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Photokinesis of Cyanobacterium *Synechocystis* sp. PCC 6803

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Motile cyanobacterium *Synechocystis* sp. PCC 6803 cells show photomovement with respect to the light stimulus. Under lateral irradiation, *Synechocystis* displays a phototactic gliding movement toward the light source by a two-dimensional random biased walk. Under vertical irradiation, *Synechocystis* decreased the frequency of mean vectorial gliding speed dependent on the applied fluence rate, whereas the deviation distribution width of the speed increased. This strongly suggests the involvement of photokinesis. Evidence for the cyanobacterial photokinesis was discussed in the previous report (Choi *et al.*, 1999. *Photochem. Photobiol.* **70**, 95-102) demonstrating that the gross scalar speed of vertically irradiating cells increased by about 50% compared with that of dark-adapted cells. In the visible wavelength range, *Synechocystis* cells showed a maximal photokinetic activity at 420 nm and a second maximal activity at 680 nm. The threshold action spectrum for the photokinesis resembles the absorption spectrum of chlorophyll with major differences in the phototaxis action spectrum at 560 nm and 660 nm. We postulate that the cyanobacterial photokinesis is powered by the energy-generating chlorophyll pigments.

key words: *Synechocystis* sp. PCC 6803, phototaxis, photokinesis, threshold action spectrum

INTRODUCTION

Cyanobacteria respond to the quantity and quality of light and search actively for a suitable environment for best survival and growth. Cyanobacteria show photomovements through type IV pili-dependent gliding motility [1, 2]. The photomovements of cyanobacteria encompass phototaxis, photokinesis, and photophobic responses [3]. Cyanobacterial phototaxis is defined as the oriented movement towards the light direction. Previously the phototaxis of *Synechocystis* sp. PCC 6803 (henceforth referred to *Synechocystis*) was photophysiologically characterized in detail [1]. Photokinesis demonstrates that the speed of movement depends on the light intensity irrespective of light direction. Cyanobacterial photokinesis has been studied in the red alga *Porphyridium cruentum* [4] and the filamentous cyanobacterium, *Anabaena variabilis* [5]. The action spectrum of photokinesis in *Phormidium* sp. correlates partly with that of photosynthesis [6]. Therefore, some part of the photosynthesis machinery could be involved in photokinesis. Photophobic response, denoted as a simple stop of movement followed by a resumption of movement in the opposite direction, is mediated by a sudden change (step-up or step-down) of light intensity [7, 8].

In the present study, we characterize the photomovement of *Synechocystis* cells with respect to the phototactic and photokinetic gliding behaviors at the single cell and population level, respectively. Data processing was performed by a computer-aided videomicroscopic system combined with analysis software Pathfinder. In addition, we will describe the possible photoreceptor involved in photokinesis, based on the comparison of cyanobacterial pigment absorption spectrum and threshold action spectrum.

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MATERIALS AND METHODS

Culture conditions and metabolic inhibitor

The motile cyanobacterium *Synechocystis* sp. PCC 6803 was donated from Pasteur Culture Collection and enriched for the actively phototactic moving species as described previously [1, 9]. The phototactic gliding cells were kept gliding toward the lateral irradiation of fluorescence white light source adjusted at 10 μmol/m^2/s on the surface of BG11-agar medium (0.4%) supplemented with 5 mM TES buffer (pH 8) at 28°C.

For experiments with a metabolic inhibitor, 1,3-dichlorophenyldimethyl urea (DCMU) was purchased from Sigma Chemical Co. (St. Louis, MO). The celll suspension was incubated for 30 min under dark conditions in presence of 10 μM DCMU followed by videomicroscopic data acquistion.

Videomicroscopic analysis of photomovement

Directionally gliding cells on 0.4% BG11-agar plate were separately collected and suspended in fresh BG11 liquid medium. Cell suspensions were then prepared for videomicroscopic recordings as described previously [1]. In brief, *Synechocystis* cells adjusted to about 150 cells on the 14" TV monitor were allowed to sink to the bottom in a cuvette with 0.15 mm depth. Then, the cells were exposed laterally to an 150 W fiber optic halogen lamp (KL1500 electronic, Schott, Wiesbaden)

as a stimulant light source equipped with a heat-absorbing quartz filter and videomicroscopic recording system (IBAS, Carl Zeiss, Eching) for the measurement of phototaxis. The photomovement was recorded for initial 5 min in the absence of the stimulus light and subsequently for 15 min under lateral irradiation with white light or monochromatic light at a constant fluence rate. The monitoring light beam of the microscope (Axioskop 50, Carl Zeiss, Eching) was filtered by an infrared bandpass filter transmitting above 800 nm which did not perturb the photomovement. For the measurement of photokinesis, cells were vertically irradiated with the built-in 50 W microscope halogen lamp connected to a neutral density filter in tandem to adjust the actinic fluence rate. The digitization process and the subsequent calculation of photomovement parameters were followed as described previously [1].

Cyanobacterial pigment analysis

Four milliliters of mid-log phase cell suspension was centrifuged at 10,000 *g* for 10 min at 4°C. The pellet was either stored at -20°C until analysis or immediately resuspended to the same volume in 20 mM sodium acetate buffer, pH 5.5. Cells were disrupted with a French pressure cell (SLM AMINCO, Urbana, IL) at 1,330 atm. Crude cell extract was precipitated with 1% (w/v) streptomycin sulfate for 30 min at 4°C and centrifuged at 10,000 *g* for 10 min at 4°C in order to eliminate membrane fragments containing chlorophyll. The amount of phycocyanin (PC) and allophycocyanin (APC) in the supernatant fraction was calculated from measurements of the optical densities at 620 and 650 nm using the following equations [10].

$$PC\ (mg/ml) = (OD_{620nm} - 0.7 \times OD_{650nm}) / 7.38$$

$$APC\ (mg/ml) = (OD_{650nm} - 0.19 \times OD_{620nm}) / 5.65$$

One milliliter of cell suspension was centrifuged at 10,000 *g* for 10 min at 4°C and the pellet was extracted with four milliliter of 90% (v/v) acetone for 1 hr at 4°C, in dim light, followed by centrifugation at 10,000 *g* for 10 min at 4°C. The amount of chlorophyll *a* and total carotenoids was calculated from the absorbance at 663, 645, and 450 nm using the following equations.

$$Chlorophyll\ a\ (g/ml) = 15.5 \times OD_{663nm} - 2.91 \times OD_{645nm}$$

$$Total\ carotenoids\ (g/ml) = 4.1 \times OD_{450nm} - 0.0435 \times chlorophyll\ a$$

Wavelength dependence of photokinesis

The effect of monochromatic light on photokinesis was measured as follows. The vertically irradiating light at 360, 420, 440, 460, 560, 660, 680, 730, and 760 nm was used as an actinic light source by appropriate combinations of each color filter (10 nm band width) and neutral density filters. During the continuous exposure to the population cells captured in a cuvette, the cells moving tracks were monitored and recorded automatically to the image analyzer. Subsequently, the photokinetic motility parameter expressed as gross scalar speed (μm/min) was calculated by the predefined algorithm Pathfinder. Wavelength dependence of photokinesis was expressed as the threshold photokinesis action spectrum by fitting the linear regression of the reciprocal of fluence rate at monochromatic wavelength. Here, we defined the threshold as the fluence rate at which the linear ascending fluence rate curve intersects the base line (13.8 μm/min) of gross scalar speed detected under dark conditions.

RESULTS AND DISCUSSION

Photomovement behavior at the single cell level

The gliding motility tracks of *Synechocystis* cells in BG11 liquid medium are shown in Fig. 1. The cells clearly showed typically phototactic gliding motility under lateral irradiation, whereas cells showed a random gliding motility under vertical irradiation. The typical motility tracks (Fig. 1C and D) and angular changes (Fig. 1E and F) of the photomovements were measured at the level of individual cells under steady-state irradiation conditions. Under dark condition, *Synechocystis* cells glided straight for a short time, followed by sudden tumbles and changed the moving track to a new direction. This motility pattern is known as a two-dimensional random walk [11]. The concept of random walk was developed while studying the motility pattern of the *Escherichia coli* chemotaxis [12]. The present results are consistent with the model of phototaxis as a biased random walk [13, 14]. Under unilateral irradiation, cells glided phototactically in a straight direction, followed by tumbles, and then reoriented towards the light source (Fig. 1A and C). During this process, the angular change in the path of cell movement was reduced (Fig. 1E) compared to vertical irradiation, indicating a biased random walk towards light. However, *Synechocystis* cells showed no preferred directional movement under vertical irradiation (Fig. 1B and D). The angular changes of the paths of cell movement (Fig. 1F) were frequently drifted from one direction to another during the photokinetic movement. The significant regular intervals between straight movement and tumbling period were not observed in the phototactic or photokinetic gliding of *Synechocystis* cells under this assay conditions.

Photokinetic behavior at population level

The distribution of vectorial gliding speeds of *Synechocystis* cells during the steady-state photoresponse time (5~15 min after the onset of stimulant white light irradiation) is shown in Fig. 2. Scattered data of vectorial gliding speeds were fitted to Gaussian curve. The mean vectorial gliding speed (*x*-axis displacement per unit time) was shown as 0.3 μm/min in darkness and 5.3 μm/min under lateral irradiation (phototaxis). Then, the whole distribution pattern was unexpectedly changed - i.e. the peak height under lateral irradiation was decreased compared to the darkness (Fig. 2A). This suggests

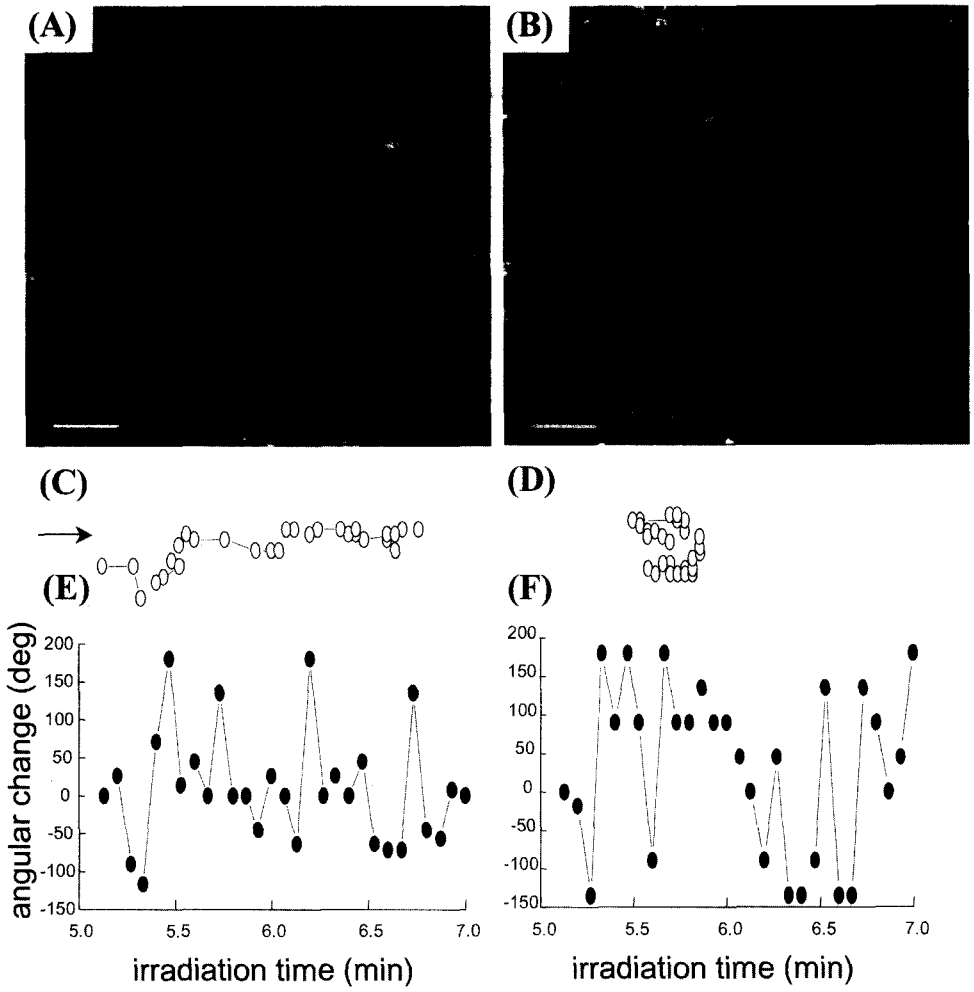

Figure 1. Cyanobacterial photomovement tracks under lateral and vertical irradiation. Top photographs are the results of overlaying 10 consecutive individual tracking images (12 interval) of *Synechocystis* cells (A, phototaxis; B, photokinesis) during 5 to 7 min after the onset of the stimulus (white light) at 3 μmol/m^2/s. Scale bar represents 10 μm. Cyanobacterial gliding photomovement diagrams of individual *Synechocystis* cells are presented as typical motility tracks (C and D) and their angular changes in those tracks (E and F), respectively, during 5 to 7 min after the onset of stimulant light irradiation. Arrows indicate the direction of the stimulus (white light).

that other photomovement components, such as photokinesis, may be involved in the lateral irradiation response. To verify the possibility of photokinesis in *Synechocystis* cells, the Gaussian curve fits were examined in darkness and under vertical irradiation conditions at two different fluence rates (Fig. 2B). As expected, there was no change of average gliding speed, however, the peak heights were decreased from 57% frequency under dark conditions to 50% and 39% at fluence rates of 5 and 25 μmol/m^2/s, respectively, under vertical irradiation (photokinesis). Thus, the gliding speeds, corresponding to the gross scalar speed defined as the integrated sum of cells' speeds in all direction, were directly calculated by a subprogram of Pathfinder. The possible inclusion of photokinesis in phototactic movement coincides well with the idea that phototaxis is a result of either photophobic or photokinetic response in *Euglena* [15].

Wavelength dependence of photokinesis

Since *Synechocystis* contains neither phycoerythrin nor phycoerythrocyanin [16], the colors of *Synechocystis* give an immediate indication of their changed contents of chlorophyll *a* and phycobilins (phycocyanin and allophycocyanin). *Synechocystis* cells cultured in liquid BG11 media till the late growth phase appear blue-green in color. The pigment contents of *Synechocystis* were quantified by measuring λ_{max} absorptions of the extracts. In intact *Synechocystis* cells, the main visible absorption spectra are attributable to chlorophyll *a* (*in vivo* λ_{max}=440, 685 nm) and phycocyanin (*in vivo* λ_{max}=640 nm), in which phycocyanin always predominates over allophycocyanin (Fig. 3). Absorption spectra of chlorophyll *a* and carotenoids were measured at room temperature by the purification of reverse phase chromatography with methanol extraction. The λ_{max} absorption peaks of the purified chlorophyll *a* peak were constantly at 430 and 665 nm, which showed a

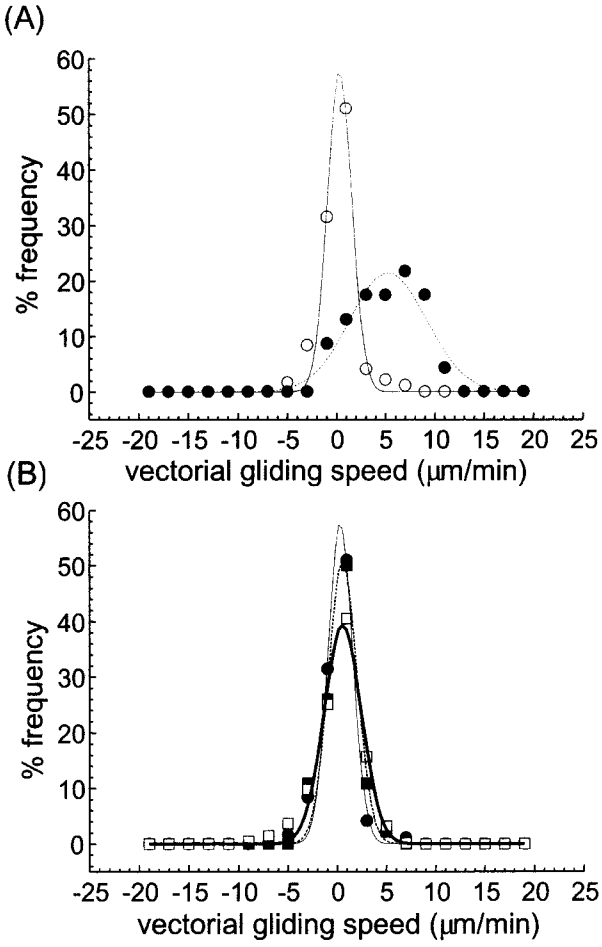

Figure 2. Distribution of vectorial gliding speed in *Synechocystis* cells under various white light conditions. The scattered data points were fitted to Gaussian curve under the dark (open circle, line) and lateral irradiation at 5 μmol/m²/s (closed circle, dashed line) (A) or under the dark (closed circle, straight line) and vertical irradiation at 3 μmol/m²/s (closed square, dotted line), or 30 μmol/m²/s (open square, thick straight line) (B). All data points were taken for 5~15 min after the onset of stimulus light. The cells over 300 were calculated by Pathfinder tracking software.

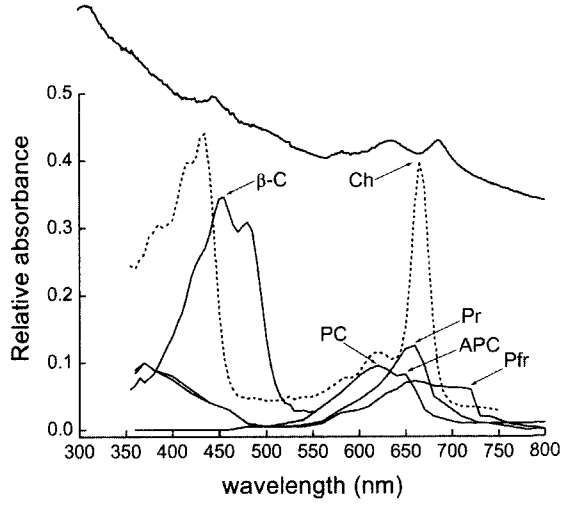

Figure 3. Absorption spectra of cyanobacterial pigments. All spectra were normalized offset for the convenient comparison. Arrows indicate the characteristic absorption peak of each pigment: chlorophyll *a* (Ch, at 665 nm from purified chlorophyll *a* and 685 nm from whole cell), β-carotene (β-C, at 455 nm from purified β-carotene), phycocyanin (PC, 620 nm from purified phycocyanin, 635 nm from whole cells), and allo-phycocyanin (APC, 650 nm from purified allo-phycocyanin). In the absorption spectra of phytochromes, red-absorbing (Pr) and far-red absorbing phytochrome (Pfr) show characteristic peaks at 660 and 730 nm, respectively [17, 18]. The upper solid line indicates the *in vivo* absorption spectrum of *Synechocystis* cells.

blue shift of 10~20 nm. Absorption spectra of phycobilins derived of water-soluble fractions of disrupted cell suspensions showed λ_{max} absorption peaks of phycocyanin and allophycocyanin at 630 and 650 nm, respectively. Reversibly photoconvertible phytochromes (adapted from the *in vitro* purified Cph1 holoprotein) showed characteristic absorption peaks at 660 and 730 nm in red- and far red-light absorbing phytochrome [17, 18].

To seek pigment(s) responsible for *Synechocystis* photokinesis, the absorption spectra of intact cells and purified phycobilins, chlorophyll *a*, and carotenoids were measured and compared to the photokinetic activity of *Synechocystis* cells at specific wavelengths. The wavelength dependency of photokinesis was measured by calculating the reciprocal of threshold fluence rate from the linear regression curves of the total scalar migration distance of *Synechocystis* (Fig. 4). The comparison of the threshold partial action spectrum with the *in vivo* absorption spectrum shows that blue (420 nm) and red (680 nm) regions are the most effective wavelength to induce photokinesis in the range of visible wavelength for photokinetic movement. The partial action spectrum of photokinesis showed apparently different pattern compared to the action spectra of *Synechocystis* positive phototaxis [1] and the dephosphorylation of a 65-kDa phosphoprotein induced by light [19]. One of the most conspicuous differences is that yellow green (560 nm) region did not induce a significant photokinesis response (Fig. 4). Therefore, the partial action spectrum of photokinesis we obtained suggests that chlorophyll *a* is the main photoreceptor pigment and the phycobilins are not involved. The notion that chlorophyll *a* is a possible photoreceptor of *Synechocystis* photokinesis is strongly supported by the fact that far-red (760 nm) light did not induce any photokinesis response (Fig. 4) since chlorophyll *a* does not absorb wavelength longer than 700 nm.

To examine whether the photosynthetic electron transport is, if any, involved in *Synechocystis* photokinesis, DCMU known as a specific metabolic inhibitor of non-cyclic photophosphorylation was added into the assay media. We observed little influence of 10 mM DCMU treatment on the photokinesis of *Synechocystis* cells at blue (420 nm) and red (680 nm) regions, which is the most effective wavelength in the visible

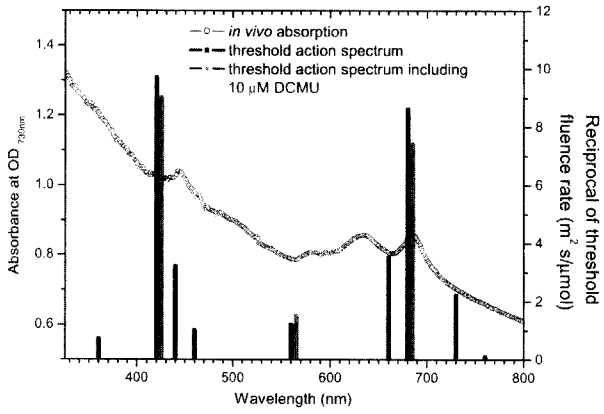

Figure 4. Threshold action spectrum of photokinesis in *Synechocystis* cells. Photon sensitivity (the reciprocal of threshold fluence rate) at each specific wavelength was determined by the x-intersect at 13.8 μm/min corresponding to the baseline detected under the dark, which was calculated by the best fitted linear regression (r^2>0.92 at all specific wavelength) from the ascending slope of fluence rate response curve. Each data of threshold fluence rate were statistically determined with more than three data points at the range of fluence rates (0.1 to 30 μmol/m²/s), representing control (black vertical lines) and DCMU-treated group (gray vertical lines). The *in vivo* absorption spectrum (upper open circle and straight line) was overlaid for the comparison of threshold action spectrum.

wavelength range (Fig. 4). These results match well with previous reports that DCMU affects neither the gliding speed of *Synechocystis* wild-type cells in liquid suspension [1] nor the gliding motility of those cells on the agar surface plate [20]. Since the *Synechocystis* photokinesis was hardly impaired by DCMU, which inhibits the linear electron transport chain between photosystem II and photosystem I just beyond the primary electron acceptor plastoquinone of photosystem II, cyclic electron transport by photosystem I might be responsible for photokinesis in *Synechocystis*. The prediction of chlorophyll *a* as a possible photoreceptor pigment of cyanobacterial photokinesis is supported by observations in *Phormidium uncinatum* [21, 22], *Phormidium cruentum* [4] and *Oscillatoria princeps* [23]. In addition, DCMU did not affect the photokinesis in non-cyanobacterial photosynthetic bacterium such as *Rhodosprillum rubrum* [24] as observed in our system. In general, the photosynthetic bacteria generate the maximum energy at the regions of blue and red light, at which major photosynthetic pigment, chlorophyll *a* absorbs. However, photoreceptors for the photomovement are not always identical to the pigments for light harvesting. Some non-photosynthetic bacteria use specific wavelengths of light just as a triggering signal for photomovement [25]. In conclusion, we suggest that the photokinetic movement is primarily involved in the energy-generation system powered by chlorophyll *a*.

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