Protoplasma (1991) 161: 17–22

PROTOPLASMA

Action spectra for phototaxis in zoospores of the brown alga *Pseudochorda gracilis*

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Received June 4, 1990
Accepted October 5, 1990

Summary. Action spectra for phototaxis in zoospores of brown alga, *Pseudochorda gracilis* (Laminariales), were examined in the wavelength range between 300 and 600 nm using the Okazaki Large Spectrograph and a video tracking system. The direction of swimming (both in percent cells swimming in parallel with the stimulating light, and in mean angle of cell movement) was dependent on the wavelength. The action spectra had two peaks at 420 and 460 nm, while light above 500 nm was not effective in changing the swimming direction of the cells.

Keywords: Phototaxis; Zoospore; *Pseudochorda gracilis*; Action spectrum; Video-tracking.

Abbreviations: TCMA tracker-cell movement analyzer system; CMA cell movement analyzer program.

Introduction

Since the life of most algae is based on photosynthesis, it is essential for them to move to a place where there are favourable light conditions. Under water, however, light intensity especially changes dramatically with depth and time of day. Most planktonic algae are able to detect the orientation and intensity of light, and to move towards or to escape from it. These phototactic responses are mostly blue-light or blue-green-light responses but the exact mechanism and photoreceptive pigments involved have yet to be elucidated (Lenci and Ghetti 1989, Nultsch and Häder 1988).

Brown algae are mostly sedentary benthic macroalgae. However, they have flagellated reproductive cells (Fig. 1) which often show obvious phototaxis. Recently, a flavin-like substance has been reported to occur in the posterior flagella of brown algal swarmers, and it has been suggested that this substance is involved in photoreception for the phototactic system (Müller et al. 1987, Kawai 1988). Although Halldal (1958) reported action spectra for phototaxis of gametes of the green alga, *Ulva*, there have been no works on action spectra for brown algal gametes or zoospores, except one by Kawai et al. 1990. This report describes measurements of the action-spectra of phototaxis in individual zoospores of the brown alga *Pseudochorda gracilis* (Laminariales, Phaeophyceae).

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Materials and methods

Cells

Sporophytes of *Pseudochorda gracilis* (Kawai and Nabata 1990) were cultured in PESI medium (Tatewaki 1966) at 5 °C, under short day conditions (8:16 LD) illuminated by white fluorescent tubes (ca. 30 μmol $m^{-2}s^{-1}$). Fertile algae were maintained at 4 °C in the dark until the experiment at Okazaki. Zoospores were released by elevated temperatures and intense illumination. Cells were concentrated using photoaccumulation, kept in a cold (4 °C) dark place and examined for their phototactic activity within 1 h after release.

Video tracking measurements

Measurements were made using the monochromatic light (at 20 nm intervals) from a large spectrograph (Okazaki Large Spectrograph; Watanabe et al. 1982, Watanabe 1985) and processed using a computerized cell-movement tracking system (TCMA; Kondo et al. 1988) in the National Institute for Basic Biology at Okazaki. Freshly released zoospores of *Pseudochorda* were placed in a very thin cuvette, made on a slide glass with coverslips (ca. 15 × 15 × 0.1 mm), on the objective stage of a microscope (Fig. 2). Zoospore movements were recorded for 24 s (16 time segments of 1.5 s: 4.5 s before, and 19.5 s after the onset of light stimulation). At least three measurements were made at each wavelength, using different preparations. Since preliminary experiment showed that light of wavelength longer than 620 nm had no effects on the movement of the zoospore, observations were made via an R-62 red cut-off filter. Fluence rates of the monochromatic lights were measured with a photon density meter, HK-

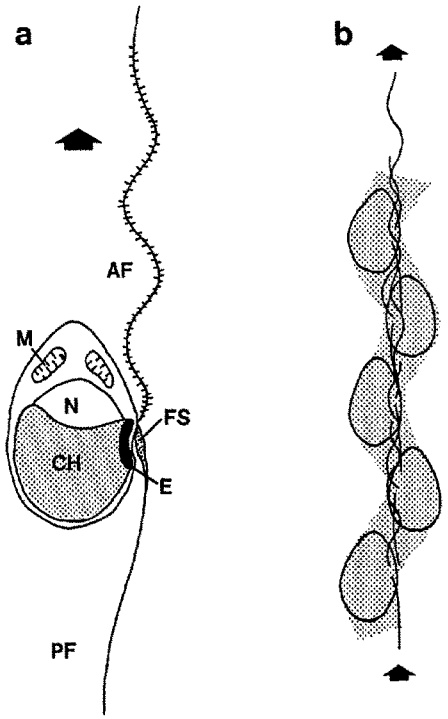

Fig. 1. Schematic representation of a zoospore of the brown alga (**a**) and its helical swimming movement (**b**). Arrows indicate the direction of swimming. *AF* Anterior flagellum, *CH* chloroplast, *E* eyespot, *FS* flagellar swelling, *M* mitochondrion, *N* nucleus, *PF* posterior flagellum. Dotted zone corresponds to the track recorded on TCMA system (see Fig. 3)

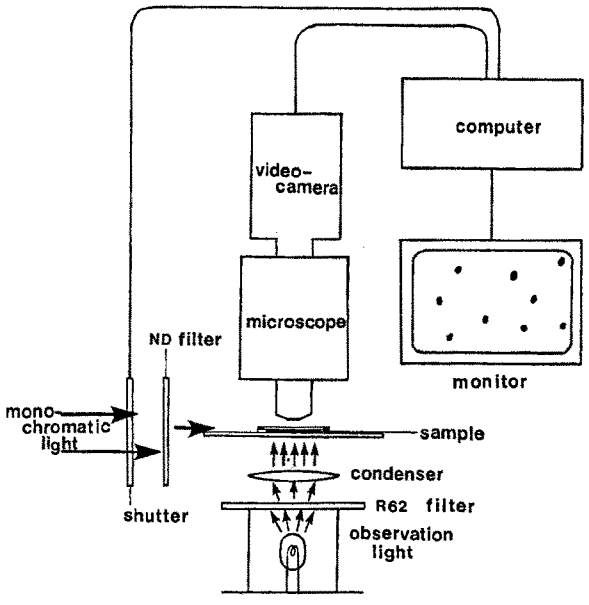

Fig. 2 Diagram of the measurement system. See text for explanation

1, costum-made by the Institute for Physical and Chemical Research, Saitama, Japan (Hashimoto et al. 1982). Neutral density filters were used to adjust fluence rate to 1×10^{15} photons $cm^{-2}s^{-1}$ at each wavelength in front of the sample cuvette. To quantify the data, the angle of movement of each cell to the light source and the distance moved in each time segment were measured manually and by a computer program (CMA; Kondo et al. 1988).

Results

Light induced changes in swimming tracks

Figure 3 shows the results of the movement of zoospores before and after irradiation with unilateral monochromatic light of 460 nm until time segment number 15. The undulating patterns occur because the brown algal swarmer rotates as it swims, around the axis of the laterally inserted anterior flagellum (Fig. 1 b). Each color shows one time segment in the chronological order of blue, red, green. In the first three time segments (Fig. 3 a, −4.5–0 s) the movements are random. After the beginning of illumination (the fourth time segment), within 3 s, most of the zoospores start to swim in the direction of the light source (Fig. 3 b). The path of the phototactically swimming cells was usually very straight (Fig. 3 c–e). The phototactic response was mostly positive, although some zoospores, less than ca. 10% of the total, showed negative phototaxis at any light conditions tested.

Wavenlength dependency of the change in velocity of swimming by the stimulating light

An apparent increase in velocity of up to about 15% was recorded after illumination between 420 and 480 nm (Table 1). This occurs because phototactically swimming zoospores swim towards the light source mainly in the plane of the objective stage, while non-responding zoospores swim with a vector component perpendicular to the light beam axis. Accordingly, the apparent two-dimensional velocity is larger in phototactically responding cells.

Action spectra for phototaxis

Figure 4 a shows, as a function of wavelength, the proportion of zoospores of which the acute angle between the swimming tracks and the optical axis of the stimulus light were within 15°. In the dark control this proportion was between 4% and 14%, while an expected proportion in randomly moving population is about 8.3%. Accordingly, the experimental error is estimated to be less than ca. 5%. This action spectrum for phototaxis clearly shows two major peaks at 420 and 460 nm. Figure 4 a shows results obtained manually and by the CMA program. Although two results correlate well to each other, the peaks obtained by the CMA program are smaller than those obtained by man-

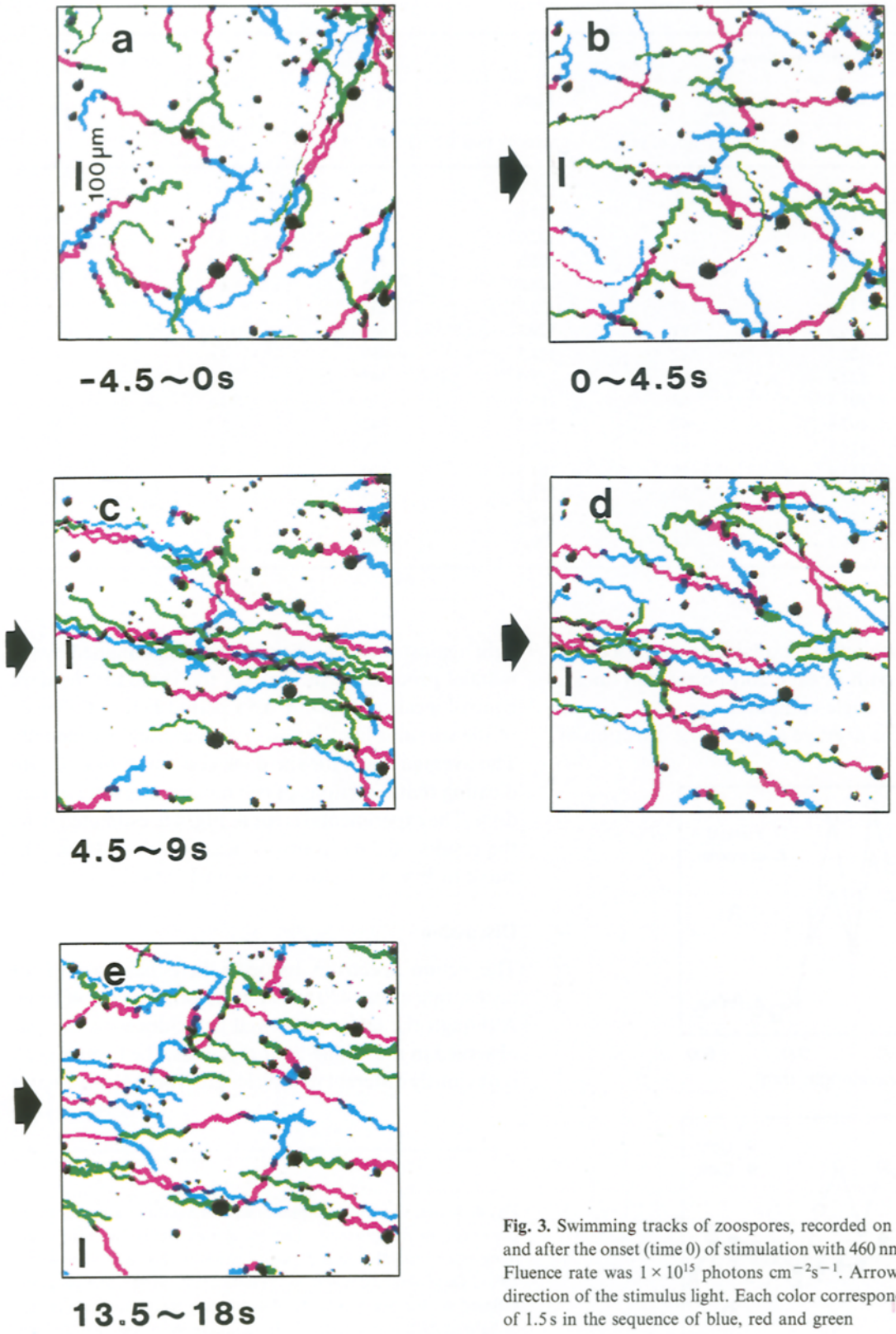

Fig. 3. Swimming tracks of zoospores, recorded on TCMA system, before and after the onset (time 0) of stimulation with 460 nm monochromatic light. Fluence rate was 1×10^{15} photons $cm^{-2}s^{-1}$. Arrows indicate the incident direction of the stimulus light. Each color corresponds to one time segment of 1.5 s in the sequence of blue, red and green

Table 1. Comparison of swimming velocity at each wavelength measured

Wavelength (nm)	Light conditions				Velocity increase in light (% of dark)
	Dark		Light		
	velocity (μm sec^{-1})	no. of cells	velocity (μm sec^{-1})	no. of cells	
300	126.9	106	126.0	331	−0.7
320	117.1	96	117.6	324	0.4
340	119.0	116	122.7	440	3.1
360	119.0	107	125.5	379	3.5
380	127.4	140	128.8	525	1.1
400	125.5	147	129.7	538	3.3
420	116.2	91	129.7	485	11.6
440	122.7	112	128.3	409	4.6
460	122.3	184	133.0	565	8.7
480	101.3	68	115.3	316	13.8
500	107.8	42	109.2	142	1.3
520	111.1	81	110.1	238	−0.9
540	113.9	19	104.5	100	−8.3
560	110.1	30	117.1	99	6.4
580	126.9	14	119.5	102	−5.8
600	115.7	24	109.7	96	0.8

ual processing, which may be explained by the fact that in many cases the path of each zoospore is not linear, producing errors in angle computations.

Figure 4 b shows the average angle to the direction of light against wavelength using the data obtained by the manual processing. In Fig. 4 b, the value for the dark control measured for the 4.5 s period before the onset of the stimulus light is also shown at each wavelength. The average angel for the dark control is 90 ± 10°, indicating that directions of cell movements were at random. The experimental error in Fig. 4 b, estimated from the results of dark controls, was less than 10%. The curve in Fig. 4 b follows those in Fig. 4 a.

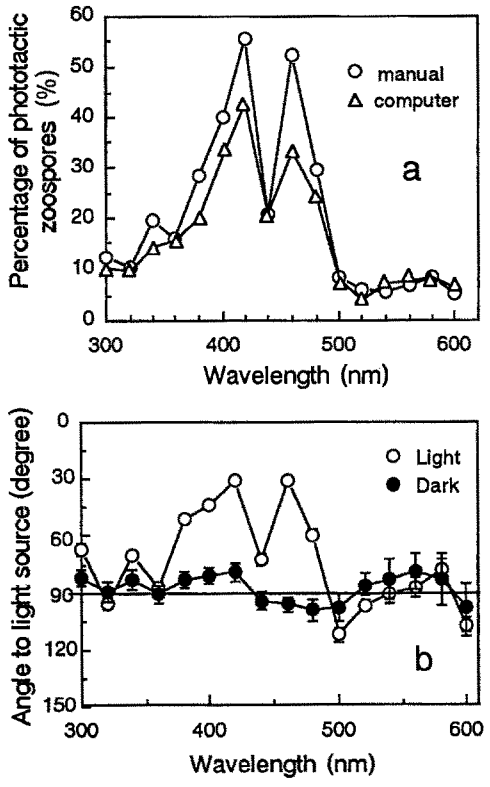

Fig. 4. Action spectrum for phototactic orientation. **a** Based on the percentage of zoospores of which the acute angle between the swimming tracks and the optical axis of the stimulus light were within 15°. ○ Data obtained manually; △ data obtained by CMA program. **b** Based on the average angle of swimming tracks to the direction of light at each wavelength. ○ In the presence of stimulating light; ● 4.5 s dark period before irradiation (dark control). Error bars indicate standard errors. Data points without error bars mean that the standard errors are less than half the size of the point marks

Discussion

The action spectrum reported here has two major peaks, one at ca. 420 nm and the other at ca. 460 nm. Although the latter peak well coincides with the one observed in most blue-light reactions, the former peak is obviously different in wavelength from another major

peak (at ca. 380 nm) found in most blue-light reactions, including phototaxis in *Euglena* (Galland and Senger 1988 a, b; Nultsch and Häder 1988; Watanabe 1988; Lenci and Ghetti 1989; Sugai and Furuya 1990).

In this connection, it is noteworthy that Kawai et al. (1990) have reported a very similar action spectrum, with major peaks at ca. 430 and ca. 460 nm, for phototaxis in male gametes of another brown alga, *Ectocarpus siliculosus*. Their experiment was done independently in a different laboratory using similar methodology (a computerized cell tracking system; Häder and Lebert 1985) to this experiment. *Ectocarpus* (Ectocarpales) is considered to be one of the most primitive members of the brown algae, while *Pseudochorda* belongs to a highly derived order of the Laminariales. It should also be noted that gametes were used in the *Ectocarpus* experiment, while zoospores were used in the *Pseudochorda* experiment. However, these two very similar action spectra reported for these two species suggest the existence of a photoreceptor system for phototaxis which is common in brown algae but somewhat different, either chemically or geometrically, from other blue-light perceiving systems, such as phototaxis in *Euglena*. It is quite interesting to note that another strikingly similar action spectrum has been reported for hair whorl formation in the gigantic unicellular green alga, *Acetabularia* (Schmid 1984).

The in vivo action spectrum for photoreactivation in *Streptomyces* (Jagger et al. 1970) and the in vitro action spectrum of the purified enzyme (Eker 1978) have a peak at ca. 445 nm and lack the obvious near-UV peak (at about 380 nm) typical of absorption spectra of riboflavin and flavoenzymes with FMN or FAD as chromophores (Galland and Senger 1988 a). Other organisms with photoreactivation action spectra similar to that of *Streptomyces* include cyanobacteria *Agmenellum* (Van Baalen and O'Donnell 1972) and *Anacystis* (Saito and Werbin 1970), the halophilic bacterium *Halobacterium* (Iwasa et al. 1988) and the green alga *Scenedesmus* (Eker 1983). The chromophore of the *Streptomyces* DNA photolyase is a 8-hydroxy-5-deazaflavin (Eker et al. 1981). The free 8-hydroxy-5-deazaflavins from *Streptomyces* have absorption spectra with a peak at 420 nm and also without the near-UV peak, at pH 8.3 (Eker et al. 1981). Therefore, it is a tempting hypothesis that the action spectra for the brown algal phototaxis and for hair whorl formation in *Acetabularia* might be explained by combining the absorption spectra of free (for 420 nm peak) and protein-bound (for 450–460 nm peak) 8-hydroxy-5-deazaflavins.

Acknowledgements

This study was carried out under the NIBB (National Institute for Basic Biology) Cooperative Research Program for the use of the Okazaki Large Spectrograph (Project No. 88–502). We thank Mr. Ichiro Mine, Miss Noriko Yonezawa and Mrs. Hisae Kiyohara for technical assistance; Dr. Ian G. Gleadall (NIBB) for critical reading of the manuscript.

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