Photomovement in *Dunaliella salina:* Fluence rate-response curves and action spectra

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Abstract. We determined the action spectra of the photophobic responses as well as the phototactic response in *Dunaliella salina* (Volvocales) using both single cells and populations. The action spectra of the photophobic responses have maxima at 510 nm, the spectrum for phototaxis has a maximum at 450-460 nm. These action spectra are not compatible with the hypothesis that flavoproteins are the photoreceptor pigments, and we suggest that carotenoproteins or rhodopsins act as the photoreceptor pigments. We also conclude that the phototactic response in *Dunaliella* is an elementary response, quite independent of the step-up and step-down photophobic responses. We also determined the action spectra of the photoaccumulation response in populations of cells adapted to two different salt conditions. Both action spectra have a peak at 490 nm. The photoaccumulation response may be a complex response composed of the phototactic and photophobic responses. Blue or blue-green light does not elicit a photokinetic response in *Dunaliella.*

Key words: Action spectra (*Dunaliella*) – Blue and blue-green light responses – *Dunaliella* – Light-induced motile responses – Photophobic responses – Phototaxis

Introduction

Plants have a remarkable ability to sense the character of the external environment through the perception of light, thereby modifying their behavior, growth or development in order to respond to environmental conditions appropriately. At the cellular level, light affects various motile phenomena. These include chloroplast movement (for reviews see Haupt 1982; Zurzycki 1980) and cytoplasmic viscosity (Virgin 1951), responses that help ensure efficient levels of photosynthetic activity. Numerous and diverse motile responses to light are found in flagellated algal cells; they allow the organisms to find the optimal position for photosynthetic efficiency in their environment (for reviews see Nultsch 1980; Häder 1988).

The inventory of photomotile responses of flagellated cells includes 1) the phototactic response, in which the cells sense the direction of light and swim toward it; 2) the photophobic responses, in which the cells sense a change in the level of irradiance with respect to time and either make an apparent stop or a 90° turn, depending on whether there is an increase (step-up) or a decrease (step-down) in the light fluence, respectively; and 3) the photokinetic response, in which the swimming speed is influenced by light. The ultimate goal of our research is to elucidate the steps involved in the signal-transduction chains that couple the light stimuli with the various elementary responses in the wall-less, unicellular, motile, biflagellate Volvocalean alga, *Dunaliella.* However, there is some question as to what is an elementary response and what is a complex response composed of repetitive elementary responses (for a review of the arguments see Colombetti and Petracchi 1989). For example, Diehn (1980) considers that the positive phototactic response in *Euglena* is not an elementary response but is just a manifestation of a series of step-down photophobic responses, where the cell responds to a decrease in irradiance by undergoing a 90° turn. In this way the cell tracks the light direction by turning every time it begins to swim away from the light. Similarly, Schletz (1976) suggests that the phototactic response in *Volvox* may not be an elementary response, but can be explained as a result of a series of step-up photophobic responses, where the cilia on the lighted side respond to an increase in light and stop beating. Consequently, the colony turns toward the light.

As a first step in unraveling the elements of the signal-transduction chains that lead to the various light-activated motile responses in *Dunaliella,* we used action
spectroscopy to establish whether or not a response is an elementary response, and to identify the possible photoreceptor pigments that may activate the responses. In the present paper, we characterize the spectral requirements (380–600 nm) of the step-up photophobic, the step-down photophobic and the phototactic responses of Dunaliella using studies with single cells that are facilitated by computer-assisted video microscopy. We show that the phototactic response in Dunaliella is an elementary response and not based on photophobic responses as found in Euglena and Volvox and extrapolated to other organisms. We also characterize the spectral requirements of the photoaccumulation response of populations and suggest that the photoaccumulation response may be a complex response composed of two or more of the above elementary responses.

Material and methods

Culture of Dunaliella cells. Unless otherwise stated, Dunaliella salina (Dunal.) Teod. (strain 1644 from the University of Texas Culture Collection, Austin, USA) was grown to a density of about 5 × 10^5 cells m^−1 (mid-logarithmic phase) in aerated modified AS100 medium (J. Brand, Botany Department, University of Texas, Austin, USA; personal communication). The modified medium included MgSO_4·7H_2O, 9.9 mM; KCl, 8.05 mM; NaNO_3, 11.77 mM; CaCl_2, 2.05 mM; 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris base), 8.26 mM; KH_2PO_4, 367 μM; H_3BO_3, 554 μM; H_2SO_4, 180 μM; ZnCl_2, 2.31 μM; Na_2EDTA, 161.16 μM; FeCl_3, 17.98 μM; (NH_4)_2SO_4, 252 mM; CoCl_2, 5 mM; MnCl_2, 2 mM, and 10% NaCl. The medium was titrated with HCl to pH 8.08. The cells were grown in growth chambers (model MIR-150; Sanyo Electric Co., Tokyo, Japan) at 28°C under continuous irradiation from fluorescent lamps (model FL20SS·W/18; Toshiba Lighting and Technology, Tokyo) with an energy fluence rate of 8.6 W·m^−2 as measured with a United Detector Technology (Santa Monica, Calif., USA) model 81; Optimeter equipped with a radiometric sensor. A subpopulation of the same strain of cells, which was conditionally grown for many years to grow on a low-salt medium, was obtained from Dr. K. Hara (Tsukuba University, Tsukuba, Japan). These cells were grown to a density of 5 × 10^8 cells m^−1 (mid-logarithmic phase) in GPM medium (Loeblich 1975) at 25°C under the same continuous irradiation as the strain-1644 cells. The GPM medium includes 75% sea water (approx. 2.3% NaCl).

Measurement of photoaccumulation. In large-scale qualitative experiments, 200 ml of cells were placed in clear plastic rectangular chambers, and the monochromatic light from the Okazaki Large Spectrograph, National Institute for Basic Biology, Okazaki, Japan (Watanabe et al. 1982) was focused on various levels with two double convex lenses (see Fig. 1). The energy fluence rate was measured with an Epply (Newport, R.I., USA) temperature-controlled thermocouple connected to a nanovoltmeter (TR 8513B; Takeda Riken, Tokyo). Photoaccumulation, in this paper, is defined as the accumulation of a population of cells in the region of the chamber closest to the light source when the light beam is parallel to the long axis of the chamber.

For the small-scale, quantitative experiments, monochromatic light (spectral full width at half maximum = 6 nm) was obtained from the spectrograph using a quartz optical light guide. Photon fluence rates were varied by inserting custom-made neutral-density filters into the light path. Photon fluence rates were measured with a photon density meter (model HK-1; RIKEN, Saitama, Japan). In these experiments, 269 μl of cells (5 × 10^6 cells m^−1) were placed in a glass cuvette (38 mm long, 3 mm in diameter) that was immediately inserted in a phototaxometer, similar to that designed by Feinleib and Curry (1967). Briefly, the measuring beams were produced by red-light-emitting diodes (Radio Shack, Fort Worth, Tex., USA) placed perpendicular to, and at the front and back of the cuvette. The red beams were directed to the opposite side of the cuvette, where two cadmium sulfide (CdS) cells were placed. The changes in the resistances of the two CdS cells were amplified through two operational amplifiers. The outputs of these amplifiers were then compared and the voltage difference was recorded. Photoaccumulation, which causes an increase in optical density on the lighted side and a decrease in optical density on the shaded side of the cuvette, was recorded on a pen recorder (type 3066; Yokogawa Electric Works, Tokyo) and the rate of photoaccumulation was determined from the phototaxometer output in mV·min^−1. During an experiment the cells were shielded from all light except the actinic light. Experiments usually lasted between 1 and 2 min and were performed at 28°C under a dim red safelight. The photon fluence rates were corrected for the transmittance of the glass cuvette and the cell suspension by measuring the relative transmittance with a Hitachi (Tokyo) 557 spectrophotometer. The fluorescence of the cells and cuvettes as measured with a Hitachi MPF-4 fluorescence spectrophotometer was negligible (data not shown).

The tracker system. The tracker system is a computer-assisted video microscope that records, digitizes and displays the swimming tracks of approx. 50 cells per field (Kondo et al. 1988). The tracker system consists of a 2.54-cm silicon vidicon camera (C 1000–2; Hamamatsu Photonics, Hamamatsu, Shizuoka, Japan). The camera control unit includes a Hamamatsu M 1005 binarization board and a Hamamatsu M 998 I/O buffer. The digitized images (National Television Systems Committee fields) are fed into a BIWAC DMS–808 computer (Digi-Tek Laboratory, Ootsu, Shiga, Japan) every 1/60 s. Depending on the experiment, 60 or 120 fields are combined into 1 superfield and 8–16 superfields are collected. Each superfield represents 1 or 2 s. The superfields are printed consecutively with a color printer (Model G 500, Mitsubishi Electric Corp., Tokyo; or Model JP 80; Seiko Epson Corp., Shiojiri, Nagano, Japan). The video-processed image is also viewed on a graphics display terminal (model JCC–M1401 II; Japan Computer Corp., Tokyo). Two hundred megabytes of superfield data were collected and analyzed for the experiments reported here. Only cells that remained in the field for the entire experiment were analyzed.

The video camera was attached to an inverted microscope (IMT-2; Olympus Corp., Tokyo) equipped with bright-field optics. The infrared observation beam originated from a 12 V/50 W tungsten-halogen lamp and was passed through a Hoya (Tokyo) IR 83 glass filter. The heat filter was removed from the collecting lens apparatus. The light was then passed through a phase-contrast condenser with an ultra-long working distance (numerical aperture (N.A.) = 0.3). The cells were observed with a 4 × SPLAN PL objective lens (N.A. = 0.13), a 1.5 × optivar and a 5 × projection lens. All the lenses are commercially available (Olympus, Tokyo). A custom-built electronic shutter, controlled by the computer, was mounted on the microscope in order to regulate the actinic monochromatic light. The line voltage was filtered with a stabilized D.C. power supply (Model PAB 32–2; Kikusui Electr ions Corp., Kawasaki, Kanagawa, Japan) before it was connected to the microscope.

The monochromatic light (spectral full width at half-maximum approx. 1 nm) used for the single-cell experiments was obtained from the spectrograph. The photon fluence rate was measured with the HK–1 photon density meter (RIKEN). During the experiments, the cells were shielded so that they were only exposed to actinic light and the infrared observation beam.

For single-cell experiments, the cells were placed in 15 mm wide, 10 mm long, 0.17 mm deep optically clear chambers. The chambers were covered with a cover glass to prevent the entry of oxygen and the occurrence of oxygen taxis. The imaging light was focused on cells in the center of the chamber.

Some of the experiments reported here were repeated on a Zeiss (Micro-Med Instruments, Walden, NY, USA) IM 35 inverted microscope connected to a commercially available Motion Analysis video processing and analyzing system (Motion Analysis Corp., Santa Rosa, Calif., USA). In this case the optical path included a