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Changes in the photosynthetic apparatus of diatoms in response to low and high light intensities

Received: 25 October 2000 / Accepted: 28 December 2000 / Published online: 25 August 2001
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Abstract The centric diatom Cyclotella cryptica and two strains of the pennate diatom Phaeodactylum tricornutum were grown under low and high light intensities (300 lux and 3,000 lux) over 4–6 weeks. Growth was monitored by repetitive cell count. The culture media were replaced weekly to avoid morphological and biochemical alterations caused by nutrient depletion. The ultrastructure of the cells was examined by transmission electron microscopy. Alterations in the light-harvesting antenna systems were investigated by Western immunoblotting. Both diatoms reduced the plastid area, i.e. decreased the amount of thylakoid lamellae, under high light intensity. The thylakoids still ran in groups of three with parallel orientation within the chloroplasts. The girdle band lamellae were not affected. The amounts of storage compounds and vacuoles increased. SDS-PAGE of total cell protein followed by Western immunoblotting with antisera directed against subunits of the light-harvesting antenna systems of C. cryptica (cc-antiserum) and the cryptophyte Cryptomonas maculata (ccm-antiserum) revealed that both diatoms reduced the amount of antenna polypeptides under increased light intensity. The cc-antiserum immunodecorated two bands with relative molecular masses ($M_r$) of 18,000 and 22,000 in C. cryptica. Both decreased under high light conditions to 67.2 ± 6.1%. Five to seven bands in the $M_r$ range of 14,000–27,000 were recognized in P. tricornutum. They decreased to $83.5.3\%$. Furthermore, the immunolabeling pattern for both strains differed under the two light regimes. The cma-antiserum immunodecorated two polypeptides with $M_r$ of 24,000 and 23,000 in C. cryptica, while both strains of P. tricornutum had five polypeptides in the $M_r$ range of 14,000–24,000 that showed some differences in staining intensities between the two strains and in response to the light intensity applied.

Keywords Cyclotella cryptica · Phaeodactylum tricornutum · Bacillariophyceae · Chloroplast ultrastructure · Light-harvesting system

Introduction

Only a limited number of diatom species have been investigated and used in biochemical, ultrastructural, and molecular studies to unravel the organization of the photosynthetic apparatus of this major algal group. Most of the work has been done with the pennate species Phaeodactylum tricornutum. Early reports dealt with the isolation of fucoxanthin chlorophyll a/c binding polypeptides (Fcps), the proteins that constitute the light-harvesting complexes (LHs) in diatoms, and with genes encoding Fcps (fcp genes) [1, 6, 7, 8, 9, 10, 11, 17, 18]. Over the last few years, knowledge on Fcps and fcp genes has expanded, and information has become available for several other diatoms, such as Cyclotella cryptica, Odontella sinensis, Skeletonema costatum, and Thalassiosira weissflogii [3, 4, 5, 14, 16, 25].

The effects of light intensity on the photosynthetic apparatus of P. tricornutum and C. cryptica have been described [7, 8, 17, 18, 22]. Morphological changes accompanying these biochemical changes, however, have not been investigated, and ultrastructural studies in this context are sparse. Pysniak and Gibbs [19] investigated the localization of Fcp and photosystem I (PSI) in P. tricornutum by immunocytochemistry, whereas Jeffrey and Veski [13] described morphological changes in cells of
**Stephanopyxis turris** cultured under different light climates. They found that the thylakoids became distorted and no longer ran parallel under high light (HL) conditions. Rosen and Lowe [23] described smaller chloroplast volumes and less thylakoid surface density for *C. meneghiana*, when this alga was exposed to light and nutrient stress simultaneously. A study dealing with the morphological differences that are exclusively caused by HL is missing. The intention of the current study is to close the gap between the data available from biochemical experiments and these ultrastructural findings. We applied low light (LL) and HL intensities to cultures of *C. cryptica* and *P. tricornutum* for 4–6 weeks. During this time, care was taken that they did not run into nutrient deprivation. Afterwards, LL- and HL-grown cells were compared by means of transmission electron microscopy, pigment content, and Western immunoblotting.

**Materials and methods**

Cultures, culture conditions, and growth measurements

*Cyclotella cryptica* Reimann (strain 1070–1) and *Phaeodactylum tricornutum* Bohlin (strains 1090–1a and 1090–6) were obtained from the “Sammlung von Algenkulturen” (SAG) at Göttingen, Germany, and grown in medium 6 [24]. Cultures were kept at 15 °C in Erlenmeyer flasks of 100–5,000 ml culture volume without aeration. The cultures were grown for 4–6 weeks either at 300 lux (low light, LL) or 3,000 lux (high light, HL) provided by white fluorescent lamps. During the experiments a light/dark regime of 16:8 h was applied. The culture medium was replaced weekly to avoid effects caused by nutrient depletion. Light intensity was measured using a BBC Goerz Metrawatt MX4 luxmeter. Growth was measured by repetitive cell counting using a Fuchs-Rosenthal counting chamber and a Zeiss photomicroscope III operating at 400x magnification.

Spectroscopy, pigment extraction, and pigment estimation

Absorbance spectra of cell suspensions and of pigment extracts were recorded at 20 °C with a U-3000 spectrophotometer (Hitachi, Tokyo, Japan). The spectra were recorded from 400 to 740 nm. The band width was set to 2 nm, and the registration speed was 300 nm/min. Pigments were extracted either with 90% (v/v) acetone or with 90% (v/v) methanol and the concentration of chlorophyll a (chl a) was estimated using the equations of Jeffrey and Humphrey [12] or Wilkinson [29]. The reference cuvettes contained the solvent used for the extraction.

SDS-PAGE and Western immunoblotting

Total cell protein of *C. cryptica* and *P. tricornutum* was used. The cell suspensions were normalized to either equal amounts of chl a (*C. cryptica*) or to the same OD at 674 nm (*P. tricornutum*). Cells were harvested by centrifugation, resuspended in the appropriate amounts of sample buffer to give 5 μg chl a/ml or 1 OD₆₇₄nm/ml, respectively, and boiled for 2–4 min at 100 °C. Then, the protein samples (25–100 ng chl a or 20–40 μl of suspensions with 1 OD₆₇₄nm) were loaded. SDS-PAGE was performed according to [15] using 15% separating gels and 5% stacking gels. Gels were run in duplicate at a constant current of 8 mA overnight or at 35 mA for 6–8 h during the day. Western blotting was performed in a Biorad Trans-blot SD semidyey transfer cell according to Towbin et al. [27]. The proteins were transferred onto Protran nitrocellulose transfer membrane (Schleicher and Schuell, Dassel, Germany) at a constant voltage of 20 V for 1.5 h. For immunodecoration, the blots were preincubated in Tris-buffered saline (TBS; 10 mM Tris-HCl, pH 7.5, 50 mM NaCl) saturated with skim milk powder for 1 h and washed in TBS followed by incubation with the first antibodies (serum diluted 1:1000 in TBS with 2% [w/v] bovine serum albumin [BSA]) for 2 h. The antibodies raised against the major LHC of the cryptophyte *Cryptomonas maculata* (emac-antiserum) and directed against the main LHCs of the diatom *C. cryptica* (cc-antiserum) were described earlier [21, 22]. After several washings with TBS, the nitrocellulose membranes were incubated with peroxidase-conjugated goat anti-rabbit IgG (diluted 1:1,000 in TBS with 2% BSA) provided by Sigma (Munich, Germany). The membranes were washed again and stained with 4-chloro-1-naphthol and H₂O₂. Images of the blots were recorded with a Herolab EASY CCD camera (type 429 K) in an illumination chamber (Herolab RH-3) and stored as digitized files. The results were documented using the enhanced analysis system EASY (Herolab, Wiesloch, Germany). For quantitative investigations on the immunodecorated protein bands, the EASY Image Plus software package was used.

**Electron microscopy**

For ultrathin sectioning, cells were embedded in Epon according to Spurk [26]. As a modification, 2% (w/v) tannic acid was included as fixative during the OsO₄ fixation step [28]. After the dehydration in a graded acetone series, the samples were infiltrated in resin/acetone mixtures and pure resin. Here, incubation times were prolonged to 12 h for each step. Ultrathin sections were cut with a Reichard-Jung Ultarcut E ultramicrotome. The sections were post-stained with lead citrate [20] and examined in a Zeiss EM109 electron microscope operated at 50 kV. For estimating the percentage of the chloroplast areas from the total areas of thin sectioned cells, photocopies were made from positive prints. Cell areas were cut out and weighed and then the areas of the chloroplasts were estimated by cutting and weighing.

**Results**

**Growth and pigmentation**

Cultures of *Cyclotella cryptica* and *P. tricornutum* were grown under LL and HL, respectively. At the end of the experiments the cell densities were measured and defined amounts of cells were subjected to quantitative pigment extraction with either acetone or methanol. The results are compiled in Fig. 1. LL-grown cultures of both diatoms were generally darker brownish colored than HL-exposed ones. This observation reflected a stronger pigmentation under LL conditions and was not caused by higher cell densities. Cell counting revealed that LL-grown cultures of *C. cryptica* and *P. tricornutum* strain 1090–6 had reached approximately 50% of the cell densities that were measured for HL-grown cultures. Moreover, *P. tricornutum* strain 1090–1a exhibited a four-fold higher cell density under HL conditions (Fig. 1A). Pigment extraction with either acetone or methanol revealed that the pigment yields were lower for both diatoms when acetone was used. Only HL-grown cells of *C. cryptica* deviated from this general observation. Acetone was thus less suited for extraction than methanol. When acetone was used as extraction solvent, the chl a concentrations of HL-grown cultures of both strains of *P. tricornutum* reached approximately 25% of the value calculated for LL-grown cultures, while HL-grown cells of *C. cryptica*
contained approximately 50% of the amount of chl a estimated for LL-grown ones. When methanol was used as solvent, the yield of chl a increased up to 46% for HL-grown cells of *P. tricornutum* strain 1090–6 and decreased to 34% for *C. cryptica* of the values measured for LL-grown cultures (Fig. 1B). In vivo absorption spectroscopy and spectra of pigment extracts confirmed and extended these results. Figure 1C shows (thin line) that LL-grown cells of *C. cryptica* had absorbance maxima at 673, 633, and 437 nm, and shoulders at 460 and 500 nm. The absorbances at 673 and 437 nm are mainly caused by chl a, whereas those at 460 and 500 nm are due to chl c and carotenoids, respectively. The peak at 633 nm is due to chl a and chl c. The in vivo absorption spectrum of LL-grown cells of *P. tricornutum* matched that of *C. cryptica* and had the same maxima and shoulders. The maxima, however, shifted to 674, 633, and 439 nm (Fig. 1D, thin line). The spectra of HL-grown cells of both diatoms, in contrast, showed dramatic changes. The peaks and shoulders were either less or more pronounced or had even disappeared. The shoulder at 500 nm became a peak in *P. tricornutum* and the maximum at 674 nm shifted to a higher wavelength. The maximum at 633 nm seemed to disappear for *C. cryptica* and to increase in *P. tricornutum* (Fig. 1C, D, thick lines) when the spectra were normalized at 674 nm. Absorption spectra of pigment extracts of both diatoms which were normalized at 663 nm revealed that the absorption at 633 nm and 663 nm had not changed significantly (Fig. 1E, F). The changes in pigmentation observed in the in vivo spectra thus reflected differences in the amounts of carotenoids rather than in the amounts of chl a and chl c. Thus, HL-grown cells of both diatoms seemed to harbor more carotenoids than LL-grown ones. This can be deduced from the spectra of pigment extracts that had higher absorbance values between 400 and 550 nm (Fig. 1E, F, thick lines).

Electron microscopy showed the ultrastructure of cells and details of the chloroplasts of *C. cryptica* and
P. tricornutum (Figs. 2 and 3, respectively). As there were no obvious differences for the two P. tricornutum strains, they are not distinguished further here. LL-grown cells of both algae had the typical features known for diatoms. The cells were surrounded by a silicified frustule. Nuclei, mitochondria, dictyosomes, membranes of the endoplasmic reticulum, and vacuoles are visible in the cells. Either two large (P. tricornutum) or several small (C. cryptica) chloroplasts were the dominating organelles, comprising 34±2% and 35±7% of the sectioned area of cells of C. cryptica and P. tricornutum, respectively (Figs. 2A, B and 3A). The chloroplasts were densely packed with thylakoid membranes, which, as known for diatoms, were predominantly arranged in threes. A girdle lamella encircled the rim of the chloroplast (Figs. 2C and 3A).

Both diatoms responded to HL conditions by increasing the area covered by vacuoles and reducing the chloroplast area (Figs. 2D, E and 3B). The sectioned chloroplast areas decreased to approx. 12±4% in C. cryptica and 18±2% in P. tricornutum. The thylakoid membranes still ran in groups of three, but the overall amount of thylakoids was reduced significantly. The girdle lamella was still present.

Western immunoblotting

Total cell protein of LL- and HL-grown cells of the two strains of P. tricornutum and of C. cryptica were subjected to SDS-PAGE followed by Western immunoblotting. Equal amounts of chl a or cell suspensions equalized to the same OD674 values were loaded. The results are shown in Fig. 4. When the blots were incubated with the cc-antiserum (Fig. 4A), two bands with $M_r$ of 18,000 and 22,000 and a set of five to seven bands in the $M_r$ range of 14,000–27,000 were recognized from the total cell protein of C. cryptica and P. tricornutum, respectively. Quantitative estimations revealed that HL-grown cells of C. cryptica and P. tricornutum showed only 67.2±6.1% and 83±5.3%, respectively, of the signal intensities for Fcp that were measured for LL-grown cells. Thus, P. tricornutum responded to a lesser
and both polypeptides decreased simultaneously under HL conditions (Fig. 4A, lanes 1, 2). When Western blots were incubated with the cmao-antiserum, the banding pattern changed dramatically (Fig. 4B). Thus, both strains of *P. tricornutum* had almost identical banding patterns, i.e. five polypeptides in the *M*~r~ range of 14,000–24,000, which showed some differences in staining intensities between the two strains (indicated by asterisks in Fig. 4B, lanes 3, 6) and in response to the light intensity applied. Only two polypeptides, with *M*~r~ of approximately 24,000 and 23,000, were immunodecorated in *C. cryptica*. The *M*~r~ 24,000 band was found in LL-grown cells, whereas the *M*~r~ 23,000 band was decorated in HL-grown cells (indicated by arrowheads in Fig. 4B, lanes 1, 2).

**Discussion**

Cells of *C. cryptica* and of two strains of *P. tricornutum* were grown under LL and HL conditions and the morphological and biochemical effects were investigated simultaneously by transmission electron microscopy, pigment analyses, and Western immunoblotting. Previous work on these and related algae has focused exclusively on ultrastructural or biochemical changes.

Both algae respond to increased light intensity with an increase in the amount of carotenoids and a reduction of the concentrations of chl *a* and chl *c*. This result is in accordance data of Friedman and Albete [7, 8], who registered for *P. tricornutum* a reduction of chl *a* and, simultaneously, a loss of the reaction centers of the two photosystems and of LHC under HL conditions. Owens and Wold [18] and Owens [17] obtained similar results but registered an increase of a “major light-harvesting antenna” concomitant with a decrease of a “minor antenna” under HL conditions. Friedman and Albete [7, 8], Owens and Wold [18] and Owens [17] did not use an immunological approach but isolated pigment protein complexes by detergent-mediated solubilization of thylakoid membranes followed by sucrose density gradient centrifugation. Thus, their results may correspond to the data obtained by Western immunoblotting for *P. tricornutum*. Both strains of *P. tricornutum* differed from one another with respect to the polypeptides that could be decorated by the two antisera used. Both strains, however, responded to changing light climates by changing the amounts and thus ratios of the light-harvesting polypeptides that could be immunodecorated. The fact that the signal intensities of several immunodecorated bands of *P. tricornutum* decreased or increased in response to the light climate might be taken as direct evidence for the existence of defined light-harvesting subunits that become induced or repressed by light. In previous publications, either two or three polypeptides have been described for *P. tricornutum* that could be immunodecorated by a homologous antiserum directed against the LHC of this alga [6, 8]. The number of bands reported here exceeds this number drastically.
and shows that the antenna system of *P. tricornutum* harbors many more polypeptides and thus is encoded by several more *fep* genes than has been expected. Currently, six *fep* genes have been cloned and sequenced from *P. tricornutum*. They are clustered in two loci separated by short intergenic regions and are most probably located on the same chromosome [2]. The existence of multigene families encoding Feps has been shown in detail for *C. cryptica* and *Skeletonema costatum* [3, 4, 25].

For *C. cryptica*, reduced pigment content under HL growth conditions has already been measured. The protein bands with $M_r$ of 18,000 and 22,000, which were decorated by the ee-antisera, and of 24,000 and 23,000, which were decorated by the ecm-antisera, match those shown by Rhiel et al. [22]. Rosen and Lowe [23] reported a similar finding for the pigment content of *Cyclotella meneghiniana* when this alga was exposed to light and nutrient stress simultaneously. The morphological changes accompanying these biochemical changes have not been investigated in these two algae. Jeffery and Vesik [13] investigated morphological changes in cells of *Stephanopyxis turris* cultured under different light climates and found that the thylakoids became distorted, tended to be twisted and out of plane and no longer ran parallel under HL conditions. Rosen and Lowe [23] described smaller chloroplast volume and less thylakoid surface density for *C. meneghiniana* when this alga was exposed to light and nutrient stress simultaneously. Based on our findings, we assume that light stress alone causes the same effects. For *C. cryptica* and *P. tricornutum*, the amount of thylakoids in the chloroplasts became reduced, too, thus resulting in an overall reduced chloroplast area. This is in line with the biochemical data demonstrating a loss of chl a and thus of reaction centers of the two photosystems and of LHC under HL conditions. The parallel orientation of thylakoids remaining in both algae under the light regimes applied might be characteristic for *C. cryptica* and *P. tricornutum* or, probably, higher light intensities or prolonged exposure might result in the distortion described for *S. turris*.

Acknowledgements The authors express their gratitude to Renate Kort and Michael Pilzen for technical assistance. The financial support of the Deutsche Forschungsgemeinschaft (Rh 20:3–1 and Rh 20:3–2) is gratefully acknowledged. We hereby declare that the experiments comply with the current laws of Germany.

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