynthesis” [15]
since it carries information not only on the pigment compositions of PS II and PS I but also on
the dynamic interactions and regulations of complex photosynthetic sub-processes. For
fluorometry we used suspensions of living cells (at 277 K) as well as frozen cell suspensions
(at 77 K). Chl \textit{a} fluorescence emission spectra and their inverted 2nd derivatives were used in
order to identify the pigment compositions of PS II and PS I and, correspondingly, Chl \textit{a}
fluorescence excitation spectra in order to identify the light harvesting pigments that supply
electronic excitation to reaction center complexes of PS II and PS I [16].

In this report, we present evidence in support for two distinct subpopulations of MAAs in
\textit{P. antarctica} and in RSD cells, one cytosolic and the other plastidic and, further, for a
photosynthetic light harvesting role for the plastidic MAAs. We also show, in terms of 2nd
derivative analysis of fluorescence spectra, a diminished presence of PS II in the RSD
kleptoplasts, compared to the \textit{P. antarctica} chloroplasts. And lastly, on comparing PS II-
driven O2 evolution (a known generator of reactive oxygen species, ROS; see chapters in
Demmig-Adams et al., ref. 17) we find the PSII of kleptoplasts substantially less active than
the PS II of chloroplasts. On the basis of these findings, we propose that the reported
functional longevity of RSD kleptoplasts [10] can be rationalized in terms (a) to a lower
exposure to ROS and (b) to a lower dependence on nucleus-encoded light harvesting Chl a
proteins (LHC) because of the supplementary light harvesting by plastidic MAAs.

2. Materials and Methods

2.1. Cell cultures and preparations

A unialgal culture of \textit{P. antarctica} was created by picking alga colonies and transferring
them, through two washes in sterile medium, to fresh filter sterile f2 + silica medium (culture
medium) [18]. A culture enriched for RSD was created by collecting the cells from near the
surface of a mixed RSD and \textit{P. antarctica} culture. The resulting culture was more than 95%
enriched in RSD dinoflagellate cells. \textit{P. antarctica} and RSD cells were re-cultivated from the
enriched cultures by inoculating cells into culture medium at 0–4 \textdegree C in an illuminated
incubator with a photoperiod of 12 h light, 12 h dark.
To extract cell suspensions with the water and organic solvent miscible aprotic solvent dimethylformamide (DMF; 19, 20), the suspensions were centrifuged, DMF was added to the residue, and the resulting DMF extract was obtained after a second centrifugation.

Total Chl (Chl a+Chl c2) concentration was determined according to Ritchie [21].

2.2. Absorbance 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 BaSO4. The spectra were scanned from 300 nm to 700 nm, at a speed of 200 nm min⁻¹. DMF extracts were measured with a Perkin Elmer 557 (PerkinElmer, Inc., Waltham, MA, USA), UV-visible scanning spectrophotometer. Displayed spectra are normalized to equal peak heights (= 1) at 680 nm, in the case of cell suspensions, and at 664 nm in the case of the DMF extracts. Normalized spectra of DMF extract were used to derive the respective 2nd derivative inverted absorption spectra.

2.3. Measurements of Chl a fluorescence

Assay samples for fluorometry were prepared by injecting 200 µl cell suspension into quartz capillary tubes (2.5 mm internal diameter). Prior to freezing to 77 K with liquid nitrogen, the cells were dark adapted for 30 min, at 2-4 °C. Fluorescence was excited either at 436 nm (Δλ = 10 nm; Chl a-selective excitation) or at 345 nm (Δλ = 10 nm; MAAs-selective excitation). Chl a fluorescence emission and excitation 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. Fluorescence emission spectra were scanned with a detection bandwidth of Δλ= 2.5 nm, and with a Corning CS 2-60 cut-off filter to prevent stray exciting light from entering the measuring monochromator. Excitation spectra were scanned with a Δλ = 5 nm bandwidth by Chl a fluorescence detection either at 683nm for spectra recorded at 277 K, or at 689 nm for spectra recorded at 77, with Δλ = 5 nm.

To quench selectively the Chl a fluorescence that originates PS II, the cells were treated with p-nitrothiophenol under illumination with red light (680 nm, Δλ = 5 nm), prior to freezing to 77 K and to recording the fluorescence spectrum, as described by Kobayashi [22, 23]. The red light was obtained from the exciting monochromator of the Hitachi F-2500 spectrofluorometer.
2.4. Photosystem II activity

PS II activity was determined in terms of photosynthetic O₂ evolution, by measuring the rate photosynthetic electron transport across PS II, from water, as electron donor, to phenyl-p-benzoquinone (PBQ) as post-PSII electron acceptor at 2°C [24]. Assays were performed at 4°C (277 K) with a Clark-type oxygen electrode (DW1; Oxygraph, Hansatech, King’s Lynn, U.K.) fitted with a slide projector to provide saturating actinic illumination to samples. The reaction mixture (1 ml) contained cells (RSD or *P. antarctica*) and 1 mM PBQ. Oxygen evolution rates are expressed in μmolO₂ mg (total Chl)-1 h⁻¹.

3. Results

The absorption and pigmentation characteristics of *P. antarctica* and RSD cells are compared in Figs. 1(A-C). The two absorption spectra in Fig. 1A appear generally similar, except for the lower abundance of MAAs in the RSD cells compared to *Phaeocystis*. To obtain more quantitative values for the abundances of MAAs relative to Chl a in the two cell types, we calculated absorbance ratios of MAAs vs Chl a (A_{MAAs}/A_{Chl a}) from the spectra in Fig. 1A, in the case of the alga and the dinoflagellate cell, and from those in Fig. 1B, in the case of their DMF extracts. The relative abundance of MAAs in *P. antarctica* cells, calculated from the absorbance ratio A_{MAAs}/A_{Chl a} = A_{325}/A_{440}, is ca. 2.11. In the RSD cells, the ratio A_{MAAs}/A_{Chl a} = A_{340}/A_{440}, drops to ca. 0.84, namely to about 40% of the relative abundance ratio in *P. antarctica* cells. This dramatic drop in the relative abundance of MAAs upon the internalization of the alga cell by the dinoflagellate may suggest the co-existence of two subpopulations of MAAs in the alga, one cytosolic and more prone to digestion or loss in the dinoflagellate and the other plastidic (chloroplasts and kleptoplasts) and less prone to digestion.

Further corroborating evidence, for the existence of two distinct subpopulations of MAAs in *P. antarctica* and in RSD cells, is provided by comparing the relative MAAs abundances in the DMF extracts (Fig. 2B). In the DMF extract of *P. antarctica*, the relative abundance ratio (calculated from A_{MAAs}/A_{Chl a} = A_{325}/A_{436}) is ca. 0.24, while in the DMF extract of RSD cells (calculated from A_{MAAs}/A_{Chl a} = A_{337}/A_{436}) it is ca. 0.59. It is important to mention here, that DMF extracts chlorophylls quantitatively [19, 20], so the calculated relative MAAs abundance ratios of cell suspensions and of DMF extracts are comparable. These results
show, therefore, that DMF extracts only a small fraction (about 11%) of the MMAs of the P. antarctica cells (which are mostly cytosolic) while, in contrast, it extracts about 70% of the MAAs present in the RSD cells (which are mostly plastidic).

Detailed information about overlapping bands in an optical spectrum can be obtained from an inverted plot of its second derivative [25, 26]. In such plots, individual absorption or fluorescence bands are narrower, while the band maxima locations are unchanged. This information, together with reasonable estimates of spectral half-band widths, allows the deconvolution of a composite spectrum into individual overlapping contributions. Fig. 1C displays such inverted second derivative plots of the spectra the DMF extracted pigments in Fig. 1B. The broad bands of the latter spectra are resolved in Fig. 1C to individual absorption contributions which are assigned (after Zapata et al., ref. 27) as follows: Chl $a$ (431 nm, 664 nm); Chl $c_2$ (452 nm, 580 nm, 625 nm); fucoxanthin (452 nm); β-carotene and xanthophylls (484 nm).

Figure 2 displays fluorescence emission spectra of RSD and P. antarctica cells, excited either at 345 nm (absorbed mostly by MAAs-specific absorption [28-30] but also by short-wavelength tails of Chls $a$, $c_2$; black lines) or at 436 nm (Chl $a$-specific absorption; grey lines), and recorded either at 277 K (A and C, cells active) or at 77 K (B and D, cells frozen). With either excitation, Chl $a$ fluorescence peaks at the same wavelength, namely at 683 nm in the case of active cells (A and C) and at 689 in the case of the frozen cells (B and D).

In interpreting the fluorescence spectra on Fig. 2, it is important to notice that a solution of pure Chl $a$ in diethyl ether absorbs at 350 nm ~24% of the light it absorbs at 428 nm (at the Soret peak; see e.g., ref [31]). In view of that, it is quite interesting to notice that by exciting RSD cells at 345 nm the generated Chl $a$ fluorescence is ~70% of that generated by excitation at 436 nm at 277 K (Fig. 2A) and ~82% of the same at 77 K (Fig. 2B). In either case, the expectation would be expected ~ 24%. A likely explanation for the unexpectedly stronger fluorescence at the 345 nm excitation is the presence a non-chlorophyll sensitizer that contributes to the detected Chl $a$ fluorescence signal. A most reasonable candidate for this sensitizer are the MAAs.

In contrast, in the case of P. antarctica cells, excitation at 345 nm generates ~26% of the Chl $a$ fluorescence generated at 436 nm at 277 K (Fig. 2C) and ~60% of that at 77 K (Fig. 2D). These results may be explained by the fact that, in P. antarctica cells, MAAs are present in the cytoplasm (mostly) as well as in the chloroplasts. Not only the cytoplasmic MAAs cannot transfer excitation to Chl $a$, but they also block a fraction of the 345 nm excitation
from reaching the chloroplastic MAAs. Altogether, these results suggest that the UV-absorbing MAAs in the chloroplasts transfer electronic excitation to the Chls \textit{a in vivo}.

Figure 3 displays inverted second derivative plots of the 77 K fluorescence spectra of \textit{P. antarctica} (black line) and of RSD cells (grey line), excited at 436 nm (Chl \textit{a} absorption, A) and at 345 nm (MAAs absorption, B). These plots show clearly that the fluorescence bands of the algal cells and of the RSD cells are composites, each comprising two individual emission sub-bands which are centered at 682-683 nm (Fi-683) and at 689 nm (Fi-689). The salient observation of this experiment (see Fig. 3B) is that the MAAs of RSD cells are competent in exciting both the Fi-683 and Fi-689 sub-bands, although quite remarkably, to different extents: MAAs in RSD kleptoplast are less competent in exciting Fi-683 than the MAAs in algal cells, whereas with respect to Fi-689, both the alga chloroplast MAAs and the RSD kleptoplast MAAs are equally competent.

Figure 4 displays excitation spectra of the F683 and the F689 Chl \textit{a} fluorescence bands emitted by \textit{P. antarctica} cells at 277 K (A) and at 77 K (B) and by RSD cells emitted at 277 K (C) and at 77 K (D) respectively. The displayed spectra appear, more or less, similar, except that an excitation band at 520 nm is clearly resolved in the 77 K spectra of \textit{P. antarctica}. (B and D). The excitation bands are tentatively assigned as follows [32, 33]: A\textit{340} (MAAs); A\textit{375} (MAAs); A\textit{440} (Chl \textit{a}); A\textit{465-520} (fucoxanthins, \(\beta\)-carotene, and Chls \textit{c}2 and \textit{c}3). Of particular interest is the ability of MAAs to sensitize Chl \textit{a} fluorescence via electronic excitation transfer, possibly by other pigments, as suggested by Sivalingam [34].

According to Kobayashi \textit{et al.} [22], \(p\)-nitrothiophenol (\(p\)-NTP) inhibits electron donation to PSII when added to chloroplasts in light, and at the same time it increases the Chl \textit{a} fluorescence that PS II emits (as detected in the 77 K emission spectra). On the other hand, when added to chloroplasts in the dark, \(p\)-NTP had no effect on the PSII activity and its fluorescence. \(p\)-NTP has no effect on PS I when added to chloroplasts either in light, or in the dark [22]. In the experiment of Fig. 5, we used the \(p\)-NTP addition phenomenology, in order to assign the Fi-683 and Fi-689 sub-bands (resolved in the 77 K fluorescence spectra of \textit{P. antarctica} and of kleptoplast-hosting RSD cells, see Fig. 3) to PS I and PS II. Emission spectra of untreated (black line) and of \(p\)-NTP-treated \textit{P. antarctica} cells (grey line) are shown in Fig. 5A and corresponding spectra of RSD cells in Fig. 5B. All spectra are normalized to 1 at 689 nm.

According to Fig. 5A, and to the inset in it, the treatment of \textit{P. antarctica} cells with \(p\)-NTP led to an increase of Fi-683 and had no effect on Fi-689 compared to Fi-689. On this basis, we assign Fi-683 to PS II and Fi-689 to PS I. In the RSD cells (Fig. 5B), whose Chl \textit{a}
fluorescence is centered at 689 nm, a treatment with \( p \)-NTP caused no stimulation of fluorescence. We do, therefore, assign, the F689 band of the kleptoplast RSD cells to PS I. Apparently, in the latter cells, PS II is much diminished, although not altogether missing, as indicated by the presence of a F683 emission in the inverted 2nd derivative kleptoplast fluorescence spectra shown in Fig. 3, A and B.

If the PSII activity is indeed diminished in the RSD cells, as the fluorimetric data indicate, then this should be reflected in their photosynthetic oxygen evolution activity. To confirm this expectation, we compared the oxygen evolution activities of \( P. antarctica \) and of RSD cells. In our experiment, we assayed photosynthetic electron transport from water (as electron donor) to phenyl-\( p \)-benzoquinone (as post-PS II) electron acceptor. The results are presented in Table 1, which shows the measured PS II activity RSD cells to be about 17\% of that of \( P. antarctica \) cells. We conclude, from these results, the role of oxygenic PS II is severely diminished in the dinoflagellate-hosted kleptoplasts, compared to that of the alga prey chloroplasts.

4. Discussion

In this research we studied and compared the oxygenic photosynthesis of \( P. antarctica \) and of RSD cells which host kleptoplasts derived from \( P. antarctica \) chloroplasts. Specifically, we investigated changes in light harvesting photosynthetic pigments and the photosynthetic oxygen evolution activities (PS II activities) that accompany the conversion of active algal chloroplast to active dinoflagellate kleptoplast.

As the emission spectra (Figs. 2 and 3) and the excitation spectra (Fig. 4) of Chl \( a \) fluorescence show, the UV absorbing MAAs are capable of exciting Chls \( a \) of PS II and PS I, both in the alga chloroplast and in the dinoflagellate kleptoplast and, therefore, they must be included among the photosynthesis light harvesting pigments of these organisms. This result stands in conflict with the report of Moisan & Mitchell [23] who detected no Chl \( a \) fluorescence on exciting \( P. antarctica \) with UV light and concluded that MAAs-absorbed light is photosynthetically inactive. The discrepancy can be rationalized from the fact that Moisan and Mitchell [23] measured Chl \( a \) fluorescence at 730 nm (in the far red tail of the emission band, cf. Fig. 2) while we measured it at its peak emission (689 nm).

According to the classical theory of Th. Förster [35], the efficiency of intermolecular resonance electronic excitation transfer depends on two conditions: (a) the overlap between the donor emission spectrum and the acceptor absorption spectrum (both expressed in the
frequency domain and normalized); and (b) the close proximity (the so-called Rö law) between the interacting molecules. Of the MAAs that have been identified in *P. antarctica* (mycosporine-glycine, mycosporine-glycine-valine and shinorine; ref. [36]) only shinorine has been reported to fluoresce; ref. [37, 38]). This, perhaps, relates to shinorine being a cyclohexenimine derivative, while the other two MAAs are cyclohexenone derivatives. When excited at 350 nm, shinorine emits weak fluorescence centered around 400 nm and extending to beyond 500 nm [39], therefore, its fluorescence overlaps completely with the Soret absorption band of Chl *a* (at 436 nm). The fact that we do see Chl *a* fluorescence by exciting MAAs indicates a close proximity of the MAAs to Chl *a* hinting to the presence of the MAAs within the plastids.

In their review, Shick and Dunlap [39] consider the localization of MAAs in chloroplasts a possibility that had to be proven. Our results indicate the presence of two distinct subfractions of MAAs, one in the cytosol and another in the plastids, that is further supported by the absorption spectra of whole cells and of their DMF extracts (Figs. 1, A & B). In *P. antarctica*, the major MAAs fraction is cytosolic. This fraction is expected to screen out UV light, but not to sensitize Chls *a* in the plastids. The plastidic MAAs fraction, on the other hand, is expected to sensitize Chl *a* fluorescence both in alga chloroplasts and in RSD kleptoplasts, although more in the latter case since there are no cytosolic MAAs to screen off MAAs-selective excitation.

The water-splitting and oxygen-generating function of PS II is known to be associated with the generation of ROS, such as singlet oxygen ($^1$O$_2$) and superoxide radical (O$_2^-$) which cause various kinds of damage to photosynthetic complexes and membranes (see chapters in Demmig-Adams et al., ref. 17). To a lesser extent, ROS are also formed in PS I. According to Shick and Dunlap (39), in addition to screening off UV light, the MAAs function as antioxidants, by scavenging for ROS formed during photosynthesis. In this task, the plastidic MAAs may be particularly important by being close to the PS II complexes.

The possibility to distinguish between the 77 K Chl *a* fluorescence emissions of PS II and PS I enabled us to explore the changes in the light-harvesting pigment complements of the two photosystems that take place upon conversion of an active algal chloroplast to an active dinoflagellate kleptoplast. As evident from the inverted second derivatives plots on Figs. 3A and 3B, in kleptoplasts the excitation share of the PS II is substantially lower than in chloroplasts. This may imply a subdued role for PS II and, correspondingly, an enhanced role for PS I in the kleptoplast. Indeed, as the experiment on Table 1 confirms, the photochemical activity of PS II (assessed in terms of photosynthetic O$_2$ evolution activity) is severely
diminished (< 20%) in the kleptoplast-hosting RSD cells compared to the chloroplast hosting
*P. antarctica* cells. Thus, the kleptoplast seems to primarily operate its PS I in order to meet
its requirements in low potential reducing compounds and high energy triphosphates. RSD
may be viewed, therefore, as efficient photo-heterotrophs, whereas the *Phaeocystis* prey are
efficient photo-autotrophs.

In conclusion, we suggest that the relative longevity of the RSD kleptoplast can be
rationalized (a) in terms of a reduced role of PS II and (b) in terms of a lower dependence on
nucleus-encoded and cytosol-synthesized light harvesting Chl *a, c* protein complexes, due to
supplementary light harvesting for photosynthesis by MAAs.

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References


Figure Legends

Fig. 1. (A) Absorption spectra of *P. antarctica* (black line) and of kleptoplast-hosting RSD cells (grey line). (B) Absorption spectra of DMF extracts of *P. antarctica* cells (black line) and of kleptoplast-hosting RSD cells (grey line). (C) Inverted 2nd derivatives of the spectra in Fig. 1(B). Spectra in A are normalized to equal peak heights (= 1) at 680 nm and in B are normalized to equal peak heights (= 1) at 664 nm; the normalized spectra in B were used to derive the spectra in C. Arrows indicate absorption peaks.

Fig. 2. Chl *a* fluorescence spectra of kleptoplast-hosting RSD cells (A and B) and of *P. antarctica* cells (C and D). The spectra were excited using either Chl *a*-selective excitation (436 nm, grey lines), or MAAs-selective excitation (345 nm, black lines) and were recorded either at 277 K (A and C, cells active), or at 77 K (B and D, cells frozen).

Fig. 3. Inverted 2nd derivative plots of the 77 K fluorescence emission spectra of *P. antarctica* (black line) and of kleptoplast-hosting RSD cells (grey line), excited either at 436 nm (Chl *a* absorption, A); or at 345 nm (MAAs absorption, B). Fluorescence emission spectra, normalized to equal peak heights at 689 nm, were used to obtain the derivative spectra.

Fig. 4. Chl *a* fluorescence excitation spectra of kleptoplast-hosting RSD cells (A & B) and of *P. antarctica* cells (C & D). The spectra in A & C were recorded at 277 K (Chl *a* fluorescence detected at 683 nm, with Δλ = 5 nm) and those in B & D at 77 K (Chl *a* fluorescence detected at 689 nm, with Δλ = 5 nm). Excitation was scanned from 300 nm to 640 nm, with Δλ = 5 nm). Arrows indicate the peaks in the spectra.

Fig. 5. Effects of treating *P. antarctica* cells (A), and of kleptoplast-hosting RSD cells (B) at 277 K, with *p*-nitrothiophenol under red light (680 nm, Δλ = 5 nm) on their Chl *a* fluorescence spectra at 77 K. Black lines, untreated cells (control); grey lines, treated cells. Inset to Fig. 5A: Difference fluorescence spectrum of *P. antarctica* cells (ΔF = F(+p-NTP) – F(−p-NTP)). Fluorescence was excited at 436 nm (Δλ = 10 nm).
Table 1
Photosynthetic oxygen evolution activity by Photosystem II of *P. antarctica* and of kleptoplast-hosting RSD cells.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Oxygen Evolution Activity(^1) (μmol O(_2) mg (total Chl))(^{-1})h(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. antarctica</em></td>
<td>114.33 ± 4.02</td>
</tr>
<tr>
<td>RSD-kleoplasts</td>
<td>20.02 ± 6.08</td>
</tr>
</tbody>
</table>

\(^1\) Activity values are means ± s.e (n = 3)
Chl a fluorescence (inverted 2nd derivative)

A

689 nm

683 nm

λexc=436 nm

B

689 nm

682 nm

λexc=345 nm

Wavelength, nm