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Hagen, Christoph; Braune, Wolfram; Björn, Lars Olof

Published in:
Journal of Phycology

DOI:
10.1111/j.0022-3646.1994.00241.x

1994

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J. Phycol. **30,** 241–248 (1994)

FUNCTIONAL ASPECTS OF SECONDARY CAROTENOIDS IN *HAEMATOCOCCUS LACUSTRIS* (VOLVOCALES). III. ACTION AS A "SUNSHADE"[1]

Christoph Hagen, Wolfram Braune[2]

Institute of General Botany, Friedrich Schiller University Jena, Am Planetarium 1, D-07743 Jena, Germany

and

Lars Olof Björn

Section of Plant Physiology, Lund University, Box 7007, S-22007 Lund, Sweden

ABSTRACT

We investigated the protection from photoinhibition by different developmental stages of Haematococcus lacustris *[Girod] Rostafinski using chlorophyll fluorescence measurements of single cells and suspensions. An overall correlation between higher cellular content of secondary carotenoids and the capacity to withstand excessive irradiation was observed in flagellated cells and aplanospores of* H. lacustris. *Low-light-reversible spreading of extrachloroplastic secondary carotenoids occurred in the periphery of the cell during strong irradiation. This process resulted in increased shading of the cup-shaped chloroplast as demonstrated by a decrease in chlorophyll fluorescence. Extrachloroplastic accumulation of secondary carotenoids in* H. lacustris *can be interpreted as a specific adaptation to habitats that exhibit strong insolation.*

Key index words: carotenoids; chlorophyll fluorescence; Chlorophyta; Haematococcus lacustris; *image analysis; photoinhibition; secondary carotenoids*

In algae, secondary carotenoids (SCs) are carotenoids (Car) that accumulate outside the chromatophores, usually in large quantity, under stress conditions (Grung et al. 1989). The ability to synthesize SCs occurs in algae living under extreme conditions (Geisert et al. 1987, Albertano et al. 1991, Johnson and An 1991). For instance, *Chlamydomonas nivalis* is a species of the "cryovegetation" that causes "red snow" in high mountains and polar regions (Czygan 1970). The habitats of SC-synthesizing algae are especially characterized by the rapid variation of abiotic factors (Schneider 1989) and strong insolation (Czygan 1970, Hoffmann 1989). Similar ecological findings led to the suggestion that accumulation of Car might protect cells from injury induced by excessive irradiation (Mainx 1927, McLean 1967, Fogg 1991); however, some studies suggest that SCs have no useful function (Egger 1967).

Ben-Amotz et al. (1989) indicated that a higher capacity to withstand photoinhibitory treatment is related to shading by β-carotene accumulated in intrachloroplastic "oily globules" of *Dunaliella bardawil*. Although direct comparison between this process and extrachloroplastic accumulation of SCs in *Haematococcus lacustris* [Girod] Rostafinski was rejected by Boussiba and Vonshak (1991), the SCs in lipid vacuoles of this green alga (Yong and Lee 1991, Kobayashi et al. 1992) were considered to provide the same shading mechanism as that suggested by Ben-Amotz et al. (1989) in *Dunaliella*.

The studies of Ben-Amotz et al. (1989) and Yong and Lee (1991) are based on measurements of photosynthetic oxygen evolution after strong light exposure that caused photoinhibition. The physiology of this process is the focus of recent photosynthesis research (Barber and Andersson 1992, Horton and Ruban 1992, Osmond et al. 1993, Van Wijk and Van Hasselt 1993). *In vivo* chlorophyll (Chl) fluorescence induction kinetics in a dark–light transition have been proven to be of value in analyzing reversible inhibition of photosynthetic activity (Hor-

[1] Received 16 August 1993. Accepted 8 December 1993.
[2] Author for reprint requests.

ton and Bowyer 1990). The so-called saturation pulse method makes continuous analysis of photochemical and nonphotochemical Chl fluorescence quenching during this transition possible (Schreiber et al. 1986). Photochemical quenching is caused by charge separation at photosystem II reaction centers, whereas nonphotochemical quenching includes a number of nonradiative deexcitation processes in photosystem II (Horton and Bowyer 1990). These energy dissipation processes are associated with a reversible decrease in the photochemical efficiency of photosystem II and can apparently protect against stronger and more sustained decreases in the photon efficiency of photosynthesis (Horton and Ruban 1992).

To investigate the photoprotective function of SCs in *H. lacustris* in more detail, *in vivo* Chl fluorescence measurements were performed in cell suspensions and single cells of different developmental stages of the alga. Differences in the SC content of the cells were achieved by 1) precultivation under low or high irradiance (aplanospores), 2) germination of aplanospores containing low and high amounts of SCs, which led to green and red flagellates, respectively, and 3) limitation of the nitrogen source during germination (cf. Hagen et al. 1993b). Involvement of strong light-induced spreading of SC-containing lipid vacuoles into the cell periphery as protection against photoinhibitory irradiation is also discussed.

MATERIALS AND METHODS

Haematococcus lacustris [Girod] Rostafinski (no. 192.80 of the culture collection of the University of Göttingen, Germany) was grown autotrophically at 21° C (± 2° C) in Erlenmeyer flasks under continuous white light (daylight fluorescent lamps, 12.5 $W \cdot m^{-2}$) in a medium described by Hedlich (1982) with addition of 0.3 μM thiamine. The flasks were not shaken or aerated. The inoculum of this standard batch culture always came from a stock suspension of aplanospores that was at least 3 months old. Flagellates differing in SC content were grown as described before (Hagen et al. 1992) after germination in fresh medium under 7 $W \cdot m^{-2}$ of white light. They were obtained either from 1) red aplanospores exposed for 48 h under normal (Chl/Car: 0.55) and nitrate-deprived (Chl/Car: 0.17) conditions and 2) green aplanospores grown for 4 days (Chl/Car: 1.14, 1.32). Green aplanospores were precultivated for 2 weeks after addition of fresh medium in 12.5 $W \cdot m^{-2}$ and then for another 10 weeks in 0.5 $W \cdot m^{-2}$ of white light. To induce rapid SC accumulation, one part of this suspension was irradiated with 16.5 $W \cdot m^{-2}$ of white light for 2 weeks. Nitrogen deprivation of the medium was achieved by isoosmotic exchange of KNO_3 by KCl.

Pigmented cells were counted using a Fuchs-Rosenthal counting chamber. Total irradiance was measured (unless stated otherwise) by means of a miniaturized thermopile (Physical and Technical Institute, Jena, Germany). Pigment extraction was performed after sonication of the cells with acetone (100%). Pigment contents and *in vitro* Chl/Car ratios were calculated according to Lichtenthaler (1987).

The *in vivo* Chl/SC ratio (see below) is based on a specific absorption shoulder of SC at 540 nm. This shoulder could only be found in spectra obtained from suspensions and single cells (Hagen et al. 1993a). The height of this shoulder was not directly used as a dimension for the SC content because of systematic errors initiated by cell growth (Kirk 1975). When the *in vivo* Chl/SC ratio was calculated, the reference was used as the numerator to 1) indicate changes in the early period of SC accumulation more sensitively and 2) minimize the influence of the reference wavelength (linear instead of hyperbolic impact).

Cytophotometry. The photometric device of a light microscope (Fluoval, Zeiss, Jena, Germany) was used for absorption measurements of single cells *in vivo* (Krug 1980). *In vivo* Chl/SC ratios were calculated as follows: $Chl/SC = [A_{red\ max} - A_{725}]/[A_{540} - A_{725}]$ (A_{725}, A_{540}, $A_{red\ max}$: absorption of one cell measured at 725 nm, 540 nm, and the red absorption maximum, respectively).

Computer-aided microscopic image analysis. The suspension of aplanospores used in the experiments was grown as described for red aplanospores with the exception that exposure to stronger light did not exceed 7 days. Before measurement, the suspensions were adapted to 4.5 $W \cdot m^{-2}$ of white light for 24 h. This cultivation resulted in aplanospores that were not filled up completely by SC-containing lipid vacuoles. Variations of the intensity of irradiation using the microscopic lamp were achieved by neutral density filters. The video signal of a black and white CCD camera (VS 450, Stemmer, Munich, Germany; including an interference filter 540 nm from Zeiss) mounted on top of a light microscope (Amplival, Zeiss, Jena, Germany) was processed by a commercially available frame grabber board (PIP-1024B, Matrox, Quebec, Canada) and analyzed using image-processing software (DIAS 3.1, Tower-Soft, Jena, Germany). The program included standard modules of image processing: preprocessing; optical isolation of the cell from the background by threshold operations; calculation of size, shape, and gray value parameters of this cell; and measurement of the averaged gray value cross-section along the cellular diameter (5-pixel band; 1 pixel = 0.2 μm). The resulting gray values were inverted, and the logarithm was calculated for each point. Data are presented after smoothing (gliding mean, ±10 pixels).

Absorption spectroscopy of suspensions. The algal samples were suspended in agar (0.15–0.2%, w/v) and measured in 0.5-cm cuvettes covered on the front side with a strong scattering film of silica. Measurement was performed in the second sample position of a spectrophotometer (Specord UV/VIS, Zeiss, Jena, Germany) according to the "opal glass method" described by Shibata et al. (1954). *In vivo* Chl/SC ratios were calculated as follows: $Chl/SC = [A_{red\ max} - A_{750}]/[A_{540} - A_{750}]$ (A_{750}, A_{540}, $A_{red\ max}$: absorption of the sample measured at 750 nm, 540 nm, and the red absorption maximum, respectively).

Microfluorometry of single cells. The same equipment as for cytophotometry was used (epifluorescence). The photomultiplier was shielded by an orange glass filter (OG4, Zeiss, Jena, Germany). After 5 min predarkening, the cell was exposed to 40 or 80 $W \cdot m^{-2}$ of blue broad-band light (photographic shutter, voltage-stabilized halogen lamp, filter combination: KP490, B226, B228, B229, B424, G264, D484; Zeiss, Jena, Germany). Fluorescence induction kinetics were monitored over 3 min by means of a chart recorder (slow kinetic) and a storage oscilloscope (C8-13, GUS) for detection of basic fluorescence (F_o) during the fast kinetic. The parameter describing the rate of the fluorescence decrease to an almost steady-state level reached after 3 min of excitation (Rfd) was calculated according to Lichtenthaler et al. (1986).

Pulse amplitude-modulated fluorescence. The modulated *in vivo* Chl fluorescence (cf. Schreiber et al. 1986) of algal samples was recorded (unless stated otherwise) after 10 min predarkening in a suspension cuvette at room temperature using the pulse-modulated Chl fluorometer system PAM 101-103 (Walz, Effeltrich, Germany). Chlorophyll content of the algal samples never exceeded 20 $\mu g \cdot mL^{-1}$. Dark-adapted basic fluorescence (F_o) was determined by a weak, modulated light of 0.1 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (1.6 kHz, automatic switching to 100 kHz during actinic and saturation light). Dark-adapted maximal fluorescence (F_m) was determined by a saturating 500-ms flash of 1000 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (FL103; DT Cyan short-pass filter, Balzers, Liechtenstein; white light). The accuracy of F_m was checked by the shape of the fluorescence rise as described in the manual of the

pulse fluorometer software DA100 (version 1.9, Walz). After a lag phase of 60 s, a fluorescence transient of 3-min duration was induced by continuous actinic light of 50 μmol photons·m^{-2}·s^{-1} (102-L, 650 nm). To analyze quenching mechanisms, saturation pulses were triggered every 10 s. Light-adapted basic fluorescence (F_o') was measured at the lowest level after switching off actinic light. The nonphotochemical quenching coefficient (q_N) was calculated as described in Hagen et al. (1992). Normalized variable fluorescence (i.e. photosystem II photochemical efficiency; Havaux 1993) was calculated as $F_v/F_m = (F_m - F_o)/F_m$. For nomenclature, see Van Kooten and Snel (1990).

Red and blue light flash experiments were performed during photoinhibitory treatment as follows. Twenty-four hours before the experiment, suspensions of green and red aplanospores were centrifuged and adapted to 4.5 W·m^{-2} of white light after resuspension in nitrate-deprived medium to avoid germination (0.2% agar, w/v; cell density before measurement: 8×10^5 cells·mL^{-1}; Chl content did not exceed 20 μg·mL^{-1}). Aliquots of both aplanospore suspensions were exposed to strong white light (470 W·m^{-2}, halogen lamp combined with heat-protecting filter W302 from Zeiss, Jena, Germany) in 0.5-cm cuvettes at room temperature. After 5 min predarkening of these samples, F_o was determined by weak modulated light of 0.04 μmol photons·m^{-2}·s^{-1} (1.6 kHz, automatic switching to 100 kHz during actinic flashes). Then, a 400-ms blue flash was applied (FL103/E; DT Cyan shortpass filter, Balzers, Liechtenstein; KP560, Zeiss, Jena, Germany; 122 W·m^{-2}). After 60 s, the sample was excited by a 400-ms red flash (102-L, 650 nm, 20 W·m^{-2}). For each flash, the normalized variable fluorescence (F_v/F_m) was calculated as already mentioned using the same level of F_o. To avoid systematic errors, the order of red and blue flashes was changed during repetitive measurements.

Fluorescence excitation spectroscopy. Steady-state fluorescence excitation F_{470nm}/F_{600nm} ratios (emission wavelength: 680 nm) were measured in a 1-cm quartz cuvette at room temperature with a red sensitive photomultiplier (M12 FC51, Fernsehelektronik, Berlin, Germany) mounted on a spectrofluorometer (Aminco-Bowman, Silverspring, Maryland). The slit apertures of both monochromators were adjusted to give a 5-nm bandpass. The algal sample was diluted to a cell density of 2×10^5 cells·mL^{-1} and measured after a 5-min exposure to the excitation light. Three measurements of the sample were averaged. The chlorophyll content did not exceed 5 μg·mL^{-1}.

Photoinhibitory treatment of flagellate suspensions. Suspensions of flagellates (cell density: 3×10^5 cells·mL^{-1}; Chl content did not exceed 15 μg mL^{-1}; light path through the suspension: 0.5 cm) were exposed in Petri dishes at 20° C to red and blue broad-band photoinhibitory light (light source: xenon high pressure lamp; red light: KG 3, RG 610, Calflex C from Schott, Mainz, Germany, D482 from Zeiss, Jena, Germany, 400–700 nm at 1225 μmol photons·m^{-2}·s^{-1}, 600–700 nm at 1151 μmol photons·m^{-2}·s^{-1}; blue light: KG 3, BG 12/2 mm, Calflex C from Schott, Mainz, Germany, 400–700 nm at 1210 μmol photons·m^{-2}·s^{-1}, 400–500 nm at 1125 μmol photons·m^{-2}·s^{-1}). Photon fluxes were measured using a spectroradiometer (Optronics Model 742, Orlando, Florida). Changes in cell density and cellular Chl or Car content were not detected during photoinhibitory irradiation (maximal for 10 h).

RESULTS

In vivo Chl fluorescence measurements provide direct information about absorption efficiency of the photosynthetic apparatus (Buschmann 1986). If SCs shade Chl in the blue wavelength region by their absorption, a specific lowering of Chl fluorescence excited by blue light versus red light should be obtained during the synthesis of SCs. Indeed, this dependence of the corresponding steady-state Chl fluorescence excitation ratio on the degree of SC accumulation was observed (Fig. 1).

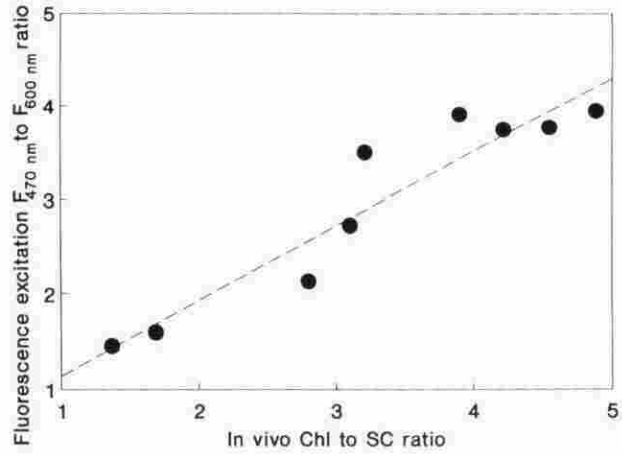

FIG. 1. Steady-state fluorescence excitation F_{470nm}/F_{600nm} ratio measured at room temperature versus *in vivo* Chl/SC ratio detected by absorption spectroscopy in suspensions of SC-accumulating *Haematococcus lacustris*.

To obtain cells containing different amounts of SCs for this experiment, aplanospores were precultivated for 2 weeks after addition of fresh medium in 12.5 W·m^{-2} and then for another 10 weeks in 4.5 W·m^{-2} of white light. After that, germination of these low-light-adapted green aplanospores was induced after addition of fresh medium (0.2% agar, w/v) under different irradiances (4.5, 9.5, 14, and 19 W·m^{-2}, white light). Apart from the starting sample, samples were taken from each light treatment after 43 and 140 h, times that represent different developmental states of the culture. The *in vivo* Chl/SC ratio of these samples was positively correlated with the fluorescence excitation F_{470nm}/F_{600nm} ratio (Fig. 1).

To determine whether or not shading by SCs increases the capacity to withstand high-light treatment, photoinhibitory processes were studied in flagellates and aplanospores differing in SC content. An increase of F_o after 1.5-h photoinhibitory irradiation with blue light occurred only in green flagellate suspensions (Fig. 2). This was accompanied by a decrease of F_v/F_m (Fig. 2) indicating photoinhibition (Öquist and Huner 1993). Besides adaptation processes, especially in green samples, the stronger inhibition by blue light compared to red light in these suspensions was conspicuous (Fig. 3).

Analysis of the nonphotochemical quenching coefficient q_N during fluorescence-induction kinetics revealed further information about the mechanism of higher resistance to photoinhibition in SC-containing flagellates. Before photoinhibitory treatment, green flagellates used in our experiments showed generally a lower q_N than red flagellates. An increase of nonphotochemical quenching during 10 h exposure to red photoinhibitory irradiation was observed in red flagellates, but not during blue light

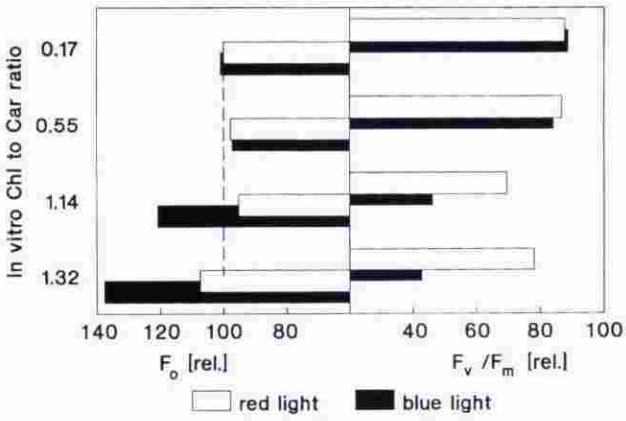

FIG. 2. Relative changes of dark-adapted basic fluorescence (F_o) and normalized variable fluorescence (F_v/F_m) in relation to the *in vitro* Chl/Car ratio in suspensions of flagellates treated for 1.5 h by red or blue photoinhibitory irradiation. The corresponding value of the sample before photoinhibitory treatment equals 100%.

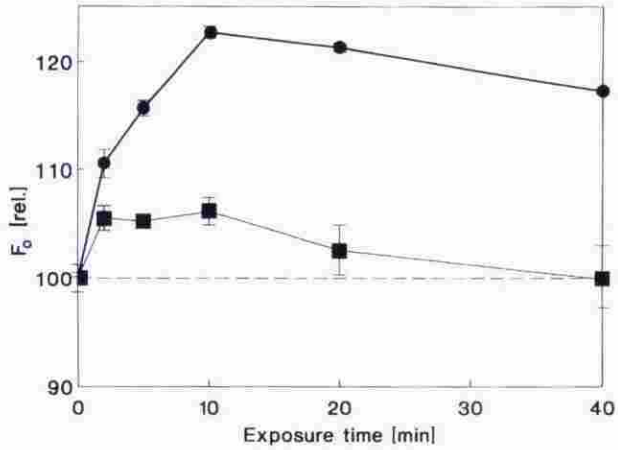

FIG. 4. Relative changes of dark-adapted basic fluorescence (F_o) during irradiation with white light (470 W·m^{-2}) in green (●) and red (■) aplanospore suspensions. Value of the corresponding samples at the beginning of the experiment equals 100%. Bars indicate ±SE.

irradiation of red flagellates or in green flagellates at all.

We were also interested in determining whether or not higher resistance to photoinhibition detected by oxygen evolution measurements in red aplanospore suspensions (Yong and Lee 1991) can be correlated to Chl fluorescence measurements. Immediately after photoinhibitory irradiation, green and red aplanospore suspensions were predarkened and measured as described for red and blue light flash experiments. The increase in F_o (Fig. 4) indicates stronger inhibition in green aplanospores (Havaux et al. 1991; see Fig. 2). The corresponding F_v/F_m values decreased faster in green aplanospore samples during photoinhibitory irradiation.

In discussing mechanisms with higher capacity to withstand excessive irradiation in SC-containing cells, Yong and Lee (1991) reported evidence supporting the view that a strong light-induced spreading of SCs can prevent photoinhibition by apparently acting as a light filter. To describe that spreading quantitatively, computer-aided microscopic image analysis was applied to single cells during high-light treatment. Changes of the gray value cross-section along the diameter of a single aplanospore were measured during irradiation with 290 W·m^{-2} of white light (40 min) and recovery under 13 W·m^{-2} (100 min). Absorption in the cell periphery continued to increase during the first 20 min of low-light recovery (Fig. 5). In this cell, recovery was achieved almost completely after 100 min. These changes can also be expressed using cellular gray value features calculated by the software system (Fig. 6). The reversible increase of the mean cellular gray value during spread of the SC-containing lipid vacuoles (Fig. 6) might be explained by decrease of the "pack-

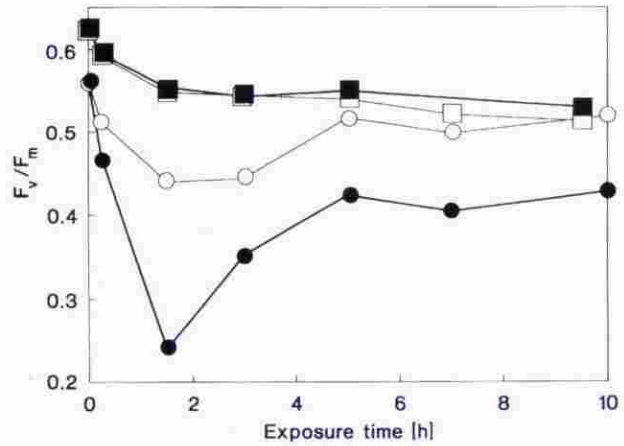

FIG. 3. Changes of normalized variable fluorescence (F_v/F_m) in red (□■) and green (○●) suspensions of flagellates during irradiation with red (□○) and blue (■●) photoinhibitory light (see Fig. 2).

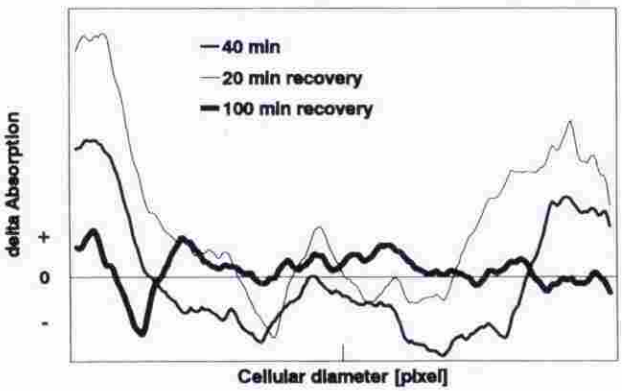

FIG. 5. Changes of absorption measured along the cellular diameter in a single SC-containing aplanospore during strong light treatment (290 W·m^{-2}, 40 min), followed by recovery (13 W·m^{-2}). Differences were calculated by subtraction of corresponding absorbance data measured at the beginning of the experiment.

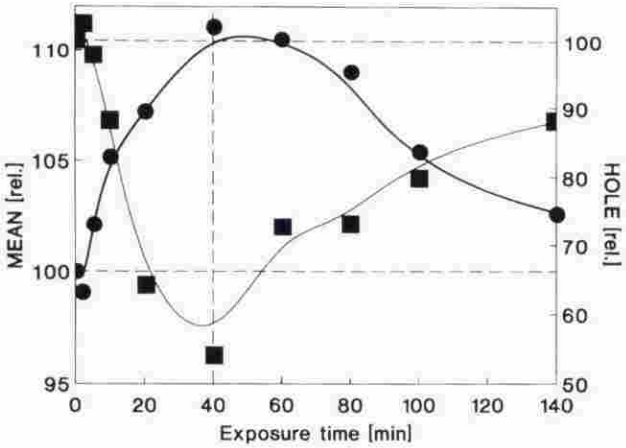

FIG. 6. Changes in cellular gray value features of a single SC-containing aplanospore during the experiment described in Figure 5 (MEAN, [●]: averaged gray value of the cell; HOLE, [■]: number of pixels within the cell shape that exhibit a gray value below MEAN). Corresponding value measured at the beginning of the experiment equals 100%. Vertical broken line marks beginning of recovery at 40 min.

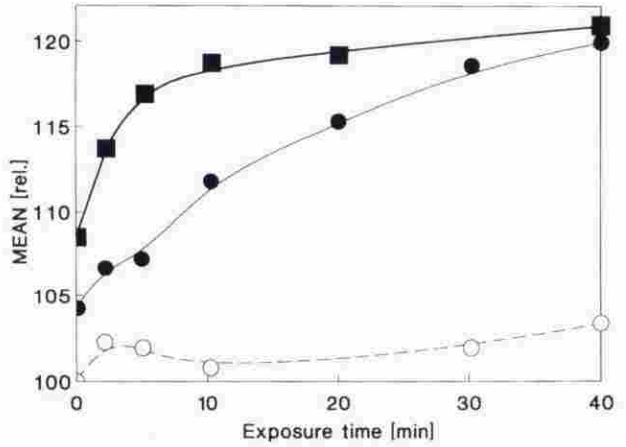

FIG. 7. Changes of the averaged cellular gray value (MEAN) in a single SC-containing aplanospore during exposure to 13 W·m^{-2} (○), then to 63 W·m^{-2} (●), and, after 40 min of recovery (13 W·m^{-2}), to 550 W·m^{-2} (■). Value measured at the beginning of the experiment equals 100%.

age effect" and the corresponding increase in specific absorption of the pigment molecules (Osborne 1986). The decline of the parameter HOLE (Fig. 6) describes the increasing optical homogeneity of the cell during SC spreading.

SC spreading increases with increasing irradiance (Fig. 7). At first, one single aplanospore was measured during irradiation with the recovery light only. Then, the same cell was exposed to 63 W·m^{-2} of white light. After 40 min of recovery, the cell was irradiated with 550 W·m^{-2} of white light. Furthermore, recovery seems to be light-dependent, because it was accelerated by low light compared with dark recovery (data not shown).

To undertake further attempts to study the mechanism of this putative pigment movement, 1) only the left half of an aplanospore (Fig. 8a) and, after recovery, 2) the complete cell (Fig. 8b) were irradiated with 550 W·m^{-2} of white light. The orange "halo" effect occurring near the cell membrane, and normally accompanying SC spreading, could only be observed in the irradiated region. The phenomenon was limited to the irradiated area (Fig. 8a), especially after 10 min of recovery (13 W·m^{-2}). The increase of absorption in the cell periphery corresponds to an absorption decrease in the central region of the cell during strong light exposure (Fig. 8b). There, the SC-containing lipid vacuoles surround the nucleus before strong light irradiation (Santos and Mesquita 1984; Fig. 8b, gray value cross-section).

Consequences of the SC spreading, in particular for the absorption efficiency of the photosynthetic pigments, can be demonstrated by an extraordinarily strong Chl fluorescence quenching as demonstrated in a single aplanospore measured by microfluorometry (blue actinic light, 40 W·m^{-2}; Fig. 9a). The cell was characterized by SC-containing lipid vacuoles arranged around the nucleus. Before the experiment, a sample was taken from a brownish 8-week-old standard batch culture (12.5 W·m^{-2}, white light) and adapted to low light (4.5 W·m^{-2}) for 48 h in a microscope slide chamber. Between each measurement, the microscope slide was irradiated by ca. 400 W·m^{-2} of white light for 5 min before predarkening. Between the fifth and sixth measurements, the cell was adapted again to 4.5 W·m^{-2} for 4 h. The fluorescence decrease (Fig. 9a) was not accompanied by a corresponding decrease of photosynthetic efficiency, as indicated by the unchanged Rfd value (Lichtenthaler et al. 1986).

To study this phenomenon in greater detail, different cells of a similar low-light-adapted suspension (4.5 W·m^{-2} of white light) were measured by microfluorometry (blue actinic light, 80 W·m^{-2}) and cytophotometry (Fig. 9b). A new microscopic slide was prepared for each cell. Irradiation between measurements was omitted. The extent of fluorescence quenching seemed to be positively correlated with the degree of SC accumulation of the corresponding aplanospore. The results obtained by microfluorometry cannot be compared directly with the fluorescence measurements already described. Besides the pulse modulation principle, we used red light for F_o measurements in cell suspensions.

An increase in the shading activity due to SC spreading, particularly in the blue wavelength region, was observed during photoinhibitory treatment of aplanospores (Fig. 4). If normalized variable fluorescence detected in these samples with either a single blue or red light flash is compared, the corresponding ratio decreased to a higher extent in red aplanospores during strong light exposure (Fig. 10). That indicates a decreasing Chl fluorescence excitation efficiency, especially of the blue actinic light

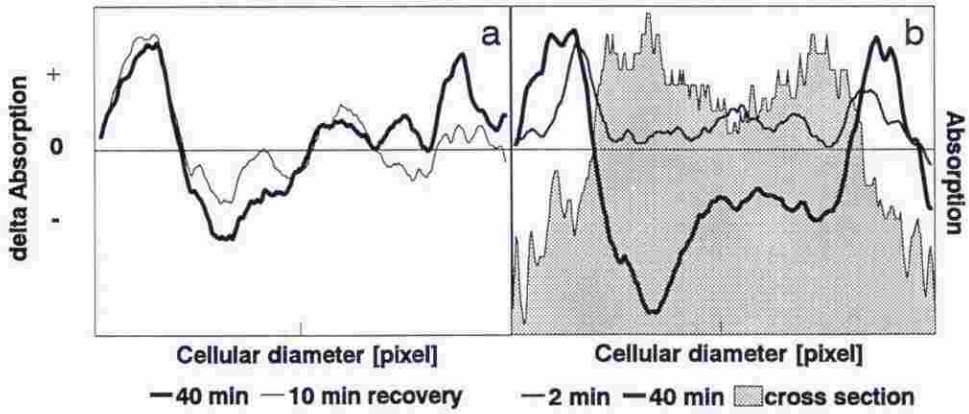

FIG. 8a, b. Changes of absorption measured along the cellular diameter in a single SC-containing aplanospore a) during 40 min of strong light irradiation (550 $W \cdot m^{-2}$) of the left half of the cell and b) after 30 min of recovery (13 $W \cdot m^{-2}$) during 40 min of strong light irradiation (550 $W \cdot m^{-2}$) of the complete cell. Differences were calculated by subtraction of corresponding absorbance data measured at the beginning of the experiment (depicted as gray value cross-section in b).

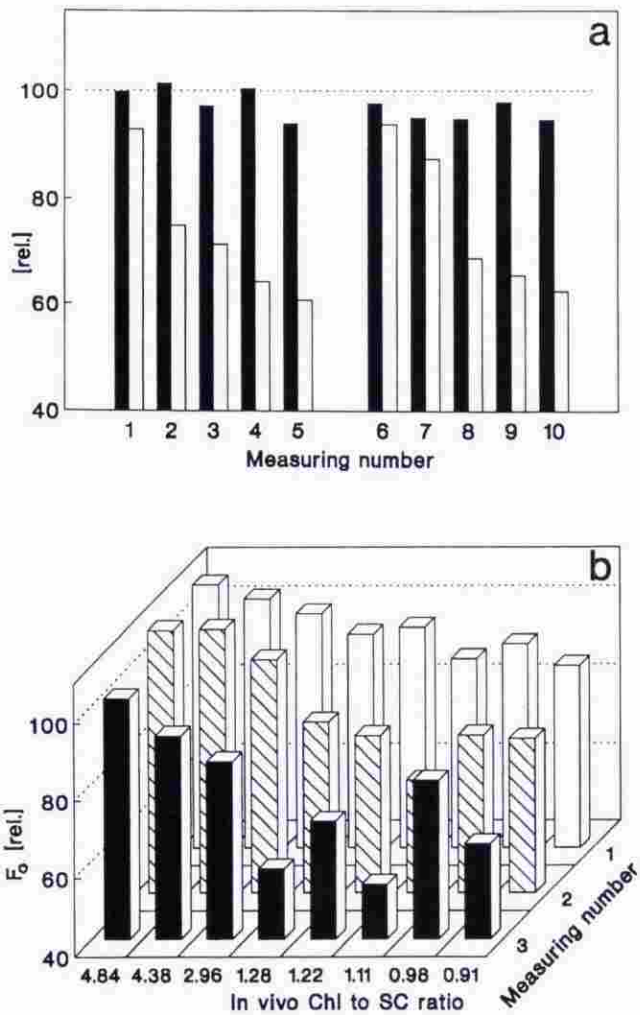

FIG. 9a, b. Decrease of Chl fluorescence during repetitive microfluorometric measurements in single aplanospores. a) Comparison of basic fluorescence (F_o, open bars) and rate of fluorescence decrease (Rfd, filled bars). b) Dependence of the decrease of F_o on the degree of SC accumulation determined by cytophotometry. The corresponding value of the cell at the beginning of the experiment equals 100%.

during photoinhibitory treatment in SC-containing cells.

DISCUSSION

Our results suggest that accumulation of SC in *H. lacustris* should be discussed as an adaptation to the specific ecological situation in its habitat. This freshwater alga ("Blutregenalge": Czygan 1970) can be found especially in shallow ponds, rook pools, puddles, and other small and periodic waters (Pocock 1959, Schneider 1989). Apart from formation of resting cells, protection from "bleaching" by stronger light is of special importance in these habitats (Fryxell 1983) that lack shaded regions.

An overall decrease of photosynthetic light absorption by Chl (and probably also by primary Car in the photosynthetic apparatus) in the blue wavelength region was detected in SC-accumulating sus-

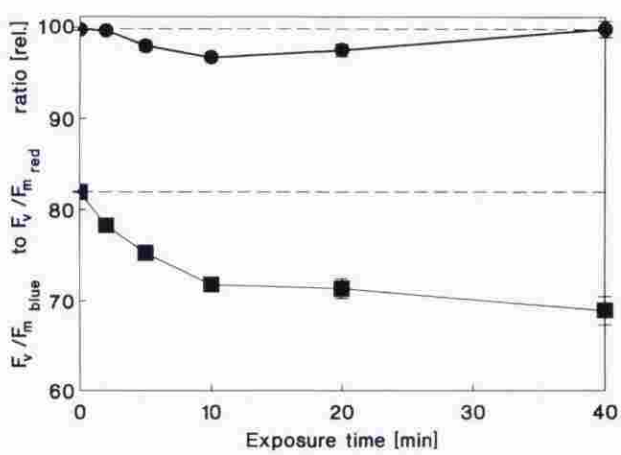

FIG. 10. Changes of the ratio of normalized variable fluorescence (F_v/F_m) detected with blue or red actinic light, during irradiation with white light (470 $W \cdot m^{-2}$) in green (●) and red (■) aplanospore suspensions. Broken lines mark the corresponding values measured in samples at the beginning of the experiment. Bars indicate ±SE.

pensions of *H. lacustris*. This agrees with results obtained by low-temperature fluorescence spectroscopy of SC-accumulating aplanospores (Hagen et al. 1993a). The low cell density of samples used in the experiments indicates that this shading due to SC absorption might act not only by mutual competitive absorption in a population of cells but also within the single SC-containing cell.

Protection against photoinhibition in SC-containing flagellates and aplanospores is suggested as a physiological role of this absorptive behavior. A higher sensitivity of green aplanospores to photoinhibitory treatment was demonstrated by Chl fluorescence measurements and confirms the results reported by Yong and Lee (1991). We found that blue light of the same photon flux as red light was more effective in inducing photoinhibition in green flagellates. Thus, light-dependent SC spreading seems to be of special value for the survival of *H. lacustris* in its habitat. The cells can adapt to extreme increases in irradiation by lowering blue light absorption of the photosynthetic apparatus. Together with low-light reversibility, SC migration in the cell periphery might help to maintain photosynthetic activity during periods of moderate insolation.

The increase of excitation energy-dissipating processes, indicated by an increase of nonphotochemical fluorescence quenching in red flagellate suspensions during photoinhibitory treatment, indicates that other protecting mechanisms in addition to SC shading are involved. Thus, an increased activity of the Mehler reaction reported in green algae (Neubauer and Yamamoto 1992, Rees et al. 1992) might lead to an increased capacity of nonradiative excitation energy dissipation in *H. lacustris*. Ketocarotenoids included in proteins of the photosynthetic apparatus as reported in *Eremosphaera viridis* (Vechtel et al. 1992) could protect against overexcitation and photooxidative damage. However, 1) the dependence of the Chl fluorescence decrease during strong light exposure on the degree of SC accumulation and 2) the decreasing Chl fluorescence excitation efficiency of blue actinic light during photoinhibitory treatment in SC-containing cells can hardly be explained with processes other than an active spreading of the SC in the cell periphery, finally covering the complete cell and shading the cup-shaped chloroplast. Synthesis of SC did not occur during this intracellular pigment distribution (Yong and Lee 1991).

The hypothesis of a shading function of SC is further supported by the fact that the increase of q_N in red flagellates was observed during irradiation with red light only. Blue light of the same photon flux did not cause this increase, although it was more effective in inducing photoinhibition in green samples. However, more experimental work should be conducted to study the involvement of protecting mechanisms other than SC shading. Thus, a more specific Car determination (Hagen et al. 1993b) could help to determine whether or not changes in the xanthophyll cycle components are correlated with a higher capacity to withstand photoinhibitory treatment (cf. Sharma and Hall 1993).

Active use of the supposed shading function of SC range was reported in several organisms. Mainx (1927) observed microscopically strong light-induced transport of "haematochrome droplets" from the center into the periphery of the cells of *Euglena sanguinea* and *vice versa* in low light. Similar changes were reported in *Stentor igneus* (Mainx 1927). In *H. lacustris*, light direction-dependent (Reichenow 1910) and light intensity-dependent (Elliot 1934) distribution of SC within the cells was observed.

We can only speculate about the mechanism of SC spreading. Mainx (1927) reported fast transport of SC-containing lipid vacuoles along "preformed channels" in *Euglena sanguinea*. The rate of spreading in *H. lacustris* seems to be light intensity-dependent. Chlorophyll fluorescence decrease due to spreading of SC, induced by blue excitation only, might indicate special efficiency of the blue wavelength region. The process seems to be restricted to the irradiated area. Some data also indicate partial involvement of chloroplast movements. The continuing absorption increase in the outer part of the cell during the first period of recovery might be explained by the increasing concentration of SC in the cellular periphery due to fast "relaxation" of the chloroplast, which contracts rapidly in strong light (see Fig. 8b, 2 min).

Further studies are needed to get more and unambiguous results on the mechanism of SC spreading. Besides cellular experiments using confocal laser microscopy, conditions should be elaborated that allow studies of the phenomenon in suspensions of cells, for instance, using advanced techniques of absorption difference spectroscopy (dual wavelength mode, Ulbricht sphere). This is of special interest for investigation of the involvement of photoreceptor and cytoskeletal activity in the mechanism of SC spreading.

The kind help of J. F. Bornman is gratefully acknowledged. C.H. has been supported as a scholar of the "Studienstiftung des deutschen Volkes." The work is part of a project supported by the "Deutsche Forschungsgemeinschaft."

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