Volume 336, number 3, 516–520 FEBS 13473 December 1993

Photosynthesis modulates the sign of phototaxis of wild-type *Chlamydomonas reinhardtii*

Effects of red background illumination and 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea

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Received 5 November 1993; revised version received 15 November 1993

We have found that the sign of phototaxis, i.e. the direction of phototactic migration either towards or away from the light source, of wild-type *Chlamydomonas reinhardtii* depends on its photosynthetic activity. This accounts for the frequently reported transient positive phototaxis that precedes a negative phototaxis of wild-type cells, as well as the earlier observations that preillumination affects the sign of phototaxis. The bases for our conclusion are as follows. (1) The transient nature of phototaxis was preferentially observable with blue-green actinic light rather than with green actinic light. (2) Red background light induces negative phototaxis under the actinic-light conditions in which, without background light, *Chlamydomonas* cells show exclusively positive phototaxis. (3) Both the effect of red background light and the transient change in the sign of phototaxis were inhibited by 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea, a specific inhibitor of photosynthesis. The conclusion modifies the accepted view that photosynthesis does not link with the phototaxis of this microorganism, thus constituting a necessary part in elucidating mechanisms of algal phototaxis.

Phototaxis; *Chlamydomonas reinhardtii*; Photosynthesis; Rhodopsin; Phytochrome

1. INTRODUCTION

The unicellular flagellate green alga *Chlamydomonas* exhibits oriented movement responses with reference to a light beam; the oriented swimming responses toward and away from the light source are called positive and negative phototaxis, respectively. Recent studies have revealed and confirmed that photobehavioral responses of *C. reinhardtii* are mediated by rhodopsin-like protein species [1–6]. However, detailed mechanisms of phototaxis, including those controlling the sign of phototaxis, remain unclear [7–9].

The sign of phototaxis of wild-type *Chlamydomonas* cells depends on both growth stages of cell cultures and actinic light intensities [8,10] (for a recent review, see pp. 208–216 of [11]). In wild-type cells, positive phototaxis is usually observed at low or moderate light intensities and at higher light intensities they show negative phototaxis. The sign of phototaxis also depends on other factors such as concentrations of cations in the medium [8,12,13], and is modulated by prestimulus irradiance or light conditions just before phototactic experiments [10,14,15]. In addition, *Chlamydomonas* cells often show a transient positive phototaxis followed by a strong negative phototaxis [6,8,10,15–17].

Although possible involvement of photosynthesis, either directly or indirectly, in mechanisms of photomovements in various flagellate algae had been discussed in the old literature [14,18], Stavis and Hirschberg indicated that photosynthesis is not required for phototaxis of *Chlamydomonas* [12]. Since then, phototaxis of this alga has long been assumed to be independent of photosynthesis [19]. This view appeared to be further substantiated by recent successful studies on *Chlamydomonas* rhodopsin [1–6], thus it became natural for us to surmise that the transient positive phototaxis and the modulatory effects of preillumination on the sign of phototaxis were consequences of the adaptation phenomena of photosensory transduction processes, widely seen in archaebacterial and animal rhodopsin systems.

We report here, however, evidence that photosynthesis indeed affects the sign of phototaxis of *C. reinhardtii*.

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Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; *C. reinhardtii*, *Chlamydomonas reinhardtii*; Pr, red-absorbing form of phytochrome; Pfr, far-red absorbing form of phytochrome; ATP, adenosine triphosphate.

2. MATERIALS AND METHODS

Wild-type *Chlamydomonas reinhardtii* 137c mt^+ was a gift from So-ichi Nakamura of Ryukyu University, Naha, Okinawa, Japan. The cells from agar plates were transferred to 1.5 ml of liquid high-salt medium [20] supplemented with trace metals [21] and kept at 23°C. After 1–2 days, the suspension was added to 100 ml of the medium in a 300 ml Erlenmyer flask and grown on a 12:12 h light/dark cycle for 3–5 days under a white fluorescent lamp (2000 lux). Before experiments, phototactically accumulated cells were pipetted out, diluted with 30 to 50 ml of fresh medium to a cell density of $1–2 \times 10^5$ cells/ml, and mildly agitated in a 100 ml flask on a rotary shaker (170 rpm) under a fluorescent lamp (300 lux) for at least 2 h. To minimize the effect of circadian rhythms, phototactic experiments were carried out for less than 4 h a day.

Phototaxis of the cells was assayed in a $10 \times 10 \times 0.5$ mm (in inside dimensions) quartz chamber on a microscope (Y2B or TMD, Nikon) connected with an infrared-sensitive video camera (XC-77, Sony Corp., Tokyo). Ten microliters of the cell suspension were put on the center of the chamber and covered with a coverslip so that the suspension forms a disk owing to its surface tension. The whole area of the cell suspension was illuminated from underneath with monitoring (background) light from a 12 V, 100 W tungsten halogen lamp passed through condenser lenses (Ph4 of Nikon phase contrast II optics) and appropriate long-pass filters (Y-52, R-60 from Toshiba, IR-76 from Hoya, and/or a heat-absorbing filter from Master Co. Ltd.). Actinic light from a 100 W tungsten-halogen lamp was passed through a heat absorbing (Master Co. Ltd., Tokyo), a long pass (Y-44 or L-39, Toshiba) and a narrow band interference (KL-51 or KL-47, Toshiba) filters, and delivered horizontally through an electronic shutter (C-79, Chuo Precision Industrial Co., Tokyo). Light intensities (fluence rates) were measured with a calibrated thermopile (MIR-100Q, Mitsubishi Oil Chemicals Co.).

A computerized automated method [3] was used for time-resolved quantitation of the phototaxis of individual cells. The software was modified so that the phototactic orientation can be determined as a function of time during a single period of actinic irradiation. Before each measurement, cells in the chamber were adapted for 12.5 min to dim red light ($\lambda > 600$ nm, 10–20 lux) and subsequently for 2.5 min to the background light specified in the text. Each specimen was subjected only once to the actinic light, and data were accumulated over 9–10 measurements for the calculation of the phototactic indexes described previously [9]. Statistical errors involved in the phototactic indexes fell roughly within ± 0.05, as the indexes were calculated according to swimming vectors of 500–1200 individual cell tracks.

Chemicals were obtained from Nacalai Tesque Co. (Kyoto). DCMU was used as an ethanolic solution.

3. RESULTS

3.1. *Effect of background illumination on the sign of phototaxis*

Throughout the experiment, we used wild-type 137c mt^+ cells at logarithmic growth phase; these cells, when examined with natural light coming in through the room window, generally showed positive and negative phototaxis in response to weak (< 500 lux, i.e. < ca. 2×10^{12} photons/mm^2·s) and intense (> 1000 lux) light, respectively. However, when the cells in the phototactic chamber were kept 10–50 min in the dark and observed with infrared monitoring (background) light ($\lambda > 760$ nm), we noticed that they tended to show exclusively positive phototaxis to green (510 ± 13 nm) actinic light over a wide range of actinic intensity (10^{11}–5×10^{12} photons/mm^2·s). Moreover, when the background light was abruptly changed from infrared to red ($\lambda > 620$ nm), these accumulated cells started swimming away from the light source within a few seconds. Thereafter, accumulation at the opposite side of the chamber was observable for minutes, indicating that the change in the sign of phototaxis was not transient. In addition, these cells started swimming back again towards the actinic light source, shortly after the background light was switched again from red to infrared ($\lambda > 760$ nm). This reversible change in the sign of phototaxis was repeatable at least for 4 cycles. But at later times both the migration of cells as a whole population and the resultant biased distribution became obscure.

Stable positive phototaxis was observable for more than half an hour under infrared background illumination, when the green actinic light was applied at a relatively low intensity (e.g. 10^{12} photons/mm^2·s). In contrast, the positive phototaxis was transient and followed by a strong negative phototaxis, when blue-green (470 ± 12 nm) actinic light at increased intensities was applied as described below.

3.2. *Time-dependent positive and negative phototaxis at various fluence rates*

In Fig. 1, positive and negative phototaxis indexes, which represent the fraction of the cells swimming towards (closed symbols) and away from (open symbols) the light source, respectively, are plotted against the time after onset of actinic irradiation. Under red background illumination, clear negative phototaxis was observed throughout the period of blue-green actinic irradiation at the intensity of 1.5×10^{13} photons/mm^2·s (Fig. 1A). In contrast, the cells exhibited transient positive phototaxis, when infrared background light was illuminated instead of red background light (Fig. 1B). With increasing intensity of the blue-green actinic light, we observed stronger negative phototaxis that followed the transient positive phototaxis (Fig. 1C,D). This suggested that both the red background illumination and the increase in the intensity of the blue-green actinic light had analogous effects on the sign of phototaxis.

3.3. *Effect of photosynthetic inhibitor DCMU*

It is well established that in *Chlamydomonas*, most effective wavelength regions for photosynthesis are blue (390–470 nm) and red (620–690 nm), and thus green light is less effective (e.g. cf. [22]). Our observations described above are therefore consistent with the supposition that the transience of positive phototaxis stemming from the appearance of the subsequent negative phototaxis is a consequence of increased rate of photosynthesis, since the transience is more easily observable with the blue-green actinic light than with the green actinic light. Thus we examined the effects of the photosynthetic inhibitor DCMU on the transient positive phototaxis under infrared background illumination (Fig. 1E,F). The photosynthetic inhibitor significantly

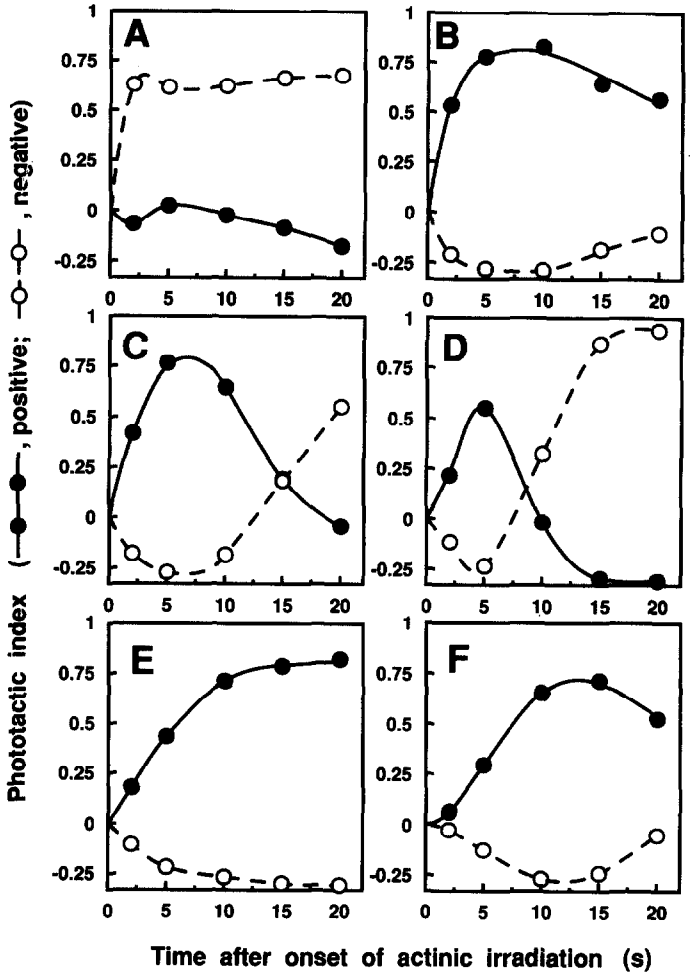

Fig. 1. Time-dependent *Chlamydomonas* phototaxis. Positive (I_+) and negative (I_-) phototaxis indexes were defined as $I_\pm = (F_\pm - 0.25)/0.75$, where F_+ and F_- represent fractions of cells swimming toward and away from the actinic light source, respectively [9]. Cells were observed with red ($\lambda > 620$ nm, 130 W/m^2, for panel A) or infrared ($\lambda > 760$ nm, 30 W/m^2, for panels B–F) background light. Intensities of actinic light (470 ± 12 nm) were 1.5×10^{13} photons/mm^2·s (panels A and B), 4.5×10^{13} photons/mm^2·s (panels C and E), and 1.5×10^{14} photons/mm^2·s (panels D and F). (Abscissa) Time after onset of actinic irradiation. (Panels E and F) 15–40 min after addition of 10^{-4} M DCMU.

inhibited the negative phototaxis that had to appear during the later period of the actinic irradiation. The fact indicated that the appearance of the transient phototaxis and the subsequent negative phototaxis is linked with the photosynthetic activity of the *Chlamydomonas* cells.

3.4. *A test for the possible involvement of phytochrome*

To test whether the effect of the red background light is linked with photosynthesis, or mediated by the plant red/far-red photoreceptor phytochrome [23] presumably occurring in this unicellular green alga [24], different combinations of optical filters were examined for background illumination. Transmittance spectrum of one (curve a of Fig. 2F) matched the absorption peak of the red-absorbing form (Pr) of pea phytochrome (660 nm [23]). Assuming that the far-red absorbing form (Pfr) of the putative *Chlamydomonas* phytochrome has a similar absorption maximum to that of pea phytochrome (730 nm), we chose another filter (curve b) to provide more effective far-red light for the excitation of Pfr than does the other (curve c). The far-red background light passed through the filter b (Fig. 2B), however, elicited an intermediate effect between those elicited by the red and far-red background lights through filters a and c (Fig. 2A and C, respectively), suggesting that the photoexcitation of the putative Pfr had no effect. This is in line with the observation that, in the experiment described in section 3.1, abrupt positive phototaxis was elicited not only by the sudden change of the background light from red into infrared, but simply by turning off the red background light. These facts strongly suggested that the background light effect were ascribable to the absorption by chlorophylls (650–690 nm) rather than by phytochromes. This is further supported by the observed inhibitory effect of DCMU on the appearance of negative phototaxis under red background illumination (Fig. 2D,E).

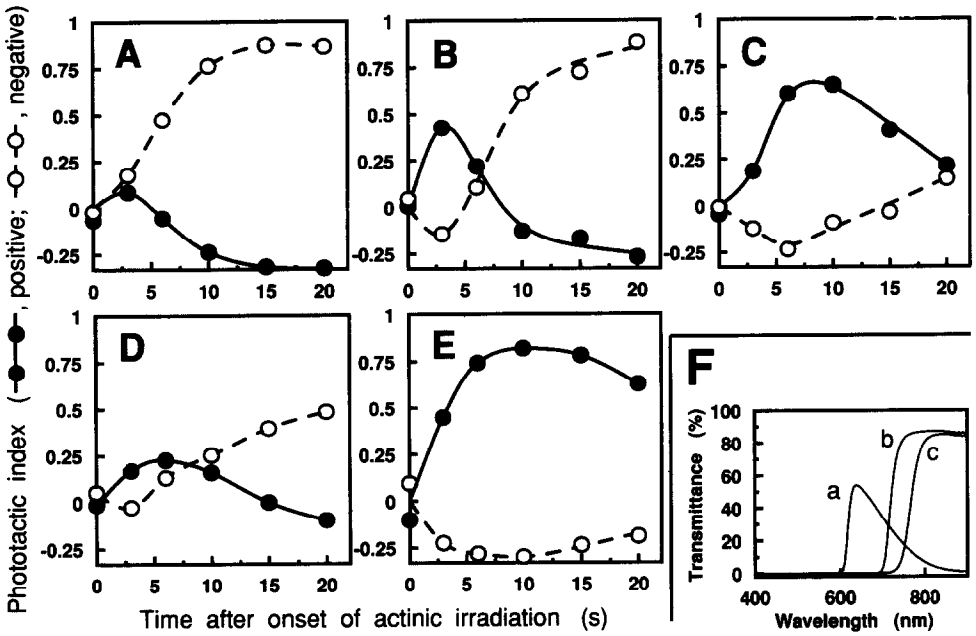

Fig. 2. Effects of background light on time-dependent phototactic orientation. Symbols at time zero designate the indexes before actinic irradiation. Actinic light: 470 ± 12 nm, 4.5×10^{13} photons/mm^2·s. (A) Red background light (see a in F) at 50 W/m^2. (B) Infrared background light I (see b in F) at 56 W/m^2. (C) Infrared background light II (see c in F) at 30 W/m^2. (D) Red background light at 50 W/m^2, 15–40 min after addition of 2×10^{-5} M DCMU. (E) Red background light, 15–40 min after addition of 8×10^{-5} M DCMU. (F) Transmittance spectra of optical filters that provide the red background light (a), the infrared light I (b) and the infrared light II (c).

4. DISCUSSION

Our results described above have demonstrated that photosynthesis plays an important role in regulating the sign of phototaxis in wild-type *C. reinhardtii*, namely, responsible for both the appearance of transient positive phototaxis and the observed background light effect on the sign of phototaxis. Consistently, neither such strong effect of red background light as in wild-type strains nor the prominent transient phototactic response was observed ([6] and our unpublished result) in carotenoid-deficient strains whose chlorophyll content is very low [2–5]. Whereas the primary photoreceptor for phototaxis of *Chlamydomonas* is evidently a rhodopsin (see introduction), photosynthesis affects the sign of phototaxis.

The mechanism by which photosynthesis controls the sign of phototaxis, however, remains as an open question. We have previously reported that a carotenoid-deficient blind mutant strain exhibited exclusively negative phototaxis after reconstitution of its photoreceptor with retinal (native chromophore) but showed positive phototaxis after reconstitution with retinal analogs [9]. Here, we should again point out that changes in angular velocity of the *Chlamydomonas* cell body that rotates during swimming might affect the sign of phototaxis because they may collapse the timing between the detection of the light signal and the phototactic correction of the direction of swimming [7–9]. Thus, in wild-type cells, a possibility exists that the increase in cellular ATP level as a consequence of photophosphorylation increases the angular velocity of the cell body as a consequence of a possible increase in linear swimming velocity, thereby affecting the sign of phototaxis. However, we think this unlikely because the abrupt directional change appeared much faster than we observed a decrease or increase in the swimming velocity of the cells after a sudden change in the background illumination (data not shown). We speculate that a more plausible mechanism so far is that the change in the phototactic direction is caused by a light-induced rapid flux of some ionic species through chloroplast membranes. For example, light-induced Ca^{2+} influx has been demonstrated in spinach chloroplasts [25,26], and if it also takes place in *Chlamydomonas* cells, phototaxis should possibly be affected because Ca^{2+} is an important ion for the photomovement responses of *C. reinhardtii* [8,12,13,27–30]. The role of Ca^{2+} in the appearance of the transient positive phototaxis was also discussed in a recent report [6].

Acknowledgements: This study was supported in part by Grants-in-Aid for Scientific Research (04302065, 04305006) from the Ministry of Education, Science and Culture, Japan and NIBB cooperative research program for the OLS (92-530).

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